Availability of Iron in Enriched Soda Crackers[1]

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ABSTRACT

The availability of iron in soda crackers enriched with ferrous sulfate and reduced iron was tested in hemoglobin-depleted rats. Availability assessments were based on the extent of repletion of hemoglobin in response to test diets fed for 15 days. All test diets contained 20 p.p.m. of iron furnished by enriched crackers or bread crumbs. While rats on the control diet (unenriched crackers; iron, 7 p.p.m.) continued to become progressively more anemic during the repletion period, those fed test diets gradually recovered. Hemoglobin recovery was most rapid in rats fed ferrous sulfate and less than half as rapid in those fed reduced iron. No differences were observed in iron availabilities between enriched breads and crackers. Exclusion of soda in cracker-making or its addition to cracker crumbs, or the addition of iron to cracker crumbs instead of to the flour used, had no appreciable effect on iron availability. Compared to bread, the process of cracker-making increased the pH appreciably and reduced the solubility of iron under simulated gastric conditions, but the availability of the iron remained unaffected.

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To reduce or prevent the prevalence of iron-deficiency anemia in the human population of this country (1,2), the U.S. Food and Drug Administration (3) has proposed that the content of iron in enriched wheat flour be increased to 40 mg. per lb. While knowledge of the content of iron in a food, enriched or otherwise, is important, it is imperative that the extent of availability of iron in that food also be known in order to fully assess its potential as a source of iron. Many factors affect the availability of iron in food. We earlier reported (4) on the availability of iron in bread as affected by the source of iron used. The consideration that the availability of iron in enriched soda crackers might be reduced because of the possible formation, during cracker-making, of carbonates and/or oxides of iron, prompted the present studies. The availability of iron in soda crackers enriched with ferrous sulfate and with reduced iron was thus tested using hemoglobin-depleted rats.

MATERIALS AND METHODS

Weanling male rats of Sprague-Dawley strain weighing about 50 g. initially were housed in stainless-steel cages and fed a low-iron diet (Table I) for 5 weeks when their hemoglobin (Hb) levels dropped to less than 5 g. per 100 ml. Hb was determined on tail blood (amputated tail tip) by the Cyanomet-hemoglobin method (5) and hematocrit (Hct) by microcapillary centrifugation.

Finely powdered ferrous sulfate (iron, 30.3%) and reduced iron (reduced by hydrogen; iron, 96%) intended for commercial enrichment were used to enrich flour at a level representing the maximum (16.5 mg. iron per lb.) presently permitted by the enriched flour standard of identity. Bread and crackers were made, using enriched flour, by the sponge-and-dough procedures according to the formulas shown in Table II.

Sucrose in the low-iron diet was replaced with appropriate amounts (about 50 g.) of air-dried, finely ground bread or cracker crumbs to obtain diets (Table III) containing 20 p.p.m. of iron. Diet prepared with unenriched crackers had an iron content of 7 p.p.m. and served as the control. Deionized water and diets, premixed

TABLE I. COMPOSITION OF LOW-IRON DIET

Ingredients	g.
Dried skim milk[a]	30.0
Vitamin diet fortification mixture[a]	2.0
Corn oil	4.0
Sodium chloride	1.0
Calcium carbonate	1.0
Monosodium phosphate	1.0
Trace minerals[b]	0.43
Sucrose	60.57
	100.00
Iron, p.p.m.	3.0
Protein (N X 6.25)	10.0

[a]From Nutritional Biochemicals, Cleveland, Ohio. Vitamin mixture contained ascorbic acid.

[b]Contained the following (mg.): $MgSO_4 \cdot 7H_2O$, 414; $MnSO_4 \cdot H_2O$, 15; $CuSO_4$, 1.3; $ZnCl_2$ 2.5; and KI, 0.024.

