Bioavailability of Different Sources of Dietary Iron Fed to Pitman-Moore Miniature Pigs[1]

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ABSTRACT Prevention of Fe deficiency was studied with eight Pitman-Moore miniature pigs in each of seven groups. From 5 to 33 days of age the animals received identical cereal–milk diets except for Fe content: diet 1, no added Fe (8 ppm); diet 2, ferrous sulfate; diet 3, catalytically reduced Fe; diet 4, electrolytic Fe powder; diet 5, sodium Fe pyrophosphate; diet 6, ferripolyphosphate powder; diet 7, disodium Fe EDTA. Diets 2–7 provided 64 to 69 ppm of supplemental Fe. Body weight and gain in weight of pigs fed diet 1 did not differ significantly from those of pigs fed diets 2–7; however, gain in body weight between 21 and 28 days of the feeding trial was significantly less in pigs fed diets 1 or 5 than in pigs fed diets 2, 6 or 7. Final Hb and hematocrit values were less than initial values in pigs fed diets 1, 3, 4 and 5. The change in Hb, hematocrit and total Hb Fe of pigs fed diets 1, 3 or 5 differed significantly from those of pigs fed diets 2, 6 or 7. The efficiency with which supplemental Fe was incorporated into Hb ranged between 27 and 30% in pigs fed diets 2, 6 or 7, was 21% in pigs fed diet 4 and approximately 10% in pigs fed diets 3 or 5. Pigs fed diet 1 differed from those fed the other diets in the following manner: liver weight was significantly less; weights of spleen, heart and kidney were significantly greater; less of an intraperitoneal injection of ^{59}Fe was retained in Hb and in organs; of the retained ^{59}Fe, significantly less was found in Hb and significantly more in tissues. Fe status as measured by Hb and hematocrit appeared to be inversely correlated with ^{59}Fe concentration in liver, spleen, kidney and heart. J. Nutr. *104:* 619–628, 1974.

INDEXING KEY WORDS Fe bioavailability · Hb formation · ^{59}Fe metabolism · infant cereals

All commercially prepared strained cereals for infants currently marketed in the United States are fortified with sodium iron pyrophosphate and/or electrolytic Fe powder at levels ranging from 50 to 100 mg of elemental iron (Fe) per 100 g of dry product. The Fe-fortified cereals have been fed to infants for many years; however, bioavailability of the specific forms of Fe used to supplement these cereals has rarely been evaluated in experimental designs based on prevention of anemia. The miniature pig experiment reported here compares the bioavailability of various forms of Fe in rapidly growing animals. Forms of Fe used in commercial production of infant cereals are compared with three experimental forms of Fe in a cereal–milk mixture similar to that commonly fed to infants.

MATERIALS AND METHODS

Fifty-six Pitman-Moore pigs were weaned at 5 days of age and allowed ad libitum access to diet (table 1) and distilled water throughout the 28-day feeding period. Proximate analysis of the diet by calculation was (in %): carbohydrate, 38.4; protein, 26.9; fat, 25.8; ash, 6.0; water, 2.7; crude fiber, 0.2. Since it has been shown by Theuer et al. (1, 2) that the availability of supplemental iron depends upon the man-

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TABLE 1

Composition of diets (%)

Whole milk powder[1]	94
High-protein cereal[2]	6
Vitamins[3]	+
Trace minerals[4]	+
Elemental Fe added at 100 mg/100 g from the following sources:	
Diet 1–No added Fe	
Diet 2–$FeSO_4$ (FS)[5]	
Diet 3–Catalytically reduced Fe (RI)[6]	
Diet 4–Electrolytic Fe powder (EI)[7]	
Diet 5–Sodium Fe pyrophosphate (SIP)[8]	
Diet 6–Ferripolyphosphate powder (FP)[9]	
Diet 7–Disodium Fe EDTA (DIE)[10]	

