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Bioavailability of Iron from Iron Phosphates in Cereals and Infant Foods

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Bioavailability of iron from ferric orthophosphate and sodium iron pyrophosphate (SIP) used in the fortification of breakfast cereals and infant foods was determined by the rat repletion assay. The relative biological value (RBV%, $FeSO_4$ = 100%) of iron from ferric orthophosphate added to breakfast cereals ranged from 33% to more than 60%. The RBV of iron from SIP added to breakfast cereals and infant cereals was found to lie between 14 and 40%. The bioavailability of iron from SIP in two soy-based infant formulas was higher (48 and 57%), indicating the favorable effect of processing. An experimental cereal containing wheat, corn syrup, and honey fortified with SIP gave a value of 78%. When the same specimen of SIP was added to the rat diet containing the unfortified cereal, an RBV of 73% was obtained. The variable bioavailability of iron from these iron phosphates needs to be investigated in regard to their physical-chemical characteristics which affect the bioavailability.

Ferric orthophosphate and sodium iron pyrophosphate are used as iron source additives in breakfast cereals, infant cereals, and in some infant formulas mainly because they do not impart any color to the final product and the keeping quality of the foods is not adversely affected. Questions have been raised, however, regarding the bioavailability of iron added to foods in these forms (Rios et al., 1975; Fritz et al., 1975). The bioavailability of iron from ferric pyrophosphate and sodium iron pyrophosphate was reported to improve due to processing involved in the manufacture of diets for weight control (Hodson, 1970) and infant formulas (Theuer et al., 1971). Information on the bioavailability of iron added to breakfast cereals and infant foods in the form of iron phosphates is not available. It was therefore decided to determine the availability of iron from cereal products and infant formulas fortified with iron phosphates by using the rat repletion assay (Shah and Belonje, 1973).

MATERIALS AND METHODS

The rat repletion assay as described before (Shah and Belonje, 1973) was employed to determine the relative biological value (RBV) of iron from cereal products and infant formulas. Ferrous sulfate was used as the standard source. Many of the products were tested at one level of feeding. For screening purposes, this was found by Coccodrilli et al. (1976) to be quite satisfactory. The single level of feeding was chosen in such a way that the anticipated response would be close to that of the middle level of the standard source. The foods assayed with their major ingredients and the iron content are given in Table I. For the determination of iron 2 g of each food was ashed in muffle furnace at 450 °C using 50% nitric acid (Baker analyzed) as ash-aid. The ash was dissolved in 25% hydrochloric acid (Baker analyzed) and the iron content was determined by atomic absorption spectroscopy.

The cereal products and the freeze-dried infant formulas were added to the basal diet in place of starch. Breakfast cereals formed 13–33% of the diet and the freeze-dried infant formulas made up about 30% of the diet, whereas the infant cereals ranged from 3.5 to 7.2%. These food products, along with some other iron sources were tested in six separate experiments. The basal diet contained: casein, 20%; sucrose, 40%; corn starch, 25%; corn oil, 10%; vitamin mix [Momcilovic et al., 1976; except pyridoxine hydrochloride was increased to 0.7 g/kg of mixture according to NRC (1972) recommendation], 1%; mineral mix [excluding iron, g/kg of salt mixture according to NRC (1972) recommendations: sodium chloride, 20.607; sodium carbonate, 10.125; potassium sulfate, 45.347; potassium carbonate, 43.570; magnesium carbonate ($4MgCO_3 \cdot Mg(OH)_2 \cdot 5H_2O$), 40.435; manganese sulfate ($MnSO_4 \cdot H_2O$), 3.846; sodium selenite, 0.0022; cupric carbonate ($CuCO_3 \cdot Cu(OH)_2 \cdot H_2O$), 0.235; zinc sulfate ($ZnSO_4 \cdot 7H_2O$), 1.319; potassium iodide, 0.005; calcium carbonate, 150.632; calcium phosphate monobasic ($CaH_4(PO_4)_2 \cdot H_2O$), 406.835; dextrose, 277.040], 4%. The iron content of the basal diet was found by analysis to vary from 4.7 to 8.0 μg/g, the major sources being casein and the calcium salts in the mineral mixture. The iron contents of the diets containing the standard source ($FeSO_4$) and the cereals were determined as described above and found to be within ±10% of the expected values. Based on the reported iron contents of the ingredients of the cereal products and on probable proportions of the major ingredients, the contribution of the endogenous iron in the breakfast cereals ranged from 6 to 15% of the total iron and in the infant cereals it varied from 1 to 8%. The proportions of endogenous iron in infant formulas L and M were 28 and 14%, respectively. Thus in all the products, except infant formula L, at least 85% of the total iron was contributed by the iron phosphate concerned.

