reaction with lysine. This is supported by the fact that even the minute amounts of residual carbohydrates in the isolated protein are sufficient to exert a catalytic effect.

LITERATURE CITED

Agr. Res. **17**, 4 (1969).
Arroyo, P. T., Lillard, D. A., *J. Food Sci.* **35**, 769 (1970).
Budzikiewicz, H., Djerassi, C., Williams, D. H., "Mass Spectrometry of Organic Compounds," Holden-Day, San Francisco, Calif., 1967, p 276.
Gehrke, C. W., Kuo, K., Zumwalt, R. W., *J. Chromatogr.* **57**, 209 (1971).
Gehrke, C. W., Stalling, D., *Separ. Sci.* **2**, 101 (1967).
Gruenwedel, D. W., Patnaik, R. K., *J. Agr. Food Chem.* **19**, 775 (1971).
Miller, E. L., Carpenter, K. J., Milner, D. K., *Brit. J. Nutr.* **19**, 547 (1965).
Pierson, E., Giella, M., Tishler, M., *J. Amer. Chem. Soc.* **70**, 1450 (1948).
Roach, D., Gehrke, C. W., *J. Chromatogr.* **43**, 303 (1969).
Schonberg, A., Moubacher, R., *Chem. Rev.* **50**, 201 (1952).
Shemer, M., Wei, L. S., Perkins, E. G., *J. Food Sci.* **38**, 112 (1973).
Wainwright, T., McMahon, J. F., McDowell, J., *J. Sci. Food Agr.* **23**, 911 (1972).
Zumwalt, R. W., Roach, D., Gehrke, C. W., *J. Chromatogr.* **53**, 171 (1970).

Received for review July 24, 1973. Accepted October 30, 1974. Supported in part by the Illinois Agricultural Experiment Station, University of Illinois, Urbana, Ill. 61801.

Effect of Dietary Carbohydrates and Fats on Inorganic Iron Absorption

Ezzat K. Amine[1] and D. Mark Hegsted*

The effects of modifying the dietary carbohydrate and fat upon the availability of iron to rats were studied. In general, iron utilization was greatest with diets containing lactose, less in diets containing sucrose, and least with diets in which the carbohydrate was supplied as starch. However, the effect of the carbohydrate was not uniform when iron sources of differing availability were tested. Diets high in fat favored iron utilization and iron absorption was greater in diets in which the fat was supplied as coconut oil than in those in which the fat was supplied as corn oil.

It is now well known that the availability of dietary iron is dependent not only upon the nature of the iron source in the diet but upon the nature of the diet with which the iron is consumed (Moore and Dubach, 1951; Layrisse *et al.*, 1968; Pla and Fritz, 1970; Amine *et al.*, 1972; Amine and Hegsted, 1974). In previous work (Amine and Hegsted, 1971) we found that the absorption of radioiron was markedly influenced by the kind of dietary carbohydrate. The studies reported here were undertaken to explore this effect further and to evaluate the effects of modifying the kind and amount of dietary fat upon the absorption of dietary iron.

MATERIALS AND METHODS

The composition of the diets used in these studies is indicated in Table I. The kind and amount of carbohydrate and fat in the diet were modified in the various experiments as indicated below. Such diets (Amine and Hegsted, 1971) contain less than 10 ppm of iron.

In the first experiment the diet contained glucose as the source of carbohydrate and 5, 15, or 30% of fat supplied as either coconut oil or corn oil. The absorption of radioiron was studied by the technique previously described (Amine and Hegsted, 1971). Briefly, groups of six female rats (Charles River Breeding Laboratories, Wilmington, Mass.) that were moderately iron deficient were provided with the indicated diets low in iron for 1 week. They were then fasted overnight and given 2 g of the appropriate diet containing 20 μg of iron as ferric ammonium citrate and 0.2 μCi of ^{59}Fe as $FeCl_3$. Two hours later when all or nearly all of the food had been eaten the animals were placed in a whole body counter to determine the amount of ^{59}Fe consumed. The animals were continued on the diets for 9 days after which they were counted again. The difference in counts corrected for physical decay of the isotope was taken as a measure of the iron retained.

