Bioavailability of Iron from Ferric Pyrophosphate

Hiroshi Tsuchita,*,† Atsuko Kobayashi,† Tadashi Kojima,† Tamotsu Kuwata,† Tomihiro Noguchi,‡ Kaoru Koto,‡ and Tsuyoshi Takahashi†

Central Research Institute, Meiji Milk Products Company, Ltd., 1-21-3 Sakae-cho, Higashimurayama-shi, Tokyo 189, Japan, and Soka-Factory, Kanto Chemical Company, Inc., Inari, Soka-shi, Saitama 340, Japan

The iron bioavailability from ferric pyrophosphate was investigated by hemoglobin repletion assay. Three iron sources were prepared from the aqueous suspension of ferric pyrophosphate: mixed with skim milk and dehydrated (FP1), directly dehydrated (FP2), and FP2 mixed with skim milk and redehydrated (FP3). The relative bioavailability of FP1 was 100 of ferrous sulfate by slope ratio analysis. The relative hemoglobin value of FP2 varied from 66 to 82. Mixing with skim milk and heating improved the bioavailability of ferric pyrophosphate. The effect of skim milk and the physicochemical properties of FP2 on the iron bioavailability are discussed.

Many iron sources that exhibit the best bioavailability adversely affect food quality by accelerating lipid oxidation or by producing an unfavorable color or flavor. In iron fortification of milk and milk products, compatible and nonreactive iron compounds are needed. Ferric pyrophosphate is considered to be one of these compounds, but it showed a relative bioavailability of 45 of ferrous sulfate by the hemoglobin repletion technique with anemic chicks and rats (Fritz et al., 1970). The low iron availability is a critical defect in iron supplementation for infant formula, since it often represents the sole source of dietary iron during the first few months of life (Cook and Bothwell, 1984). On the other hand, several investigations have been undertaken concerning the effects of food components and food processing on the bioavailability of ferric pyrophosphate. For instance, Theuer et al. (1971, 1973) investigated the effects of sterilization upon the availability of supplemental iron added to infant formulas. Relative iron availability of ferric pyrophosphate added to a milk-based formula was increased from 75% to 125% of standard ferrous sulfate (Theuer et al., 1973). When used in iron supplementation of infant formulas, two different forms of ferric pyrophosphate (aqueous suspension and dry powder) are available in Japan. However, very little is known about the difference in iron availability between these two forms of ferric pyrophosphate. We found in our preliminary experiment that the bioavailability of ferric pyrophosphate supplemented to powdered infant formula varied according to the method of the iron source preparation. For example, no definite iron bioavailability was observed when powdered ferric pyrophosphate was added to the powdered infant formula, although a higher value was obtained when ferric pyrophosphate suspension was treated with skim milk and dried. The purpose of this research was to evaluate the influence of physicochemical properties of powdered ferric pyrophosphate on the iron bioavailability and to determine the relative bioavailability of ferric pyrophosphate treated with skim milk.

MATERIALS AND METHODS

Preparation of Ferric Pyrophosphate. Ferric pyrophosphate was prepared by adding sodium pyrophosphate solution (22.3 g of $Na_4P_2O_7 \cdot 10H_2O$ dissolved in 220 mL of water) to ferric chloride solution (18.0 g of $FeCl_3 \cdot 6H_2O$ dissolved in 60 mL of water). The precipitate was collected on a Büchner funnel from the reaction mixture and washed with water. The ferric pyrophosphate suspension was obtained by redispersing the precipitate in 250 mL of water. The ferric pyrophosphate suspension containing 12.4 g of $Fe_4(P_2O_7)_3$ was mixed with 6200 g of 10% reconstituted skim milk at 50 °C, warmed to 70 °C, and held for 10 min, and the mixture was dried under vacuum (FP1). Powdered ferric pyrophosphate was obtained from the ferric pyrophosphate suspension by vacuum-drying (FP2). The weight loss on ignition (500 °C, 1 h) of FP2 varied from 12% to 26% according to the drying condition. To study the influence of water content on iron bioavailability, FP2(16%), FP2(19%), and FP2(26%), where the percentage in parentheses represents the weight loss on ignition, were used in experiment 1. In experiment 2, FP2(12%) was selected because of the lowest weight loss on ignition. FP2(12%) was pulverized with a mortar and pestle and sifted through a 100-mesh sieve. One gram of FP2(12%) was dispersed in 500 g of 10% reconstituted skim milk at 50 °C, warmed to 70 °C, and held for 10 min, and the mixture was dried under vacuum (FP3).