In the case of breakfast cereals N and O the parallel line assay model (Shah and Belonje, 1973) did not fit the hemoglobin data after 2 weeks of repletion. However, the slope ratio model (Amine et al., 1972) was found to be suitable. The RBV and the 95% fiducial limits were calculated according to this model.

RESULTS AND DISCUSSION

Typical data for food intake during 2 weeks of repletion, the body weights and the hemoglobin levels at the beginning and at the end of repletion are summarized in Table II. Initially, hemoglobin levels of the rats in the

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Table I. Foods Tested by the Rat Repletion Assay

product type	iron source	sample	major ingredients	iron content (by analysis), μg/g
breakfast cereal	FO[a]	A	rice	120
		B	corn, corn syrup, molasses	155
	SIP[b]	C	oats	300
		D	corn, oats, honey	230
infant cereal	SIP	E	rice	710
		F	wheat, oats, corn, soy	560
		G	soy	590
		H	barley	1160
		I	barley, mixed fruits	1040
		J	wheat, oats, corn, skim milk powder, yeast	1020
		K	rice	810
infant formula[c]	SIP	L	soy	70
		M	soy	105
breakfast cereal[d]	SIP	N	wheat, corn syrup, honey	1330
		O	sample N, unfortified[f]	

[a] Ferric orthophosphate ($FePO_4$). [b] Sodium iron pyrophosphate ($Na_8Fe_4(P_2O_7)_5 \cdot xH_2O$). [c] Freeze-dried. [d] An experimental breakfast cereal supplied by G. Coccodrilli, Jr., General Foods Corporation, White Plains, NY. [f] The sodium iron pyrophosphate, which was used in the fortification of cereal N, was added to the diets to give the same iron levels.

Table II. Typical Data for a 2-Week Repletion Assay

source	iron added, μg/g	hemoglobin, g/100 mL		body weight, g		food intake, g
		initial	final	initial	final	
basal	0	5.3 ± 0.5[a]	4.9 ± 0.2	178 ± 15	227 ± 19	179 ± 13
$FeSO_4$	6	5.3 ± 0.6	6.2 ± 0.6	175 ± 19	226 ± 19	185 ± 8
	12	5.4 ± 0.6	8.4 ± 0.7	182 ± 10	253 ± 15	211 ± 17
	24	5.4 ± 0.6	10.5 ± 0.7	177 ± 16	251 ± 14	222 ± 12
rice cereal						
K in Table I	20	5.3 ± 0.5	7.0 ± 0.9	176 ± 12	236 ± 16	191 ± 16
	40	5.3 ± 0.5	9.3 ± 0.8	168 ± 19	231 ± 24	196 ± 15
	80	5.3 ± 0.5	11.7 ± 0.6	179 ± 20	255 ± 25	221 ± 16

[a] Mean ± standard deviation.

Table III. Relative Biological Value (RBV, $FeSO_4$ = 100%) of Iron from Iron Phosphates in Cereals and Infant Foods

product type	iron source	sample	manufacturer no.	iron from cereal, μg/g	RBV, %	95% fiducial limits
breakfast cereal	FO[a]	A	1	40	33	25-44
		B[b]	1	40		
	SIP[c]	C	2	40	14	10-19
		D	2	40	26	20-34
infant cereal	SIP	E	3	40	23	18-30
		F	4	40	22	17-29
		G	4	40	22	17-29
		H	5	40	17	13-29
		I	5	40	23	18-30
		J	3	20, 40, 80	63	54-73
		K	3	20, 40, 80	40	32-49
infant formula[d]	SIP	L	6	20	48	40-58
		M	6	30	57	47-69
breakfast cereal[e]	SIP	N	7	12, 24, 36	78[f]	72-84
		O	7	12, 24, 36	73[f]	68-79

[a] Ferric orthophosphate ($FePO_4$). [b] The hemoglobin response was above the response to the highest standard, viz. 25 μg/g as ferrous sulfate. [c] Sodium iron pyrophosphate ($Na_8Fe_4(P_2O_7)_5 \cdot xH_2O$). [d] Freeze-dried. [e] An experimental breakfast cereal. [f] By slope ratio assay model.

different diet groups were quite uniform because the allocation of the animals to the groups was made on the basis of their hemoglobin levels. The same was not true for initial body weights, although the differences between the initial weights were small. The variation in food intake and body weight gain of the rats in the different diet groups did not have any appreciable effect on the RBVs for the iron sources tested. This observation was similar to our earlier report in which only elemental iron powders were assayed (Shah and Belonje, 1973).

