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SOME PHYSIOLOGICAL ASPECTS OF IRON TRANSPORT - STUDIES ON THE FORTIFICATION OF SUGAR WITH IRON

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A Thesis Submitted to the Faculty of Medicine
University of the Witwatersrand, Johannesburg
for the Degree of Doctor of Philosophy (Medicine)

Johannesburg 1976

-7-1-1977 68905

ACKNOWLEDGEMENTS

I am indebted to Professor T.H. Bothwell, Professor R.W. Charlton, Dr. Sean Lynch and Dr. John Torrance for their invaluable advice and help; to Mrs. Shirley Lichtigfeld, Miss Fawzia Khan and Mr. Peter Smith for their indispensable technical assistance; and to the South African Medical Research Council and South African Sugar Association for the financial support which made this research possible. Finally, I should like to thank Marlene Hynd for typing this manuscript and Mrs. Val Rough for her expert photography.

PUBLICATIONS

Much of the data presented in this thesis has been published in the following articles:

'Studies in the fortification of cane sugar with iron and ascorbic acid' (1975)
British Journal of Nutrition, 34: 141-152.

'The effect of tea on iron absorption' (1975)
Gut, 16: 193-200.

'The mechanism of the inhibition of iron absorption by tea' (1975)
South African Journal of Medical Sciences, 40: 110-116.

ABSTRACT

An attempt was made to fortify sugar with iron in order to prevent the development of iron deficiency. It was possible to add various iron salts and ascorbic acid to sugar without discolouring the vehicle even after storage for many months under hot humid conditions. The absorption of iron from fortified sugar and cereal meals was then measured in human volunteers using either an "extrinsic tag" to label the iron compound or intrinsically labelled food iron. Iron absorption from ferrous sulphate was found to be good in the presence of adequate ascorbic acid. When sugar fortified with ferrous sulphate was added to tea or coffee, however, there was such a marked colour change that sugar fortified in this way could be ruled out as an effective vehicle for dietary iron supplementation. No such discolouration occurred with ferric orthophosphate, however, and absorption studies were thus undertaken with sugar containing ferric orthophosphate and ascorbic acid. The absorption was poor, in striking contrast to previous results using salt as the vehicle for this compound. The reason for these discrepant results was found to be the need to apply heat if the iron in ferric orthophosphate is to be converted into an absorbable form by ascorbic acid. There are thus real problems in using sugar as a vehicle for the fortification of the diet with iron and ascorbic acid. The addition of ascorbic acid alone to a vehicle such as sugar may, however, be adequate to improve iron nutrition.

A formal investigation was then undertaken into the effect of tea on iron absorption. Tea was found to inhibit the absorption of iron from iron salts and cereal meals even in the presence of adequate ascorbic acid. Tea also inhibited iron absorption from uncooked haemoglobin but had no effect on either cooked haemoglobin or crystallised haem. The effect on inorganic iron was found to be related to the tannin content of the tea.

probably via the formation of poorly absorbed iron-tannin complexes within the gastro-intestinal lumen. The effect on haemoglobin may be related to the astringent properties of tannin on the globin moiety. These results suggest that tea may be an important factor in the production of iron deficiency in many parts of the world. Furthermore, tannins are present in many vegetable foods and their role in modifying iron absorption generally may be extremely important.

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CHAPTER 1

INTRODUCTION

Man's need for iron stems from its important role in cellular energy production. In health, the amount absorbed from the diet replaces losses of iron from the body; iron deficiency occurs when the balance between these two processes is upset. Several stages in the development of the condition are recognised. Initially the demands of the body are met by iron mobilised from stores. Only limited iron is in the storage form, however, and when storage depots have been drained, "prelatent iron deficiency" (Heinrich et al., 1971) or "iron depletion" (Committee on Iron Deficiency of the American Medical Association, 1968) is said to occur. If negative iron balance continues the amount of iron circulating in the plasma decreases and the stage of "iron deficiency without anaemia" is reached (World Health Organisation Expert Committee, 1972). Finally, the supply of iron to the bone marrow becomes insufficient for the production of red blood cells, and anaemia develops.

1.1 THE PREVALENCE OF IRON DEFICIENCY

Estimates of the prevalence of iron deficiency vary greatly depending on the criteria by which it is diagnosed.

1.1.1 Prelatent and Latent Iron Deficiency

Measurement of storage iron is a sensitive method of detecting iron deficiency in its early stages. Great technical difficulties are encountered, however, and until very recently, the histological assessment of stainable iron in the bone marrow was the best available method (Rath and Finch, 1948). Bone marrow aspiration is an invasive procedure, however, unsuited to large epidemiological surveys, and has been used only rarely. Nevertheless, when it has been used the prevalence of iron deficiency has been found to be high, and of 269 randomly selected Swedish women of childbearing age assessed in this way, 33 per cent were found to be totally lacking in storage iron (Hallberg, 1970). Other workers have estimated the prevalence of prelatent iron deficiency by measuring the non-haem iron content of specimens of liver taken at autopsy. A global study, using this technique, demonstrated most importantly a geographical variation in the content of iron stores (Charlton et al., 1970). The lowest levels found were in

tropical areas, especially India and New Guinea (Disler et al., 1973), suggesting that iron deficiency is more common in these areas.

Owing to the difficulties encountered in quantitating iron stores, attempts have been made to estimate the prevalence of pre-latent iron deficiency by measuring such peripheral indices as serum iron, total iron binding capacity, and red cell protoporphyrin. These parameters correlate poorly with storage iron, however, and if all patients with serum total iron binding capacities in excess of 64.80 µmol/l were treated for iron deficiency, 35 per cent of the patients without stainable iron in the bone marrow would be excluded from treatment, while 30 per cent of those treated would have adequate stores (Garby, 1973). An exciting recent advance, however, is the availability of a radio-immunoassay for serum ferritin (Addison et al., 1972; Miles et al., 1974). This is a simple test needing only a small amount of blood, and initial results show an excellent correlation with iron stores (Jacobs et al., 1972; Cook et al., 1974; Lipschitz et al., 1974). Even with this sensitive test, however, significant problems arise in the interpretation of results. Before the prevalence of iron deficiency can be calculated, the criteria for the diagnosis of the condition must be fixed. This is extremely difficult as irrespective of the level chosen, some degree of misclassification appears inevitable (vide infra).

