

The Effect of Achylia Gastrica on Iron Absorption *

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The importance of gastric secretion in iron absorption remains unsettled although the problem has been studied for many years. Early clinical studies in which iron absorption was measured by the rate of hemoglobin regeneration in iron-deficient subjects suggested that gastric acidity plays a role in the absorption of food iron (1-3), therapeutic doses of inorganic iron salts, and ferrum reductum (4-6). Defective absorption of dietary iron has been demonstrated in achlorhydric subjects by a chemical balance technique (7), but several workers using serum iron tolerance curves as an index of absorption have reported that achlorhydria does not reduce the assimilation of large doses of inorganic iron salts (8-10). More recent data obtained by isotopic methods are conflicting, and in many instances the number of observations is too few to support firm conclusions. Some studies have indicated that achlorhydria does not reduce the absorption of large doses of ferrous salts (11), hemoglobin iron (12, 13), or iron from a standard meal (14), whereas others have shown that the absorption of iron in bread (15) or a standard meal (16) is markedly affected by achlorhydria, at least in iron-deficient subjects. A comparable defect in achlorhydric subjects without anemia has not been found, but studies have been recorded on only six patients (14, 17), and the techniques employed may not have been adequate for the demonstration of diminished iron absorption because the percentage of absorption in the control subjects in these studies was very low.

In the present study iron absorption tests were performed on nine subjects with achylia gastrica who were not anemic by using a test meal of white bread of low iron content so that the percentage of absorption of labeled iron was relatively high in the control group of normal subjects. In six of the subjects with achylia gastrica the effect of normal and of neutralized gastric juice on iron absorption was determined.

Methods

Iron absorption test. After an overnight fast 2 μC of $\text{Fe}^{59}\text{SO}_4$ SA, 19 to 34 mc per mg) diluted in 150 ml of iron-free water was given orally with 20 g of white bread. Nothing further was given by mouth for 3 hours. Ascorbic acid was not added to the test dose, and it was not acidified. To reduce the iron content of the test dose a batch of bread was prepared from nonenriched flour and baked in aluminum pans. The loaves were sliced, the crust was removed, and 20-g portions were wrapped in Parafilm and stored at -20°C until they were used. The iron content of a 20-g portion of this bread was 170 μg . The tracer was given along with bread rather than after incorporation during the baking process because Steinkamp, Dubach, and Moore (18) obtained the same level of absorption with the two methods.

The absorption of the tracer was determined by a fecal excretion technique and the fecal radioactivity measured in a large well scintillation detector as previously described (19). Stool collections were continued until three successive specimens contained less than 1% of the administered dose. A 5-minute counting time was employed for each sample with a counting error of less than 1% for specimens containing more than 1% of the administered dose. Radioactivity remaining on the glass container used to administer the dose was measured in the large well detector and an appropriate correction applied. A minimum of three stools was examined for occult blood during the collection period to ensure that none of the absorbed iron which had been incorporated into hemoglobin was lost by the fecal route. Iron absorption tests were carried out in the Special Investigation Unit of the Kingston General Hospital to assure completeness of fecal collection.

A comparison was made of the fecal excretion technique and the technique based on the uptake of radioiron by red blood cells in 19 subjects. Blood volume was pre-

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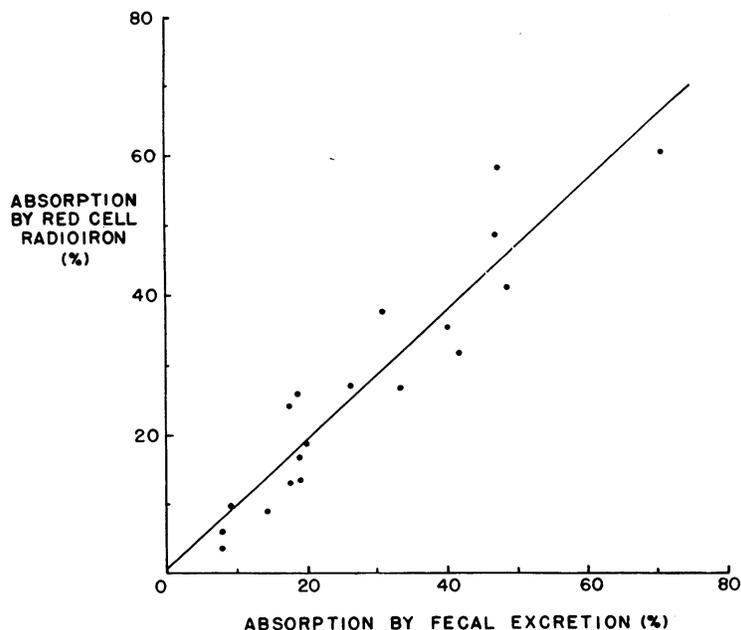