In the second experiment the availability of several forms of iron in diets containing different carbohydrate was determined using the "prophylactic assay" previously described (Amine and Hegsted, 1974). Thirty-nine groups of five female weanling rats were used. Three groups received the diets without added iron which were made with either starch, sucrose, or lactose as the carbohydrate source. Three additional groups were used to investigate each iron source with each type of diet. These groups received three arbitrarily selected levels of iron supplied as either ferrous sulfate, reduced iron, sodium iron pyrophosphate, or ferric orthophosphate. All the diets contained 15% of a partially hydrogenated vegetable oil. The animals were fed the diets for a 3-week period. They were weighed at intervals and bled after 3 weeks to determine hemoglobin (Crosby *et al.*, 1954) and hematocrit.

The third experiment also utilized five female animals per group and investigated the effects of varying the kind of carbohydrate and fat on the availability of ferrous sulfate. The diets were made with either starch, sucrose, or a lactose–starch mixture (2:1). The diets contained either 5

Table I. Composition of the Diets

Ingredients	%
Carbohydrate (starch, sucrose, or lactose)	44.2–64.2
Casein (vitamin free)[a]	20.0
Salt mix (iron free)[b]	5.0
Vitamin mix[c]	0.5
Choline chloride	0.3
Dietary fat[d]	5.0–25.0
Cellulose	5.0

[a] General Biochemicals, Chagrin Falls, Ohio. [b] Jones and Foster (1945). Modified by the addition of 0.05 g of sodium selenite and 0.05 g of chromium acetate/2043 g of iron-free salt mix. [c] Hegsted *et al.* (1967). [d] Spry, Lever Brothers Co., New York, N.Y.

Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts 02115.
[1] Present address: Department of Nutrition, High Institute of Public Health, Alexandria, Egypt.

Table II. Effect of Dietary Fats on Inorganic Iron Absorption by Iron Deficient Rats

Dietary modification, %	% ^{59}Fe retained[a]
Coconut oil, 5	37.6 ± 3.1a
Coconut oil, 15	39.2 ± 3.9a
Coconut oil, 30	45.5 ± 3.0b
Corn oil, 5	25.0 ± 2.3c
Corn oil, 15	39.5 ± 2.2ad
Corn oil, 30	42.3 ± 1.8e

[a] Mean ± standard error. Values followed by the same letter are not significantly different.

or 25% of corn oil or coconut oil. Each diet was fed without added iron or with 10 or 20 mg of iron/kg of diet added as ferrous sulfate. As in the previous experiment the animals received the diets for 3 weeks and were then bled for the estimation of hemoglobin and hematocrit.

RESULTS

Experiment 1. As shown in Table II the absorption of radioiron under these conditions was favored by the high fat diets and especially by the saturated fat—coconut oil. The difference between the oils was particularly marked when the level of fat in the diet was relatively low.

Experiment 2. The levels of iron provided in each of the experimental diets and the terminal body weights, hemoglobin, and hematocrit values are shown in Table III. As shown in Figure 1 when the diets made with the same carbohydrate were compared, the dose–response lines obtained with the various iron sources appeared to be linear and to have a common intercept. They thus fulfilled the criteria of an adequate slope-ratio assay (Amine *et al.*, 1972; Amine and Hegsted, 1974) and the slopes of the response lines may be used to compare potencies of the various iron sources. In Figure 1, for example, reduced iron had a potency of 30.9% compared to ferrous sulfate (0.148/0.480 × 100 = 30.9). The estimated potencies of each of the iron sources compared to ferrous sulfate with each diet are shown in Table IV.

