

# The effect of tea on iron absorption

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**SUMMARY** The effect of tea on iron absorption was studied in human volunteers. Absorption from solutions of  $\text{FeCl}_3$  and  $\text{FeSO}_4$ , bread, a meal of rice with potato and onion soup, and uncooked haemoglobin was inhibited whether ascorbic acid was present or not. No inhibition was noted if the haemoglobin was cooked. The effect on the absorption of non-haem iron was ascribed to the formation of insoluble iron tannate complexes. Drinking tannin-containing beverages such as tea with meals may contribute to the pathogenesis of iron deficiency if the diet consists largely of vegetable foodstuffs.

Evidence is mounting that the absorption of iron from individual food items is profoundly affected by the composition of the meal as a whole. For example, egg iron is very poorly absorbed, but the percentage is considerably increased by drinking orange juice (Callender, Marney, and Warner, 1970), while a smaller percentage of the iron in soybean is absorbed if black beans form part of the meal (Martinez-Torres and Layrisse, 1973). Our interest in tea was aroused during a study of the absorption of iron from maize meal porridge served with sugar containing ferrous sulphate and ascorbic acid. When tea was drunk with the meal the absorption figures seemed lower than might have been expected. The possibility that tea might inhibit iron absorption did not appear to have been studied, and, since it is a popular drink in a number of countries where iron deficiency is a major nutritional problem, it was decided to undertake a formal investigation.

## Materials and Methods

### SUBJECTS

The volunteers who took part in the present investigation were multiparous Indian housewives living in a municipal housing scheme at Chatsworth near Durban. It has been established previously that iron deficiency is common among such individuals (Mayet, Adams, Moodley, Kleber, and Cooper, 1972). Their mean age was 40 years (range 26-60

years). Written consent was obtained after the nature of the investigation had been explained to them.

On two consecutive mornings after an overnight fast the subjects drank a solution of one of the iron compounds or ate one of the standard meals. The iron compound or food iron was labelled with  $2.5 \mu\text{C } ^{55}\text{Fe}$  on the one morning and with  $2.5 \mu\text{C } ^{59}\text{Fe}$  on the other, and 200 ml of either warm tap water or tea was drunk immediately afterwards. Nothing more was eaten or drunk for the next four hours. Two weeks later the subjects reassembled after again fasting overnight and a specimen of blood was collected for measurement of the  $^{55}\text{Fe}$  and  $^{59}\text{Fe}$  content, haemoglobin concentration, serum iron concentration, unsaturated iron-binding capacity, and serum ferritin concentration. They then drank 50 ml tap water containing  $1.7 \mu\text{mol}$  ascorbic acid and  $0.54 \mu\text{mol}$  iron as  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  labelled with  $2.5 \mu\text{C } ^{59}\text{Fe}$ . No further food or drink was allowed for four hours. Measurement of the  $^{59}\text{Fe}$  content of a second blood sample collected after a further 14 days enabled the absorption of this 'reference iron salt' to be calculated by difference, and provided an index of each individual's absorbing capacity.

### IRON COMPOUNDS

Every subject received  $0.54 \mu\text{mol}$  iron on each of the two mornings. In different experiments the iron was administered as  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ , as  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  together with  $1.7 \mu\text{mol}$  ascorbic acid, as rabbit haemoglobin, or as crystallized rabbit haem. The

iron salts were freshly dissolved in 50 ml tap water, but the haemoglobin and the haem were administered in 50 ml preserved tomato juice or in gravy. Radioactive haemoglobin was prepared by injecting male New Zealand white rabbits intramuscularly with 200  $\mu\text{c}$  of either  $^{59}\text{Fe}$  or  $^{55}\text{Fe}$ . After some weeks blood was obtained from a marginal ear vein. The erythrocytes were separated by centrifugation and washed three times with sterile isotonic sodium chloride solution. They were suspended in distilled water, frozen to  $-20^\circ\text{C}$  and thawed, and membranes were separated from the haemoglobin solution by centrifugation at  $2500 \times g$ . Radioactive haemoglobin in a dosage of 2.5  $\mu\text{c}$  was used in each study. It was mixed with either unlabelled haemoglobin or mince to provide 0.54  $\mu\text{mol}$  iron per person. Haem was extracted from the haemoglobin by the method of Labbe and Nishida (1962). Radioactive haem was mixed with unlabelled haem to provide 0.5  $\mu\text{mol}$  iron and 2.5  $\mu\text{c}$  per person.