[1] Extra Grade, Lot 1248, North Star Dairy, St. Paul, Minn. [2] Gerber Products Co. Ingredients: soya flour, 60–64% (to adjust protein level to 35%); oat flour, 23%; wheat flour, 11%; sugar, soya oil, calcium carbonate, lecithin, thiamin, riboflavin, niacin, 4%. Proximate analysis (in %): carbohydrate, 42.5; protein, 35.0; ash, 7.4; water, 6.3; fat, 6.2; crude fiber, 2.6. [3] mg/kg diet: vitamin B-12, 0.022; niacin, 7.0; *dl*-α-tocopheryl acetate, 387 (194 IU). [4] mg/kg diet: Cu ($CuSO_4$), 4.5; Mn ($MnSO_4$), 20.0; Zn ($ZnSO_4$), 200; I (KI), 0.2. [5] $FeSO_4 \cdot 7H_2O$, food grade, Mallincrodt Chemical Works, St. Louis, Mo. [6] Catalytically reduced Fe (carbon monoxide), nominally extra fine (20–40 μ), Mallincrodt Chemical Works, St. Louis, Mo. [7] Electrolytic Fe powder (0–40 μ), Glidden Metals, Cleveland, Ohio. [8] Stauffer Chemical Co., Chicago, Ill. [9] Ferripolyphosphate whey protein powder #93010, Eastern Marketing & Nutrition Research Division, USDA, Philadelphia, Pa. [10] (Sequestrene), Geigy Chemical Co., Ardsley, N. Y.

ner in which it is incorporated into the diet, the six forms of supplemental Fe were incorporated into the high protein cereal [2] during processing. Analysis of the diets revealed the following amounts of Fe (diets 1–7, mg Fe/kg): 8, 68, 66, 66, 64, 64 and 69. Of the total Fe in diets 2–7, it is estimated that approximately 12% was naturally occurring whereas 88% was added in processing. The Fe content of these diets was intended to be slightly less than the 80 mg/kg level estimated by the National Research Council (3) to meet the Fe requirement of the baby pig.

The pigs were maintained individually in stainless steel cages measuring 75 × 90 × 70 cm, each equipped with a rubber-covered electric heating pad, feeder and waterer.

Samples of blood were taken from the tail at 0, 7, 14, 21, 24 and 28 days of study. On day 14 of the experiment each pig was given a 10 μCi intraperitoneal injection of ^{59}Fe ($FeCl_3$; 5.13 mCi/mg Fe) in 2 ml of 0.9% NaCl. After a 12-hour fast on day 29 of the study a blood sample was obtained from the anterior vena cava and the pigs were then exsanguinated. The liver, spleen, kidneys, heart, adrenals, thyroid and samples of psoas muscle and intestinal mucosa were weighed and frozen for further analysis.

Hemoglobin (Hb) was measured by the cyanmethemoglobin method [3] and hematocrits were determined after centrifugation of blood in heparinized capillary tubes. Automated methods were used for determining serum concentrations of Fe, cholesterol and total protein.[4] Atomic absorption spectrophotometry was employed for determining concentrations of Fe in the diets and in tissue.[5] Serum concentrations of triglyceride were determined according to the procedure of Lofland (4). Separation of serum proteins into their fractions was carried out by electrophoresis on cellulose acetate as described previously (5). Activity of ^{59}Fe in blood and tissue was determined with a gamma well counter.[6]

The data were analyzed as a two-way analysis of variance for sex and diet effects as outlined by Snedecor (6). Because statistically significant sex-related differences were not noted in any of the parameters

[2] Supplied by Dr. George A. Purvis, Gerber Products Co., Fremont, Mich.

[3] Hycel Cyanmethemoglobin Kit, Hycel, Inc., P. O. Box 36329, Houston, Tex.

[4] Technicon Instruments Corp., Tarrytown, N. Y., AutoAnalyzer Model QB. These methods are described in detail in the Technicon Handbook as follows: Fe, N-62p; cholesterol, N-24a; and total protein, N-14b.

[5] Atomic Absorption Spectrophotometer, Model 303. "Analytical Methods for Atomic Absorption" 1971 Perkin-Elmer Corp., Norwalk, Conn.

