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Studies on Fe Complexes Produced by Yeast. III. Characteristics of Fe Absorption from an Fe(II)–Oligosaccharide Complex in the Rat Gastrointestinal Tract

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Gastrointestinal Fe absorption from an Fe(II)–oligosaccharide complex (designated Bl-c) produced in wine by yeast and showing high hematopoietic activity in rats was examined. Oral administration to rats of ^{59}Fe-labeled Bl-c (10—250 μg Fe/body) resulted in effective Fe absorption and incorporation into hemoglobin, particularly at high doses (both parameters at 250 μg Fe/body were about 3 times and twice the values with $FeSO_4$ and ferrous ascorbate, respectively, used as reference compounds). When Bl-c was injected into rat gastrointestinal loops, the duodenum showed the highest Fe absorption. Experiments with duodenal loops and everted rat duodenal sacs showed that both Fe tissue uptake and transfer to the serosal side from Bl-c tended to saturate at high concentrations, depended on the temperature and pH of the mucosal medium, and were affected by metabolic inhibitors. This suggests that a special transport system other than passive diffusion may be involved in gastrointestinal Fe absorption from Bl-c and contribute to the high absorption of Bl-c. Gel filtration patterns of Fe transferred into the serosal medium before and after glycosidase treatment and its infrared spectra indicated that most of the Fe transferred from the Bl-c-added mucosal medium (except at quite low concentrations) was an oligosaccharide–Fe complex similar to intact Bl-c.

Keywords——ferrous complex; oligosaccharide; gastrointestinal absorption; ferrous transport system

Introduction

Previously, we separated from wine an Fe(II) complex produced by *Saccharomyces cerevisiae* and revealed that (a) the complex, designated Bl-c, had a molecular weight of around 1500, and its ligand was an oligosaccharide composed of an uronic acid and neutral sugars; (b) the complex, when administered orally to rats, showed high gastrointestinal Fe absorption and incorporation of Fe into hemoglobin; and (c) the high Fe solubility of the complex at intestinal pH might be a reason for the high Fe absorption.[1,2]

Regarding the mechanism of Fe absorption, a special transport system, other than passive diffusion, has been considered to be involved,[3-8] and recent studies on nonheme Fe have referred to the presence of specific Fe-binding sites on intestinal brush-border membranes and to the characterization of a carrier-mediated Fe transport system.[9-16] Heme Fe and some Fe complexes and chelates administered orally have also been reported to enter the mucosal cells or even the blood stream in the form of Fe bound to ligands.[6,17-19] Thus, the Fe transport system and kinetics seem to vary with the chemical form of Fe. Accordingly, in order to elucidate factors favoring Fe absorption from Fe compounds, it is indispensable to investigate not only luminal factors (solubilities and forms of Fe compounds in the digestive tract) but also mucosal factors (Fe transport system and kinetic parameters in the digestive tract).

In this study, characteristics of Fe absorption from Bl-c in the rat gastrointestinal tract in relation to mucosal factors were examined *in vivo*, *in situ* (ligated gastrointestinal loop method), and *in vitro* (everted duodenal sac method), using $FeSO_4$ and ferrous ascorbate as reference compounds.

Materials and Methods

Preparation of ^{59}Fe-Labeled Fe Complexes——Wine containing ^{59}Fe-labeled Fe complexes was prepared, and the fraction designated Bl-c was separated and purified by column chromatography, as reported previously.[1)]

Animals——Six-week-old male Jcl: SD rats (CLEA Japan, Inc.) were deprived of food overnight before use.

***In Vivo* Absorption**——(i) Preparation of Test Solutions: ^{59}Fe-Labeled Bl-c prepared as above and two reference compounds ($FeSO_4$ and ferrous ascorbate) labeled with ^{59}Fe by the previously described method[1)] were adequately mixed with nonlabeled Fe compounds to make test solutions containing 10—250 μg Fe/about 5 μCi/ml.

(ii) Administration and Determination of Fe Absorption and Incorporation into Hemoglobin: The *in vivo* experiment was performed as reported previously.[1)] Each test solution was administered into the stomach in a volume of 1 ml per 160 g body weight. The amounts of ^{59}Fe absorbed through the gastrointestinal tract and incorporated into hemoglobin were calculated, using the equations shown previously,[1)] from data on fecal excretion (0—168 h) and blood ^{59}Fe levels (at 168 h), respectively.

***In Situ* Absorption (Ligated Gastrointestinal Loop Method)**——Rats were laparotomized under Na pentobarbital anesthesia, and a 5-cm loop (a ligated part of the stomach, duodenum, jejunum, ileum, or colon) was prepared in each animal according to the method of Levine and Pelikan.[20)] Each test solution prepared as in the *in vivo* experiment was injected into the loop in a volume of 0.5 ml. Ten or 30 min later, the loop was excised, and the contents were washed into 100 ml of cold physiological saline. Radioactivities in a portion of the washing and in the loop tissue were determined, and the value after subtracting these values from the ^{59}Fe dose was taken as the amount of ^{59}Fe transferred into the body.

***In Vitro* Absorption (Everted Duodenal Sac Method)**——The duodenum was excised from each rat, and an everted duodenal sac (tissue weight, 0.50 g) was prepared while cooling in ice by the method of Wiseman.[21)] A fine stainless steel tube was inserted into one end of the sac, and through it, 0.5 ml of the serosal medium (0.1 M NaCl, 5 mM KCl, 0.1 mM $MgSO_4$, 0.3% glucose, and 40 mM Hepes buffer (pH 7.0)) was injected into the sac. The mucosal medium used was of the same composition as the serosal medium, unless otherwise described. The mucosal medium containing each ^{59}Fe–Fe compound (4 ml) was placed in a 10-ml plastic tube, which was held at a constant temperature of 4, 25, or 37 °C in a bath and aerated with a mixture of 95% O_2 and 5% CO_2 from 10 min before the start of incubation through the experiment. In the experiment with metabolic inhibitors, 2,4-dinitrophenol and/or Na iodoacetate were added to the mucosal medium. At specified time points after the start of incubation of the sac, 50 μl aliquots of the mucosal and serosal media were taken, and ^{59}Fe activities in both media were determined. In the experiment to examine the time courses of Fe concentration, changes in volume of the media were minimized by returning the samples used for determination into the respective media before the next sampling time. After 90 min of incubation, the sac was removed, and the Fe-transported serosal medium was collected from the sac. The sac was washed well in 100 ml of cold physiological saline, and the amount of ^{59}Fe taken up by the sac tissue was determined. The determined value was corrected by subtracting the ^{59}Fe uptake by the extracellular space of the tissue obtained beforehand with ^{14}C-inulin (Amersham Japan) under the same experimental conditions.[22)]