1.1.2 Iron Deficiency Anaemia

Surveys based on estimation of haemoglobin concentration or haematocrit are simple and economical, and although they reflect anaemia rather than iron deficiency, these are probably related in a significant proportion of cases (Foy et al.,1952). The same difficulties of interpretation apply to these parameters too, however. Any population appears to consist of at least two subpopulations, one physiologically iron deficient and the other iron replete. The frequency distribution curves of the haemoglobin concentrations of these two groups overlap widely, and thus no single haemoglobin concentration can be used to differentiate the one from the other (World Health Organisation, 1972). Thus if 1074.6 µmol iron is given daily

to randomly selected menstruating women, 17 per cent of Swedes will respond haematologically (Garby et al., 1969) as will 35 per cent of Thai women (Garby, 1973). Among the responders, however, will be many women with "normal" haemoglobin concentrations, and many so called anaemic women will show no response. Those studies in which the prevalence has been estimated purely on the basis of haemoglobin concentrations should thus be treated with reserve. Nevertheless, using the criteria suggested by the World Health Organisation Expert Committee in 1972, "anaemia" was found in 20 per cent of Welsh women (Garby, 1973), 25 per cent of Swedish women (Garby, 1973), 8 per cent of White American women and 20 per cent of American Negroes (McDonough et al., 1965). In tropical countries the estimates are much higher and in Asia, approximately 10 per cent of men, 20 per cent of non-pregnant women, 40 per cent of pregnant women and 92 per cent of children aged less than two years are thought to suffer from iron deficiency anaemia (Cowan and Bharucha, 1973). In Latin America, an investigation in seven countries showed that anaemia was present in 3.9 per cent of men, 17.3 per cent of non-pregnant females and 38.5 per cent of pregnant females (Cook et al., 1971), and in malaria free villages in Thailand the condition occurred in 70 per cent of the females and 60 per cent of the males (Garby, 1973). In South Africans of Indian extraction the results are similar, and anaemia was found in 40 per cent of pregnant women (Mayet, 1966). It has been suggested that the most realistic estimate of the prevalence is obtained by considering this data mathematically, on the basis of probability (Garby, 1970), i.e. the probability of any subject with a particular haematocrit or haemoglobin concentration, being anaemic. Thus 82 per cent of patients with haematocrits of less than 32 per cent are likely to be affected, while this is true of only 3.2 per cent of those with haematocrits of greater than 40 per cent. The current controversy must not be taken as an indication that there is little iron deficiency in the world, however, as it should be remembered that in Sweden, a country with one of the highest standards of living in the Western world, 17 per cent of women of childbearing age benefitted haematologically by taking iron tablets. Nevertheless, it must be stressed that

relatively small changes in diagnostic criteria will lead to substantial changes in prevalence estimations.

It is also important to realise that the estimations are all based on the "normal" rather than the physiologically ideal. Whether a higher haemoglobin concentration, for example, will lead to a longer lifespan, is completely unknown. Furthermore the prevalence in females has been calculated on the basis that the normal haemoglobin concentration is lower than males (World Health Organisation, 1972). Although it is obvious that menstrual blood loss, negative iron balance in pregnancy and androgens in males are the probable reasons for this, the supposition that females can function as well as males at a lower haemoglobin concentration has no good physiological basis. The prevalence in females may thus be far higher than indicated.

1.1.3 Relationship of Symptoms to Haematological Parameters

This debate has led many to argue that estimates of prevalence should rather be based on symptoms related to iron deficiency, as the ultimate importance of the syndrome relates only to the disturbance in health which it causes (Crosby, 1974).

Although iron deficiency per se is not thought to cause any symptoms, in one study oral iron was found to have a beneficial effect on non-anaemic iron deficient women (Beutler et al., 1960). This finding has never been repeated, however, and more recently no correlation was found between bone marrow iron content and physical work capacity (Ericsson, 1970). However, it must be stressed that little is known of the effect of iron deficiency on the function of other iron containing energy generating compounds such as the cytochromes. Preliminary data suggest that it may in fact be of great importance (Dallman, 1974).

Various attempts have been made to correlate the degree of anaemia with symptoms. In 1954, a study of 360 housewives showed no difference in haemoglobin levels between those complaining of the classic symptoms of anaemia such as weakness, palpitations, tinnitus and dizziness and those who were asymptomatic (Berry and Nash, 1954). This impression was confirmed by subsequent, much larger studies, in which no correlation was found between symptoms and moderate anaemia

(i.e. down to levels of 8 g/dl), (Elwood and Wood, 1966; Wood and Elwood, 1966; Morrow et al., 1968; Elwood et al., 1969.) There are several possible reasons for this rather surprising paradox. Firstly the body has a great capacity to compensate for anaemia by increasing oxygen delivery to the tissues. This is accomplished partly through an increase in the cardiac output, and partly by decreasing the affinity of haemoglobin for oxygen, the latter being associated with a rise in the red cell content of 2,3-diphosphoglyceric acid and adenosine triphosphate (Torrance et al., 1970; Garby, 1973.) An inverse relationship has recently been demonstrated between the concentration of 2,3-diphosphoglyceric acid in the erythrocyte and symptoms through a wide range of haemoglobin concentration (Garby, 1973). These compensatory mechanisms have limits, however, and although an anaemic individual may be able to cope easily with a sedentary occupation, his efficiency may decrease in the face of severe exercise. An excellent correlation has in fact been shown between the haematocrit and physical fitness as measured by the Harvard Step Test (Viteri and Torun, 1974). Furthermore oxygen consumption has been found to decrease by 50%/g haemoglobin/dl in healthy males (Grimby et al., 1973). It must be stressed too that patients with diseases of the cardiovascular or respiratory systems may even find difficulty in coping with sedentary occupations if anaemic. The poor correlation found between symptoms and haematological indices may thus reflect the poor discriminant ability of the questions rather than their lack of association.