Animals and Diets. Sprague-Dawley male weanling rats (Japan SLC Inc., Shizuoka Prefecture, Japan), housed in individual aluminum cages, were allowed ad libitum a low-iron basal diet and deionized water for 4 weeks in a temperature-controlled (23 ± 2 °C) room with 50% humidity and a 12–12 h light–dark cycle (lights on from 6:00 a.m. to 6:00 p.m.). The composition of the low-iron basal diet is shown in Table I, and this diet contained by analysis 5 mg of iron/kg. Tail blood was drawn to determine hemoglobin (Hb) and hematocrit (Ht) levels during the iron depletion period. The rats were then divided into groups of eight animals each, on the basis of similar hemoglobin status (Hb <6 g dL). Three experiments were carried out as follows.

Experiment 1. Iron Bioavailability from Ferric Pyrophosphate. Five groups of anemic rats were fed diets supplemented with ferrous sulfate (FS), FP1, FP2(16%), FP2(19%), and FP2(26%), respectively, at 25 ppm as iron. All iron sources were pulverized with a mortar and pestle and sifted through a 100-mesh sieve (particle size <149 μm) before mixed into basal diets. Powdered skim milk was mixed with FS or FP2 to yield the same iron salt/milk solid ratio as FP1 before it was added to the basal diet. Blood samples were taken from the tail at 0, 4, 7, 11, and 14 days of study to determine Hb and Ht levels. The relative Hb value, the ratio of Hb value from each iron source to that from FS on the 14th day, was calculated. After a 14-day iron repletion period, animals were fasted overnight and anesthetized by intraperitoneal injection of sodium pentobarbital (40 mg/kg of body weight), and blood samples were taken from the aorta ventralis to determine serum iron and total iron binding capacity (TIBC).

Experiment 2. Effect of Skim Milk on Iron Bioavailability. Four groups of anemic rats were fed diets supplemented with

† Meiji Milk Products Co.
‡ Kanto Chemical Co.

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Table I. Composition of Basal Diet

ingredient	%	ingredient	%
casein	20.0	soybean oil	5.0
dl-methionine	0.3	mineral mixture[a]	3.5
cornstarch	35.0	vitamin mixture[b]	1.0
sucrose	30.0	choline bitartrate	0.2
cellulose powder	5.0		

[a] The mineral mixture based on the AIN-76 mineral mixture (AIN, 1977) except for iron contained the following (g/kg of mixture): $CaHPO_4$, 500.0; NaCl, 74.0; tripotassium citrate monohydrate, 220.0; K_2SO_4, 52.0; MgO, 24.0; manganese carbonate, 3.5; zinc carbonate, 1.6; copper carbonate, 0.3; KIO_3, 0.01; $Na_2SeO_3 \cdot 5H_2O$, 0.01; $CrK(SO_4)_2 \cdot 12H_2O$, 0.55. [b] The vitamin mixture was prepared according to the AIN-76 vitamin mixture (AIN, 1977).

FS, FP1, FP2(12%), and FP3, respectively, at 25 ppm as iron. Powdered skim milk was mixed with FP2(12%) by the method described above. Blood samples were taken from the tail at 0, 3, 7, 10, and 14 days of study to determine Hb and Ht levels. The relative Hb values were calculated as described above. Serum iron and TIBC were measured by use of the same procedure as in experiment 1.

Experiment 3. Slope Ratio Analysis of FP1. Seven groups of anemic rats were used. One group received the basal diet. Three other groups received the basal diet supplemented with FP1 (particle size <149 μm) to provide 6, 12, and 24 ppm of iron. For the remaining three groups, standard FS mixed with powdered skim milk was added to the basal diet to provide 6, 12, and 24 ppm of iron. Blood samples were taken by use of the same procedure as in experiment 2. The linear regression line, $y = ax + b$, for each iron source (y is Hb on the 14th day of iron repletion and x is the iron level in the diet) was calculated. The relative iron bioavailability of FP1 was expressed as the ratio of the slope of the regression line for FP1 to that for FS. Serum iron and TIBC were measured by use of the same procedure as in experiment 1. The liver and spleen were removed, weighed, and frozen for subsequent determination of tissue iron concentration.