TABLE II. FORMULATION OF BREAD AND CRACKERS

	Bread		Crackers	
	Sponge g.	Dough g.	Sponge g.	Dough g.
Flour	70.0	30.0	60.0	40.0
Yeast	2.5	...	0.2	...
Yeast food	0.5	... z	...	...
Sugar	...	6.0	...	...
Salt	...	2.0	...	1.25
Lard	...	3.0	12.5	...
Monoglycerides	...	0.5	...	...
Sodium bicarbonate	...	...	...	0.725
Water	44.8	19.2	26.8	...
	Sponge time: 210 min. Floor time: 20 min. Interm. proof: 10 min. Pan proof: 55 min. Baking (450° F.): 20 min.		Sponge time: 19 hr. at 72° F. Dough time: 5 hr. at 83° F. Sheeted out dough was cut into crackers and baked (425° F., 7 min.)	

with water to form a slurry to minimize spillage, were offered ad libitum to diet groups of eight rats. Weight gain and food intake records were kept for individual rats. Hb and Hct were measured at 5-day intervals for 15 days following the 5-week depletion period. Total Hb regenerated during the 15-day repletion period was calculated based on the blood volume of rats (6.7 ml. per 100 g. body weight) corresponding to their individual body weights and Hb concentrations at days 0 and 15.

Iron in the iron sources, in bread and cracker crumbs, and in the resultant test diets was determined (ashed samples) by the a, a′dipyridyl method (6). To examine the solubility of iron under simulated gastric conditions, a suitable sample of bread and cracker crumbs was extracted with pepsin-HCl (0.5% pepsin in 0.1N HCl; pH, 1.15) for 90 min. at 37°C. in a Dubnoff shaker (30 oscillations per min.). Total iron in the clear filtrate was determined by the above method. The pH of bread and cracker crumbs was determined by the AACC method (6).

RESULTS AND DISCUSSION

Table III summarizes the results of Hb and Hct regeneration in anemic rats fed, for 15 days, isoproteinous diets containing 20 p.p.m. of iron furnished by enriched bread and crackers. Dietary iron, though marginal when fed at 20 p.p.m. level, induces Hb regeneration at a satisfactory rate and permits a reasonably accurate assessment of relative iron availabilities. Because of differences in body weight of rats and in their diet consumption and thus iron intakes, relative bioavailabilities were calculated based on the gain, in 15 days, of Hb expressed as grams per milligram of iron consumed rather than Hb concentration (grams, percent) alone.

The differences observed in iron availabilities between ferrous sulfate and reduced iron agree closely with the results reported earlier (4) and also by others

TABLE III. AVAILABILITY OF IRON IN ENRICHED SODA CRACKERS[a] (15-DAY EXPERIMENT)