Characterization of Fe Transferred into the Serosal Medium——The ^{59}Fe-transferred serosal media obtained with the three Fe compounds after incubation in the *in vitro* experiment were subjected to gel filtration on a Sephadex G-25 column (Pharmacia Fine Chemicals, Inc.), and their elution patterns were compared with those of the respective intact compounds. With respect to Bl-c, elution patterns after enzymatic treatment were also examined; *i.e.*, 200 μl each of the ^{59}Fe-transferred serosal medium (PH 7.0) and intact Bl-c solution (10 μg Fe/ml 0.1 M NaCl–40 mM Hepes buffer (pH 7.0)) was incubated with glycosidases (10 units each of α-amylase and α-glucosidase from *Bacillus* species and yeast, respectively; Sigma Chemical Co.) or trypsin (10 units, derived from the bovine pancreas; Sigma) at 30°C for 1 h and applied to a Sephadex G-25 column. In addition, the ^{59}Fe-transferred serosal medium obtained with Bl-c was applied to Sephadex G-25 and G-50 columns (Pharmacia) in that order, and the radioactive fraction appearing in a position corresponding to that of intact Bl-c was obtained. Its infrared (IR) spectrum was determined by the KBr disc method with a JASCO FTS-20 infrared spectrometer (Fourier-transform type).

Results

In Vivo Absorption (Dose Dependence of Fe Absorption and Incorporation into Hemoglobin)

Figure 1 shows the amounts of Fe absorbed through the gastrointestinal tract and incorporated into hemoglobin in rats after oral administration of Bl-c and the two reference

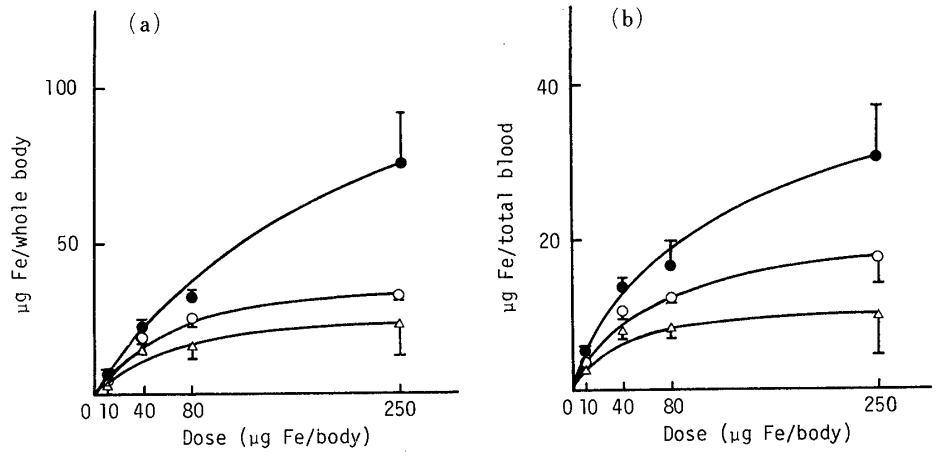

Fig. 1. *In Vivo* Fe Absorption (a) and Incorporation into Hemoglobin (b) in Rats after Oral Administration of Fe Compounds

●, Bl-c; △, $FeSO_4$; ○, ferrous ascorbate. Each point with a bar represents the mean ± S.E. (n=3—5).

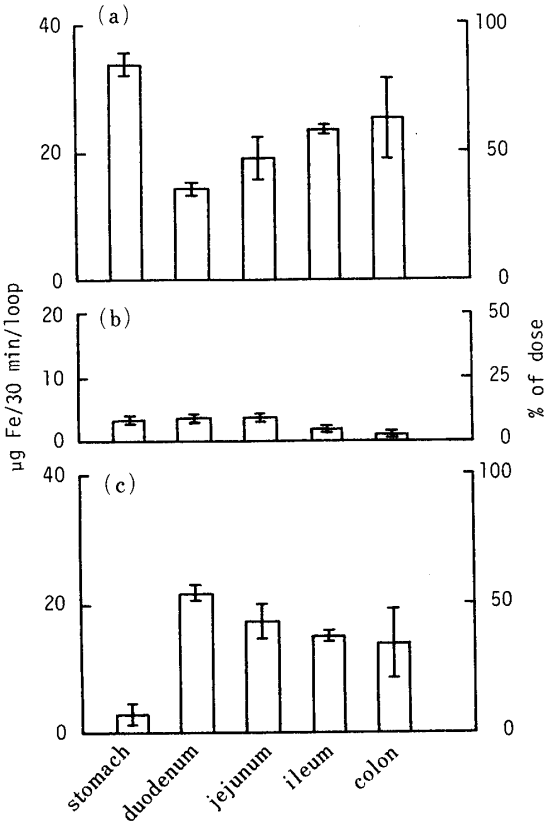

Fig. 2. *In Situ* Fe Absorption at 30 min after Injection of Bl-c into Loops Prepared at Different Sites of the Rat Gastrointestinal Tract

(a), residual Fe in loop; (b), tissue uptake; (c), transfer into body.

Bl-c solution (40 μg Fe/ml), 0.5 ml, was injected into each ligated loop. Each column with a bar represents the mean ± S.E. (n=3).