Comparisons across diets containing the different carbohydrates were relatively unsatisfactory. As shown in Figure 2 when the dose–response lines for the same iron

Table III. Mean Terminal Values and Standard Errors for Hemoglobin, Hematocrit, and Body Weight of Rats in Experiment 2

Iron source	Iron dose, ppm	Dietary carbohydrate	Body wt, g	Hemoglobin, g/100 ml	Hematocrit, %
Basal diet	0	Starch	114.8 ± 10.5	5.2 ± 0.4	21.8 ± 1.7
Ferrous sulfate	5	Starch	128.8 ± 2.3	7.6 ± 0.2	30.4 ± 1.0
	10		132.4 ± 3.5	9.3 ± 0.4	36.4 ± 1.5
	15		134.0 ± 5.5	11.4 ± 0.7[a]	43.2 ± 5.5[a]
Reduced iron	5	Starch	116.0 ± 4.6	5.5 ± 0.4	23.8 ± 1.1
	15		128.0 ± 6.1	5.7 ± 0.5	25.4 ± 2.2
	25		130.4 ± 2.1	6.5 ± 0.1	28.0 ± 1.5
Sodium iron pyrophosphate	15	Starch	126.4 ± 5.5	7.0 ± 0.4	29.2 ± 1.9
	30		125.4 ± 6.4	7.8 ± 0.3	31.6 ± 0.8
	45		123.6 ± 4.4	9.8 ± 1.3	37.4 ± 3.7
Ferric orthophosphate	20	Starch	139.2 ± 4.8	7.9 ± 0.7	30.8 ± 1.2
	40		129.6 ± 5.2	10.9 ± 0.8	41.4 ± 0.8
	60		122.0 ± 8.4	11.4 ± 0.7	41.2 ± 1.7
Basal diet	0	Sucrose	105.8 ± 6.4	5.8 ± 0.4	26.2 ± 1.9
Ferrous sulfate	5	Sucrose	97.2 ± 0.8	7.6 ± 0.3	28.6 ± 0.7
	10		133.2 ± 2.2	9.0 ± 0.2	34.6 ± 1.1
	15		128.6 ± 6.4	10.6 ± 0.4	39.0 ± 1.1
Reduced iron	5	Sucrose	109.0 ± 6.3	6.3 ± 0.8	28.4 ± 3.0
	15		106.2 ± 5.0	6.9 ± 0.7	31.0 ± 2.7
	25		105.6 ± 3.9	8.4 ± 0.5	34.6 ± 2.6
Sodium iron pyrophosphate	15	Sucrose	115.8 ± 7.1	8.1 ± 0.4	30.2 ± 1.5
	30		140.8 ± 4.6	9.1 ± 0.8	35.0 ± 3.9
	45		137.6 ± 3.7	9.8 ± 0.8	37.2 ± 1.5
Ferric orthophosphate	20	Sucrose	143.0 ± 4.6	8.5 ± 0.5	32.8 ± 1.8
	40		146.0 ± 4.8	12.0 ± 0.4	42.6 ± 1.1
	60		137.2 ± 5.8	13.9 ± 1.0[a]	46.0 ± 1.2[a]
Basal diet	0	Lactose	125.4 ± 3.4	7.9 ± 0.6	31.2 ± 0.4
Ferrous sulfate	5	Lactose	120.4 ± 3.9	10.3 ± 0.5	38.2 ± 0.4
	10		127.8 ± 4.3	12.7 ± 0.4	47.0 ± 2.3
	15		129.6 ± 4.1	13.1 ± 0.1[a]	45.4 ± 1.8[a]
Reduced iron	5	Lactose	116.6 ± 4.1	8.3 ± 0.3	35.2 ± 2.2
	15		128.4 ± 4.9	10.4 ± 0.8	41.2 ± 2.6
	25		137.6 ± 0.8	11.6 ± 0.3	42.4 ± 0.7
Sodium iron pyrophosphate	15	Lactose	118.0 ± 5.4	10.0 ± 0.5	38.4 ± 1.7
	30		126.0 ± 5.9	11.3 ± 0.4	41.8 ± 1.2
	45		127.4 ± 2.9	13.0 ± 0.4	44.6 ± 0.9
Ferric orthophosphate	20	Lactose	125.0 ± 6.6	10.0 ± 0.5	40.6 ± 2.5
	40		129.0 ± 4.7	13.3 ± 0.6	44.6 ± 1.5
	60		127.0 ± 3.2	12.8 ± 0.2[a]	46.6 ± 1.8[a]

[a] Data not included in the estimate of potency.