FIG. 1. COMPARISON OF PERCENTAGE OF IRON ABSORPTION MEASURED BY FECAL EXCRETION AND RED CELL UPTAKE OF Fe^{59} .

dicted by the formula of Allen and co-workers (20), and it was assumed that 75% of the absorbed iron had been incorporated into circulating hemoglobin by day 10. The coefficient of correlation between the results obtained by the two methods was 0.94, the regression line slope 0.93, and the error of the estimate 21% expressed as the coefficient of variation (Figure 1). In view of the error inherent in the estimation of blood volume and the additional error introduced by assuming that the percentage of incorporation of absorbed tracer is the same in all subjects, the fecal excretion technique was chosen in preference to red cell uptake for the measurement of iron absorption. The relatively high percentage of absorption of the tracer from the test meal and the high sensitivity of the detector used for assaying fecal activity overcome the objections raised by Bothwell and Finch (21) to the use of the fecal excretion technique.

Preparation of gastric juice. Gastric juice was collected from normal subjects during histamine stimulation and divided into two parts. One portion was stored at $-20^{\circ}C$, and the other was neutralized with 1 N NaOH before storage. In iron absorption tests in which gastric juice was employed the radioiron was given with 90 ml of gastric juice and 60 ml of iron-free water.

Selection of subjects. Nine control subjects were selected from co-operative psychiatric patients who were free from organic disease and who were found on investigation to have a hemoglobin concentration above 13.0 g per 100 ml, normal peripheral blood smear, vitamin B_{12} absorption above 10% (22), gastric acid as determined by either the augmented histamine test (23) or the Diagnex Blue test with histamine stimulation, and

normal serum iron, unsaturated iron-binding capacity, and transferrin saturation (24).

The group with achylia gastrica was composed of nine patients with treated pernicious anemia who had been given parenteral vitamin B_{12} for periods of 3 months to 10 years and who were in complete hematological remission. The diagnosis of pernicious anemia was based on a history of megaloblastic anemia responding to vitamin B_{12} alone, vitamin B_{12} absorption less than 5.0% (22) which increased to more than 10% with intrinsic factor, and pH of gastric juice greater than 7.0 after maximal histamine stimulation. The patients were considered in hematological remission if the hemoglobin concentration, peripheral blood smear, serum iron, unsaturated iron-binding capacity, and transferrin saturation fulfilled the criteria set out for the control group.

The age and sex distribution and the mean values for hemoglobin concentration, hematocrit, and unsaturated iron-binding capacity were similar in the two groups (Table I). The mean serum iron and the saturation of transferrin were slightly lower in the patients with achylia, but the difference was not statistically significant ($p > 0.20$).

Results

Iron absorption in achylia gastrica. In the control group iron absorption determined by our technique ranged from 19 to 49% with a mean of 35.2% ($SD \pm 12.0$), whereas in the achylic group absorption varied from 8 to 39%, with a mean of

TABLE I
*Hematological data and the results of iron absorption tests in normal and achylic subjects**

Subject	Sex	Age	Hgb	Hct	Serum iron	UIBC	Transferrin saturation	Iron absorption
		<i>yrs</i>	<i>g/100 ml</i>	<i>%</i>	<i>μg/100 ml</i>	<i>μg/100 ml</i>	<i>%</i>	<i>%</i>
Normal								
1	F	40	13.5	38	163	270	38	49.2
2	M	75	15.5	48	118	241	33	48.9
3	M	51	13.2	41	88	263	25	47.5
4	M	48	15.0	45	65	230	22	41.8
5	F	68	13.8	37	55	255	18	40.2
6	M	49	15.4	43	78	240	25	26.4
7	F	56	16.3	44	164	193	46	24.4
8	F	57	13.6	42	88	313	22	20.1
9	M	59	14.7	45	114	301	28	18.7
Mean		56	14.6	43	104	256	29	35.2 ± 12†
Achylic								
1	M	74	15.0	45	78	308	20	39.0
2	M	63	13.9	38	65	285	19	34.4
3	F	51	14.8	44	60	240	20	18.7
4	M	66	14.5	41	63	308	17	17.7
5	M	52	14.5	43	52	272	16	13.3
6	F	64	13.5	40	98	197	33	8.0
7	F	47	13.3	42	163	151	52	9.1
8	M	62	15.8	46	79	345	19	17.5
9	F	71	13.0	41	58	265	18	20.3
Mean		61	14.3	42	80	263	24	19.8 ± 9.9†

* Abbreviations: Hgb = hemoglobin; Hct = hematocrit; UIBC = unsaturated iron-binding capacity.