Fe compounds. With all Fe compounds, both parameters increased with increasing doses and tended to saturate at high doses. The values in the Bl-c group were higher than those in the two other groups, and the differences widened with increasing doses. At 250 μg Fe/body, each value in the Bl-c group was about 3 times and twice the values in the $FeSO_4$ and ferrous ascorbate groups, respectively.

In Situ Absorption (Ligated Gastrointestinal Loop Method)

Absorption Site——Bl-c was injected into loops each prepared in different parts of the rat gastrointestinal tract, and the amounts of Fe remaining in the loop, taken up by the loop

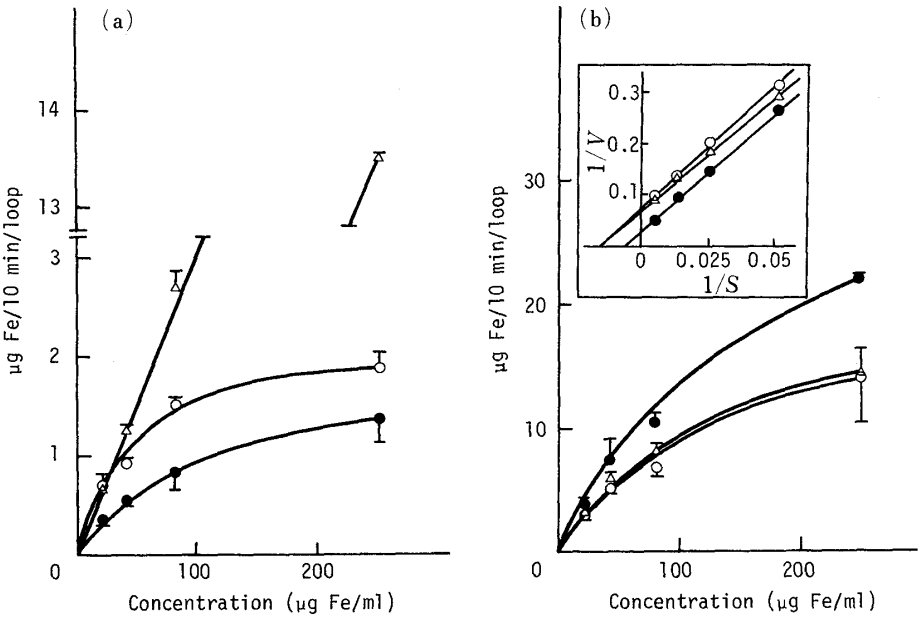

Fig. 3. *In Situ* Fe Absorption at 10 min after Injection of Fe Compounds into Rat Duodenal Loops

(a), tissue uptake; (b), transfer into body.
●, Bl-c; △, $FeSO_4$; ○, ferrous ascorbate. Each point with a bar represents the mean ± S.E. ($n=3$).

tissue and transferred into the body were compared among the gastrointestinal parts (Fig. 2). The residual Fe amount in the loop was the highest in the stomach, decreasing in the colon, ileum, jejunum, and duodenum in that order, and the tissue uptake was higher in the duodenum and jejunum. As for the Fe amount transferred into the body, the duodenum showed the highest value, followed by the jejunum, ileum, and colon in that order. The amount transferred from the stomach was very small.

Concentration Dependence of Fe Absorption——Figure 3 presents the amounts of Fe taken up by the loop tissue and transferred into the body after injection of various concentrations of Bl-c and the two reference Fe compounds into ligated rat duodenal loops. The amounts taken up by the tissue in the Bl-c and ferrous ascorbate groups showed a tendency to saturate at high concentrations. In the $FeSO_4$ group, no saturation was seen, and at 250 μg Fe/ml, 11% of the dose injected (13.8 μg Fe) was distributed into the loop tissue. The Fe transfer into the body had a tendency to saturate at high concentrations in all groups, and the Fe absorbed from Bl-c was higher, as *in vivo*, than the values from the two reference Fe compounds. In the Lineweaver–Burk plots of the Fe transfer into the body, the y-intercept of Bl-c was smaller (maximum transfer rate, 31 μg Fe/10 min/loop) than the values of the reference compounds. The apparent K_m value for Bl-c was 152 μg Fe/ml.

In Vitro Absorption (Everted Duodenal Sac Method)

Time Course and Temperature Dependence——Figure 4 shows time courses of Fe absorption from Bl-c added to the mucosal medium into an everted rat duodenal sac under incubation at 4, 25, and 37 °C. The Fe concentration in the mucosal medium decreased with time in a temperature-dependent manner. The Fe transfer into the serosal medium increased nearly linearly with time during the 90-min incubation period, and at 90 min the amount of Fe transfer at 37 °C was 2.4 times the value at 4 °C. The tissue uptake at 37 °C was 2.9 times the value at 4 °C. Under these experimental conditions, the Fe taken up by the tissue accounted for more than 80% of the amount eliminated from the mucosal medium in 90 min of incubation at

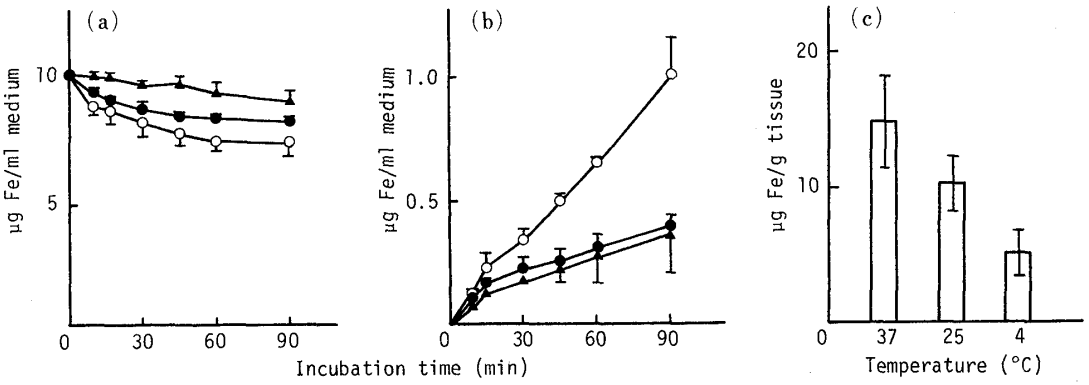

Fig. 4. Temperature Dependence of *in Vitro* Fe Absorption from Bl-c-Added Mucosal Media into Everted Rat Duodenal Sacs

(a), mucosal medium; (b), serosal medium; (c), tissue (at 90 min).
Each point or column with a bar represents the mean ± S.E. ($n=3$). Incubation conditions: initial Bl-c concentration in mucosal medium, 10 μg Fe/ml; mucosal medium, mixture of 0.1 M NaCl, 5 mM KCl, 0.1 mM $MgSO_4$, 0.3% glucose, and 40 mM Hepes buffer (pH 7.0).
○, 37 °C; ●, 25 °C; ▲, 4 °C.