Nevertheless, even if there is no unequivocal evidence that moderate anaemia leads to a disturbance in health, there seems little doubt that a haemoglobin concentration of less than 8 g/dl is associated with clinical symptoms (Garby, 1973). As levels as low as 8 g/dl occur in between 10 per cent and 40 per cent of the inhabitants of some tropical countries (Sood et al., 1968), it is reasonable to conclude that at least in certain parts of the world, significant iron deficiency is extremely common and that iron deficient individuals would benefit by treatment of the condition.

1.2 AETIOLOGY OF IRON DEFICIENCY IN THE POPULATION

Adequate iron nutrition depends on a balance between iron loss and iron assimilation, and disturbance of either of these processes may contribute to the development of iron deficiency in any individual. It is, therefore, clear that deficiency is most likely to occur at times of greatest need i.e. infancy, pregnancy and adolescence. It is obvious that the female in her reproductive years is far more likely to become deficient than the adult male.

1.2.1 Iron Loss as a Cause of Deficiency

In the healthy adult, skin iron losses have been shown to vary little even under conditions of extreme heat and sweating (Green et al., 1968). Almost the only physiological condition under which the loss of iron is sufficient to cause deficiency is pregnancy. Approximately 7164 µmol of iron must be mobilised during pregnancy, three-quarters of which is taken up by the foetus. The iron requirements of the pregnant female therefore increases to between 53.7 and 71.6 µmol daily. The chances of a pregnant woman becoming iron deficient are thus exceptionally high, and this is even more likely if iron stores have been depleted by several inadequately spaced previous pregnancies. In poor communities a high birth rate is almost invariably coupled with an inadequate diet, to produce an ideal setting for the development of iron deficiency.

When blood loss occurs, however, iron losses become extremely significant. Thus a repeated loss of more than 80ml blood per menstrual period is thought to lead to iron deficiency (Rybo, 1973) and such losses occur regularly in 11 per cent of "healthy" females in Western society (Hallberg et al., 1966). In tropical areas, some 600 million individuals harbour hookworms and bleed chronically from the gastrointestinal tract (Roche and Layrisse, 1968) and an inverse relationship has been shown between hookworm loads and mean haemoglobin levels in rural communities in Venezuela (Layrisse and Roche, 1964). The same situation obtains with heavy infestations of other parasites such as Schistosoma Haematobium and Trichiuris Trichiura (Martinez-Torres and Layrisse, 1973).

Furthermore such common conditions as peptic ulceration, hiatus hernia and carcinomata of the gastrointestinal tract may frequently be asymptomatic and persist with blood loss for long periods before treatment is given. Finally, iron deficiency may result from chronic ingestion of salicylates or other anti-inflammatory drugs, the mean daily blood loss rising from 0.5 ml to 2.6 ml daily in patients taking soluble aspirin (Baird et al., 1970).

Nevertheless if iron absorption from the diet were sufficiently flexible, excessive losses would be buffered by increased absorption. In many cases, however, even physiological iron losses cannot be equalled by iron retained from the diet. Clearly inadequate iron assimilation from food is at least partly responsible for much iron deficiency.

1.2.2 Iron Absorption from Food

A substantial amount of information is now available regarding the absorption of iron from food. Unfortunately much of the data is uninterpretable in terms of contemporary concepts. Thus early workers in this field investigated iron absorption by studying the haematological response of iron deficient animals fed on various foodstuffs (Free and Bing, 1940; Miller and Louis, 1945; Pye and MacLeod, 1946; Ruegamer et al., 1946; Subrahmanyan et al., 1950; Sen,1952). Results from these experiments conflict and as man may not handle iron in the same way as other animals, such data have little significance.

Initial human studies using chemical balance techniques (Johnston et al., 1948 ; Johnston et al., 1949; Schlaphoff et al., 1949; McMillan and Johnston, 1951; Hussain and Patwardhan, 1959) can be criticised too in that the difference between oral intake and faecal iron loss is small, complete collection of faeces difficult, and the differentiation between excreted and unabsorbed iron impossible (Josephs, 1958).

The evolution of methods for biologically labelling foodstuffs provided a more accurate device for measuring iron absorption. In many early studies, however, labelled foodstuffs were either given together with a "standard meal" or beverages such as tea or coffee were allowed as desired (Moore and

Dubach, 1951; Walsh et al., 1955; Chodos et al., 1957; Schulz and Smith, 1958; Halkett et al., 1958; Callender and Warner, 1968; Callender et al., 1970; Callender and Warner, 1970; Ashworth et al., 1973). Recent work has suggested that all dietary iron enters one of two physiologically "common pools" in the gastrointestinal tract, (Bjorn-Rasmussen et al., 1972; Cook et al., 1972) one pool containing all non-haem iron and the other all haem iron. Absorption depends on the interaction between inhibitors and stimulators of iron absorption within each pool. Absorption from the non-haem pool is in addition affected by the contents of the haem pool, being higher if the haem pool also contains iron. While the measurement of iron absorption from a standard meal is not without significance, as will be discussed later, the data are rather difficult to interpret in terms of a single foodstuff.