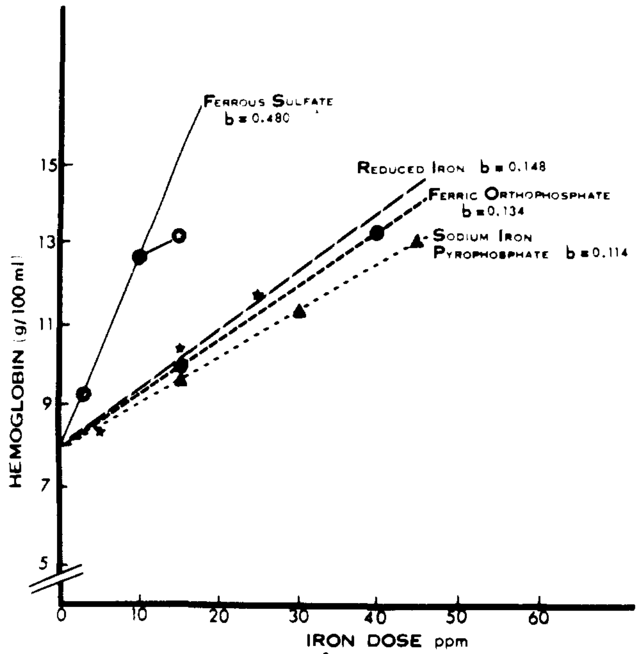

Figure 1. Dose-response lines obtained with various iron sources in the diet containing lactose; *b* = slope of the regression line. The apparently aberrant value obtained at the highest level of ferrous sulfate was omitted in calculating the slope of the line.

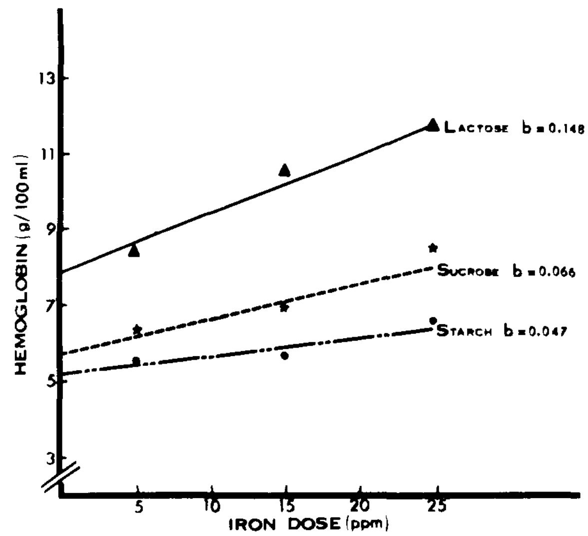

Figure 2. Dose-response lines obtained with reduced iron when the diets contained different carbohydrates; *b* = slope of the regression line.

Table IV. Estimates of the Relative Availability of Different Iron Sources Fed with Different Carbohydrates in Experiment 2

Iron source	Starch, %	Sucrose, %	Lactose, %
Ferrous sulfate	100.0	100.0	100.0
Reduced iron	11.4 ± 6.0[a]	14.5 ± 5.6	30.9 ± 4.8
Sodium iron pyrophosphate	23.7 ± 3.0	30.9 ± 5.2	23.8 ± 3.0
Ferric orthophosphate	33.9 ± 3.8	49.2 ± 7.6	27.9 ± 3.6

[a] Mean ± standard error.