† Mean ± SD.

19.8% (SD ± 9.9), and in all but three subjects the absorption was less than 20% (Table I). The difference between the mean absorption in each group is statistically significant at the 1.0% level.

Effect of gastric juice on iron absorption in achylia gastrica. In six patients with achylia gastrica selected because of their continued availability for study the mean absorption of iron from bread alone was 21.8%, and with the addition of normal gastric juice to the test meal a greater than twofold increase in absorption occurred in all but one subject. The mean absorption, 50.6%, was significantly greater than the mean absorption with bread alone ($p < 0.005$) (Figure 2). The difference between the mean absorption with normal gastric juice in this group of six patients with achylia gastrica and the absorption of bread iron alone in control subjects was not statistically significant.

The effect of neutralized gastric juice was quite variable. There was at least a twofold increase over the absorption with bread alone in Cases 4 and 5, only a slight increase in Cases 1, 3, and 6,

and a considerable reduction in absorption in Case 2. The mean absorption with neutralized gastric juice was 28.9%, which was significantly lower than with normal juice ($p < 0.05$) but not different from the absorption with bread iron alone in these subjects ($p > 0.30$).

Discussion

In this paper achlorhydria is used in a broad sense to denote reduced gastric acid secretion as demonstrated by the conventional histamine technique, Diagnex Blue test, or the augmented histamine test in patients in whom gastric secretion of intrinsic factor may or may not have been adequate. Achylia gastrica is used to describe the defect in gastric secretion in patients with adult pernicious anemia.

The apparent conflict between observations in the literature concerning the effect of gastric secretion on iron absorption can be largely resolved if it is postulated that gastric secretion plays a role in the absorption of iron from certain types of

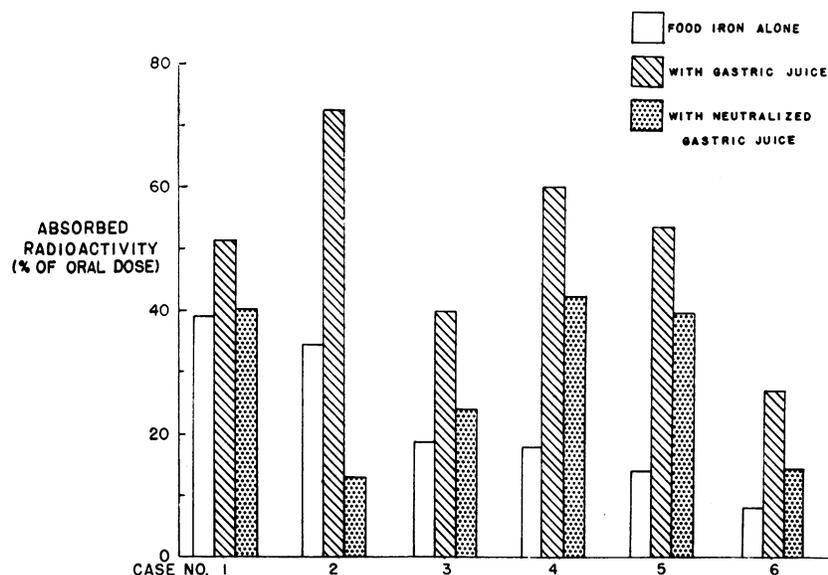


FIG. 2. EFFECT OF GASTRIC JUICE ON THE ABSORPTION OF FOOD IRON IN SUBJECTS WITH ACHYLIA GASTRICA.

food (1-3, 7, 15, 16) but plays no part in the absorption of larger doses of inorganic iron salts (8-11) or of hemoglobin iron (12, 13). The only work at variance with the postulate that gastric secretion plays a role in the absorption of food iron is the study of Pirzio-Biroli, Bothwell, and Finch (14), who observed that four subjects with achylia gastrica and a larger group of control subjects both absorbed 5.3% of iron from a standard meal. The low percentage of absorption from their test meal, however, may have masked defective absorption in the achylic subjects; Goldberg and associates using a comparable test meal reported 57.5% absorption of iron in iron-deficient subjects and clearly reduced absorption in subjects with achlorhydria (18.5%). Although previous work indicates that acid secretion plays no part in the absorption of large doses of inorganic iron salts (8-11), in three of these studies iron absorption was measured by serum iron tolerance curves that have been shown by several investigators to correlate poorly with results obtained by more refined methods (25-28), and further studies on this point are desirable. That different mechanisms may be involved in the absorption of different forms of iron is apparent in the studies of iron absorption in patients after partial gastrectomy in whom it has been found that the absorption of iron salts is normal (27, 29) and