#### IRON IN BREAD

Two loaves of white bread were baked, using 70-80% extraction flour. Sufficient  $^{59}\text{FeCl}_3$  or  $^{55}\text{FeCl}_3$  was mixed into the dough together with the yeast so as to provide 0.54  $\mu\text{mol}$  iron and 2.5  $\mu\text{c}/100$  g bread, the quantity consumed by each subject. The bread was eaten without butter or jam.

#### IRON IN RICE WITH POTATO AND ONION SOUP

Sufficient rice intrinsically labelled with  $^{55}\text{Fe}$  by hydroponic culture (Hussain, Walker, Layrisse, Clark, and Finch, 1965; Layrisse, Cook, Martinez, Roche, Kuhn, Walker, and Finch, 1969) was mixed with carrier rice to provide 2.5  $\mu\text{c}$   $^{55}\text{Fe}$  and 45 g dry rice per person. The rice was soaked overnight in water and then boiled until no excess water remained. It was divided into equal portions by weighing and eaten with the potato and onion soup. Soup for 10 subjects was prepared by frying 1 kg peeled potatoes and 500 g onions in 2 table-spoons of sunflower seed oil. After adding 850 ml water and 5 g curry powder it was brought to the boil and allowed to simmer for 30 minutes. During this time 5.37  $\mu\text{mol}$  iron as  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  labelled with 25  $\mu\text{c}$   $^{59}\text{Fe}$  was added together with 56.79  $\mu\text{mol}$  ascorbic acid. The thick soup was thoroughly mixed in a Waring blender, divided into equal portions, and eaten with the rice. The meal thus contained  $^{55}\text{Fe}$  as the label for the intrinsic rice and iron and  $^{59}\text{Fe}$  as the label for the added  $\text{FeSO}_4$ .

In a second experiment the meal of rice with potato and onion soup was prepared in the same way except that no intrinsically labelled rice was used; on the one morning the extrinsic label in the soup was  $^{59}\text{Fe}$  and on the other  $^{55}\text{Fe}$ .

#### HAEMOGLOBIN IRON IN MINCED LAMB

Enough minced lamb to provide a total of 0.54  $\mu\text{mol}$  iron per person (including that present in the haemoglobin gravy) was fried in oil and divided into equal portions by weighing. Isotopically labelled rabbit haemoglobin solution providing 2.5  $\mu\text{c}$  per individual was added to the frying pan which had been used to cook the mince, and simmered for 15 minutes to make a gravy. Equal portions were poured over the fried mince helpings.

#### TEA

A commercial brand of tea widely used by the people of Chatsworth (Pot O'Gold, O.K. Bazaars Ltd) was selected. The 200 ml drunk by each individual was prepared from 5 g dry tea. In some studies 40 ml pasteurized cow's milk was added. When the effect of tea with milk was compared with that of tea without milk, an extra 40 ml water was added to the latter to make the volumes the same.

#### ISOTOPIC AND CHEMICAL METHODS

Blood samples (10 ml) and aliquots of standard iron solutions and foods were prepared for differential radioactive counting by the method of Katz, Zoukis, Hart, and Dern (1964). The quantities of  $^{55}\text{Fe}$  and  $^{59}\text{Fe}$  in the processed samples were determined by means of a liquid scintillation system (Insta-Gel, Packard Instrument Company, Downers Grove, Illinois) and a Packard Tri-Carb AAA spectrometer (model 3375), which automatically adjusted for quenching. The counting efficiency was 24% for  $^{55}\text{Fe}$  and 42% for  $^{59}\text{Fe}$  at optimal gain and window settings. The  $^{59}\text{Fe}$  activity in the 4 ml blood samples collected immediately before the 'reference iron salt' was administered, and two weeks later was assessed (against suitable standards) by means of a Packard Auto-Gamma Tri-Carb (model 3001) spectrometer. All figures for percentage absorption were calculated on the assumption that 100% of the absorbed radioactivity was present in the haemoglobin of circulating red cells, and that the blood volume of each subject was 65 ml/kg. We calculated that if the whole of each test dose had been retained, the total radiation dose averaged over a period of 13 weeks would have been approximately 20% of the permissible whole body burden for continuous exposure in the case of  $^{59}\text{Fe}$  and 0.2% in the case of  $^{55}\text{Fe}$  (International Commission on Radiological Protection, 1960).