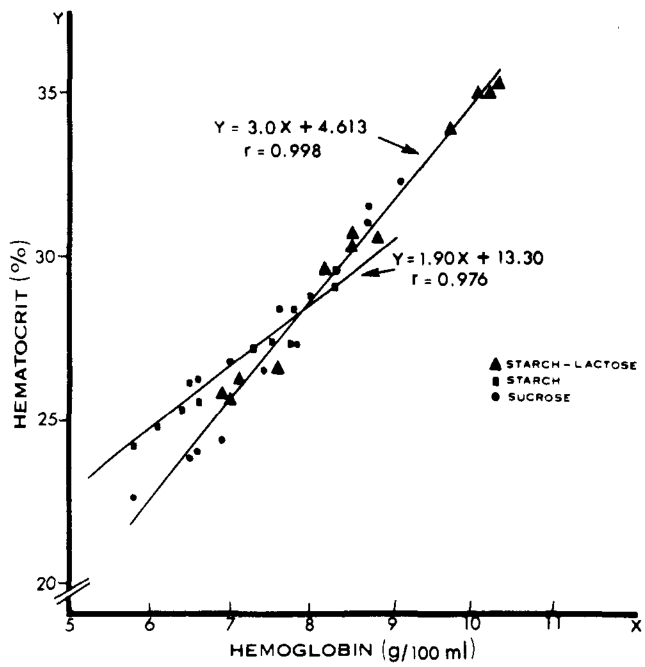

Figure 3. As shown in this diagram, the relationship between hemoglobin and hematocrit was apparently affected by the carbohydrate in the diet (experiment 3).

source are compared, the intercepts are quite different. With the diets containing sugar, especially lactose, which favor iron absorption, the animals became less anemic even with the diet low in iron. Presumably, the diet affects the utilization of the residual iron in the diet although a possible effect upon the retention of body iron is not ruled out by the data available.

Experiment 3. The terminal body weights, hemoglobin, and hematocrit values obtained in the animals fed the various diets are shown in Table V. Since the high fat diets were of greater caloric density and the grams of food consumed would be expected to be less with such diets, the iron content was expressed on a caloric basis. When this was done the analysis of variance of the hemoglobin (Table VI) and the hematocrit values indicated that the data fulfilled the criteria of an adequate slope-ratio assay (Finney, 1964; Hegsted *et al.*, 1968; Amine and Hegsted, 1974). The estimated potencies of ferrous sulfate in the various diets were therefore compared to that of the diet containing lactose-starch which was the most effective of the 12 diets studied. These comparisons are shown in Table VI also.

Substantial differences in potency for some of the diets were found when hematocrit was used as the measure of response indicating that hemoglobin and hematocrit were not equivalent measures of response to dietary iron. The relationships between the average hemoglobin and hematocrit values were therefore compared. As shown in Figure 3, although the correlations are high the relationship appears to be different with the diet containing starch and those containing either of the sugars—sucrose or lactose. Although this explains why strictly comparable results were not obtained using hemoglobin and hematocrit as measures of response to dietary iron, the reason for it is not understood. Similar differences in the diets used in experiment 2 were not found.

DISCUSSION

These data confirm the now well-known differences in the availability of different iron sources. It should be understood that the differences in the availability of the iron sources are also not necessarily characteristic of these products. The availability of reduced iron, for example, depends upon particle size (Pla and Fritz, 1971; Cook *et al.*, 1973; Amine and Hegsted, 1974). Unfortunately, the

Table V. Mean Terminal Values and Standard Errors for Hemoglobin, Hematocrit, and Body Weight of Rats in Experiment 3

