THE INFLUENCE OF DIET ON IRON ABSORPTION

II. The Interrelation of Iron and Phosphorus*

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(Received for publication, April 12, 1949)

In an accompanying paper (1) it was shown that rats fed a diet composed of corn grits and lard absorbed and deposited in the liver large amounts of iron when excessive amounts of ferric citrate were added to the diet. In contrast, animals which received a diet of Purina dog chow containing the same amount of ferric citrate, showed an insignificant increase in liver iron. The increase in iron in the livers of the rats was not due to a redistribution of body iron concurrently with the large loss of body weight. This was indicated by the fact that animals fed the corn grit diet without added iron lost a comparable amount of weight but showed only a slight increase in liver iron. Furthermore, the liver iron in some animals which received the corn grit diet for long periods has been found to exceed the total body iron of normal rats. On the basis of these data it has been concluded that a normal intestinal block to iron absorption fails under certain conditions.

The basal diet of 80 per cent corn grits and 20 per cent lard plus haliver oil which was used, is obviously deficient in several nutrients, as indicated by the rapid loss in weight of the animals whether iron was added or not, and by an inspection of the published data upon the nutrient content of corn grits (2). This paper presents the studies designed to determine the dietary factors primarily involved in the failure of animals fed the corn grit and lard diet to control iron absorption.

EXPERIMENTAL

Experimental conditions were essentially the same as previously described (1). Groups of adult albino rats were housed together in large cages and fed the various diets *ad libitum*.

* Supported in part by grants-in-aid from the Milbank Memorial Fund, New York, the Nutrition Foundation, Inc., New York, the Williams and Waterman Fund of the Research Corporation, New York, the American Meat Institute, Chicago, Swift and Company, Chicago, and the United States Public Health Service, Bethesda; and assisted by the Joint Program of the Office of Naval Research, and the Atomic Energy Commission.

Both sexes of rats have been used although all the rats in any particular experiment were of the same sex. There were from four to six rats in each group and they usually received the diets for periods ranging from 5 to 7 weeks, although in a few studies the experimental time was prolonged. All animals were weighed twice weekly.

The animals were etherized, the chest cavities opened, and the animals were bled either by heart puncture or by cutting the vena cava. When this was done promptly before the heart stopped beating, most of the blood was removed from the liver, and perfusion of this organ appeared unnecessary and was not done. The liver was then removed, sections taken for histological examination, and the remainder analyzed for total iron. Chemical and microscopic methods were the same as used previously (1).

The two basal diets used were the corn grit and lard diet (CGL—80 per cent corn grits and 20 per cent lard and haliver oil, 1 drop per animal per week) and Purina dog chow. Iron salts and the other materials studied were added to these diets as indicated. When large amounts, over 4 per cent, of any material were added to the corn grit diet, an equal reduction in the amount of corn grits was made.

RESULTS

The corn grit diet (CGL) contains only small amounts of several vitamins and minerals. It is also low in protein and the protein present is of poor quality. The addition of vitamins to this diet containing iron was essentially without effect upon the amount of iron deposited in the liver or the amount of weight lost during the study (Table I). The addition of casein may have been partially effective in preventing iron accumulation in the liver. However, when a complete salt mixture (3) was added there was only an insignificant rise in liver iron. Both casein and salt mixture reduced the loss in body weight to the same degree. Since the vitamin supplements were without effect upon the absorption and storage of iron, these were added to the diet in subsequent work. This supplemented diet is indicated as CGL-B.

The effect of casein and salt mixture was tested again in the next experiment over a longer period of time (Experiment 3, Table I). It was again apparent that the salt mixture was much more effective than casein but over the 70 to 84 day period it did not completely prevent an increase in liver iron.

In attempting to determine which constituent of the salt mixture was primarily involved, calcium and phosphorus supplements were tried. The corn grit–lard diet is extremely low in both of these minerals, containing approximately 0.01 per cent calcium and 0.04 per cent phosphorus. The salts containing phosphorus largely prevented the accumulation of liver iron at the levels fed, while the addition of calcium carbonate appeared to have no effect. The possibility that protein or amino acids might influence iron absorption was also considered since these have been shown to have an effect upon the absorption of calcium (4–6). The data suggest that cystine, methionine, and glutamic acid supplements may have increased the absorption or deposition of iron although gelatin did not. In one later study the effect of cystine was less marked and the problem has not been pursued further. These data are suggestive, but

TABLE I

The Effect of Various Supplements to the Corn Grit Diet and Other Diets upon the Deposition of Liver Iron