TABLE I. Effects of Metabolic Inhibitors on *in Vitro* Fe Absorption from Bl-c (1 μg Fe/ml)-Added Mucosal Media into Everted Rat Duodenal Sacs after a 90-min Incubation at 37 °C

Inhibitor	Concentration of inhibitor (mM)	Elimination from mucosal medium (μg Fe/15 min/ml medium)	Tissue uptake (μg Fe/90 min/g tissue)	Transfer into serosal medium (μg Fe/90 min/ml medium)
Control	—	0.23±0.02 (0)	2.23±0.13 (0)	0.25±0.04 (0)
2,4-Dinitrophenol	0.2	0.13±0.05 (42.9)	1.02±0.12 (54.3)	0.04±0.00 (83.9)
	0.5	0.13±0.03 (40.7)	1.58±0.15 (29.1)	0.04±0.02 (82.3)
Na iodoacetate	1	0.11±0.00 (49.6)	0.95±0.27 (57.2)	0.02±0.01 (92.7)
	3	0.13±0.02 (44.7)	0.82±0.27 (63.2)	0.02±0.01 (93.1)
2,4-Dinitrophenol + Na iodoacetate	0.5 + 3	0.14±0.02 (36.7)	0.64±0.14 (71.3)	0.02±0.00 (92.3)

Each value represents the mean ± S.E. ($n=3$). The value in parentheses represents percent inhibition compared with the control.

all temperatures, and the amount transferred into the serosal medium was less than 10%.

Effects of Metabolic Inhibitors——The effects of 2,4-dinitrophenol, an oxidative phosphorylation inhibitor, and Na iodoacetate, a sulfhydryl inhibitor, on *in vitro* Fe absorption from Bl-c were examined (Table I). The rate of Fe elimination from the mucosal medium and Fe tissue uptake and transfer into the serosal medium decreased upon addition of the metabolic inhibitors. The inhibition (%) of elimination rate and tissue uptake ranged from about 40 to 70%, while the transfer was markedly inhibited, with inhibition values of about 83% (2,4-dinitrophenol) and about 93% (Na iodoacetate).

Concentration Dependence in Relation to Temperature and Metabolic Energy Dependence——Table II shows the results of an examination of the concentration dependence of Fe absorption from Bl-c and the two reference compounds added to the mucosal medium into an everted rat duodenal sac with incubation under various conditions.

When the everted duodenal sac was incubated at 37 °C with various concentrations of Bl-c added to the mucosal medium, the Fe elimination rate, tissue uptake, and transfer into the serosal medium tended to saturate at high concentrations. At 4 °C, all three parameters increased almost linearly with increasing concentrations, without showing saturation.

TABLE II. Concentration Dependence of *in Vitro* Fe Absorption from Fe Compound-Added Mucosal Media into Everted Rat Duodenal Sacs after a 90-min Incubation at 37 and 4 °C (without a Metabolic Inhibitor) and with a Metabolic Inhibitor (at 37 °C)

Fe compound	Concentration (μg Fe/ml)	Elimination from mucosal medium (μg Fe/15 min/ml medium)			Tissue uptake (μg Fe/90 min/g tissue)			Transfer into serosal medium (μg Fe/90 min/ml medium)		
		37 °C	4 °C	+Inhibitor[a)]	37 °C	4 °C	+Inhibitor	37 °C	4 °C	+Inhibitor
Bl-c	1	0.23± 0.02	0.03±0.00	0.13± 0.02	2.23± 0.13	0.41± 0.00	0.82± 0.27	0.25±0.04	0.01±0.00	0.02±0.01
	10	1.44± 0.13	0.76±0.05	1.13± 0.56	15.03± 3.63	5.27± 1.74	6.88± 0.33	1.02±0.16	0.37±0.15	0.35±0.00
	40	5.25± 1.24	1.93±0.58	4.07± 1.15	33.53± 3.17	7.28± 1.02	22.43± 2.74	2.89±0.14	0.81±0.15	2.03±0.26
	250	22.82± 3.17	11.97±2.08	17.26± 8.86	132.92± 8.71	27.21± 1.92	111.00±21.06	14.22±2.73	4.89±0.84	10.32±0.72
$FeSO_4$	1	0.18± 0.14	0.02±0.01	0.09± 0.00	1.50± 0.79	0.43± 0.05	0.31± 0.10	0.02±0.01	0.00±0.00	0.00±0.00
	10	1.37± 0.25	0.56±0.04	0.78± 0.30	18.34± 1.96	12.85± 1.82	3.09± 0.45	0.54±0.02	0.15±0.02	0.02±0.00
	40	6.08± 2.30	1.47±0.34	5.31± 3.06	52.18±10.75	22.30± 4.67	24.81± 9.87	1.77±0.63	0.22±0.03	0.03±0.01
	250	66.38±16.70	23.21±4.43	48.53±12.56	252.00±48.20	110.75± 3.27	211.03±25.16	5.70±3.90	1.81±0.04	1.30±0.71
Ferrous ascorbate	1	0.19± 0.07	0.03±0.00	0.09± 0.02	1.17± 0.05	0.50± 0.10	0.91± 0.23	0.15±0.06	0.00±0.00	0.01±0.00
	10	1.17± 0.60	0.74±0.26	1.25± 0.07	10.66± 0.11	7.32± 0.78	9.07± 2.56	0.87±0.31	0.23±0.14	0.25±0.04
	40	4.02± 1.31	1.75±0.07	3.93± 0.20	41.15± 5.96	14.45± 1.24	28.31± 6.80	2.56±0.62	0.71±0.22	1.28±0.07
	250	22.87± 2.78	16.33±3.00	20.51± 5.57	143.84± 4.61	59.56±21.08	146.22±19.56	11.30±2.17	6.07±2.10	10.84±0.63

a) 3 mM Na iodoacetate was added to the mucosal medium. The other incubation conditions were the same as in Fig. 4. Each value represents the mean ± S.E. (n = 3—5).