Dietary carbohydrate	Dietary fat	Iron dose, mg/1000 kcal	Body wt, g	Hemoglobin, g/100 ml	Hematocrit, %
Starch	25% coconut oil	0	100.5 ± 7.3	6.1 ± 0.3	24.8 ± 0.9
		2.07	116.6 ± 6.0	7.8 ± 0.4	27.3 ± 0.8
		4.14	111.2 ± 4.3	8.3 ± 0.5	29.5 ± 1.1
Starch	5% coconut oil	0	115.3 ± 6.3	6.6 ± 0.1	25.5 ± 0.6
		2.61	126.5 ± 4.9	7.3 ± 0.2	27.2 ± 0.7
		5.22	134.0 ± 4.5	8.3 ± 0.3	29.0 ± 0.3
Starch	25% corn oil	0	112.7 ± 4.8	5.8 ± 0.2	24.2 ± 0.6
		2.07	79.3 ± 3.8	6.5 ± 0.1	26.1 ± 0.5
		4.14	117.6 ± 2.7	7.8 ± 0.1	28.3 ± 0.3
Starch	5% corn oil	0	74.7 ± 3.7	6.4 ± 0.2	25.3 ± 0.6
		2.61	103.0 ± 3.4	6.6 ± 0.2	26.2 ± 0.4
		5.22	113.5 ± 3.5	7.5 ± 0.3	27.3 ± 0.8
Sucrose	25% coconut oil	0	115.0 ± 5.1	6.6 ± 0.2	23.7 ± 0.8
		2.07	126.5 ± 4.6	8.0 ± 0.1	28.7 ± 0.7
		4.14	125.0 ± 5.2	9.1 ± 0.2	32.3 ± 0.9
Sucrose	5% coconut oil	0	101.5 ± 6.2	6.9 ± 0.2	24.3 ± 0.7
		2.61	110.0 ± 7.1	7.8 ± 0.2	27.3 ± 0.8
		5.22	112.8 ± 4.8	8.7 ± 0.1	31.5 ± 0.6
Sucrose	25% corn oil	0	119.3 ± 4.5	5.8 ± 0.5	22.6 ± 1.6
		2.07	133.0 ± 4.8	7.0 ± 0.3	26.7 ± 1.3
		4.14	127.5 ± 3.6	8.7 ± 0.3	31.0 ± 1.1
Sucrose	5% corn oil	0	92.1 ± 4.1	6.5 ± 0.3	23.8 ± 1.4
		2.61	83.0 ± 6.8	7.4 ± 0.6	26.5 ± 0.6
		5.22	95.5 ± 4.8	7.6 ± 0.1	28.3 ± 0.5
2:1 lactose–starch	25% coconut oil	0	115.3 ± 6.2	7.0 ± 0.5	25.5 ± 0.9
		2.07	116.5 ± 4.7	8.8 ± 0.5	30.5 ± 1.5
		5.22	116.0 ± 3.6	10.3 ± 0.6	35.2 ± 1.3
2:1 lactose–starch	5% coconut oil	0	98.2 ± 2.6	7.6 ± 0.7	26.5 ± 1.8
		2.61	114.8 ± 4.1	8.5 ± 0.3	30.7 ± 1.5
		5.22	113.3 ± 4.1	10.1 ± 0.7	35.0 ± 2.0
2:1 lactose–starch	25% corn oil	0	110.5 ± 3.6	6.9 ± 0.3	25.8 ± 1.0
		2.07	104.0 ± 2.7	8.2 ± 0.4	29.5 ± 1.8
		4.14	110.7 ± 5.7	9.7 ± 0.4	33.8 ± 1.2
2:1 lactose–starch	5% corn oil	0	64.7 ± 2.9	7.1 ± 0.1	26.2 ± 0.5
		2.61	82.0 ± 3.6	8.5 ± 0.6	30.3 ± 1.7
		5.22	85.6 ± 4.8	10.2 ± 0.9	35.0 ± 2.0

sample used in these studies was not well characterized. Previous work (Amine and Hegsted, 1974) has shown that processing may also modify the availability of the iron in a fortified product, either increasing or decreasing the availability of the total iron in the final product.