[6] Picker Magna Scaler III A, Model 6041 with well counter equipped with a NaI scintillation detector, Picker Nuclear, North Haven, Conn.

TABLE 2

Effect of dietary Fe source on performance of Pitman-Moore miniature pigs[1]

Parameter	Diet number 1 (O)	2 (FS)	3 (RI)	4 (EI)	5 (SIP)	6 (FP)	7 (DIE)	SEM	Critical value[2] $P < 0.05$
Body weight (g)									
Initial	1388	1361	1381	1327	1239	1372	1321	79.4	349
Final	5676	6424	5789	6030	5508	6303	6433	303.1	1331
Daily gain in wt (g)									
wk 1	45	38	35	49	50	49	66	11.4	50
wk 2	166	176	156	161	167	164	167	13.8	61
wk 3	192	216	204	209	178	222	217	15.7	69
wk 4	220	304	245	263	223	280	289	12.6	56
Average (28 days)	155	182	159	169	154	178	184	10.0	44
Gain (g/kg feed)	857	833	889	904	886	834	817	28.4	125
Fe intake (mg)									
Total	42	415	330	343	311	378	431	20.6	90
Supplemental	—	363	288	299	270	329	378	20.0	88

[1] Eight animals in each treatment group. [2] Tukey, J. W. (7) Difference between treatment means greater than critical value statistically significant ($P < 0.05$).

measured, only data pertaining to diet effects are presented in tables 2 to 6. Tests of multiple comparison were calculated according to the method of Tukey (7).

RESULTS

Performance. Data on body weight, gain in weight and intake of Fe during the 28-day feeding period are presented in table 2. There were no significant differences in initial or final body weights between treatment groups. However, a treatment-related effect on gain in body weight was apparent by week 4 of feeding. Pigs not receiving a supplemental source of dietary Fe (diet 1) and those fed diet 5 gained significantly less in body weight between 21 and 28 days of feeding than did pigs fed diets 2, 6 and 7. In addition, pigs fed diet 2 gained significantly more weight between days 21 and 28 of the study than did pigs fed diet 3. Source of supplemental Fe was without effect on feed efficiency.

Although final body weights did not differ significantly among treatment groups, larger pigs generally consumed greater quantities of food and, hence, Fe than did smaller pigs (table 2). Pigs fed diets 2 and 7 consumed signficantly more supplemental Fe during the 28-day feeding period than did pigs fed diet 5. Pigs fed diet 7 also consumed more supplemental Fe than did pigs fed diet 3.

Blood and serum chemical values. The influence of dietary Fe source on concentration of Hb in blood was evident in some treatment groups by day 7 of the study. Hematocrit values closely approximated the changes seen in concentration of Hb. Data presented in table 3 compare initial and final blood values for Hb, hematocrit and total Hb Fe. Analyses of serum Fe, total Fe binding capacity (TIBC) and percentage saturation of transferrin were not undertaken until the end of the study.

The amount of Fe included in diets 3, 4 and 5 was not sufficient to maintain initial levels of Hb or hematocrit. Final concentrations of Hb in blood of pigs fed no added Fe (diet 1) did not differ significantly from those of pigs fed diets 3 and 5; however, the final hematocrit of pigs fed diet 1 was significantly less than that of any other treatment group.

Since there was a maximum range of 1.0 g in initial concentrations of Hb and a range of 3.0 in initial hematocrit between some of the treatment groups, it seems relevant to express final Hb concentration and hematocrit in terms of change from initial levels. There was no significant difference in change in Hb concentration or hematocrit between the pigs fed the no-added-Fe diet and those fed diets 3 and 5. No significant changes in Hb concentra-

TABLE 3

Effect of dietary Fe source on blood and serum Fe status of Pitman-Moore miniature pigs