Experiment	Group	Time	Basal diet	Supplement to diet*		Liver iron			Weight change
				Kind	Amount	Chemical		Histological‡	
		days			*per cent*	*total mg.*	*mg./100 gm.*		*per cent*
	1	33	CGL	—	—	1.17	19.4	1	−23
	2	33	"	—	—	4.73	69.6	2–4	−31
	3	33	"	Nicotinic acid	0.008	4.17	63.1	3–4	−33
2	4	33	"	B complex	§	4.16	62.0	2–4	−30
	5	33	"	Purified casein	8.0	2.26	42.7	0–1	−25
	6	33	"	Complete salt mixture‖	4.0	1.65	23.9	0–1	−26
	7	33	"	Alpha tocopherol	50 mg./wk.	4.25	67.4	2–4	−33
	1	70–84	CGL-B¶	Purified casein	8.0	5.66	70.7	2–4	−29
	2	70–84	"	Salt mixture	4.0	3.51	41.3	1–2	−15
	3	30–38	"	$CaCO_3$	1.35	3.30	54.9	2–3	−33
	4	30–38	"	$CaHPO_4$	2.4	1.96	30.2	1–2	−11
3	5	30–38	"	K_2HPO_4	1.6	2.18	32.1	0–2	−12
	6	30–38	"	Cystine	0.05	6.65	121.0	4–6	−30
	7	30–38	"	Methionine	0.3	8.08	137.0	3–6	−34
	8	30–38	"	Glutamic acid	2.0	6.60	100.4	4–6	−32
	9	30–38	"	Gelatin	8.0	2.88	48.8	2–4	−31
	2	35	Rice	(Corn grits in CGL-B replaced by rice)	—	6.23	68.4	4	−10
	3	35	Synthetic	Synthetic low P	—	2.14	34.4	2	−15
4	4	35	CG-B	(Lard in CGL-B replaced with corn grits)	—	8.64	108.0	4–5	−4.6
	5	35	CGL-B	Low P salt mixture	4.0	4.08	60.9	2–3	−16
	6	35	"	Low salt mixture and casein	4.0 8.0	4.32	43.6	1–3	−8
11	1	62	CGL-B	Complete salt mixture and casein	4.0 10.0	1.73	13.4	0	+58
	1	38	CGL-B	—	—	9.80	159.0	8	−28
13	2	38	Corn	(Corn grits in CGL - B replaced by corn meal)	—	6.91	112.0	3–6	−10

* All diets contained 2 per cent ferric citrate powder (U.S.P. VIII) except for group 1 in Experiment 2.

‡ No visible iron or only occasional cells or Kupffer cells with iron as is observed in animals receiving the Purina diet plus 0.31 per cent iron as ferric citrate. Increasing amounts of visible iron in the liver cells indicated by higher numbers.

§ Per 100 gm. of diet: 400 μg. thiamine, 400 μg. pyridoxine, 800 μg. riboflavin, 2.5 mg. calcium pantothenate, 4.0 mg. nicotinic acid, 20 μg. biotin, 20 μg. folic acid, 0.25 gm. inositol, and 0.5 gm. choline chloride.

‖ *J. Biol. Chem.*, 1941, **139**, 459.

¶ CGL plus thiamine, pyridoxine, riboflavin, calcium pantothenate, nicotinic acid, and choline chloride as indicated above.

attention has been focused primarily upon the conditions which prevent iron absorption upon the corn grit diet rather than upon those factors which may have the opposite effect.

In Experiment 4 several different diets were fed to determine whether a diet high in corn grits was essential in order to obtain excessive iron absorption or whether other low phosphorus diets would produce the same result. Group 2 received a diet similar in composition to the usual CGL-B diet except that all the corn grits were replaced by polished rice. Group 3 received a purified low phosphorus diet. This diet had the following percentage composition: casein 10, lard 20, glucose 60, cod liver oil 1, low phosphorus salt mixture 4, and the usual complement of synthetic vitamins. The low phosphorus salt mixture was made by omitting the $CaHPO_4$ and K_2HPO_4 from the complete salt mixture and adding sufficient KCl to replace the potassium thus removed. These diets permitted accumulation of iron in the livers although to a lesser degree than was usually obtained with diet CGL-B. It was concluded that while the selection of a corn grit diet was fortunate in that it allowed the maximum iron absorption of any of the diets listed, corn grits in the diet are not necessary for this effect since other low phosphorus diets produced similar results. (See also group 2, Experiment 13, in which corn meal was fed in place of corn grits.)