The RBVs of iron from iron phosphates used in the fortification of breakfast cereals and infant foods are given in Table III. Breakfast cereals A and B containing ferric orthophosphate were products of the same manufacturer but the response to B was higher (14.0 ± 1.0 g of hemoglobin) than that for the standard diet containing 25 μg/g of iron from ferrous sulfate, indicating the RBV of iron was at least 60%. This was much higher than the 33% RBV for the cereal A. It is likely that the difference was due to the interaction of the ingredients in the cereals or a difference in processing. It is also probable that the two lots of ferric orthophosphate were not identical. The RBV of different specimens of ferric orthophosphate has been reported by Coccodrilli et al. (1976) and by Harrison et

al. (1976) to be highly variable. Cereals C and D fortified with sodium iron pyrophosphate (SIP) were products marketed by another manufacturer. The RBV for D was slightly higher than that for C. Infant cereals E, J, and K containing SIP were produced by the same manufacturer, but since E was bought much earlier than J and K, the source of SIP could have been different. Interestingly the RBV of iron from these cereals varied from 23 to 63%, indicating that the SIP used was probably not of the same quality. The bioavailability of iron from infant cereals F and G (manufacturer 5) was comparable to that of iron from cereal E (manufacturer 3). All these cereals fortified with SIP were poor sources of iron. However, all forms of SIP were not of poor quality. The SIP in product J was probably the best and that in K was of intermediate quality.

The differences in the bioavailability of iron from ferric orthophosphate and from SIP could be due to variation in the chemical composition of the specimens, the shape and size of the particles, or their solubility in dilute hydrochloric acid or gastric or intestinal juices (British Ministry of Health, 1968; Fritz et al., 1975; Harrison et al., 1976; Coccodrilli et al., 1976). The relationship between these parameters and the bioavailability of iron from iron phosphates needs further investigation. As in the case of iron powders (Shah et al., 1977), there is a need to develop an in vitro chemical test which can distinguish between an acceptable and a poor quality ferric orthophosphate or sodium iron pyrophosphate.

Processing has been reported to increase the bioavailability of iron from ferric pyrophosphate added to liquid infant formula products (Theuer et al., 1971) and of iron from ferric orthophosphate added to weight control dietaries (Hodson, 1970). However, the processing involved in the manufacture of cereal products does not involve conditions which would be conducive to promoting the reduction of ferric ion to the ferrous state (Steele, 1976). Iron source additives and vitamins are usually added at a stage beyond which the product is subject to minimum heat treatment. This is confirmed by the RBV of SIP in breakfast cereals N and O. In the case of N the effects of processing were present but the RBV of iron from SIP was not affected. Thus it was evident that the particular specimen of SIP used in these cereals was of a very good quality.

The bioavailability of iron added to soy-based infant formulas L and M was higher than all the cereal products assayed except the experimental cereals N and O. Most probably this improvement in availability was the result of processing, as reported by Theuer et al. (1971), in the case of ferric pyrophosphate.

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Volatile Constituents of *Castanopsis* Flower

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The volatile constituents of *Castanopsis* flower (*C. caspidata* Schottky var. Sieboldii Nakai), which had not been studied prior to this report, have been investigated by gas chromatography–mass spectrometry. Fifty-six compounds were identified in the oil which was obtained from this flower by the distillation–extraction method (Likens and Nickerson apparatus). The compounds reported here were 11 monoterpenes, 4 sesquiterpenes, 21 aliphatic compounds, 12 aromatic compounds, and 7 heterocyclic compounds. Major compounds of this oil were salicylaldehyde, *o*-aminoacetophenone, methyl salicylate, linalool, and nonanal.

Castanopsis caspidata Schottky var. *Sieboldi Nakai* (*Sudajii* in Japanese) grows wild over central and southern Japan and is commonly found in the forest. Some grow to heights of 20 m. The flower (spike form) blooms from May to June, and the plant bears nuts which are edible in autumn. This plant is a member of Fagaceae family which includes *Castanea* (chestnut tree). The flowers of *Castanopsis* and *Castanea* have similar characteristic odors, and recently those odors have received much attention as they are used to give an animal note to fragrances.

In this study, the volatile constituents of *Castanopsis*

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