Parameter	Diet number 1 (O)	2 (FS)	3 (RI)	4 (EI)	5 (SIP)	6 (FP)	7 (DIE)	SEM	Critical value $P < 0.05$
Hb (g/100 ml)									
Final	6.2	11.3	7.6	9.4	7.7	10.5	10.8	0.38	1.7
Initial	10.2	9.6	10.5	10.3	10.3	9.5	9.9	0.52	2.3
F–I	−4.0	1.6	−2.8	−0.9	−2.6	1.0	0.9	0.67	2.9
Hematocrit (%)									
Final	19.7	33.2	24.6	29.4	24.1	32.7	33.7	0.97	4.2
Initial	32.4	30.1	32.8	32.2	32.6	29.8	31.0	1.72	7.6
F–I	−12.7	3.1	−8.2	−2.8	−8.5	3.0	2.7	2.05	9.0
Total Hb Fe (mg)[1]									
Final	98	204	125	159	119	188	195	11.0	48
Initial	44	40	44	42	40	41	41	3.0	13
F–I	53	163	81	117	80	147	154	10.9	48
F–I[2]	—	110	28	64	27	94	101	NA[3]	NA
Fe utilization (%)									
Total[4]	126	39	25	34	26	38	36	5.6	25
Supplemental[5]	—	30	10	21	10	28	27	NA	NA
$FeSO_4 \cdot 7H_2O$ = 100	—	100	33	70	33	97	90	NA	NA
Serum Fe (μg/100 ml)	38	83	59	49	28	65	100	20.1	88
Serum TIBC (μg/100 ml)	301	323	330	299	283	308	332	19.9	87
Transferrin saturation (%)	12.4	25.2	15.6	15.7	10.0	21.1	25.5	4.1	18

[1] Calculated on the assumption that blood volume, expressed as % of body weight (W in kg) = 9.5 $W^{-0.068}$ and Hb contains 3.35 mg Fe/g. [2] Corrected for response of no-added-Fe group. [3] Not applicable—statistical analysis not done. [4] Percentage of total Fe intake incorporated into Hb Fe. [5] Percentage of supplemental Fe intake incorporated into Hb Fe.

tion or hematocrit were observed between pigs fed diets 2, 4, 6 or 7.

On the basis of the assumptions that blood volume of swine (% of body weight) is 9.5 $W^{-0.068}$ (8), where body weight (W) is expressed in kg, and that Hb contains 3.35 mg Fe/g (9), it is possible to calculate total Hb Fe and to estimate the percentage of dietary Fe incorporated into Hb. After 28 days of feeding, quantities of total Hb Fe were significantly greater in pigs fed diets 2, 6 and 7 than in pigs fed diets 1, 3 and 5. Total Hb Fe of pigs fed diet 4 for 28 days did not differ significantly from that of pigs fed diets 2, 6 and 7 for a similar period of time. The same statistically significant treatment effects were noted when expressed in terms of the change in total Hb Fe.

Percentage utilization of dietary Fe for formation of Hb can be calculated by dividing the difference in initial and final total Hb Fe by the total weight of Fe consumed during the feeding study (table 2). Obviously, pigs not fed a supplemental source of Fe utilized body stores of Fe to form Hb since the increment in Hb Fe during the study exceeded dietary intake. Correcting for the estimated contribution of naturally occurring Fe in the diet, it can be seen that pigs fed diets 3 and 5 incorporated into Hb approximately 10% of the supplementary Fe consumed. The efficiency of Fe utilization by pigs fed diets 2, 6 and 7 ranged from 27 to 30%. The corresponding value for pigs fed diet 4 was 21%. Comparison of Fe utilization against diet 2 (diet 2 = 100) revealed the sources of Fe in diets 3 and 5 to be approximately one-third as available as that contained in diet 2. Fe added to diets 6 and 7 appeared to be 90 to 97% as available as Fe added to

TABLE 4

Effect of dietary Fe source on serum chemical values of Pitman-Moore miniature pigs

Serum (100 ml)	Diet number 1 (O)	2 (FS)	3 (RI)	4 (EI)	5 (SIP)	6 (FP)	7 (DIE)	SEM	Critical value $P < 0.05$
Protein (g)									
Albumin	2.77	3.05	2.88	2.84	2.90	3.12	3.16	0.068	0.30
Globulin	2.46	2.09	2.42	2.29	2.42	2.19	2.28	0.085	0.37
Total	5.22	5.20	5.30	5.14	5.32	5.31	5.45	0.125	0.55
Cholesterol (mg)	126	138	117	130	128	143	122	7.7	34
Triglyceride (mg)	80	99	136	134	107	100	173	18.3	80
Phospholipid (mg)	63	72	64	63	66	69	59	7.0	31

diet 2, and Fe added to diet 4 appeared to be 70% as available as that added to diet 2.