that there is a defect in the absorption of iron from a standard meal (14, 30), from eggs (31), and from bread (15, 32). There is also evidence for the presence of more than one mechanism in the recent work of Turnbull, Cleton, and Finch (33) which strongly suggests that iron is absorbed from hemoglobin as a porphyrin complex. In contrast to the absorption of ferrous salts, the absorption of hemoglobin iron is not decreased by food or by phytate or increased by ascorbic acid, and it is not surprising that gastric secretion plays no part in the absorption of this form of dietary iron.

The observation in the present study that the administration of normal but not of neutralized gastric juice completely corrects the defective absorption of food iron in achylic subjects indicates that the enhancing effect of gastric juice is related to pH, but this effect could be due to substances in gastric juice other than hydrochloric acid, such as pepsin or a reducing agent. Many workers have postulated that an acid medium promotes iron absorption by dissolving and ionizing food iron and also by delaying the formation of insoluble or undissociated iron complexes at the higher pH of the small intestine (8, 34-36), and support for this concept is derived from animal studies which have shown that acidity promotes the absorption of inorganic iron from isolated

small intestinal loops (37, 38). Pepsin may promote the absorption of food iron by releasing iron bound to protein (39) and by enhancing the reducing properties of protein due to release of sulfhydryl groups. Recent work has shown that a number of amino acids increase the absorption of iron from isolated intestinal loops in the rat (40), and it is possible that pepsin might also enhance iron absorption by increasing the concentration of amino acid residues in the upper small intestine. Because pepsin requires an acid medium for its action, it is difficult to separate the effect of the enzyme from that of acidity. Reducing agents in gastric juice may explain its enhancing effect on the absorption of dietary iron, but again it is difficult to separate their effect from that of acidity because reducing substances perform more efficiently in an acid medium (35). The recent report of Beutler, Fairbanks, and Fahey (41) that small concentrations of gastric juice prevent the precipitation of ferrous iron at pH 8.5 suggests that there is a factor in gastric secretion apart from acidity that may promote the absorption of food iron. Apparently, the ability of gastric juice to augment the absorption of food iron could be due to a combination of factors rather than to any single property.

The work of Moore (17, 42) has been widely quoted as proving that acidity plays no part in the absorption of food iron. Moore studied the absorption of radioiron biosynthetically incorporated into eggs and found that the addition of sufficient hydrochloric acid to the test dose to reduce the pH to less than two did not enhance iron absorption in two subjects with hypochromic anemia. No tests of gastric function were reported in these patients, and the number of observations is really insufficient to draw conclusions concerning the effect of acidity per se on iron absorption. In addition iron in eggs is less well absorbed than iron in other foods probably because it is tightly bound in organic complexes (21), and it may be unwise to apply the results obtained with a single food in a very small number of subjects to all types of dietary iron.

There are two factors that make it difficult to apply the results of the present study to normal dietary conditions. In the first place the amount of carrier iron in the test meal is much smaller

than in a normal diet. Nevertheless, if a defect exists on a low intake, it is likely to be present with a high intake although the relative magnitude of the defect may be different. Secondly, the composition of the diet is of prime importance in determining the absorption of food iron (21) and the finding of a defect in iron absorption with bread does not prove that it exists with other foods or that it is even present when other foods are given with bread. Although the results of the present investigation indicate that there is a defect in the absorption of iron from bread in achylic subjects, the role of normal gastric juice in the absorption of iron under normal dietary conditions requires further investigation.

Summary

The absorption of radioiron from a test meal of white bread containing 170 μg of iron was determined in nine patients with treated pernicious anemia and in nine control subjects by measuring fecal radioactivity in a large well-type scintillation counter. The mean iron absorption in the achylic group was 19.8% compared with 35.2% in the controls. The difference between the means is statistically significant at the 1% level. A significant increase in absorption was observed when normal gastric juice was given with the test dose of food iron to six of the achylic subjects. Although the effect of adding neutralized gastric juice to the test dose was somewhat variable, the mean absorption with neutralized gastric juice was significantly lower than with normal gastric juice but not different from the absorption with bread alone.

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