Analytical Method. Hb was measured by the cyanomethemoglobin method, and Ht was determined after centrifugation of blood in a heparinized capillary tube. Serum iron and TIBC were measured by the standard method of the International Committee for Standardization in Hematology (ICSH) (ICSH, 1978a,b). Diet samples and animal tissue were wet ashed with 1 N nitric acid in a muffle furnace at 550 °C for 24 h. Ashed samples were dissolved with concentrated hydrochloric acid and diluted with deionized water for iron analysis by an atomic absorption spectrophotometer (Shimadzu, AA-640-13): The recovery of iron was 100%. Iron(III) was determined by a volumetric method (Kolthoff and Belcher, 1953), phosphorus by a spectrophotometric molybdovanadate method (Heckman, 1965), and chloride by AgCl nephelometry (Boltz et al., 1978). The X-ray diffraction pattern of a powder sample was taken with monochrofiltered Cu Kα radiation by using Rigaku Denki Geigerflex X-ray diffractometer RAD IIIA. Thermogravimetry (TG) was carried out on a Seiko Instrument & Electronics TG/DTA 30. The infrared (IR) spectrum was obtained with a Shimadzu Model IR-420 spectrophotometer, the samples being analyzed as a KBr pellet (13-mm diameter) prepared with 1.5 mg of sample in 400 mg of anhydrous KBr.

Results are expressed as the mean and the standard error of the mean. Data were analyzed statistically by one-way analysis of variance. When significant F ratios were found ($P < 0.05$), individual means were compared by the least significant difference test.

RESULTS AND DISCUSSION

Physicochemical Properties of FP2 and Iron Bioavailability. The weight loss of powdered ferric pyrophosphate after ignition at 800 °C for 1 h is standardized below 20% (*Food Chemical Codex*, 1981). When prepared by the method described above, loss on ignition (at 500 °C) varied from 12% to 26%. The chemical components and the molar ratio (Fe_2O_3/P_2O_5) of FP2(16%) were similar to those of FP2(26%) except for H_2O content (Table II). The X-ray diffraction spectrum obtained from FP2(16%) after heating for 1 h at 750 °C was identical with that of FP2(26%), and they were in good agreement with ASTM data for $Fe_4(P_2O_7)_3$ (*Powder Diffraction* File, 1983) (Figure 1). Small peaks for $FePO_4$ and $NaFeP_2O_7$ were also identified in FP2(16%) as well as FP2(26%). No crystalline phase was found in the spectra obtained from samples heated below 500 °C (Figure 1). This indicates that powdered ferric pyrophosphate was not crystalline but amorphous material. From the differential scanning calorimetric (DSC) curves of FP2(16%) and FP2(26%) (−90 to 20 °C), no endothermic peak due to free water was observed (the data are not shown). The IR spectrum of FP2(16%) was almost identical with that of FP2(26%) (Figure 2), showing the absorption of the O–H stretching vibration and the H–O–H bending vibration of the water at 3400 and 1620 cm^{-1}, respectively. These absorptions were shifted due to the strong hydrogen bond comparing with the absorption of water vapor, in which the O–H stretching vibrations appeared at 3756 and 3652 cm^{-1} and the H–O–H bending vibration appeared at 1595 cm^{-1}. It is indicated that water in powdered ferric pyrophosphate

Table II. Comparison of Chemical Analysis of FP2

	FP2 (19%), %	FP2 (26%), %
Fe_2O_3	32.30	30.88
P_2O_5	43.90	41.25
Na_2O	1.35	1.23
Cl	0.50	0.30
H_2O	20.26	26.28
molar ratio Fe_2O_3/P_2O_5	0.654	0.666