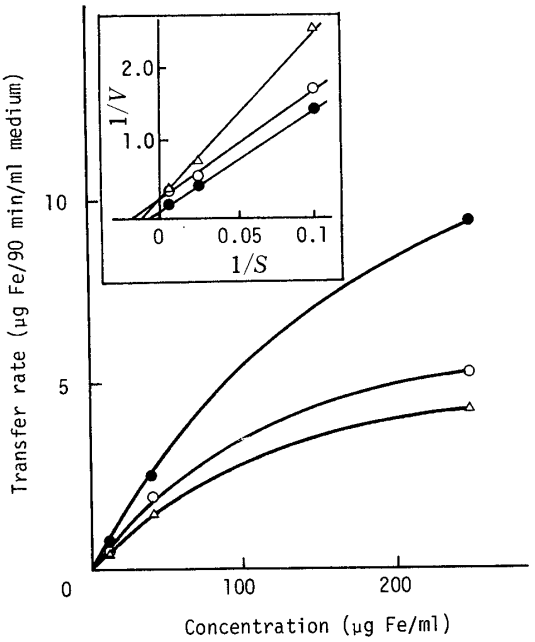

Fig. 5. Concentration Dependence of *in Vitro* Fe Transfer (as a Temperature-Dependent Component) into Serosal Media from Fe Compound-Added Mucosal Media

●, Bl-c; △, $FeSO_4$; ○, ferrous ascorbate. Each value, expressed as the difference between the values at 37 and 4 °C (without a metabolic inhibitor) shown in Table II, represents the mean of three determinations.

Accordingly, the temperature dependence of Fe absorption was greater at lower concentrations of Bl-c. When Na iodoacetate was added to the mucosal medium, all parameters were affected more greatly with lower concentrations of Bl-c. Among the three parameters, the transfer into the serosal medium was most inhibited; the inhibition at the initial Bl-c concentration of 1 μg Fe/ml medium was 93%, while the value at 250 μg Fe/ml medium was only 27%. The results with the two reference Fe compounds were similar to those with Bl-c, except the elimination rate and tissue uptake of Fe from $FeSO_4$. In the case of $FeSO_4$, these parameters increased linearly with increasing concentrations; particularly at high concentrations, Fe was precipitated during incubation, and a part of the precipitate adhered to the sac tissue.

These results show that a saturable, temperature-dependent component is involved in the process of Fe transfer from the three Fe compounds. The component appears to be affected, at least partly, by a metabolic inhibitor (Na iodoacetate). Figure 5 shows this component of the Fe transfer into serosal medium expressed as the difference between the 37 and 4 °C values shown in Table II. The amount of the component was the highest with Bl-c, followed by ferrous ascorbate and $FeSO_4$ in that order. In the Lineweaver–Burk plots, the *y*-intercept of Bl-c was smaller than the values for ferrous ascorbate and $FeSO_4$, which were nearly equal. The apparent K_m value for Bl-c was 182 μg Fe/ml.

pH Dependence——The three parameters of Fe absorption from Bl-c and the two reference Fe compounds were determined with the mucosal media adjusted to pH 6.0—8.0 (Fig. 6). With all Fe compounds, the elimination rate from the mucosal medium and tissue uptake were high at acid pH. With Bl-c and ferrous ascorbate, the transfer into the serosal medium reached the maximum at pH 7.0, whereas with $FeSO_4$, the transfer showed the maximum at pH 6.0, decreasing markedly at pH 7.0 and 8.0.

Characterization of Fe Transferred into the Serosal Medium in the *in Vitro* Absorption Experiment

The chemical form of Fe transferred into the serosal medium in the absorption experiment with an everted rat duodenal sac was examined.

Figure 7 shows elution patterns on Sephadex G-25 of Fe transferred into the serosal medium from the mucosal medium containing Bl-c or either reference Fe compound. In the

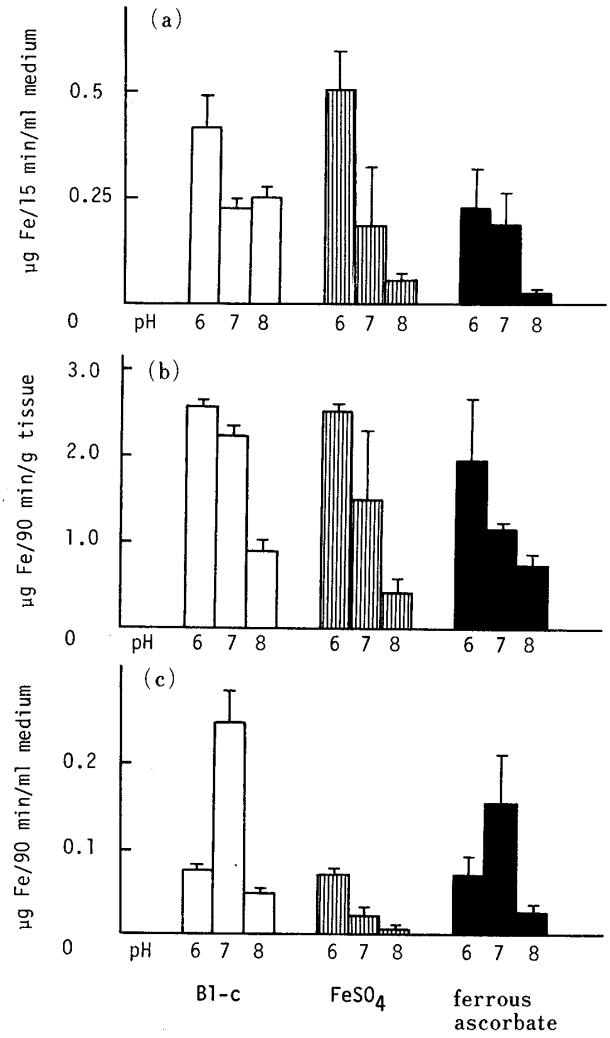

Fig. 6. pH Dependence of *in Vitro* Fe Absorption from Fe Compound-Added Media into Everted Rat Duodenal Sacs after 90-min Incubation at 37 °C

(a), elimination from mucosal medium; (b), tissue uptake; (c), transfer into serosal medium.