	Iron Source							
	Ferr. sulfate		Reduced iron					None
Diet No.[b]	A(BRD)	B(CRA)	C(BRD)	D(CRA)	E(CRA)	F(CRA)	G(CRA)	H(CRA)
Dietary iron[c], p.p.m.	20	20	20	20	20	20	20	7
Body weight gain, g.	110	109	105	97	95	100	94	72
	±7	±7	±15	±13	±13	±12	±8	±13
Diet intake, g.	221	197	208	179	183	191	181	150
	±12	±17	±20	±18	±18	±19	±13	±16
Hemoglobin, g./100 ml.								
0 day	4.72	4.75	4.79	4.72	4.74	4.73	4.73	4.72
	±1.11	±1.13	±1.07	±0.86	±0.81	±0.75	±0.81	±0.77
5 day	5.54	5.71	4.22	4.27	4.09	4.35	4.06	3.56
	±0.59	±0.62	±0.64	±0.75	±0.16	±0.44	±0.53	±0.22
10 day	6.69	6.36	4.48	4.18	4.36	4.32	4.21	3.14
	±0.37	±0.54	±0.65	±0.51	±0.21	±0.39	±0.26	±0.29
15 day	8.48	8.36	5.63	4.94	4.94	5.06	5.00	2.99
	±0.40	±0.91	±0.68	±0.61	±0.57	±0.45	±0.96	±0.19
Hematocrit, %								
0 day	23.6	24.2	24.0	23.0	23.7	24.1	23.1	24.4
	±3.3	±2.9	±3.1	±3.3	±3.3	±2.2	±3.2	±2.6
5 day	27.4	27.7	23.1	21.5	21.3	21.7	20.9	18.6
	±1.5	±1.1	±1.8	±2.9	±1.0	±1.2	±1.9	±2.0
10 day	33.4	31.5	23.9	23.3	22.8	23.3	23.1	18.1
	±3.1	±3.0	±1.8	±2.4	±2.2	±1.2	±3.0	±1.2
15 day	37.6	38.1	28.4	27.9	27.2	27.0	26.9	20.3
	±2.0	±3.4	±1.4	±2.7	±2.3	±1.7	±3.5	±3.1
Iron consumed, mg.	4.428	3.931	4.153	3.576	3.654	3.789	3.627	...
	±0.246	±0.331	±0.435	±0.364	±0.364	±0.400	±0.240	
Hemoglobin gain[d], g.	0.913	0.839	0.419	0.338	0.327	0.365	0.312	...
	±0.132	±0.126	±0.140	±0.075	±0.059	±0.053	±0.088	
Hemoglobin gain,	0.206	0.214	0.099	0.094	0.090	0.096	0.086	...
g./mg. iron consumed	±0.026	±0.028	±0.026	±0.015	±0.018	±0.009	±0.024	
Relative iron availability[e], %	100	104	48	46	44	47	42	...
Solubility of iron[f], %	28.6	22.4	22.4	9.6	11.4	8.8	25.9	18.8
pH[g]	5.3	8.0	5.3	8.2	5.9	6.8	8.1	8.1

[a] All values represent average of eight rats ± standard deviation. Unless indicated otherwise, all data cover the entire 15-day repletion phase.

[b] All diets contained iron-enriched bread (BRD) or crackers (CRA) excepting diet G (iron added to finished cracker crumbs) and H (unenriched crackers). All cracker diets also contained soda in the formula, except diet E (no soda) and diet F (soda added to finished cracker crumbs).

[c] Sucrose in low-iron diet was replaced with about 50 g. of bread or cracker crumbs to obtain diets of 20 p.p.m. of iron. Unenriched crackers used in formulating diet H.

[d] Total gain in Hb in 15 days as calculated based on blood volume of rats and their Hb concentration at days 0 and 15.

[e] Based on Hb gain expressed as grams per milligram of iron consumed.

[f] In bread or cracker crumbs under simulated gastric conditions.

[g] Of bread or cracker crumbs.

(7,8). Whereas in rats fed reduced iron the increase in Hb and Hct levels did not occur until after day 10 of feeding, those fed ferrous sulfate showed a more rapid increase in their Hb and Hct levels, apparently because of higher assimilation of iron. A higher Hb level in rats fed bread diets as against cracker diets (diets A and C versus B and D) apparently resulted from higher intakes of iron rather than from differences in availabilities, since relative availabilities differed very little. Sodium bicarbonate, as used in cracker-making, did not appear to interfere with the availability of iron. The characteristic high availability of iron from ferrous sulfate remained unaffected when enriched flour was used in the making of crackers as in the making of bread (diets A versus B). This appeared to be the case also for reduced iron (diets C versus D). Elimination of sodium bicarbonate from the cracker formula or its addition to cracker crumbs instead of to the dough did not affect iron availability (diets D versus E and F). Apparently transformation of iron to carbonates and/or oxides which are very poorly available (7-9) did not occur during cracker-making. Availability of iron was also unaffected when iron was added directly to finished cracker crumbs instead of to the flour used (diets D versus G).

The relative order of solubility of iron, under simulated gastric conditions, showed little relationship to relative bioavailabilities. The process of cracker-making, however, did reduce the solubility of iron (Table III) since the iron in bread and also in samples in which iron was added to the cracker crumbs instead of to the flour used was appreciably more soluble. The changes in pH appeared not to affect iron availability. The data thus seem to suggest that iron in enriched soda crackers remains as well available as in enriched bread in spite of a decrease in its solubility and increased pH.

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