Serum iron concentrations were measured by a modification (Bothwell and Finch, 1962, p. 18) of the method of Bothwell and Mallett (1955) in which sulphonated bathophenanthroline was used as the colour reagent. The unsaturated iron-binding capacity was determined by the method of Herbert, Gottlieb, Lau, Gevirtz, Sharney, and Wasserman

Haematological Data					Percentage Absorbed		
Haemoglobin (g/dl)	Serum Iron ( $\mu\text{mol/l}$ )	UIBC ( $\mu\text{mol/l}$ )	Percentage Saturation Transferrin	Serum Ferritin ( $\mu\text{g/l}$ )	Water	Tea	Reference Iron Salt
<i>FeCl<sub>3</sub></i>							
9.9	6.27	93.62	6.3	—	3.7	2.3	25.6
13.6	19.69	60.50	24.5	77	6.9	2.5	8.5
10.9	8.41	77.33	9.8	8	7.5	3.6	23.5
13.9	16.11	68.20	19.1	41	7.7	4.8	5.8
12.8	17.90	60.14	22.9	15	9.5	2.0	24.3
15.5	14.50	82.52	14.9	16	21.0	12.2	33.7
11.0	6.27	92.90	6.3	2	23.1	12.3	65.3
11.8	13.25	71.42	15.6	39	25.9	7.8	42.3
11.9	14.68	74.82	16.4	8	51.7	7.9	42.4
14.0	13.07	72.14	15.3	33	60.0	6.2	31.6
Mean							
12.5	13.02	75.36	15.1	26.4	21.7 (SD $\pm$ 19.7)	6.2 (SD $\pm$ 3.9)	30.3 (SD $\pm$ 17.3)
<i>FeSO<sub>4</sub> + ascorbic acid</i>							
14.1	22.73	48.69	31.8	7	4.0	4.0	
13.7	14.50	62.83	18.8	2	5.9	4.9	
14.1	9.85	47.44	17.1	2	9.8	3.9	
14.4	13.78	57.46	19.3	88	24.3	4.2	
12.9	20.94	57.82	26.6	92	33.4	8.5	
12.9	11.64	81.45	12.5	74	37.0	12.4	
9.5	6.09	77.51	6.1	21	40.3	23.1	
14.1	17.54	47.79	22.6	23	42.4	12.6	
14.8	18.08	67.48	21.1	8	52.7	23.1	
12.5	9.49	89.50	9.6	5	59.0	15.7	
Mean							
13.3	14.46	63.80	17.0	32.0	30.9 (SD $\pm$ 19.3)	11.2 (SD $\pm$ 7.5)	

Table I Effect of tea on absorption of iron from solutions of FeCl<sub>3</sub> and FeSO<sub>4</sub> + ascorbic acid