Each column with a bar represents the mean ± S.E. ($n=3$). The incubation conditions were the same as shown in Fig. 4, except for the pH values of the mucosal medium (Mes buffer was used at pH 6.0).

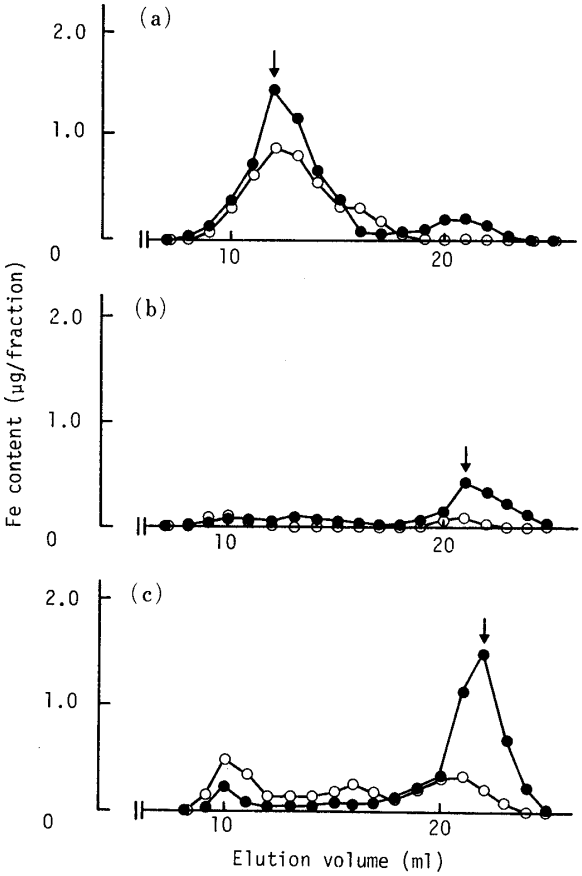

Fig. 7. Gel Filtration on Sephadex G-25 of Serosal Media Containing Fe Transferred from the Fe Compound-Added Mucosal Media after Incubation with and without a Metabolic Inhibitor

(a), Bl-c; (b), $FeSO_4$; (c), ferrous ascorbate.

○, with inhibitor (3 mM Na iodoacetate was added to the mucosal medium); ●, without inhibitor. Arrows show elution volumes of the respective intact Fe compounds.

Each sample (serosal medium) was collected after a 90-min incubation at 37 °C. Initial concentration of each Fe compound in mucosal medium, 250 μg Fe/ml; the other incubation conditions were the same as in Fig. 4. Chromatographic conditions: column size, 1 cm i.d. × 37 cm; solvent, 0.1 M NaCl–40 mM Hepes buffer (pH 6.5); flow rate, 17 ml/h.

case of incubation without the metabolic inhibitor (Na iodoacetate), the main peaks from the three Fe compounds appeared at positions almost corresponding to those from the respective intact compounds (shown by arrows in Fig. 7), though the intact $FeSO_4$ and ferrous ascorbate could not be distinguished from each other in terms of the elution position. On the chromatogram of Bl-c, an Fe peak, besides the main peak, was noted in the low-molecular-weight region in a relatively small amount (less than 10% of the total Fe transferred); this low-molecular-weight peak also appeared with lower initial concentrations of Bl-c (1—40 μg Fe/ml), but the amounts were far smaller than those of the main peaks, except at 1 μg Fe/ml

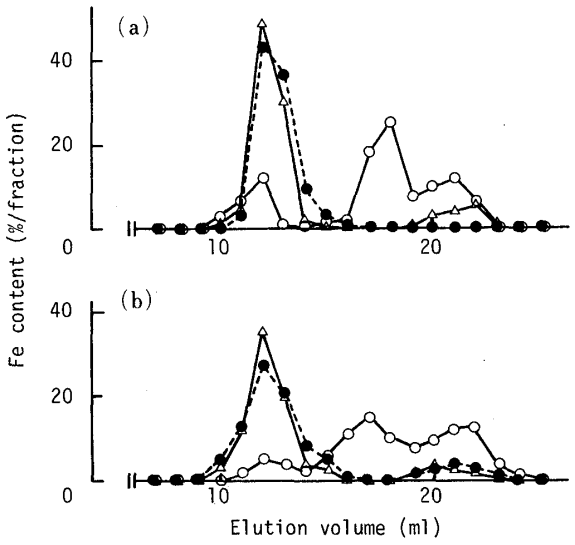

Fig. 8. Gel Filtration on Sephadex G-25 of Bl-c and the Serosal Medium Containing Fe Transferred from the Bl-c-Added Mucosal Medium[a] after Enzymatic Treatment

(a), Bl-c; (b), Fe-transferred serosal medium.
a) The serosal medium was collected after a 90-min incubation at 37 °C (initial Bl-c concentration in mucosal medium, 250 μg Fe/ml).
●, before enzymatic treatment; ○, after treatment with glycosidases; △, after treatment with trypsin. The chromatographic conditions were the same as in Fig. 7.