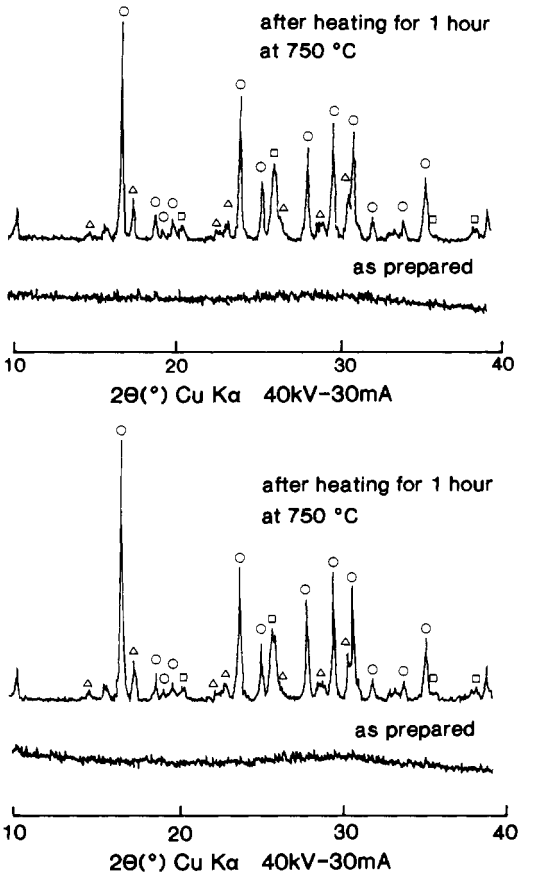

Figure 1. X-ray diffraction patterns of dehydrated ferric pyrophosphate suspension (loss on ignition, 16%) [FP2(16%)] (top) and dehydrated ferric pyrophosphate suspension (loss on ignition, 26%) [FP2(26%)] (bottom): (○) $Fe(P_2O_7)_3$; (□) $FePO_4$; (△) $NaFeP_2O_7$.

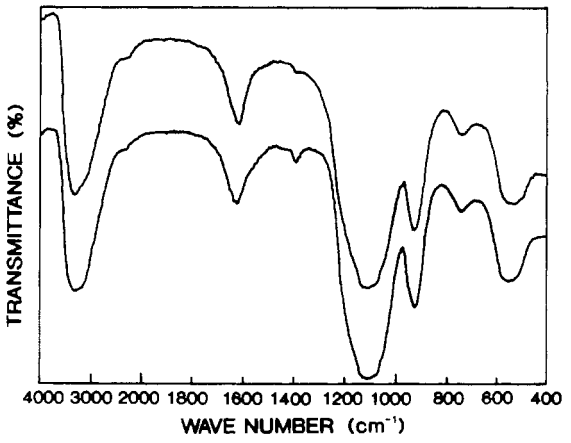

Figure 2. IR spectra of dehydrated ferric pyrophosphate suspension (loss on ignition, 26%) [FP2(26%)] (top) and dehydrated ferric pyrophosphate suspension (loss on ignition, 16%) [FP2(16%)] (bottom).

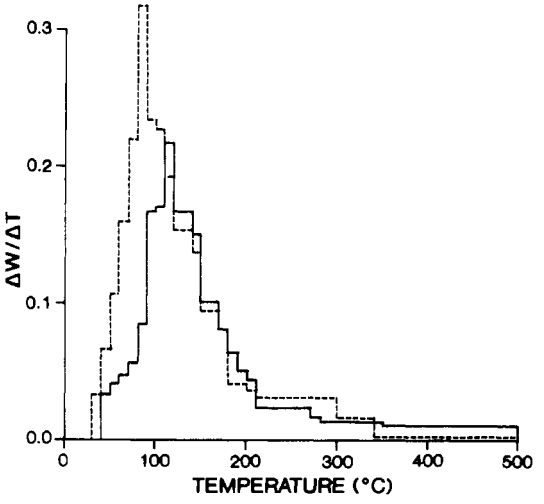

Figure 3. DTG curves of dehydrated ferric pyrophosphate suspension (loss on ignition, 16%) [FP2(16%)] (solid line) and dehydrated ferric pyrophosphate suspension (loss on ignition, 26%) [FP2(26%)] (broken line). ΔW = [wt loss at each range of temp (g)/sample wt (g)] × 100. ΔT = 10 °C.

was not free water but binding water. Derivative thermogravimetric (DTG) curves calculated from the TG curves of FP2(16%) and FP2(26%) are presented in Figure 3. The weight loss distribution of FP2(26%) lay at lower temperature than that of FP2(16%). It seems likely that each FP2 had its own DTG curve depending on the water content. It is suggested from these findings that the differences in water content of powdered ferric pyrophosphate could be related to those in the amorphous structure.

As shown in Figure 4, the FP2(16%) group gave a lower Hb value than the FP2(19%) or the FP2(26%) group at each day of study; significant differences were observed at 11 and 14 days. There was a significant difference in Ht increase between the FP2(16%) and the FP2(26%) groups, although the difference between the FP2(16%) and the FP2(19%) groups was not statistically significant. The differences in serum iron and TIBC were not clearly observed among the FP2 groups (Table III). There were no significant differences in these parameters between the FP2(19%) group and the FP2(26%) group. The FP2(12%) group gave the lowest Hb value at each day of study in experiment 2 (Figure 5). The Ht increase and the serum iron of the FP2(12%) group were lower than those of the FP3 group, although the difference was not