Haematological Data					Percentage Absorbed		
Haemoglobin (g/dl)	Serum Iron ( $\mu\text{mol/l}$ )	UIBC ( $\mu\text{mol/l}$ )	Percentage Saturation Transferrin	Serum Ferritin ( $\mu\text{g/l}$ )	Tea	Tea with Milk	Reference Salt
12.4	22.55	78.94	22.2	26	2.5	4.3	28.5
12.4	18.26	75.90	19.4	34	3.3	3.1	35.1
12.4	15.75	55.85	22.0	23	4.3	10.8	45.6
12.1	14.86	59.61	20.0	22	5.2	9.3	51.3
12.4	20.94	85.20	19.7	39	15.7	16.1	78.9
12.1	17.36	73.39	19.1	26	16.5	8.1	46.4
11.8	9.31	83.59	10.0	<2	22.4	42.1	73.9
11.2	13.96	93.08	13.0	2	36.5	33.6	90.5
Mean							
12.1	16.62	75.70	18.2	21.7	13.3 (SD $\pm$ 12.0)	15.9 (SD $\pm$ 14.3)	56.3 (SD $\pm$ 22.2)
<i>Water</i>							
14.0	17.54	51.19	25.5	102	9.2	1.8	
12.4	11.10	70.53	13.6	40	15.5	9.4	
9.0	6.62	78.22	7.8	<2	30.0	27.9	
12.1	7.88	89.68	8.1	<2	31.2	23.8	
13.4	16.83	59.61	28.7	9	42.2	10.0	
12.1	11.28	99.52	10.2	<2	42.7	33.8	
9.4	6.98	89.50	7.2	3	59.7	21.2	
11.2	22.73	72.14	24.6	13	60.4	21.4	
10.8	8.77	100.78	8.0	<2	77.3	25.0	
Mean							
11.6	12.19	79.02	14.9	19.4	40.9 (SD $\pm$ 22.2)	19.4 (SD $\pm$ 10.2)	

Table II Effects of tea and milk on absorption of iron from a solution of FeSO<sub>4</sub> + ascorbic acid

Haematological Data					Percentage Absorbed		
Haemoglobin (g/dl)	Serum Iron ( $\mu\text{mol/l}$ )	UIBC ( $\mu\text{mol/l}$ )	Percentage Saturation Transferrin	Serum Ferritin ( $\mu\text{g/l}$ )	Water	Tea	Reference Salt
12.1	16.11	39.02	29.2	124	6.0	0.5	2.5
7.0	2.69	98.09	2.7	2	2.3	0.8	76.1
11.2	13.60	52.45	20.6	18	10.3	1.1	26.2
13.6	7.70	51.37	13.0	18	10.4	1.6	25.2
12.1	16.65	59.97	21.7	11	11.6	2.7	35.2
14.0	20.05	51.55	28.0	4	13.4	3.8	60.2
10.6	6.98	99.88	6.5	7	14.2	6.6	50.3
14.0	11.81	73.03	13.9	62	15.3	8.9	10.9
Mean							
11.8	11.95	65.67	16.9	30.8	10.4 (SD $\pm$ 4.4)	3.3 (SD $\pm$ 3.0)	35.8 (SD $\pm$ 25.0)

Table III Effect of tea on absorption of iron in bread

Haematological Data					Percentage Absorbed			
Haemoglobin (g/dl)	Serum Iron ( $\mu\text{mol/l}$ )	UIBC ( $\mu\text{mol/l}$ )	Percentage Saturation Transferrin	Serum Ferritin ( $\mu\text{g/l}$ )	Intrinsic Iron	Extrinsic Iron	Extrinsic Intrinsic	Reference Iron Salt
<i>Meal followed by water</i>								
11.4	12.17	89.32	12.0	33	5.3	5.1	0.96	37.2
12.2	20.23	68.38	22.9	8	6.7	7.2	1.07	22.5
6.8	4.30	102.57	4.1	2	10.3	9.0	0.87	33.5
11.8	12.17	86.46	12.4	11	11.5	11.7	1.02	45.4
10.6	6.98	95.94	6.8	4	13.5	16.3	1.20	21.4
10.0	11.46	100.60	10.3	13	16.0	21.9	1.36	43.6
11.2	7.34	103.28	6.6	2	23.5	21.6	0.92	53.0
Mean								
10.6	10.66	92.36	10.4	10.4	12.4 (SD $\pm$ 6.1)	13.3 (SD $\pm$ 6.8)	1.05 (SD $\pm$ 0.17)	36.6 (SD $\pm$ 11.8)
<i>Meal followed by tea</i>								
14.2	20.94	49.76	29.6	100	0.5	0.6	1.20	2.9
14.8	21.48	58.71	26.8	104	0.5	0.6	1.20	3.9
14.4	13.78	65.34	17.4	68	0.8	1.4	1.70	68.2
13.2	8.77	76.97	10.2	56	1.7	2.7	1.58	11.8
12.4	13.96	75.54	15.6	12	2.2	3.4	1.54	16.6
12.8	14.86	77.51	16.0	40	3.3	3.6	1.09	35.7
13.2	13.96	94.87	12.8	2	6.1	6.1	1.00	53.0
Mean								
13.5	15.39	71.24	17.8	54.6	2.2 (SD $\pm$ 2.0)	2.6 (SD $\pm$ 2.0)	1.33 (SD $\pm$ 0.27)	27.4 (SD $\pm$ 25.5)