Although mean values for serum Fe, TIBC and percentage saturation of transferrin within each treatment group showed trends similar to those demonstrated by Hb and hematocrit, the within-treatment variance was great and statistically significant differences due to treatment were not noted.

The relation between dietary Fe source and serum concentrations of proteins and lipids is shown in table 4. Pigs with the highest Hb and hematocrit values (diets 2, 6 and 7) had significantly greater concentrations of albumin in serum than did pigs receiving no added Fe (diet 1). Serum concentrations of globulins of pigs fed diet 1 were significantly greater than those of pigs fed diet 2. Serum triglyceride concentrations of pigs fed diet 1 were significantly lower than those of pigs fed diet 7.

Organ weight and Fe content. Weight of organs is expressed in table 5 as percentage of body weight, and Fe content as μg/g

TABLE 5

Effect of dietary Fe source on organ weight (% body weight) and Fe content (μg/g) of Pitman-Moore miniature pigs

Organ	Diet number 1 (O)	2 (FS)	3 (RI)	4 (EI)	5 (SIP)	6 (FP)	7 (DIE)	SEM	Critical value $P < 0.05$
Liver									
Weight	2.55	2.62	2.64	2.79	2.40	2.62	2.79	0.046	0.20
Fe (μg/g)	45.2	47.9	43.6	61.6	49.5	47.1	48.9	9.01	39.5
Total Fe (g)	6,950	8,064	6,488	10,339	6,650	7,887	9,058	1,646.4	7,228
Spleen									
Weight	0.49	0.15	0.28	0.20	0.31	0.17	0.21	0.049	0.21
Fe (μg/g)	76.1	65.9	81.8	72.8	81.6	65.0	89.2	9.67	42.4
Total Fe (g)	2,116	615	1,297	895	1,579	610	1,351	336.6	1,478
Heart									
Weight	0.61	0.49	0.54	0.49	0.55	0.51	0.47	0.024	0.11
Fe (μg/g)	30.2	34.4	25.0	31.2	27.2	37.3	40.4	3.55	15.6
Total Fe (g)	1,004	1,154	773	900	793	1,146	1,239	123.4	542
Kidney									
Weight	0.74	0.70	0.70	0.71	0.73	0.71	0.72	0.023	0.10
Fe (μg/g)	28.5	32.3	32.3	32.9	30.2	33.7	34.4	5.42	23.8
Total Fe (g)	1,225	1,414	1,273	1,394	1,206	1,525	1,601	247.8	1,088
Psoas muscle									
Fe (μg/g)	11.9	12.8	10.0	13.1	9.8	11.4	17.6	1.60	7.0
Small intestine mucosa									
Fe (μg/g)	37.9	94.9	44.1	36.1	41.4	154.1	51.7	25.63	112.5
Underlying tissue									
Fe (μg/g)	38.0	22.5	20.2	19.3	22.2	20.1	23.2	7.98	35.0

TABLE 6

Effect of dietary Fe source on metabolism of an intraperitoneal injection of ^{59}Fe