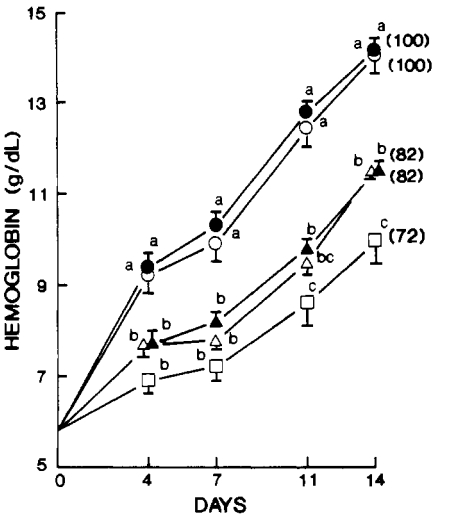

Figure 4. Experiment 1. Repletion curve of Hb: (○) dehydrated ferric pyrophosphate suspension after treatment with skim milk (FP1); (□) dehydrated ferric pyrophosphate suspension (loss on ignition, 16%) [FP2(16%)]; (△) dehydrated ferric pyrophosphate suspension (loss on ignition, 19%) [FP2(19%)]; (▲) dehydrated ferric pyrophosphate suspension (loss on ignition, 26%) [FP2(26%)]; (●) ferrous sulfate (FS). Data are expressed as means ± SE. For each experimental day, means not sharing a common superscript letter are significantly different at $P < 0.05$. The relative Hb value of each iron source is expressed in parentheses as a percentage of the Hb response to ferrous sulfate at 14 days.

Table III. Experiment 1: Relative Hb Value, Ht Increase, Serum Iron, and TIBC after 2 Weeks of Iron Repletion[a]

	rel Hb value,[c] %	Ht inc, %	serum iron, μg/dL	TIBC, μg/dL
FP1[b]	100	26.0 ± 0.5[ac]	33.8 ± 2.9	651.5 ± 38.8[a]
FP2 (16%)[b]	72	16.8 ± 1.8[b]	30.9 ± 2.8	818.4 ± 23.8[b]
FP2 (19%)[b]	82	20.1 ± 0.3[bd]	31.7 ± 3.0	324.1 ± 18.0[b]
FP2 (26%)[b]	82	21.8 ± 1.3[cd]	29.8 ± 3.0	839.0 ± 21.3[b]
ferrous sulfate	100	26.6 ± 0.9[a]	35.1 ± 3.5	755.0 ± 21.4[b]

[a] Values are means ± SE. Means not sharing a common superscript letter within a column are significantly different from each other at $P < 0.05$. [b] FP1, dehydrated ferric pyrophosphate suspension after treatment with skim milk; FP2 (16%), dehydrated ferric pyrophosphate suspension (loss on ignition, 16%); FP2 (19%), dehydrated ferric pyrophosphate suspension (loss on ignition, 19%); FP2 (26%), dehydrated ferric pyrophosphate suspension (loss on ignition, 26%). [c] Relative Hb value, see Figure 4.

Table IV. Experiment 2: Relative Hb Value, Ht Increase, Serum Iron, and TIBC after 2 Weeks of Iron Repletion[a]

	rel Hb value,[c] %	Ht inc, %	serum iron, μg/dL	TIBC, μg/dL
FP1[b]	96	26.8 ± 1.0[a]	59.8 ± 6.4[a]	764.2 ± 38.3[a]
FP2 (12%)[b]	66	15.8 ± 2.2[b]	28.1 ± 2.0[b]	937.8 ± 18.6[b]
FP3[b]	79	17.0 ± 2.0[b]	37.9 ± 5.2[b]	840.8 ± 31.1[c]
ferrous sulfate	100	29.4 ± 2.5[a]	68.8 ± 7.9[a]	736.3 ± 51.0[a]

[a] Values are means ± SE. Means not sharing a common superscript letter within a column are significantly different from each other at $P < 0.05$. [b] FP1, see footnote in Table III; FP2 (12%), dehydrated ferric pyrophosphate suspension (loss on ignition, 12%); FP3, redehydrated FP2(12%) after treatment with skim milk. [c] Relative Hb value, see Figure 5.

statistically significant. The TIBC of the FP2(12%) group was significantly higher than that of the FP3 group (Table IV). It is suggested that the water content indicated by the loss on ignition could be related to the iron bioavailability in powdered ferric pyrophosphate and that the iron availability was low when the water content was relatively small. It seems likely that the amorphous structure which was affected by the water content could be responsible for

Table V. Experiment 3: Analysis of Relative Iron Bioavailability from FP1 by Slope Ratio Analysis

iron source	regression coefficient	regression eq[a]	slope ratio[b]
ferrous sulfate	0.998	$Y = 3.86X + 3.71$	
FP1	0.996	$Y = 3.87X + 3.70$	100.4

[a] X, iron level in the diet (mg/100 g). Y, Hb on the 14th day of iron repletion (g/dL). [b] Ratio of the slope of the regression line for the FP1 to the slope of the regression line for ferrous sulfate (%). [c] FP1, see footnote in Table III.