1.2.3 Iron Absorption from Single Foodstuffs

Recently, several workers have measured iron absorption from single biologically labelled food-stuffs in fasting subjects (Fig. 1, Tables 1-4). A constant feature of all these studies was the limited availability of iron from cereals and vegetables. Thus in a large number of iron replete subjects, the mean absorption was found to be 0.9 per cent from rice, 1.3 per cent from spinach, 2.6 per cent from black beans, 3.4 per cent from maize, 4.0 per cent from lettuce, 4.2 per cent from white bread and 4.5 per cent from wholewheat bread. Iron deficient subjects fared little better and absorbed 4.7 per cent from maize, 7.1 per cent from black beans and 9.7 per cent from wholewheat bread. In addition, the absorption was low from eggs (5.5 per cent), milk (8.0 per cent) and ferritin (7.5 per cent if iron replete and 12.5 per cent if iron deficient). In iron replete subjects the absorption from liver was surprisingly poor too, being 7.0 per cent from chicken liver and 13.0 per cent from rabbit liver. This comparatively low availability presumably reflects its high ferritin content. In iron deficient subjects, however, the absorption from rabbit liver rose to 20 per cent. If meat is eaten, absorption is much higher. Thus iron replete subjects absorbed

TABLE 1

The absorption of iron from single cereals

Reference	Form of iron	Amount of iron (µmol)	Number of subjects	Iron status	% Absorption (mean)	Ratio: food iron absorption/ferrous ascorbate absorption
Layrisse and Martinez Torres, 1971	Rice	35.8	11	Normal	0.9	0.02
Layrisse et al., 1968	Maize	53.7-71.6	8	Normal	2.1	0.14
		53.7-71.6	8	Deficient	7.9	0.19
Layrisse et al., 1969		71.6	21	Normal	5.9	0.14
Layrisse and Martinez Torres, 1971		35.8-71.6	73	Normal	3.2-4.2	0.13
Layrisse et al., 1973		44.7	12	Normal	3.9	0.08
		125.3	14	Normal	1.7	0.05
		214.8	13	Normal	1.2	0.02
		393.9	12	Normal	0.5	0.02
		1109.8	7	Normal	0.4	0.01
Sayers et al., 1973		32.4	7	Deficient	3.8	0.07
Sayers et al., 1974a		71.6	5	Deficient	1.2	0.02
Hussain et al., 1965	Wheat (100% extraction)	66.2	21	Normal	4.5	0.62
		43.0	21	Deficient	7.8	0.17
Mameesh et al., 1970		53.7	9	Deficient	15.0	0.15
Sayers et al., 1973		66.2	5	Deficient	7.9	0.10
Steinkamp et al.,1955	Wheat (70-80% extraction)	53.7	9	Normal	4.2	0.33

TABLE 2

The absorption of iron from single vegetables

Reference	Form of iron	Amount of iron (µmol)	Number of subjects	Iron status	% Absorption (mean)	Ratio: food iron absorption/ferrous ascorbate absorption
Layrisse et al., 1969	Spinach	35.8	9	Normal	1.3	0.11
Layrisse et al., 1969	Black Beans	53.7-71.6	15	Normal	3.2	0.12
Layrisse et al., 1968		53.7-71.6	12	Normal	2.3	0.16
		53.7-71.6	3	Deficient	7.1	0.17
Martinez Torres and Layrisse, 1970		53.7-71.6	107	Normal	2.6	0.12
Mameesh et al., 1970	Broad Beans	50.0	9	Deficient	27.0	0.46
Layrisse et al., 1969	Soya Beans	71.6	17	Normal	17.9	0.52
Layrisse and Martinez Torres, 1971		53.7-71.6	37	Normal	6.9	0.23
Sayers et al., 1973		71.6-89.5	5	Deficient	19.8	0.27
Mameesh et al., 1970	Chickpeas	53.7	10	Deficient	36.0	0.57
Layrisse et al., 1969	Lettuce	21.7	13	Normal	4.0	0.16
Mameesh et al., 1970	Okra	33.9	9	Deficient	43.0	0.66

TABLE 3

The absorption of iron from haemoglobin

Reference	Source of haemoglobin	Amount of iron (µmol)	Number of subjects	Iron status	% Absorption (mean)
Callender et al., 1957	Rabbit (cooked)	89.5	18	Mixed	7.1
Baird and Wilson, 1959	Rabbit (raw)	89.5	21	Mixed	11.3
		35.8	40	Normal	22.0
		35.8	12	Deficient	35.0
Turnbull et al., 1962		71.6-107.4	13	Normal	8.8
		71.6-107.4	3	Deficient	11.2
		44.7- 53.7	6	Normal	12.7
		44.7- 53.7	3	Deficient	17.6
		89.5	7	Deficient	13.8
Callender, 1964		89.5	17	Normal	13.0
		89.5	27	Deficient	25.0
Bannerman et al., 1964		89.5	8	Normal	22.0
Hwang and Brown, 1963		89.5	6	Normal	20.3
Hussain et al., 1965		89.5-107.4	10	Normal	11.7
		44.7- 53.7	10	Deficient	21.3
Hallberg et al., 1966a		89.5	4	Normal	4.2
		89.5	4	Deficient	18.2
		89.5	3	Blood donors	12.7
Minnich et al., 1968		89.5	6	Normal	10.6
Layrisse et al., 1969		53.7	10	Mixed	14.1
Hallberg and Bjorn-Rasmussen, 1972		53.7	8	Normal	14.4
Layrisse and Martinez Torres, 1971		53.7	29	Mixed	12.0

TABLE 4

The absorption of iron from single organic foodstuffs

Reference	Type of food	Amount of iron (µmol)	Number of subjects	Iron status	% Absorption (mean)
Moore and Dubach, 1951	Chicken muscle	53.7-107.4	2	Normal	1.9-33.1
Heinrich et al., 1969	Rabbit muscle	53.7	10	Normal	12.0
			10	Deficient	23.0
Klavins et al., 1962	Veal muscle	35.8-71.6	10	Mixed	22.8
Kroe et al., 1963		35.8-71.6	10	Mixed	18.8
Martinez-Torres and Layrisse, 1971		35.8-71.6	107	Mixed	21.5
Hallberg and Bjorn-Rasmussen, 1972		35.8-71.6	8	Normal	12.6
Layrisse et al., 1969	Fish	23.6	19	Mixed	18.3
		17.9	4	Normal	4.9- 7.7
Layrisse et al., 1968		17.9	2	Deficient	11.5-20.1
Moore and Dubach, 1951	Chicken liver	53.7-107.4	3	Normal	7.0
		53.7-107.4	5	Deficient	20.0
Heinrich et al., 1969	Rabbit liver	53.7	10	Normal	13.0
		53.7	10	Deficient	20.0
Layrisse and Martinez-Torres, 1971		53.7	11	Mixed	14.5