Parameter	Diet number 1 (O)	2 (FS)	3 (RI)	4 (EI)	5 (SIP)	6 (FP)	7 (DIE)	SEM	Critical value $P < 0.05$
Whole blood									
(cpm × 10^3/ml)									
Final[1]	293	381	347	351	387	339	339	33	144
Initial[2]	423	581	509	522	532	528	568	62	274
F–I	−130	−200	−162	−171	−145	−188	−228	44	193
(cpm × 10^3/mg Hb Fe)									
Final	144	102	138	112	149	96	94	10	45
Initial	194	179	203	182	212	174	157	23	103
F–I	−46	−75	−60	−62	−59	−76	−62	17	77
[cpm (% dose)][3]									
Final	61.7	91.6	76.3	79.6	79.4	80.0	79.9	6.61	29.0
Initial	56.0	80.7	67.8	68.7	68.6	68.9	78.3	6.78	29.7
F–I	5.7	10.9	8.5	10.8	10.8	11.1	1.6	5.44	23.9
Organs									
(cpm × 10^3/g)									
Liver	40	25	30	29	37	25	21	3.4	15
Spleen	123	51	87	56	89	37	48	46.8	39
Kidneys	28	20	28	24	28	15	18	3.1	13
Heart	28	26	26	27	32	23	22	2.2	10
(cpm/μg Fe)									
Liver	99	59	85	61	86	59	48	12.5	55
Spleen	182	77	121	82	120	107	56	29.0	127
Kidneys	108	72	101	96	107	50	49	15.5	68
Heart	108	75	111	135	145	60	56	22.6	99
^{59}Fe retained in Hb and organs (% dose)	67.2	94.4	79.7	82.9	83.3	82.6	82.4	6.70	29.4
% retained ^{59}Fe in:									
Blood	91.5	96.9	95.6	96.0	95.3	96.8	96.6	0.67	2.9
Organs	8.5	3.1	4.4	4.0	4.7	3.2	3.4	0.67	2.9

[1] 14 days after injection. [2] 3 days after injection. [3] cpm/ml × blood volume (ml) ÷ 2.22 × 10^7.

and total organ Fe. Fe content of psoas muscle and intestinal tissue is expressed only as μg/g. Mean total organ weight may be estimated using the body weights shown in table 2.

Spleen, heart and kidney weights of pigs fed diet 1 were greater than those of pigs fed any of the other diets. Liver weights of pigs fed diets 1 and 5 were less than those of pigs fed the other diets. Statistically significant differences between organ weights of pigs in the various treatment groups may be determined from the Tukey critical values in table 5.

There were no significant treatment effects on Fe content (μg/g) of liver, spleen, heart or kidneys. The Fe content of psoas muscle of pigs fed diet 7 was significantly greater than that of pigs fed diets 3 or 5. Fe content of intestinal mucosa of pigs fed diet 6 was significantly greater than that of pigs fed diets 1, 4 and 5. No significant differences in Fe content of underlying tissue were noted.

^{59}Fe metabolism. ^{59}Fe activity in blood was determined at 3, 7, 10 and 14 days after injection and that in tissues at 14 days after injection. As shown in table 6, cpm/ml of blood and the specific activity of ^{59}Fe/mg Hb Fe decreased between days 3 and 14 after injection; however, the percentage of the dose of ^{59}Fe present in the total blood volume increased during this period.

An inverse relationship was noted between ^{59}Fe concentration (cpm/g) in liver, spleen, kidneys and heart and Fe status as measured by Hb and hematocrit. Values for cpm/μg Fe in these organs generally were proportional to cpm/g tissue. Liver and spleen of pigs fed the no-added-Fe diet (diet 1) demonstrated significantly greater