Table VI. Experiment 3: Ht Increase, Serum Iron, and TIBC after 2 Weeks of Iron Repletion[a]

iron source	iron dose, ppm	Ht inc, %	serum iron, μg/dL	TIBC, μg/dL
no added iron	0	−2.4 ± 0.3[a]	29.1 ± 2.4[a]	1029.9 ± 18.1[a]
ferrous sulfate	6	5.9 ± 0.4[b]	31.5 ± 3.0[a]	1001.5 ± 22.2[a]
	12	14.3 ± 1.0[c]	34.4 ± 2.6[a]	954.9 ± 14.1[a]
	24	27.3 ± 1.2[d]	56.1 ± 4.6[b]	777.0 ± 19.0[b]
FP1[b]	6	3.8 ± 1.1[b]	32.0 ± 2.6[a]	1033.1 ± 25.0[a]
	12	14.1 ± 0.7[c]	33.2 ± 2.4[a]	975.0 ● 26.5[a]
	24	24.3 ± 1.1[d]	54.7 ± 3.0[b]	841.7 ± 27.6[b]

[a] Values are means ± SE. Means not sharing a common superscript letter within a column are significantly different from each other at $P < 0.05$. [b] FP1, see footnot in Table III.

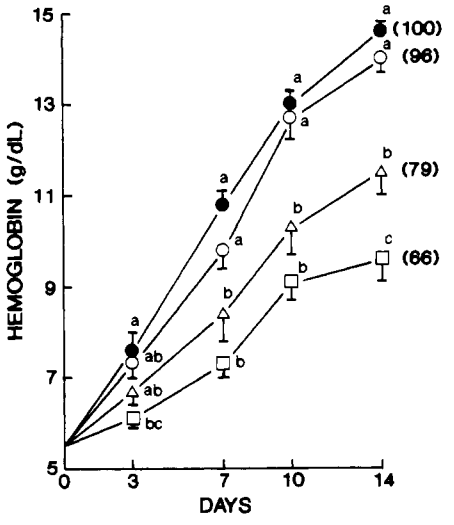

Figure 5. Experiment 2. Repletion curve of Hb: (○) dehydrated ferric pyrophosphate suspension after treatment with skim milk (FP1); (□) dehydrated ferric pyrophosphate suspension (loss on ignition, 12%) [FP2(12%)]; (△) redehydrated FP2(12%) after treatment with skim milk (FP3); (●) ferrous sulfate (FS). Data are expressed with means ± SE. For each experimental day, means not sharing a common superscript letter are significantly different at $P < 0.05$. The relative Hb value of each iron source is expressed in parentheses as a percentage of the Hb response to ferrous sulfate at 14 days.

the solubility of FP2 or the complex formation of iron with food digest in the gastrointestinal tract.

In general, iron compounds such as the pyrophosphates, orthophosphates, hydroxides, and oxides are relatively insoluble in water, compared to the sulfates and chlorides. These insoluble salts show relatively low bioavailability. Harrison et al. (1976) demonstrated that the relative bioavailability of ferric orthophosphate, which is one of the insoluble salts, was strongly influenced by particle size and solubility in 0.1 N HCl. The particle size of FP2 was less than 149 μm, although the particle size distribution was not determined. When the suspension of ferric pyrophosphate was dried, not only the change of the amorphous structure but also the flocculation of colloidal particles and a decrease in surface area were likely to occur. These changes could be responsible for a decrease in solubility of FP2.

