Reduction of Ferric Iron to the Ferrous Form During Digestion in Vitro With Saliva

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WORKERS in the field of iron metabolism agree that iron is absorbed more readily by the human being when it is in the ferrous rather than the ferric form, and some even go so far as to believe that iron can be absorbed only in the former state. Most of the iron in food is in the ferric form; thus some reduction probably takes place above or in the upper part of the small intestine from which the greatest quantities of iron are absorbed.

Kirch *et al.*[1] studied the reduction of iron in artificial gastric digestion; later Bergeim and Kirch[2] studied the reduction of iron in samples aspirated from the stomach after the ingestion of ferric chloride and selected foods. Considerable reduction was observed to have occurred. Possibly the process of reduction begins even earlier, perhaps in the mouth or in the upper part of the fundus where salivary digestion may continue under some conditions. The power of saliva to reduce some compounds has long been recognized, but no one, as far as we have been able to discover, has investigated the power of saliva to reduce ferric iron. Therefore, the present study was undertaken to investigate the action of saliva on a ferric salt and also on a ferric salt when food (bread) is present.

MATERIAL AND METHODS

A method had to be found by which both ferric and ferrous forms of iron could be determined in the same digestion mixture. Such a method has been devised by Kirch *et al.*[1] and was adapted for use in the present study. The reaction was carried out in 60-ml pear-shaped separatory funnels. Ten milliliters of 25 per cent hydrochloric acid were placed in each funnel. In rapid succession each of the following was added: 2 ml of saliva which had been incubated with ferric chloride; 10 ml of tertiary butyl alcohol; 10 ml of very cold water; 1 ml of 4 per cent potassium persulfate to those funnels in which all the iron was to be oxidized for the determination of total iron, and 1 ml of water to the other funnels; 5 ml of 20 per cent potassium thiocyanate; and finally 7.5 ml of isobutyl alcohol.

The funnels were shaken for 30 seconds, the layers allowed to separate, the water layer drawn off, and the alcohol layer poured through dry acid-washed cotton. The transmission of color was read in an Evelyn Photoelectric Colorimeter at 490 mμ with a mixture of the two alcohols for reference. Ferrous iron was calculated as the difference between total iron and ferric iron and a correction made for the ferrous iron content of the ferric chloride itself. A standard curve was made from a solution of iron wire in 1N HCl.

A stock solution of ferric chloride was made up in 1N HCl. On each test day 1 ml of the stock solution was diluted to 50 ml with glass-distilled water and added to either 12 or 14 ml of saliva. By this means the acidity of the ferric chloride was low enough that the pH of the saliva to which it was added was lowered only a little. When to 12 ml of saliva the addition of 1 ml of water was replaced by an equivalent amount of ferric chloride, the pH was lowered from 0.25 to 0.35 pH units. Care was taken to minimize the lowering of the pH because Nikiforuk[3] found that saliva had the greatest reducing effect on indicator dyes at pH

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7.5 to 8.0, slightly less at pH 7, and a rapid decline between pH 7.0 and 6.5, the area in which, according to Wessinger,[4] the pH of the saliva of most persons falls. Whether iron would be reduced in a manner similar to dyes was not known.

Four women served as subjects during May and early June. They collected saliva between 9:00 and 10:00 A.M. and again between 12:30 and 1:30 P.M. Lipstick was removed and the teeth were brushed without dentifrice at the conclusion of a meal and no collections made for at least 30 minutes. Paraffin was chewed to induce a flow of saliva. According to Nikiforuk,[3] saliva with the most "sediment (epithelial cells, bacteria, leukocytes and unidentified debris)" has the strongest reducing action on oxidation-reduction indicator dyes. Therefore, the saliva was swirled during the withdrawal of samples for analysis in order to include a representative portion of the sediment.

One milliliter of ferric chloride was placed in a test tube and 12 ml of saliva added to it. The tube was set in a water bath at 36–38°C in a darkened room for 30 minutes. The tube was swirled at the beginning and once during the incubation period. Immediately after removal from the water bath 2-ml portions were delivered into 60-ml separatory funnels and the iron content determined as rapidly as possible to avoid oxidation of the iron that had been reduced. The total iron content of the ferric chloride was 10.6 μg.

RESULTS AND DISCUSSION

The largest percentage of iron reduced by saliva in any single one of the 24 tests was 7.9, but in most tests the percentage was less than 5. The amount reduced was characteristic for the individual: The reducing power of the saliva of subject C was almost negligible, while subject B reduced an average of 7.7 per cent in the morning (Table I). In 10 of the 12 tests made with saliva collected in the morning, more iron was reduced than in samples collected in the afternoon.