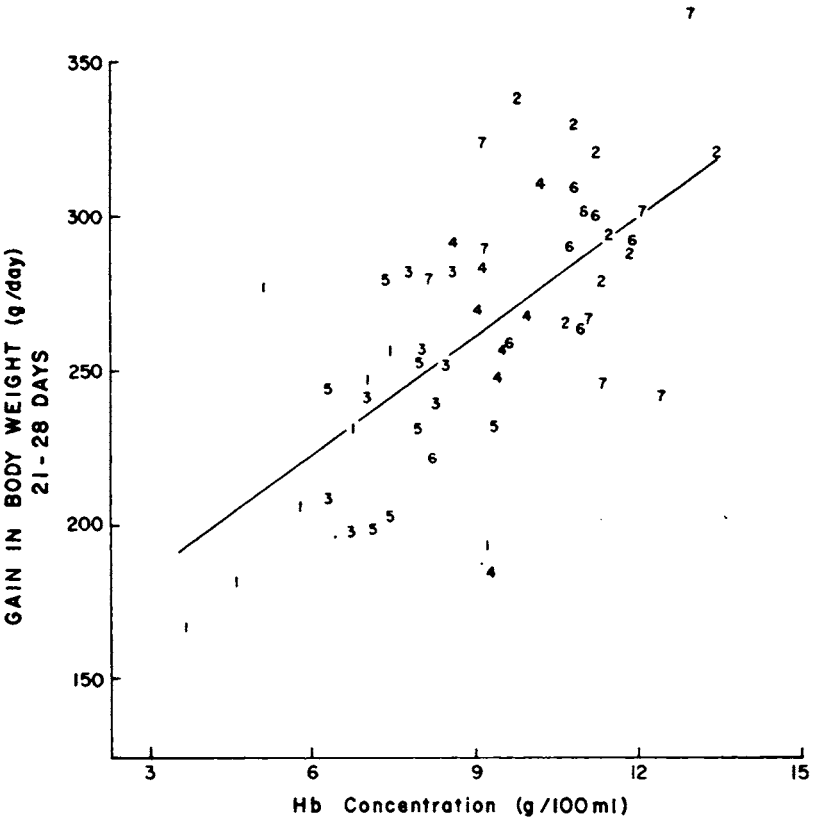

Fig. 1 Regression of gain in body weight on Hb concentration of pigs during the final week of study. The regression coefficient is 0.64 ($P < 0.01$). Each number refers to observations of one animal, the seven numbers corresponding to numbers of the diets described in table 1.

cpm/g tissue than those of pigs fed diets 2, 6 or 7. Although not statistically significant, a similar trend was noted when ^{59}Fe content was calculated on the basis of cpm/μg Fe.

Pigs fed the no-added-Fe diet retained less of the ^{59}Fe in Hb and organs (liver, spleen, heart, kidneys) than did pigs fed any of the other diets. The percentage of the ^{59}Fe dose appearing in blood was significantly less and the percentage in tissues significantly greater in pigs fed diet 1 than in those fed any of the other diets.

DISCUSSION

Matrone et al. (10) have suggested that body weight gain is a relatively insensitive indicator of iron nutritional status of the pig when concentration of Hb is greater than 5 g/100 g of blood. No significant differences in body weight gains of young pigs fed various levels of dietary iron (10 to 80 ppm) were observed by Matrone et al. (10) until day 35 of a 60-day feeding study. In the present study the effect of Fe deficiency anemia on growth performance was statistically significant by week 4 of the study. At that time gain in body weight was highly correlated ($P < 0.01$) with concentration of Hb (fig. 1).

Electrolytic iron powder (diet 4) and sodium Fe pyrophosphate (diet 5), two of three Fe preparations found to be least available in the present study, are those currently used to supplement commercially prepared infant cereals in the United States. The Fe status of pigs fed the diet supplemented with sodium Fe pyrophos-

phate (diet 5) did not differ statistically from that of pigs receiving the no-added-Fe diet (diet 1) in most of the parameters studied. Response of pigs fed the diet supplemented with catalytically reduced Fe (diet 3) was similar to that of pigs fed the diet to which sodium Fe pyrophosphate was added. Electrolytic Fe powder (diet 4), having a smaller particle size than the catalytically reduced Fe was the most available of the sources of Fe used commercially, supporting the findings of Shah and Belonje (11) that bioavailability of Fe powder is dependent upon particle size. (One manufacturer recently discontinued use of the reduced Fe source added to diet 3.)

Unfortunately, the most available forms of Fe are also the forms most likely to promote rancidity of cereals. Ferrous sulfate, a highly available Fe source against which other sources of Fe are commonly compared, is notorious in this regard. The ferripolyphosphate powder described by Jones et al. (12) was found to be a readily available source of Fe in the diet fed to miniature pigs, but apparently also possesses the undesirable property of promoting rancidity in cereal products.[7] The Fe EDTA complex is stable in this regard and because of its high availability deserves further study as a potential source of Fe in infant cereals.