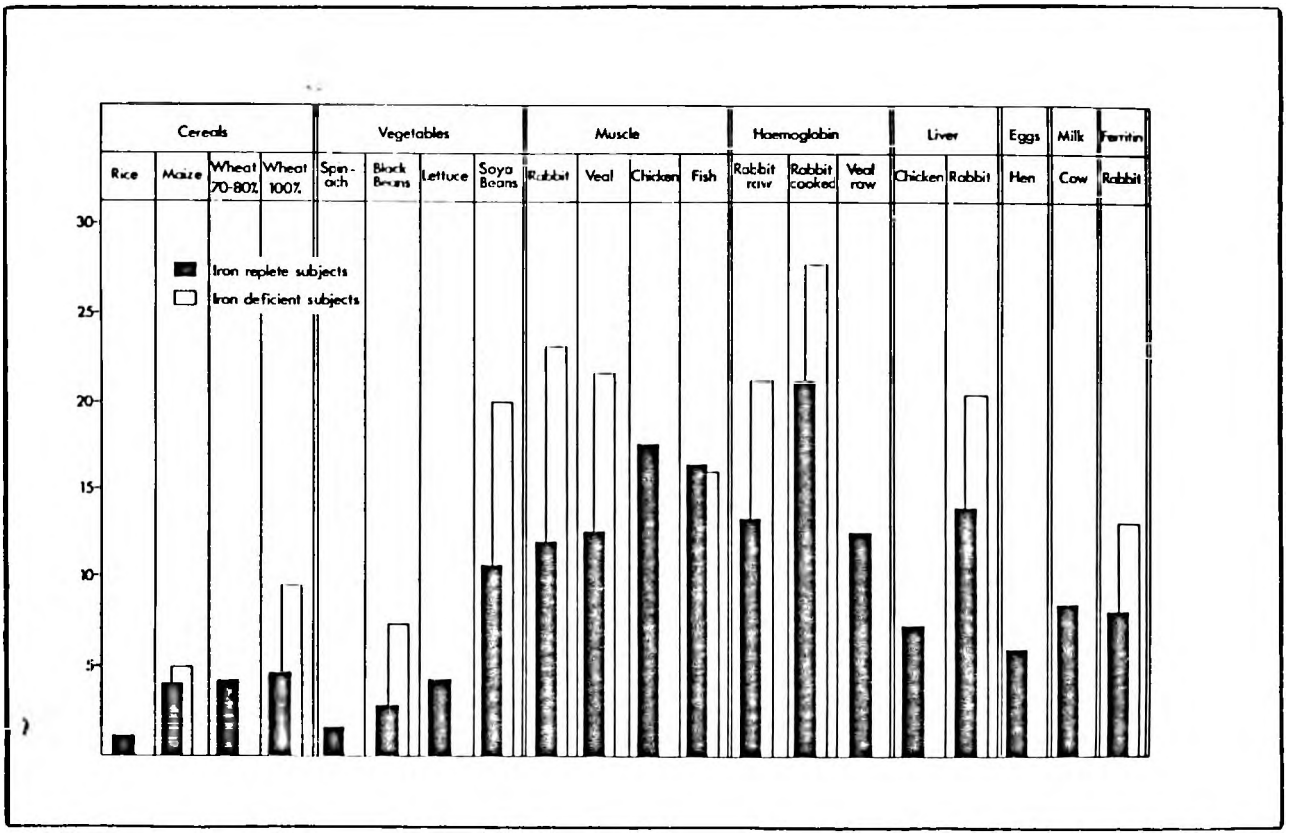

Fig. 1 Iron absorption from single foodstuffs
(Data from tables 1-4)

12.0 per cent of the iron present in rabbit muscle, 12.6 per cent of that in veal and 17.5 per cent from chicken while iron deficient subjects absorbed 21.5 per cent from veal and 23.5 per cent from rabbit. Finally, the mean absorption of iron from radioactive rabbit haemoglobin was found in iron replete subjects to be 13.1 per cent if between 71.6 and 107.4 /umol of iron was eaten, and 21.4 per cent if between 35.8 and 53.7 /umol was eaten. In iron deficient subjects these values rose to 21.5 per cent and 27.5 per cent respectively. Cooking the haemoglobin made no difference to the absorption of the iron. It appears therefore that if meat is included in the diet, iron deficiency is far less likely to develop. However, as many of the world's population, particularly those in tropical areas, derive nearly all their calories from cereals, this is small compensation.

1.2.4 Iron Absorption from the Diet as a Whole

In those people in whom iron deficiency is commonest, i.e. the inhabitants of tropical areas, total daily iron retention may for practical purposes be considered in terms of non-haem iron only. Iron assimilation then depends on the percentage of iron absorbed and the iron content of the diet. When the iron content of the largely cereal diet of South African Indians was measured from tables, a value of between 179.1 /umol and 214.9 /umol was obtained (Mayet et al., 1972). If this were true then between 10 per cent and 40 per cent of the dietary iron would have to be absorbed to satisfy the needs of all members of the community - clearly an unlikely occurrence. When, however, the total iron contant is measured chemically, much higher values have been obtained (Ramalingaswami and Patwardhan, 1949; Apte and Iyengar, 1970; Wretlind, 1970). This disparity is probably accounted for by iron which contaminates the food. This may be in the form of dirt or may be picked up from the cooking pots or utensils (Walker and Arvidsson, 1953). Iron intakes as high as 89,550 /umol daily have thus been recorded from purely cereal diets (Wretlind, 1970). What is not clear, however, is how much of this extraneous iron is available for absorption. Although, as discussed above, a physiologically "common pool" has been demonstrated,

the experiments have concerned the exchange between iron salts and intrinsic food iron. Whether this principle would apply equally to the contaminating iron compounds remains to be demonstrated. It is therefore possible that the lower values obtained using washed, uncontaminated food may more accurately reflect the available iron.