Effect of Skim Milk. In experiment 1, the Hb value of the FP1 group was greater than those of the FP2 groups at each day of study (Figure 4). The TIBC of the FP1 group was significantly greater than those of the FP2 groups, although serum iron was not significantly different among the experimental groups (Table III). In experiment 2, the FP3 group gave a higher Hb value than the FP2(12%) group at each day of study; a significant difference was observed only at 14 days (Figure 5). The Ht increase and the serum iron of the FP3 group were higher than those of the FP2(12%) group, but the differences were not statistically significant (Table IV). The TIBC of the FP3 group was significantly lower than that of the FP2(12%) group (Table IV). The Hb repletion, Ht increase, serum iron, and TIBC from the FP1 group were significantly higher than those from the FP3 group (Table IV). These findings indicate that the availability of iron from FP2(12%) was partly improved by dispersing in skim milk and redrying. However, it is suggested that redispersed FP2(12%) was not physicochemically identical with ferric pyrophosphate suspension when treated with skim milk. In experiment 3, the Hb repletion curves of the FP1 groups corresponded to those of the FS groups at each iron level (Figure 6) and the relative iron bioavailability of FP1 was found to be 100 of FS by slope ratio analysis (Table V). There was the same tendency in Ht increase as was observed in Hb repletion among the experimental groups (Table VI). Mean values for serum iron and TIBC (Table VI) showed trends similar to that demonstrated by Hb. Body weight gain (data not shown) and liver weight were not significantly different among the experimental groups except for the no iron added group. Liver iron was not significantly different among the animals fed with FP1 and FS at each iron level except for that at 6 ppm of iron (Table VII). However, liver iron contents from the FP1 (6 ppm of Fe) and FP1 (24 ppm of Fe) groups were significantly greater than that from the no iron added group. The significant effect of dietary iron level on liver iron reported by Johnson et al. (1987) was not observed clearly. This could be due to the difference in the iron state of the animals used: rats were made moderately anemic (Hb 7.8 g/dL) in their study. The weight of spleen and spleen iron from the FP1 and FS groups increased according to the iron repletion (Table VII). There was no significant difference between the animals fed with FP1 and FS at 6 and 12 ppm of iron: the reason for the lower value of the FS (24 ppm of Fe) group is not known. The relative bioavailability of FP1 (100) was also explained in terms of these parameters. The relative Hb values of the FP2 and FP3 groups were in reasonable agreement with the relative iron availabilities of 71 and 78 reported by Theuer et al. (1973). However, these values of FP2 groups were higher than that of Fritz et al. (1970). This discrepancy may be explained in terms of the difference in physicochemical state of ferric phrophosphate as described above.

Processing liquid infant formula (sterilization by standard commercial techniques) substantially improved the bioavailability of iron in ferric pyrophosphate (Theuer et al., 1973). When a soy isolate formula was used, similar sterilization treatment increased the availability of ferric pyrophosphate from 39% to 93% (Theuer et al., 1971). The process of sterilization was responsible for this improvement, not the step of dissolution or dispersion of the iron salt in the liquid formula (Theuer, 1985). Wood

Table VII. Experiment 3: Relative Wet Weight and Iron Content in Liver and Spleen[a]

iron source	iron dose, ppm	rel wet wt, g/100 g of body weight		iron content, μg/g of organ	
		liver	spleen	liver	spleen
no added iron	0	2.59 ± 0.04[a]	0.32 ± 0.03[a]	27.6 ± 1.8[a]	91.5 ± 8.0[a]
ferrous sulfate	6	2.82 ± 0.06[b]	0.32 ± 0.02[ab]	37.7 ± 2.4[ab]	101.9 ± 6.4[ab]
	12	2.76 ± 0.04[ab]	0.27 ± 0.01[abc]	32.8 ± 1.6[ab]	133.9 ± 7.1[b]
	24	2.62 ± 0.04[ab]	0.22 ± 0.01[c]	36.2 ± 2.0[ab]	122.3 ± 4.9[ab]
FP1[b]	6	2.54 ± 0.08[a]	0.32 ± 0.02[ab]	44.0 ± 5.3[bc]	105.4 ± 5.3[ab]
	12	2.62 ± 0.04[ab]	0.28 ± 0.02[abc]	39.1 ± 4.5[ab]	135.0 ± 7.8[b]
	24	2.62 ± 0.05[ab]	0.23 ± 0.01[c]	43.4 ± 3.4[bc]	193.2 ± 13.0[c]

[a] Values are means ± SE. Means not sharing a common superscript letter within a column are significantly different from each other at $P < 0.05$. [b] FP1, see footnote in Table III.