The finding that 10% of the added sodium Fe pyrophosphate and catalytically reduced Fe was incorporated into Hb is identical to the incorporation reported by Anderson et al. (13) in a study of anemic rats fed cereal–milk diets supplemented with these identical forms (same lot numbers) of iron. Incorporation of the three most available forms of Fe (diets 2, 6 and 7) into Hb ranged from 27 to 30%, values considerably lower than the 65% figure observed for ferrous sulfate in anemic rats (13).

Fritz et al. (14) reported that six samples of reduced Fe fed to anemic rats and chicks demonstrated 8 to 66% (average 37%) of the biological value of ferrous sulfate—the average value approximating that of 33% for the particular form of catalytically reduced Fe added to diet 3. The average relative biological value of three samples of sodium Fe pyrophosphate evaluated by Fritz et al. (14) was 14% (range 2 to 23%), considerably less than the 33% calculated for the sample of sodium Fe pyrophosphate added to diet 5. The relative biological value of 70% calculated for the sample of electrolytic Fe powder added to diet 4 is somewhat higher than the 46 to 51% range reported by Pla and Fritz (15) for two samples of electrolytic Fe.

It is difficult to compare the bioavailability of a particular form of Fe reported in various studies because of the known variables associated with Fe absorption. For example, interpretation of the results of an evaluation of the bioavailability of "reduced iron" would depend upon the particle size of the source of iron[8] (11), source of dietary carbohydrate[9, 10] (16) or fat[10], the species of animal studied (14, 17) and, presumably, various aspects of experimental design (e.g., prevention of anemia versus repletion). Ashworth and March (18) urge caution in comparing results of animal experiments with studies of Fe availability measured directly in infants.

Elevations in the concentration of triglycerides have been reported in the serum of anemic rats (19, 20) and chicks (20). This effect was not noted in the anemic pigs although the severity of anemia was less in the pigs fed diet 1 than in the rats or chicks.

Amine and Hegsted (20) observed significant elevations in the concentrations of total protein, albumin and globulins in plasma of anemic chicks. Concentrations of albumin were significantly less and concentrations of globulins significantly greater in serum of pigs fed diet 1 than in serum of pigs fed diets 2, 6 or 7.

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[7] Personal communication, George A. Purvis, Gerber Products Co., Fremont, Mich.

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The inverse relationship between organ weight (heart, spleen) and iron status has been observed by Matrone et al. (10) to occur in the hearts of anemic pigs and by Koivistoinen et al. (21) and Ahlström et al. (22) to occur in both heart and spleen of anemic rats. In these studies liver weight accounted for a lesser percentage of body weight in anemic than in nonanemic animals. Matrone et al. (10) reported that more of an intraperitoneal injection of ^{59}Fe was found in the tissues of anemic pigs than in those of animals with improved Fe status.

In the present study young miniature pigs absorbed modest amounts of sodium Fe pyrophosphate or catalytically reduced Fe from a cereal–milk diet. Whether the quantity of Fe absorbed by infants from infant cereals fortified with sodium Fe pyrophosphate or electrolytic Fe powder is sufficient to prevent Fe deficiency is unknown.

A recent directive by the Food and Drug Administration (23) limits Fe fortification of infant cereals to 45% of the 15 mg/day infant U. S. RDA ("recommended daily allowance") for Fe (0.45×15 mg $= 6.75$ mg) per 14 g (0.5 oz) serving unless the product is labeled as a dietary supplement. Therefore, the extent of fortification in the future will be restricted to 45 mg/100 g—less than one-half the level of Fe currently added to cereals produced by the major U. S. manufacturer of cereals for infants (24).

Because the electrolytic Fe powder with relatively small particle size (table 1) was utilized approximately twice as well as the catalytically reduced Fe or the sodium iron pyrophosphate, the electrolytic Fe powder is preferable for fortification of infant cereals. The hypothesis that infant cereals fortified with sodium Fe pyrophosphate or elemental Fe can be useful in preventing Fe deficiency in human infants needs to be tested in well-controlled clinical investigations.

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