Table IV Absorption of intrinsic and extrinsic (supplemental) iron from rice with potato and onion soup containing 100 mg ascorbic acid

Haematological Data					Percentage Absorbed		
Haemoglobin (g/dl)	Serum Iron ( $\mu\text{mol/l}$ )	UIBC ( $\mu\text{mol/l}$ )	Percentage Saturation Transferrin	Serum Ferritin ( $\mu\text{g/l}$ )	Water	Tea	Reference Iron Salt
11.4	23.63	53.16	30.8	69	2.7	0.1	0.4
12.2	12.17	51.37	19.2	15	8.8	3.4	26.1
11.2	11.28	80.19	12.3	3	9.9	3.4	50.6
10.6	8.77	83.06	9.6	12	10.6	2.8	54.6
11.0	13.07	63.19	17.1	7	12.0	2.8	68.2
9.9	6.98	80.01	8.0	3	12.3	2.4	51.9
13.3	17.54	52.27	25.1	76	13.0	0.1	14.1
12.2	22.73	52.45	30.2	30	17.2	4.8	11.5
Mean							
11.5	14.52	64.46	19.0	26.9	10.8 (SD $\pm$ 4.1)	2.5 (SD $\pm$ 1.6)	34.7 (SD $\pm$ 24.7)

Table V Effect of tea on absorption of iron from rice with potato and onion soup containing 100 mg ascorbic acid

(1967). The iron content of digested samples of food was estimated by a modification (Bothwell and Finch, 1962, p. 26) of the method of Lorber (1927). The serum ferritin concentrations were measured by radioimmunoassay using the method of Miles, Lipschitz, Bieber, and Cook (1974).

**Results**

**EFFECT OF TEA ON THE ABSORPTION OF IRON FROM SOLUTIONS OF IRON SALTS**

The drinking of tea without milk was found to inhibit the absorption of iron from a solution of FeCl<sub>3</sub> (t = 2.68, P = <0.05), and also from a solution of FeSO<sub>4</sub> containing ascorbic acid (t = 4.46, P = <0.01) (table I). Tea with milk produced much the same effect on the absorption of iron from a solution of FeSO<sub>4</sub> with ascorbic acid (t = 8.65, P = <0.001) as did tea without milk (t = 9.28, P = <0.001), the degree of inhibition being revealed by comparison with the 'reference absorption' figures (table II). When 200 ml milk without tea was drunk after the solution of FeSO<sub>4</sub> and ascorbic acid absorption was also inhibited (t = 3.44, P = <0.01).

**EFFECT OF TEA ON THE ABSORPTION OF NON-HAEM FOOD IRON**

Tea inhibited the absorption of iron from bread (t = 7.50, P = <0.001) (table III). The absorptions of the intrinsically labelled rice iron and the extrinsic iron in the potato and onion soup were closely similar whether tea was drunk with the meal or not (table IV). This observation, together with the considerable evidence from other studies that all the non-haem iron in a meal forms a common pool within the lumen of the gut (Cook, Layrisse, Martinez-Torres, Walker, Monsen, and Finch, 1972; Björn-Rasmussen, Hallberg, and Walker, 1972; Sayers, Lynch, Jacobs, Charlton, Bothwell, Walker, and Mayet, 1973; Sayers, Lynch, Charlton, Bothwell, Walker, and Mayet, 1974), indicated that an extrinsic label could be used to assess the effect of tea on the absorption of iron in the meal. Accordingly, eight further subjects consumed the rice with potato and onion soup on successive mornings, the extrinsic label being <sup>59</sup>Fe on the one occasion and <sup>55</sup>Fe on the other. When tea was drunk with the meal there was a significant inhibition of absorption (t = 6.74, P = <0.001) (table V) in spite of the presence of 100 mg ascorbic acid.