Other changes in the diet in Experiment 4 included the removal of the lard from CGL-B with an increase in the corn grits from 80 to 100 per cent; the supplementation of the CGL-B diet with the low phosphorus salt mixture to compare its effect with that of the complete salt mixture (Experiment 2, group 6); and the addition of both casein and the low phosphorus salt mixture to diet CGL-B. The low phosphorus salt mixture appears to have had little if any effect upon iron absorption and the further addition of casein was only partially effective. These results may be compared with Experiment 11, group 1, in which the complete salt mixture and casein together were completely effective in preventing a rise in liver iron.

The relation of iron absorption to phosphorus content was studied by adding iron as ferric citrate at various levels to the corn grit and the Purina diets, and by adding potassium phosphate to the corn grit diet containing iron. Fig. 1 shows the liver iron values obtained when phosphorus was added at various levels to the corn grit diet containing 0.31 per cent iron as ferric citrate. The values upon the abscissa for the phosphorus content include the small amount of phosphorus supplied by the basal diet plus the amount added as K_2HPO_4. The liver iron was directly related to the log of the phosphorus content of the diet. Also indicated upon the chart is the phosphorus content of the Purina diet, 0.87 per cent, and the liver iron value obtained in rats which received this diet containing 0.31 per cent iron. This value fits the line

obtained from the corn grit diet reasonably well, thus suggesting that the phosphorus content of the Purina diet is the determining factor in preventing excessive iron absorption upon this diet. The chart indicates that approximately 0.8 per cent phosphorus as phosphate is required to prevent a marked accumulation of liver iron when the diet contains 0.31 per cent iron as ferric citrate. This is more phosphorus than was supplied by 4 per cent of the complete salt mixture and thus it is not surprising that the salt mixture was not completely effective in preventing iron absorption. The source of the phos-

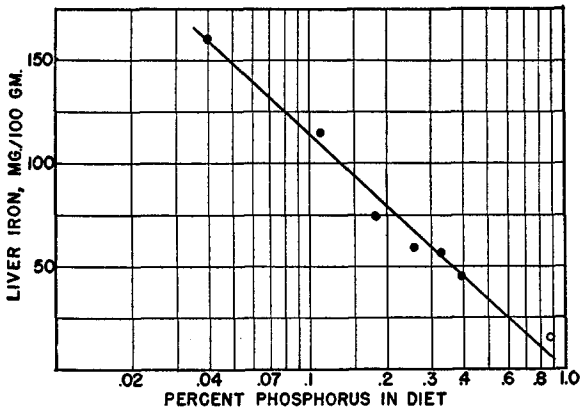

FIG. 1. The relation of liver iron to the phosphorus content of the diet. All animals received the corn grit diet containing 0.31 per cent iron as ferric citrate and the phosphorus content of the diet varied by the addition of potassium monohydrogen phosphate. The open circle represents the value obtained with the Purina diet containing the same amount of iron. Essentially the same results are obtained if total liver iron is plotted. This is also true in Figs. 2 and 3.

phorus as well as the total amount of phosphorus present in the diet is also of importance. As shown in Table II, sodium phytate and sodium pyrophosphate were much less potent than potassium phosphate, calcium phosphate, and skim milk powder when supplied at an equivalent level of phosphorus.

When different levels of iron as ferric citrate were added to the two diets, CGL-B and Purina, the results shown in Table III were obtained. In this study the iron content of the Purina diet was raised to very high levels. The largest amount tried, 1.86 per cent iron, represents 12.0 per cent of ferric citrate in the diet. The liver iron is clearly a function of the amount of dietary iron in either case, at least when a certain minimum level is exceeded. It should also be noted that with the Purina diet the level of liver iron obtained is not related to a loss in body weight. As indicated in Fig. 2, approximately 5 times as much iron is required in the Purina diet as in the corn grit diet to produce

TABLE II

The Effect of Various Phosphorous Supplements upon Iron Absorption from the Corn Grit Diet