It is also clear that contrary to the conclusion expressed by Van Campen (1970) the nature of the carbohydrate in the diet does modify the availability of dietary iron. In general, the presence of sugar in the diet, especially lactose, improved iron utilization, but this was not a uniform quantitative effect with all the iron sources. The mechanisms by which sugars improve or starch inhibits iron utilization have not been well studied. Chelation of iron by sugars, especially fructose, has been suggested as a primary mechanism (Charley *et al.*, 1963a,b; Bates *et al.*, 1972). Chelation might present iron to the gut in an absorbable form or prevent the formation in the gut of insoluble and unavailable complexes. Chelation of calcium by lactose has also been suggested as the mechanism by which lactose improves calcium utilization (Charley and Saltman, 1963), although this has also been attributed to changes in the acidity of the lower bowel due to the slow rate of absorption of this sugar (Atkinson *et al.*, 1957).

The data clearly indicate that under the conditions of these experiments modifying the kind or amount of fat did influence the availability of dietary iron. It is realized that the addition of fat to the diet, as in these experi-

Table VI. Estimates of the Relative Availability of Ferrous Sulfate When Fed with Different Carbohydrates and Fats

	Dietary carbohydrate		
Dietary fat	Starch, %	Sucrose, %	2:1 lactose–starch, %
25% coconut oil	47.6 ± 10.4	65.2 ± 11.1	100.0
5% coconut oil	32.8 ± 8.1	43.6 ± 8.5	72.6 ± 9.9
25% corn oil	23.3 ± 9.9	46.3 ± 10.4	80.4 ± 11.9
5% corn oil	13.7 ± 7.8	22.3 ± 7.9	73.8 ± 10.0

	Analysis of variance		
Source of variation	Deg of freedom	Mean squares	Significance
Due to curvature	12	0.2179	N.S.
Due to intersection	11	1.98	N.S.
Due to blanks	1	0.03	N.S.
Due to regression	12	23.14	<0.001
Error	202	0.95	

ments, results in a decreased intake of carbohydrate and protein. Thus, it is not necessarily clear whether the effect observed was due to the fat *per se* or the modification of the amount of carbohydrate and protein consumed. However, since the effect of fats of differing degrees of unsaturation was found at the same level in the diet, it would seem clear that the fat did have an effect upon iron absorption. It might be expected that the favorable effect of fat would be most likely to be shown when conditions were relatively unfavorable in the presence of large amounts of starch and this was found.

These studies further emphasize the complexity of the systems governing iron absorption. Iron deficiency appears to be common in most parts of the world and not necessarily related to iron intake. In the developing nations, the iron intake is usually relatively high, but the diets in these areas are generally characterized by being high in cereal products—often whole grain cereals—and low in fat and animal products. The inhibitory effects of cereals on iron and calcium absorption are, in part, attributable to the presence of phytates (Oberleas, 1973), but the data presented here indicate that high intakes of starch and low intakes of fat are inhibitory as well. Layrisse *et al.* (1968) have shown that the inclusion of meat in the diet improves the absorption of iron in cereals. Thus, a multitude of factors apparently combines to inhibit iron absorption including the nature of the dietary iron. The data presented here may also be relevant to the anemia in "starch eaters" (Ferguson and Keaton, 1950; Garrison and Conrad, 1967) as well as the somewhat disappointing results observed in several trials with iron enriched bread (Callender and Warner, 1968; Elwood *et al.*, 1970).

The diets of affluent countries—low in phytates and starch and high in animal products, saturated fat, and sugars—appear to combine most of the factors known to favor iron absorption. It should be noted, however, that studies of the kind reported here in which all of the dietary carbohydrate was provided by starch or sucrose, for example, provide no indication of the practical significance of the effects of those changes which might reasonably be made in acceptable diets. That is, although saturated fat and sugar improved iron absorption it is uncertain how much of either is required in a mixed diet to have a significant effect, or whether reasonable recommendations to lower the intakes of saturated fat or sugar would significantly influence iron utilization. A lack of such quantitative information is a general weakness in attempts to evaluate the effects of various factors which are known to influence iron utilization. Since it is clear that most, if not all, of these different factors interact with each other, the difficulties of arriving at reasonable conclusions are obvious.