Haematological Data					Percentage Absorbed		
Haemoglobin (g/dl)	Serum Iron (μmol/l)	UIBC (μmol/l)	Percentage Saturation Transferrin	Serum Ferritin (μg/l)	Water	Tea	Reference Iron Salt
<i>Uncooked haemoglobin</i>							
10.4	13.96	77.87	15.2	102	5.5	1.7	2.1
14.6	18.08	72.67	20.0	25	11.5	2.0	12.2
13.6	22.73	71.60	24.1	14	11.3	3.1	25.2
12.8	7.70	60.50	11.3	13	4.7	3.5	18.1
14.0	23.63	61.40	27.7	5	5.6	3.7	29.2
12.0	26.85	47.79	36.0	33	8.0	4.1	13.3
12.8	31.68	59.07	34.8	5	22.6	4.4	26.9
13.6	19.33	46.54	29.3	32	13.0	5.6	8.7
15.8	11.28	81.45	12.2	3	13.9	7.5	37.6
12.8	15.75	91.65	14.6	14	11.4	8.5	96.6
7.5	3.94	89.50	4.2	<2	23.4	10.2	74.3
8.6	6.09	75.18	7.5	<2	45.3	17.7	83.7
<b>Mean</b>							
12.4	16.75	69.60	19.7	12.8	14.7	6.0	35.7
					(SD ± 11.4)	(SD ± 4.5)	(SD ± 31.6)
<i>Cooked haemoglobin</i>							
13.2	17.54	46.18	27.5	54	2.6	3.0	2.8
11.8	15.93	46.18	25.6	32	16.7	7.5	15.2
12.8	12.35	54.77	18.4	29	10.3	7.7	12.9
13.6	19.51	36.70	34.7	31	10.0	8.1	28.2
12.4	17.36	46.36	27.2	5	11.2	11.2	48.6
11.4	12.71	57.28	18.2	<2	15.6	11.2	25.3
12.4	13.25	61.93	17.6	10	25.1	13.7	87.3
13.6	13.96	48.51	22.3	29	10.9	24.1	55.5
14.0	13.78	50.30	21.5	59	24.1	27.8	1.9
12.1	11.46	65.16	15.0	21	8.2	28.9	27.2
<b>Mean</b>							
12.7	14.79	51.34	22.8	27.1	13.5	14.3	30.5
					(SD ± 7.0)	(SD ± 9.2)	(SD ± 26.6)

Table VI Effect of tea on absorption of haemoglobin iron

Haematological Data					Percentage Absorbed		
Haemoglobin (g/dl)	Serum Iron ( $\mu\text{mol/l}$ )	UIBC ( $\mu\text{mol/l}$ )	Percentage Saturation Transferrin	Serum Ferritin ( $\mu\text{g/l}$ )	Cooked Haemoglobin	Uncooked Haemoglobin	Reference Iron Salt
14.1	31.50	22.20	58.7	5	3.8	0.9	45.3
14.6	35.44	37.77	40.4	21	8.1	4.7	22.7
15.2	42.24	40.99	51.9	21	10.5	7.8	22.3
14.4	20.23	37.95	34.8	18	11.3	4.5	15.0
14.1	16.47	40.10	29.1	25	14.6	15.2	18.3
8.2	7.34	77.87	8.6	20	15.3	5.2	28.3
14.8	31.15	55.49	36.0	2	16.0	12.5	65.2
10.6	9.85	77.87	11.2	14	16.2	4.7	28.7
13.7	15.57	54.95	22.1	118	18.2	9.9	3.5
12.4	19.33	54.06	26.3	7	23.4	15.6	32.5
Mean							
13.2	22.91	49.93	31.9	25.0	13.7 (SD $\pm$ 5.5)	8.1 (SD $\pm$ 5.0)	28.2 (SD $\pm$ 17.1)