Experiment	Group	Time	Basal diet	Iron added	Supplement		Liver iron			Weight change
					Kind	Amount	Chemical		Histological	
		days				*per cent*	*total mg.*	*mg./100 gm.*		*per cent*
	1	41	CGL-B	2 per cent Fe citrate	None	—	9.80	159.5	4	−34
	2	41	"	" "	K_2HPO_4	0.4	7.75	115.0	4	−13
	3	41	"	" "	"	0.8	5.89	74.2	2–3	−12
13	4	41	"	" "	"	1.2	4.62	59.1	2	−9
	5	41	"	" "	"	1.6	4.10	57.5	1–3	−4
	6	41	"	" "	"	2.0	2.60	40.5	1–2	−2
	1	35	CGL-B	2 per cent Fe citrate	None	—	5.10	71.8	3–4	−29
	2	35	"	" "	K_2HPO_4	1.2	1.62	20.2	0–1	−6
	3	35	"	" "	Na phytate	1.17	3.55	47.3	2–3	−11
19*	4	35	"	" "	Na pyrophosphate	1.54	3.42	45.4	2–3	−20
	5	35	"	" "	$CaHPO_4$	1.19	2.15	26.0	1–2	0
	6	35	"	" "	Skim milk powder	20.6	2.37	27.1	1–2	+7

* All supplements supplied the same amount of phosphorus in Experiment 19.

TABLE III

The Relation of Iron Level to the Amount of Iron Absorbed

Experiment	Group	Time	Basal diet	Ferric citrate added		Liver iron			Weight change
						Chemical		Histological	
		days		*per cent*	*per cent Fe*	*total mg.*	*mg./100 gm.*		*per cent*
	1	52	Purina	2	0.31	2.05	31.2	0–1	+16
	2	52	"	4	0.62	2.54	37.4	1	+17
14	3	52	"	6	0.93	4.77	69.6	1–2	+7
	4	52	"	8	1.24	7.44	94.8	2–3	+21
	5	52	"	12	1.86	13.03	171.6	3–4	+19
	1	40	CGL-B	0	0	2.56	27.3	0	−11
	2	40	"	0.25	0.039	2.90	30.7	0–1	−14
16	3	40	"	0.75	0.116	2.01	24.5	1	−17
	4	40	"	1.25	0.194	6.67	87.0	1–3	−30
	5	40	"	2.00	0.31	10.97	148.3	4	−38
	1	67	CGL-B	0	0	0.69	9.8	0	+4
	2	67	"	0.1	0.015	0.63	13.6	0–1	+3
11	3	67	"	0.4	0.062	2.65	28.4	1–2	0
	4	67	"	1.0	0.15	2.40	32.7	1	−14
	5	67	"	2.0	0.31	9.2	98.0	6	−29

an equal deposition in the liver. Thus an increase in the phosphorus content from 0.04 per cent to 0.87 per cent has decreased the absorption approximately 5 times.

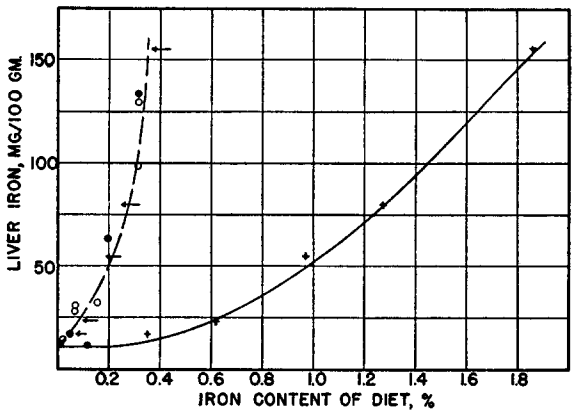

FIG. 2. The relation of the iron content of the diet to the liver iron content. Solid line, Purina diet; broken line, corn grit diet. Iron added as ferric citrate. The arrows indicate the position upon the chart which the Purina data would occupy if the iron content of the diet is divided by 5.2. It thus appears that at equal iron levels approximately 5 times as much iron is absorbed by animals receiving the corn grit diet.

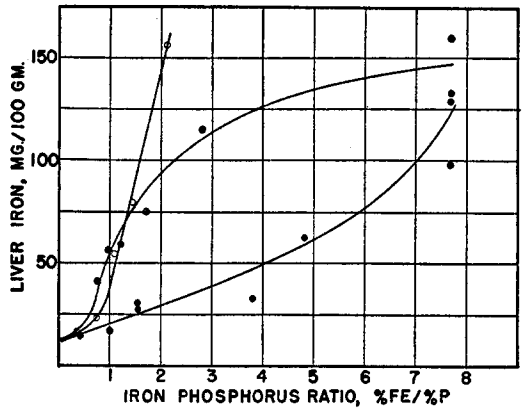

FIG. 3. The liver iron values in Figs. 1 and 2 have been plotted against the iron-phosphorus ratio of the diets. Open circles, Purina diet with various levels of ferric citrate, P constant; solid circles, corn grit diet with various levels of ferric citrate, P constant; and half-filled circles, corn grit diet with iron constant at 0.31 per cent and phosphate varied.