More data are available regarding meals containing meat. Early workers used an extrinsic tracer of ferric chloride which was added to a standard meal (Sharpe et al., 1950; Pirzio-Biroli et al., 1958; Goldberg et al., 1963; Turnbull, 1965). This method depends on the assumption that the extrinsic tag will label all the food and this has been shown to be untrue at least in the case of haem iron (Callender et al., 1957; Hallberg and Solvell, 1967). Nevertheless it must be admitted that the values found by these workers for total daily iron retention differ little from the results of contemporary workers using more modern methods. Recently, workers have used the technique of labelling the non-haem pool with an intrinsically labelled foodstuff or an extrinsic tracer in the form of an inorganic salt, and the haem pool with radioactive haemoglobin (Layrisse and Martinez-Torres, 1972; Hallberg and Bjorn-Rasmussen, 1972; Bjorn-Rasmussen et al., 1972; Bjorn-Rasmussen, 1973a; Bjorn-Rasmussen, 1973b; Bjorn-Rasmussen and Hallberg, 1974; Bjorn-Rasmussen, 1974). Thus when a typical South American meal of black beans, maize rice and meat was eaten, the mean absorption of haem iron was found to be 22.85 per cent and that of non-haem iron 4.2 per cent. Finally the absorption of both non-haem and haem iron was measured from breakfast, lunch and dinner in thirty two subjects (Bjorn-Rasmussen et al., 1974) - a whole day's diet. The mean absorption of non-haem iron was 5.3 per cent or 15.7 /umol and that of haem iron 37.3 per cent or 6.7 /umol - a total of 22.4 /umol.

1.2.5 Factors Affecting Iron Absorption

Several factors are known to influence iron absorption. While the amount of iron in storage depots, the rate of erythropoiesis and the iron content of the mucosal cell all appear

to affect iron absorption in general, the variation in the availability of iron from different foodstuffs in any single person, can only be explained on the basis of specific intraluminal factors. Thus the availability of iron from food depends largely on the physiochemical form in which it is presented to the intestinal absorptive surface. Support for this is that ferrous iron is far better absorbed than ferric iron (Moore et al., 1944; Hahn et al., 1945; Brise and Hallberg, 1962). There is good evidence to suggest that this relates, at least partly, to solubility, as ferric iron is insoluble in aqueous solutions at pH greater than 5.0 while some ferrous iron is soluble at pH 8.0. Constituents of the diet or gastrointestinal secretions which bind iron and maintain it in a soluble monomeric form at the pH of the small intestine will thus enhance absorption (Conrad, 1970). These compounds interfere with the formation of water bridges between iron molecules and so decrease polymerisation. Not all chelators stimulate iron absorption, however. Others decrease iron absorption by sequestering all six co-ordinating bonds of iron to form an insoluble complex, or have lattice building properties and so produce high molecular weight soluble polymers (Conrad, 1970). There is some evidence to suggest that polymerisation has a greater effect than precipitation on iron absorption (Benjamin et al., 1967).

Absorption then depends finally on the relative availability and avidity of competing ligands. Thus ascorbic acid is a more effective chelator for inorganic iron than are fructose or histidine because it binds iron in equimolar concentrations, while sugars and amino acids require an approximately 100-fold greater molar concentration to form a ligand (Stitt et al., 1962).

Haemoglobin is broken down to haem and globin by proteolytic duodenal enzymes and the intact metalloporphyrin is absorbed by the intestinal mucosa. The iron of the haem molecule has only two co-ordinating bonds available and is thus subject to different reactions from those of inorganic iron. Consequently absorption is largely independent of ionic iron chelators (Callender et al., 1957; Turnbull et al., 1962; Conrad et al., 1967). In solution, haem molecules have lattice building properties and polymerise to form poorly absorbed

macromolecular complexes (Conrad, 1974). The presence of small amino acids, amines or amides, increases absorption by decreasing intra-luminal polymerisation (Conrad et al., 1967).

(a) Specific factors in food that inhibit iron absorption

Poor absorption of food iron has been attributed to the presence of oxalates, phosphates and phytates, (Hegsted, et al., 1949; Sharpe et al., 1950; Apte and Venkatachalam, 1965; Peters et al., 1971; Forth and Rummel, 1973). Thus the inhibition of iron absorption by eggs seems to relate to the high content of phosphoprotein in the yolk (Halkett, et al., 1958). Calcium in low doses appears to increase iron absorption, perhaps by binding phosphates, while large amounts depress absorption (Chapman and Campbell, 1957; Dunn, 1968). In marked contrast to their effect on inorganic iron, phytate and egg yolk do not appear to affect the absorption of iron in haemoglobin (Turnbull et al., 1962; Callender et al., 1970). Absorption of iron in muscle however is reduced by the simultaneous administration of inorganic iron in the form of corn or beans (Martinez-Torres and Layrisse, 1971).

(b) Potentiators of iron absorption present in food

(i) Animal protein increases the absorption of iron salts, (Klavins et al., 1962; Brodan et al, 1968) and of the iron in black beans (Layrisse et al., 1968). Although a mixture of the constituent amino acids of fish enhances absorption in man, only cysteine can be shown to be effective when given alone (Martinez-Torres and Layrisse, 1970). In rats however, histidine and cysteine increase the absorption of inorganic iron (van Campen, 1972; van Campen, 1973).