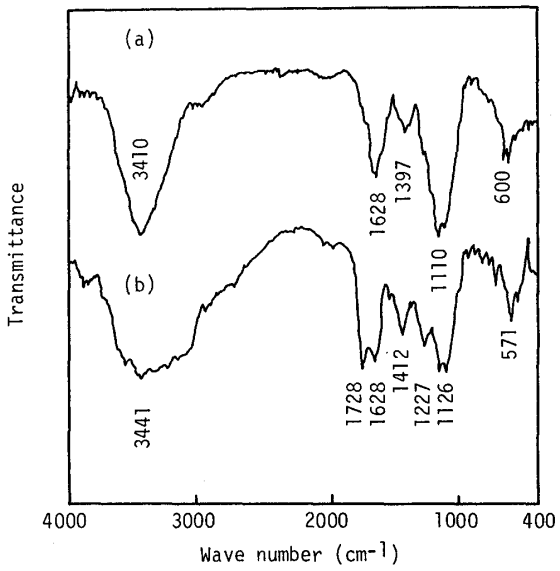

Fig. 9. IR Spectra of Intact Bl-c and Fe Transferred into the Serosal Medium from the Bl-c-Added Mucosal Medium (KBr Disc Method)

(a), intact Bl-c; (b), Fe transferred from the Bl-c-added mucosal medium (initial concentration, 250 μg Fe/ml) into the serosal medium after a 90-min incubation at 37 °C (without a metabolic inhibitor) and purified by gel filtration on Sephadex G-25 and G-50.

(60—80% of the Fe transferred). In the case of incubation with the metabolic inhibitor, the amounts of the Fe peaks in the low-molecular-weight region decreased; *i.e.*, decreases were noted in the secondary peak of Fe from Bl-c and in the main peaks from the reference Fe compounds. The main peak from Bl-c, which appeared in the high-molecular-weight region, was not affected significantly by addition of the metabolic inhibitor. When the Bl-c-added mucosal medium after 90-min incubation was similarly applied to a Sephadex G-25 column, more than 95% of Fe was eluted in the position corresponding to that of the main peak from intact Bl-c, regardless of the initial Bl-c concentration (1—250 μg Fe/ml).

Figure 8 shows elution patterns on Sephadex G-25 of Fe from Bl-c or the Fe-transferred serosal medium (obtained with Bl-c) after treatment with glycosidases or trypsin. Intact Bl-c was cleaved by glycosidase treatment at the glycosidic linkages of the ligand into fragments of lower molecular weights, but it was not affected by trypsin treatment. When the Fe-transferred serosal medium obtained with Bl-c was treated with glycosidases, the main peak corresponding to intact Bl-c was decomposed into fragments, and their elution patterns were similar to those obtained with Bl-c. The trypsin treatment did not affect the elution patterns of Fe from the serosal medium, either.

Then, the Fe-transferred serosal medium obtained with Bl-c was applied to Sephadex G-25 and G-50 columns in that order, and the IR spectrum of the peak substance corresponding to intact Bl-c was compared with that of intact Bl-c (Fig. 9). The two spectra resembled each other well; the spectral characteristics common to them were O–H stretching at 3000—3800 cm^{-1}, carbonyl absorption bands at 1600—1800 cm^{-1}, and absorption bands in the fingerprint region around 1400 and 1000—1200 cm^{-1}. In the spectrum of the serosal medium, however, some changes from the Bl-c spectrum also appeared, including new absorptions in the carbonyl absorption bands (at 1730 cm^{-1}) and at 1230 cm^{-1} and new patterns in the

fingerprint region.

Discussion

In considering the mechanism of gastrointestinal Fe absorption from Fe compounds, the Fe compounds can be classified into two groups, namely, one releasing free Fe ions within the intestinal tract and the other existing there as stable complexes or chelates. In the case of the former group such as $FeSO_4$, Fe absorption shows saturation, temperature dependence, and inhibition by metabolic inhibitors, and a special Fe transport system (carrier-mediated system) has been considered to be involved.[3-8] In this system, the first step is the binding of Fe to specific sites on intestinal brush-border membranes.[9-13] With respect to the latter group, some reports have referred to the affinity of Fe (*e.g.*, inorganic Fe salts) to binding sites on the brush-border membranes,[10,11] and some Fe compounds have been shown to enter the bloodstream and be excreted into urine in the form of Fe bound to ligands;[16,17] however, the mechanism of Fe transport from this group of compounds has not fully been clarified, except that a specific transport system for heme Fe has been identified.[7,10,18,19,23] In microorganisms, it has been reported that various stable Fe chelates are transported by a carrier-mediated system in the form of Fe bound to ligands.[24-26]

The Fe(II)–oligosaccharide complex (Bl-c) examined in this study was found to be stable over a wide pH range within the gastrointestinal tract[2] and belongs to the latter group. The present results showed concentration dependence of Fe absorption from Bl-c; this indicates that the processes of Fe uptake and transfer from Bl-c have a component which saturates at high concentrations and suggests that a special transport system may be involved in Fe absorption from Bl-c, as in the case of inorganic Fe salts and ferrous ascorbate. This view is strongly supported by the following results with an everted rat duodenal sac: (a) a temperature-dependent, saturable component was seen in the processes of Fe uptake by the tissue and transfer into the serosal medium; (b) at least a part of the component was affected by metabolic inhibitors; and (c) the Fe transfer into the serosal medium became optimal around pH 7. With inorganic Fe salts, it has been revealed that the main site of Fe absorption is the upper part of the small intestine[4,7] which is rich in Fe-specific binding sites.[7,9,10] The present finding that the upper part of the small intestine showed the highest Fe absorption from Bl-c also suggests an involvement of a carrier-mediated Fe transport system in Fe absorption from Bl-c.