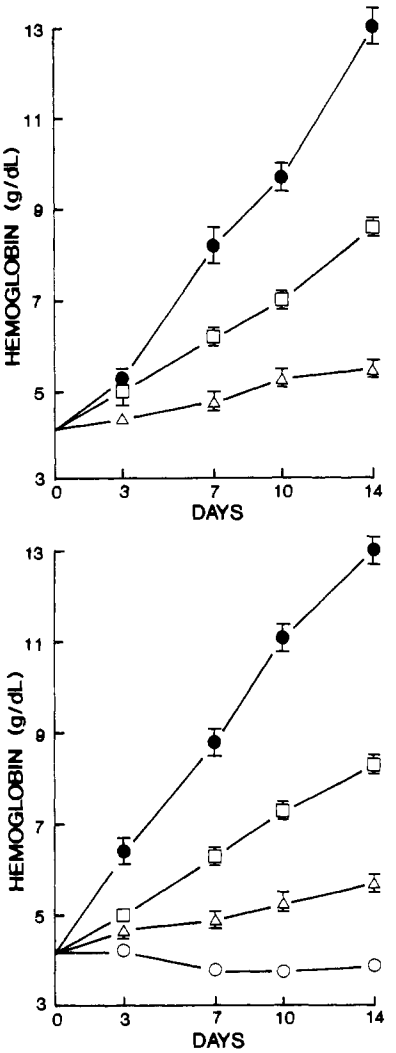

Figure 6. Experiment 3. Hb repletion curves of ferrous sulfate (bottom) and dehydrated ferric pyrophosphate suspension after treatment with skim milk (FP1) (top) at different iron doses: (○) no added iron; (△) 6 ppm; (□) 12 ppm; (●) 24 ppm. Data are expressed as means ± SE.

et al. (1978) studied the effects of heat and pressure processing (1055 g/cm^2 and 121 °C for 15 min) on the bioavailability of four inorganic iron salts by hemoglobin regeneration assay in anemic chicks. Heat and pressure treatment resulted in an increased relative biological value of ferric pyrophosphate, while the ralative biological value of ferrous sulfate remained unchanged. In the present study, to what extent the improvement of iron availability was due to the heating was not clear because a negative control group was not introduced to the experiments and the heating condition of FP1 or FP3 was moderate compared with that reported by Wood et al. (1978). However, iron–skim milk complexes were possibly formed in the preparation process of FP1 or FP3, which resulted in improved bioavailability.

The amount of iron available from the diet is often related to the relative concentrations of soluble iron and low molecular weight complexes to those agents which prevent absorption by the formation of insoluble precipitates, macromolecules, or high molecular weight complexes (Smith, 1983). Therefore, iron solubility is affected by ligands in food such as carbohydrates, proteins, and amino acids. The effects of several carbohydrates on iron absorption have been investigated. Carbohydrates presumably act as ligands for iron with a sterically favorable attachment through hydroxy groups (Lee, 1982). Bachran and Bernhard (1980) investigated the interaction of ferrous chloride with lactose using model systems. A soluble lactose–$FeCl_2$ complex, a lactose–iron gel, an insoluble lactose–$Fe(OH)_2$ adduct, and insoluble $Fe(OH)_2$ were formed; the amount of these forms was found to vary in this work. Amine et al. (1975) showed that iron utilization was greatest with diets containing lactose, but the effect was not uniform when iron sources of differing availability were tested (ferrous sulfate, reduced iron, sodium pyrophosphate, ferric orthophosphate). Relative biological values of sodium iron pyrophosphate and ferric orthophosphate were significantly reduced when β-lactose was added to the diet, while β-lactose tended to enhance the utilization of iron from ferrous sulfate (Pennel et al., 1976). However, α-lactose had no effect on the relative biological value of sodium iron pyrophosphate. Animal proteins are one of the substances that facilitate the absorption from nonheme iron. However, not all animal proteins enhance iron absorption; milk, cheese, and egg have not shown a significant effect on iron absorption in human subjects (Cook, 1976). The enhancing effect of meat on nonheme iron absorption is due to sulfydryl groups of cysteine (Taylor, 1986). Shears et al. (1987) indicated that complexation of iron occurred with the in vitro protein digest, and the carboxyl groups were identified as a site of complexation. The diets used in the present experiments had the same composition except for iron sources; there were no differences in the lactose content or the protein digestion products. The diet contained a smaller amount of lactose (0.4%) compared with that of Pennel et al. (1976) (20%). The present data were inadequate to explain the effect of lactose or protein digest, but the amount of soluble iron stabilized by these components could be considered greater in the FP1 group than in the FP2 groups.

It is recommended from the present study that ferric pyrophosphate should be used in an aqueous suspension form and treated with skim milk before drying when supplemented to powdered infant formulas. Further work to understand the relationship between the amorphous

structure and the iron bioavailability of powdered ferric phrophosphate would contribute to the iron fortification of various foods.

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