(ii) Ascorbic acid: This is the best studied potentiator of iron absorption. Original work with orange juice showed an enhanced absorption of iron in bread (Elwood et al., 1968; Callender and Warner, 1968) and in eggs, (Callender et al., 1970) and of inorganic iron

given with a standard meal (Turnbull, 1965). Ascorbic acid alone increases the absorption of both ferric and ferrous iron (Moore et al., 1939; Hopping and Ruliffson, 1963; Hoglund and Reisenstein, 1969) and non-haem iron in food (Steinkamp et al., 1955; Apte and Venkatachalam, 1965; Sayers et al., 1973; Kuhn et al., 1968; Bjorn-Rasmussen and Hallberg, 1974). In addition it increases the absorption of iron in ferritin (Kuhn et al., 1968; Heinrich et al., 1971) but does not affect absorption from haemoglobin, (Callender et al., 1957; Turnbull et al., 1962) veal or pork (Martinez-Torres and Layrisse, 1971).

(iii) Sugars: Both fructose (Charley et al., 1963; Brodan et al., 1967) and sorbitol (Herndon et al., 1958; Loria et al., 1962) have been shown to enhance absorption.

(iv) Alcohol increases the absorption of ferric iron in subjects with normal gastric acid secretion. It has no effect in subjects with achlorhydria, suggesting that in man it stimulates gastric secretion of hydrochloric acid and so promotes solubility of ferric iron (Charlton et al., 1964). This is supported by its having no effect on absorption of iron by isolated intestinal loops in rats (Tapper et al., 1968; Bush et al., 1968). Furthermore, in rats neither iron added to wine nor iron in wine made from biologically labelled grapes is better absorbed than ferrous sulphate (MacDonald and Pechet, 1964).

(c) Effect of intestinal secretions on iron absorption

(i) Hydrochloric acid: In patients with achlorhydria, absorption of ferric iron is impaired (Goldberg et al., 1963; Cook et al., 1964) and administration of weak hydrochloric acid with the iron increases its absorption (Jacobs et al., 1964). This may account for the poor absorption of ferric iron after gastrectomy (Walz et al., 1970), but the normal absorption of ferrous

iron (Smith and Mallett, 1957).
Haemoglobin iron absorption is unaffected by achlorhydria (Jacobs et al., 1964) and after gastrectomy it is normal (Baird and Wilson,1959).

(ii) Chelators in gastric juice: In the presence of gastric juice, ferric chloride remains soluble at pH 8.0, suggesting the formation of a soluble chelate. Although this binding has been said to be entirely non-specific (Swan and Glass, 1972) an iron binding glycoprotein has been isolated from gastric juice (Multani et al., 1970). This contains 180-190 iron atoms per molecule and has a molecular weight of 260,000 (Webb et al., 1973). Whether this complex has a physiological role in controlling iron absorption remains to be shown, however, and the claim that the iron binding capacity of gastric juice is lower than normal in patients with iron deficiency and idiopathic haemochromatosis (Davis et al., 1967; Luke et al., 1967; Davis et al., 1968) has found little support (Jacobs and Miles, 1968; Wynter and Williams, 1968; Powell et al., 1970). To compound the debate, gastric juice of iron deficient children has actually been shown by other workers to have a raised iron binding capacity (Russo et al., 1969).

(iii) Bile: The role of bile in iron absorption remains in doubt. Thus ligation of the common bile duct in rats has led to increased absorption from ferrous salts but decrease from ferric salts, (Webling and Holdsworth, 1966) decreased absorption of ferric iron in normal rats and a decrease in both ferric and ferrous iron in iron deficient animals (Conrad and Schade, 1968). In addition bile fed to dogs stimulates iron absorption from ferrous salts (Wheby et al., 1962). Although it was suggested that ascorbic acid is the active constituent of bile, analysis of complexes formed from inorganic iron and bile revealed differences from iron-ascorbate (Jacobs and Miles, 1970). These complexes are nevertheless soluble at high pH and would

keep iron in solution in the duodenum.

(iv) Pancreatic juice: Some patients with pancreatic insufficiency absorb iron excessively (Davis, 1961; Davis and Badenoch, 1962; Ball, 1964; Deller, 1965; Kavin et al., 1967) and this may be corrected with commercial pancreatic extract (Davis and Badenoch, 1962; Saunders et al., 1963; Davis and Biggs, 1964; Smith, 1964; Tonz et al., 1965; Deller, 1965). In normal rats the effect of pancreozymin and secretin has been variable with absorption being decreased (Davis and Biggs, 1964; Kahn, 1966; Kavin et al., 1967; Benjamin et al., 1967) or unchanged (Murray and Stein, 1967). This may be due to loose adsorption of iron to the extract rather than specific binding (Kavin et al., 1967) or the result of increased bicarbonate concentration in the duodenal lumen. There is some evidence in rats, however, for the presence of a heat labile factor in pancreatic juice which decreases iron absorption (Brozovic et al., 1966; Popovic et al., 1967).

1.3 APPROACH TO THE PREVENTION OF IRON DEFICIENCY

Limitation of any of the causes of iron deficiency should theoretically lead to an improvement in nutrition. Major practical problems stand in the way of many of the possible approaches however. Thus most of the environmental factors involved are so intimately part of the socio-economic milieu of the community, that there is little hope of any intervention programmes being rapidly effective. For example, elimination of hookworm infection would almost certainly effect an improvement in iron nutrition, but administration of anthelminthics is futile in the face of continuous reinfection. Total eradication of the parasite could only occur if there was a major modification of cultural behaviours so that the life cycle of the parasite could be interrupted (Beaton, 1974). In fact, apart from treatment of individual pathological conditions, there seems little reason to suspect that limitation of losses would be possible. It should

be mentioned, however, that oral contraceptives have been shown to decrease menstrual blood loss (Beaton et al., 1970; Cole et al., 1971). If their use continues to increase this may become an important epidemiological factor in the future.