In *in vitro* Fe absorption studies, results should be assessed carefully because the rate of Fe transfer into a serosal medium has been reported to be far lower than the *in vivo* rate.[4,7,23] In the present *in vitro* experiment, the Fe amounts transferred from Bl-c and the reference Fe compounds into the serosal media were much less than those taken up by the tissue (Table II). This was in contrast to the *in situ* result that most of Fe eliminated from the loop passed across the mucosa into the body (blood stream) (Fig. 2). The difference is presumed to have appeared because the slow process of Fe transfer to the serosal side[4,23] is more sensitive to the Fe demand from the body and physiological conditions of the mucosal tissue than the preceding, rapid process of Fe uptake by the mucosal tissue.[4,7,23,27] Accordingly, in the *in vivo* and *in situ* results with Bl-c and the reference compounds, the saturable component of Fe transfer seemed to contribute more greatly to Fe transfer into the body than in the *in vitro* situation. This is supported by the fact that the apparent K_m values for the *in situ* transfer and the temperature-dependent component of the *in vitro* transfer with Bl-c were similar to each other. On the other hand, an unsaturable component of Fe transfer *in vitro* (Table II, values at 4 °C) is considered to represent the transport by passive diffusion; this transport system has been reported to contribute much more to Fe absorption at higher concentrations *in vivo* as well as *in vitro*.[4,5,7,23]

When the chemical form of Fe transferred *in vitro* from the Bl-c-added mucosal medium into the serosal medium was examined, Fe was found to appear as two fractions: one was an Fe–oligosaccharide complex similar to intact Bl-c, which appeared mainly at high concentrations, and the other was a low-molecular-weight Fe fraction appearing at low concentrations. In the mucosal medium after a 90-min incubation with an everted rat duodenal sac, Bl-c was hardly metabolized, and the low-molecular-weight Fe fraction was not formed. Therefore, the formation of this fraction is considered to be due to the metabolism of Bl-c after uptake by mucosal cells. The addition of a metabolic inhibitor to the mucosal medium nearly completely inhibited the formation of this peak fraction; this suggests the possibility that the formation of the fraction within mucosal cells of Fe transfer therefrom may be a metabolic energy-requiring process. However, the peak amounts were small at the Bl-c concentrations of 10—250 μg Fe/ml, and it is presumed that most of the Fe transferred from Bl-c may be in the form of the complex.

In conclusion, one of the Fe transport routes from Bl-c was found to be a special system by which Fe is transported in a saturable, temperature-dependent manner, and this process is considered to contribute greatly to the high absorption activity of Bl-c, especially at large doses, as compared with that of $FeSO_4$ and ferrous ascorbate. Fe from Bl-c may be transported into the body in the form of an Fe complex with oligosaccharide similar to intact Bl-c through the special system. Further studies will be carried out on the Fe uptake by brush-border membrane vesicles from Bl-c and on the forms of Fe existing within mucosal cells after uptake.

References and Notes

1) S. Tabata and K. Tanaka, *Chem. Pharm. Bull.*, **34**, 5045 (1986).
2) S. Tabata and K. Tanaka, *Chem. Pharm. Bull.*, **35**, 3343 (1987).
3) J. Manis and D. Schachter, *Am. J. Physiol.*, **207**, 893 (1964).
4) M. S. Wheby, L. G. Jones, and W. H. Crosby, *J. Clin. Invest.*, **43**, 1433 (1964).
5) W. N. Pearson and M. Reich, *J. Nutr.*, **87**, 117 (1965).
6) H. J. Helbock and P. Saltman, *Biochim. Biophys. Acta*, **135**, 979 (1967).
7) W. Forth and W. Rummel, *Physiol. Rev.*, **53**, 724 (1973).
8) R. J. Simpson, K. B. Raja, and T. J. Peters, *Biochim. Biophys. Acta*, **860**, 229 (1986).
9) N. J. Greenberger, S. P. Balcerzak, and G. A. Ackerman, *J. Lab. Clin. Med.*, **73**, 711 (1969).
10) C. L. Kimber, T. Mukherjee, and D. J. Deller, *Am. J. Dig. Dis.*, **18**, 781 (1973).
11) T. M. Cox and M. W. O'Donnell, *Biochem. J.*, **194**, 753 (1981).
12) J. J. M. Marx and P. Aisen, *Biochim. Biophys. Acta*, **649**, 297 (1981).
13) M. W. O'Donnell and T. M. Cox, *Biochem. J.*, **202**, 107 (1982).
14) E. J. Eastham, J. I. Bell, and A. P. Douglas, *Biochem. J.*, **164**, 289 (1977).
15) R. J. Simpson and T. J. Peters, *Biochim. Biophys. Acta*, **814**, 381 (1985).
16) R. J. Simpson and T. J. Peters, *Biochim. Biophys. Acta*, **856**, 109 (1986).
17) K. Terato, T. Fujita, and Y. Yoshino, *Am. J. Dig. Dis.*, **18**, 121 (1973).
18) L. R. Weintraub, M. B. Weinstein, H.-J. Huser, and S. Rafal, *J. Clin. Invest.*, **47**, 531 (1968).
19) M. S. Wheby, G. E. Suttle, and K. T. Ford III, *Gastroenterology*, **58**, 647 (1970).
20) R. R. Levine and E. W. Pelikan, *J. Pharm. Exp. Ther.*, **131**, 319 (1954).
21) G. Wiseman, *Methods Med. Res.*, **9**, 287 (1961).
22) T. Komai, K. Kawai, and H. Shindo, *J. Nutr. Sci. Vitaminol.*, **20**, 163 (1974).
23) T. H. Bothwell, R. W. Charlton, J. D. Cook, and C. A. Finch, "Iron Metabolism in Man," Blackwell Scientific Publications, Oxford, London, Edinburgh, and Melbourne, 1979, pp. 256—283.
24) H. Rosenberg, I. G. Young, and B. R. Byers, "Microbial Iron Metabolism," ed. by J. B. Neilands, Academic Press, New York and London, 1974, pp. 67—105.
25) G. Muller and K. N. Raymond, *J. Bacteriol.*, **160**, 304 (1984).
26) D. J. Ecker, B. F. Matzanke, and K. N. Raymond, *J. Bacteriol.*, **167**, 666 (1986).
27) M. E. Conrad, L. R. Weintraub, and W. H. Crosby, *J. Clin. Invest.*, **43**, 963 (1964).