Table VII Absorption of iron from cooked and uncooked haemoglobin ingested with tea

## EFFECT OF TEA ON THE ABSORPTION OF HAEM IRON

Tea significantly inhibited the absorption of haemoglobin iron from a solution of uncooked rabbit haemoglobin in tomato juice ( $t = 3.89$ ,  $P = < 0.005$ ) (table VI). Of greater practical importance, however, was the finding that tea had no significant effect on the absorption of haemoglobin iron from the fried lamb mince with rabbit haemoglobin gravy ( $t = 0.28$ ,  $P = > 0.70$ ). The conclusion that tea did not affect the absorption of haemoglobin iron from cooked food was checked by a comparison between the absorption of cooked and uncooked rabbit haemoglobin iron administered in tomato juice and followed by a cup of tea on each occasion (table VII). The absorption of iron from the uncooked haemoglobin was significantly less than from the cooked haemoglobin ( $t = 4.66$ ,  $P = < 0.005$ ). In different experiments the absorption of haemoglobin iron without tea was very similar whether the haemoglobin was uncooked or cooked (table VII), and this is in agreement with previous reports (Callender, Mallett, and Smith, 1957; Turnbull, Cleton, and Finch, 1962). It therefore seemed justifiable to conclude that tea inhibits the absorption of haemoglobin iron only if it has not been cooked. Finally, the effect of tea on the absorption of crystallized rabbit haem was examined. No inhibition was found, the mean figures ( $\pm$  SD) in nine subjects being 11.8% ( $\pm$  3.5) with water and 10.6% ( $\pm$  5.0) with tea.

## RELATIONSHIP BETWEEN SERUM FERRITIN CONCENTRATION AND IRON ABSORPTION

Only where ferrous sulphate and ascorbic acid had been administered were there enough absorption results to permit the rate of iron absorption to be correlated with the serum ferritin concentration. The percentage iron absorptions from the different

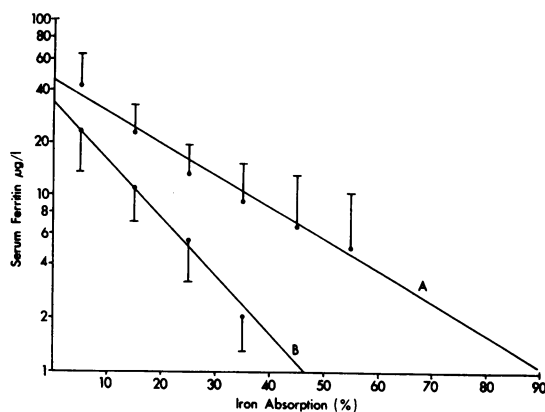


Fig 1 The relationship between the serum ferritin concentration (mean  $\pm$  2SE) and the absorption of iron from a solution of  $\text{FeSO}_4$  with ascorbic acid. The iron solution was followed by a drink of water (A) or tea (B).

experiments were grouped into decades and were plotted against the mean log serum ferritin concentrations (see fig). A straight line relationship was revealed such that  $y = -0.018x + 1.650$  ( $r = -0.67$ ,  $P = < 0.001$ ). When tea was drunk with the iron solution the slope was steeper ( $y = -0.029x + 1.488$ ) ( $r = -0.50$ ,  $P = < 0.01$ ). The difference between the regression coefficients of the two lines was statistically significant ( $t = 2.26$ ,  $P = < 0.05$ ).