Because some of the action of saliva takes place in the fundus of the stomach where air is excluded, and because the absence of oxygen might prevent reoxidation of iron reduced by the digestion medium, a few tests were made under nitrogen. The saliva of subject A was used for four such tests. In two of them the saliva was incubated for 30 minutes, and in two others for 3 hours. Neither the exclu ion of air nor the additional time increased the reduction of iron when no food was present.

The practical question is whether iron is reduced when food and saliva both are present. No doubt the reducing power of foods differs; only bread was employed in the present study. To 1 ml of ferric chloride in a 50-ml centrifuge tube, 14 ml of saliva, and 1 g of dried, ground, unenriched bread were added. Air was replaced with nitrogen in all tests except two. The tubes were incubated for 3 hours except in two tests, when incubation was terminated after 1 hour. The tubes were swirled every 10 minutes. Tubes containing 1 ml of ferric chloride and 14 ml of water were incubated at the same time for the determination of ferrous iron in the ferric chloride solution and the total iron. At the end of the incubation period the tubes containing bread were centrifuged and 2-ml portions of the supernatant liquid drawn off for the determination of iron.

When the saliva of subject A collected in the morning was combined with bread and two tests made in the presence of air, the percentages of reduction of ferric to ferrous iron were 6.7 and 7.7 (Table II); when the air was replaced with nitrogen the percentages rose to 23.5 and 33.7. Since nitrogen increased the reduction so much it was used in the rest of the tests.

The percentages of iron reduced by the saliva of all three subjects were much higher when the saliva was mixed with bread than when saliva

TABLE I
Reduction of Iron in Ferric Chloride[a] by Saliva

No. tests	Subject	Uncombined iron reduced (%)		Combined iron (μg)	
		A.M.	P.M.	A.M.	P.M.
4	A	4.7	3.2	0.3	0.2
2	B	7.7	2.0	0.2	0.1
4	C	1.0	1.2	0.6	0.6
2	D	3.9	2.7	0.5	0.2

[a] The total iron content of the ferric chloride was 10.6 μg.

TABLE II
Reduction of Iron in Ferric Chloride by Bread and Saliva

Subject	Conditions	Uncombined iron reduced (%)		Total iron (μg)		Combined iron (μg)	
		A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
A	3 hr, no N	6.7	—	9.2	—	4.4	—
	3 hr, N	23.5	—	9.2	—	3.8	—
	3 hr, no N	7.7	—	9.6	—	3.4	—
	3 hr, N	33.7	—	9.6	—	2.6	—
	1 hr, N	15.3	7.8	9.1	9.3	4.4	5.4
	3 hr, N	46.9	18.9	9.0	9.0	2.9	4.6
B	3 hr, N	42.8	28.9	9.0	9.0	1.2	3.5
C	3 hr, N	22.3	16.7	9.3	9.3	5.2	5.8

N = nitrogen.

specimens alone were used. The reductions for saliva collected in the morning and incubated for 3 hours under nitrogen ranged from 22.3 to 46.9 per cent and in the afternoon from 16.7 to 28.9 per cent (see Table II). The variability was wide among subjects and for saliva collected from the same subject on different days. Similar wide variation was found by Bergeim and Kirch[2] in gastric digestion. Wide variability might be anticipated because saliva is far from a homogenous mixture. In the present study the addition of bread caused the removal of a high proportion of iron from the solution. Kirch *et al.*[1] found a similar removal of iron by food in gastric digestion mixtures. Presumably the iron combined with the food. Whenever the amount of combined iron was high the percentage of reduction was low. More iron was combined in mixtures containing saliva collected in the afternoon than in those containing saliva collected in the morning.

Apparently the reduction of iron begins with the ingestion of food. It is interesting to speculate whether differences among people in the reducing power of the digestion medium cause variation in the amount of iron absorbed, and also whether more iron is absorbed from foods ingested in the morning.

SUMMARY

Saliva collected in both morning and afternoon from four women was incubated with ferric chloride alone and also with ferric chloride and unenriched bread. More iron was reduced by the salivas collected in the morning than by those collected in the afternoon. With ferric chloride alone the reduction was usually less than 5 per cent. The levels of reduction were characteristic of the individual. When bread was added, reductions with the morning salivas ranged from 22.3 to 46.9 per cent and with afternoon salivas from 16.7 to 28.9 per cent.

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