LITERATURE CITED

Amine, E. K., Hegsted, D. M., *J. Nutr.* **101**, 927 (1971).
Amine, E. K., Hegsted, D. M., *J. Agr. Food Chem.* **22**, 470 (1974).
Amine, E. K., Neff, R., Hegsted, D. M., *J. Agr. Food Chem.* **20**, 246 (1972).
Atkinson, R. L., Kratzer, E. H., Stewart, G. F., *J. Dairy Sci.* **40**, 1114 (1957).
Bates, G. W., Boyer, J., Hegenauer, J. C., Saltman, P., *Amer. J. Clin. Nutr.* **25**, 983 (1972).
Callender, S. T., Warner, G. T., *Amer. J. Clin. Nutr.* **21**, 1170 (1968).
Charley, P. J., Saltman, P., *Science* **193**, 1205 (1963).
Charley, P. J., Sarkar, B., Stitt, C., Saltman, P., *Biochim. Biophys. Acta* **69**, 313 (1963a).
Charley, P. J., Stitt, C., Shore, E., Saltman, P., *J. Lab. Clin. Med.* **61**, 397 (1963b).
Cook, J. D., Minnich, V., Moore, C. V., Rasmussen, A., Bradley, W. B., Finch, C. A., *Amer. J. Clin. Nutr.*, **26**, 861 (1973).
Crosby, W. H., Munn, J. I., Furth, F. W., *U.S. Armed Forces Med. J.* **51**, 693 (1954).
Elwood, P. C., Benjamin, I. T., Fry, F. A., Eakins, J. D., Brown, D. A., Dekock, P. C., Shah, J. U., *Amer. J. Clin. Nutr.* **23**, 1267 (1970).
Ferguson, J. H., Keaton, A. G., *New Orleans Med. Surg. J.* **102**, 460 (1950).
Finney, D. J., "Statistical Method in Biological Assay," 2nd ed, Hafner Publishing Co., New York, N.Y., 1964.
Garrison, F. D., Conrad, M. E., *Proc. Soc. Exp. Biol. Med.* **126**, 304 (1967).
Hegsted, D. M., *et al.*, *J. Nutr.* **92**, 403 (1967).
Hegsted, D. M., Neff, R., Worcester, J., *J. Agr. Food Chem.* **16**, 190 (1968).
Jones, J. J., Foster, C., *J. Nutr.* **24**, 245 (1945).
Layrisse, M., Torres, C. M., Roche, M., *Amer. J. Clin. Nutr.* **21**, 1175 (1968).
Moore, C. V., Dubach, R., *Trans. Ass. Amer. Physicians* **64**, 245 (1951).
Oberleas, D., in "Toxicants Occurring Naturally in Foods," 2nd ed, Strong, F. M., Ed., National Academy of Science-National Research Council, Washington, D.C., 1973, p 363.
Pla, G. W., Fritz, J. C., *J. Ass. Offic. Anal. Chem.* **53**, 791 (1970).
Pla, G. W., Fritz, J. C., *J. Ass. Offic. Anal. Chem.* **54**, 13 (1971).
Van Campen, D. R., in "Proceedings of a Workshop on Measures to Increase Iron in Foods and Diets," National Academy of Sciences-National Research Council, Washington, D.C., 1970, p 30.

Received for review September 3, 1974. Accepted November 4, 1974. This work was supported in part by grants-in-aid from the National Institutes of Health (AM-13090 and 5-K6-AM-18455) and the Fund for Research and Teaching, Department of Nutrition, Harvard School of Public Health.