## Discussion

The results of the present study indicate that tea inhibits the absorption of non-haem iron to a significant extent. The effect was seen with a solution of ferric chloride, with a solution of ferrous sulphate

plus ascorbic acid, and with the iron in bread and in a rice meal, and was similar whether the tea contained milk or not. No attempt was made to investigate the mechanism responsible for the interference with absorption, but it seems likely that it was due to the tannins in the tea. Tannins form coloured complexes with ferric iron (Finar, 1956), and a blackish discoloration was seen when  $0.54 \mu\text{mol}$  iron as  $\text{FeCl}_3$  was added to 200 ml tea. Much the same effect was produced by the quantity of ferrous sulphate with ascorbic acid used in the present study. The formation of iron complexes within the intestinal lumen may profoundly affect iron absorption (Conrad, 1970). Such complexes may be soluble or insoluble. Some of the agents which form soluble complexes facilitate iron absorption, eg, ascorbic acid, while others such as bicarbonate (Benjamin, Cortell, and Conrad, 1967) and EDTA (Brise and Hallberg, 1962) reduce the availability of the iron. If the complex is insoluble it is not absorbed. The extent to which iron can be absorbed from food is probably largely dependent on the relative concentrations of various complexing agents present in the meal. It follows that supplemental iron salts are subject to the same influences as the intrinsic iron present in individual foodstuffs, and it has repeatedly been shown that they are absorbed to a similar degree (Cook, Layrisse, Martinez-Torres, Walker, Monsen, and Finch, 1972; Björn-Rasmussen and Hallberg, 1972; Sayers, Lynch, Jacobs, Charlton, Bothwell, Walker, and Mayet, 1973; Sayers, Lynch, Charlton, Bothwell, Walker, and Mayet, 1974). Further evidence of this was obtained in the present study (table IV).

Haem iron is absorbed as such, and it is only within the mucosal epithelial cells that the iron is liberated from the porphyrin (Weintraub, Weinstein, Huser, and Rafal, 1968). Luminal chelators including ascorbic acid have been shown not to influence its absorption (Callender, Mallet, and Smith, 1957; Turnbull, Cleton, and Finch, 1962; Conrad, Benjamin, Williams, and Foy, 1967), and the significant inhibition of the absorption of uncooked haemoglobin iron by tea was therefore surprising. An agent capable of chelating ionic iron is most unlikely also to be able to form complexes with haem (Conrad, 1970), and indeed the absorption of crystallized haem was found not to be inhibited. The possibility that the inhibition of haemoglobin iron absorption by tea might be due to an effect on the globin was therefore considered. The tanning of leather is thought to involve the formation of cross-links between collagen fibres, the phenolic groups of the vegetable tannins probably attaching to the peptide bonds between the amino acids by hydrogen bonding (Haslem, 1966). Possibly the uncooked globin was 'tanned' by the tea, and thereby rendered

less susceptible to hydrolysis by the proteolytic enzymes of the digestive juices. If this occurred then less haem would be released and less would be available for absorption. The observation that tea had no inhibitory effect if the haemoglobin were cooked strengthened this possibility, since cooking denatures the globin but does not affect the absorption of haemoglobin iron (Callender *et al*, 1957; Turnbull *et al*, 1962).

Since meat is almost invariably cooked before it is eaten, little nutritional significance can be attached to the inhibition of the absorption of uncooked haemoglobin. In those communities where meat is an important dietary constituent the iron nutrition is generally satisfactory, but iron deficiency is rife when the average diet of the population consists very largely of vegetable staples. Iron is poorly absorbed from wheat, maize, or rice meals (Martinez-Torres and Layrisse, 1973; Sayers *et al*, 1973; Sayers *et al*, 1974), and tea may aggravate the nutritional problem. Tea is drunk during meals by many South Africans of Indian extraction, and this is also the custom in other parts of the world. Since preliminary observations (unpublished) indicate that coffee has a similar effect, the implications may be even wider.

An interesting peripheral observation was the inverse correlation between the serum ferritin concentration and the percentage absorption of iron (see fig). The relationship was similar to that found by Cook, Lipschitz, Miles, and Finch (1974), and confirms the value of the serum ferritin concentration as a measure of the body's need for iron. The steeper slope of the line when tea was drunk can be ascribed to the effective sequestration of a proportion of the iron in unabsorbable tannin complexes. The prevalence of iron deficiency among the group as a whole was underscored by the finding that the serum ferritin concentration was below the lower limit of normality of  $10 \mu\text{g/l}$  in a third of them (Jacobs, Miller, Worwood, Beamish, and Wardrop, 1972; Lipschitz, Cook, and Finch, 1974).

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