The experiments indicated that iron absorption could be changed by altering either the phosphorus or the iron content of the diet. It might be expected that the iron-phosphorus ratio of the diets would be the controlling factor. This was tested by plotting the liver iron values obtained in the previous experiments

against the iron-phosphorus ratio of the diets (Fig. 3). Excessive liver iron was found in most of the diets when the iron-phosphorus ratio was greater than 1 but the amount of liver iron does not appear to be correlated with this ratio. Since the phosphorus content of the Purina diet was higher than that of the corn grits diet, more iron had to be added to obtain the same iron-phosphorus ratio. The liver iron reached very high levels at lower ratios with the Purina diet than with the corn grit diet. This suggests that the absolute amount of iron in the diet is also an important factor.

Some of the data cannot be satisfactorily explained by the amount of phosphorus the diets contained. In Experiment 11 the diet supplemented with both salt mixture and casein contained only 0.4 per cent phosphorus and yet no increase in liver iron was observed. Similarly, the phosphorus content of the diet which group 6, Experiment 2 received, contained only 0.32 per cent phosphorus but was moderately effective in preventing iron absorption. These data suggest that in some instances factors other than the phosphorus content of the diet may influence either the rate or extent of iron absorption.

DISCUSSION

From the data presented, it may be concluded that the low phosphorus content of the iron-supplemented corn grit diet is the primary cause of the abnormally high iron absorption. Iron absorption can be reduced by the addition of phosphate salts to this diet. The amount of phosphorus in the Purina chow diet is sufficient to account for the fact that excessive iron absorption was not found with this diet at the levels of iron previously tried. It also appears likely that iron absorption can be increased by the addition of sufficiently large amounts of iron to any diet.

It can be noted, however, that not all the data conform to the proposition that iron absorption is simply a matter of iron and phosphorus levels in the diet. The effect of casein, both when added to the corn grit diet and as judged by the effect of the studies made with the purified diet, would appear to be greater than the expected from the phosphorus content. On the other hand, there is the suggestion in the data that some materials, *e.g.* certain amino acids, may increase the amount of iron absorbed. These other factors may play a lesser rôle in the dietary regulation of iron absorption under the conditions of our experiments.

Gillman, Mandelstam, and Gillman (7, 8) have reported massive accumulations of iron in the livers of pellagrins and malnourished people in South Africa. It would seem that both their observations in patients and our findings in animals could be classed as dietary hemochromatosis.[1] It is of interest that

[1] The term hemachromatosis is used because it is so firmly entrenched in the literature. Cytosiderosis has not been used since the implication has been made that the iron is derived by abnormal cell metabolism. Since the iron accumulates as the result of excessive iron absorption rather than from hemoglobin, a more truly descriptive term is ferrichromatosis.

in both instances very abnormal diets are involved and that the general pattern of the distribution of iron in the tissues is similar. While both of these conditions must represent a breakdown in normal regulation of iron absorption, it is impossible to make any implications of a common etiology. It is particularly difficult to relate an experiment lasting a few weeks to the clinical observations of a condition which probably takes years to develop.

Hahn *et al.* (9), and others (10, 11) have concluded that iron absorption is *normally* controlled by some mechanism which regulates the absorption of iron in proportion to body needs. However, it is clear from the present data that this is not an absolute phenomenon. It is probable that the normal mechanism is effective only when the iron-phosphorus ratio is within certain limits and at low levels of iron intakes. If the equilibrium is shifted markedly, either by large iron dosage or by a decrease in phosphorus, the normal control fails.

SUMMARY

Rats fed a corn grit diet containing large amounts of ferric citrate absorb and deposit excessive amounts of iron in their livers. Undoubtedly various factors are involved in iron absorption, but these studies indicate that the low level of dietary phosphate was primarily responsible.

The addition of phosphate salts to this diet has shown that the amount of iron deposited in the liver was inversely related to the phosphorus content of the diet.

It is possible to obtain excessive iron deposits in the livers of animals receiving a normal diet, by adding large amounts of iron salts to the diet. This is not associated with losses of body weight in these animals.

It is concluded that the absolute amount of iron and/or phosphorus in the diet as well as the iron-phosphorus ratio influences the amount of iron absorbed.

We are greatly indebted to Merck & Company, Inc., Rahway, Corn Industries Research Foundation, New York, Sheffield Farms Company, Inc., New York, Abbott Laboratories, North Chicago, and Wilson Laboratories, Chicago, for generously supplying us with materials used in these experiments.

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