The problem is therefore best approached from its other aspect, i.e. through an increase in the effective iron intake. One way of doing this would be by giving iron tablets to iron deficient people. Major problems involved in any such project however, would be the immense costs of the preliminary screening programme, the difficulty in deciding which people need treatment and the task of ensuring that medication is actually taken. Even in highly motivated people, the taking of tablets falls off as symptoms subside (Beaton, 1974). Attention has thus been turned to possible means of preventing the condition from occurring. One method that would probably be effective would be to increase the amount of meat in the diet and decrease the cereal consumption. On a world-wide basis, however, with the growing shortage and cost of red meat, this is clearly impractical. In the light of present knowledge, therefore, the cheapest, most effective way of improving iron nutrition appears to be the fortification of staple foodstuffs with iron. This is already standard practice in several countries. In Britain for example, the iron lost during the milling of wheat is replaced by raising the iron content of white flour to 29.5 μmol/100g (Ministry of Agriculture, Fisheries and Food, 1963). The American Government accepts the principle of food supplementation too, and while at present the iron content of enriched flour is fixed at 53.7 μmol/100g, recent suggestions that this be increased to 234.0 μmol/100g have been seriously considered (Federal Register, 1970). Perhaps the most serious objection to such programmes as these is that apart from infant foods which are restricted to a specific group, enriched foods will be eaten by all members of the community whether or not they are iron deficient. It has been argued that in the United States of America, fewer than 1 per cent of the population would benefit from such a programme (Crosby, 1974) and there is a real risk of iron

overload developing in some individuals. Whereas in America such an argument may have some grounds, it is almost certainly untenable in countries such as India, where iron deficiency occurs so much more commonly. It must be admitted that the addition of iron to food may be detrimental to sufferers from idiopathic haemochromatosis and their immediate families, but this condition occurs in only 0.005 per cent of all hospital admissions (Finch and Finch, 1955). Even if this figure were increased several fold, the prevalence of iron deficiency in tropical areas would be far greater. The antagonists cite, too, the condition of Bantu siderosis, as an example of iron overload occurring in healthy people directly as a result of excessive iron intake. In rebuttal it should be pointed out that the Bantu regularly consume between 1791 /umol and 3582 /umol of iron daily and develop the condition after 40-60 years. There is in addition recent evidence to suggest that the iron in Bantu Beer is far more available for absorption than the iron in its constituent cereals (Disler et al., 1974). Thus in Ethiopia, where as much as 8955 /umol iron is consumed from unfermented cereals daily (Wretlind, 1970) iron deficiency is the nutritional problem rather than iron overload. It therefore seems likely that the potential benefits of such a programme far outweigh the potential risks, at least in tropical countries.

The final question that must be asked is how successful such a programme will be if initiated. The principle of food fortification is the administration of small amounts of supplemental iron to the community to prevent the development of iron deficiency in those individuals at risk. That such an approach might be successful was suggested in one study in which female subjects were given either 179.1 /umol or 537.3 /umol iron in tablet form, or placebo after convential treatment for iron deficiency (Elwood, 1968). After six months the haemoglobin levels were significantly lower in the placebo group when compared with the treated subjects. Nevertheless with a single exception, no long term feeding study has shown the fortification of food to be beneficial

(Mackay et al., 1945; Widdowson and McCance, 1954; Elwood, 1968). Even the successful study can be criticised in that there was no control group and absorption of iron from the enriched bread would have had to be in excess of 30 per cent to account for the rise in haemoglobin. There is good evidence to suggest that these disappointing results relate to the poor availability of iron in the cereals (Callender and Warner, 1968; Callender and Warner, 1970; Elwood et al., 1968; Elwood et al., 1970; Cook et al., 1973) as the estimated daily iron absorption from 200 g iron enriched British bread is approximately 10.7 µmol, a negligible benefit.

1.3.1. Enhancement of Iron Absorption with Ascorbic Acid

It is clear, therefore, that if food is to be fortified with iron, then a potentiator of iron absorption must accompany the iron supplements. Of the compounds known to enhance iron absorption, ascorbic acid has the advantage of being readily available, non-toxic and active in extremely small quantities. Furthermore, when ascorbic acid was added before cooking to a meal of rice, the absorption of both added and intrinsic iron was found to be substantially increased (Sayers et al., 1973). This knowledge led to an attempt to fortify salt with both iron and ascorbic acid (Sayers et al., 1974a; Sayers et al., 1974b).

Two difficulties were encountered in these experiments however; firstly the coarse, cheap salt used by low socio-economic groups in many countries discolours rapidly on storage if enriched in this way; secondly, no enhancement of absorption occurs when the fortified salt is used in baking, an effect attributed to denaturation of ascorbic acid at high temperature.

1.4 AIMS OF THIS THESIS

It was the intention of this thesis to study sugar as an alternative vehicle for supplementary iron and

ascorbic acid. Sugar is consumed by all strata of society in amounts large enough to make its fortification feasible. In addition it would have the theoretical advantage of being added to food late in its preparation, rendering the ascorbic acid less vulnerable to extremes of temperature. Nevertheless, fortification of sugar would be practicable only if the appearance and taste of the sugar were acceptable to the consumer, not only immediately after mixing, but also after storage. Once this had been established, it was necessary to demonstrate enhanced absorption from cereal meals eaten with sugar enriched in this way.

Problems encountered during these experiments led to a separate investigation being carried out into the effect of tea and coffee on iron absorption. Finally the nature of the reaction between tea and iron was studied in detail.

CHAPTER 2

GENERAL MATERIALS AND METHODS

One hundred and ninety three volunteers took part in the human studies during the present investigation. All were multiparous housewives of Indian extraction, living in municipal houses at Chatsworth near Durban. Their mean age was 40 years (range 26-60).

2.1 ETHICAL CONSIDERATIONS

Before making this study, approval was obtained from the Committee for Research on Human Subjects of the Faculty of Medicine, University of the Witwatersrand. Written consent was obtained from all subjects after the nature of the investigation had been explained to them. Insofar as the radiation dosage was concerned, it was 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 per cent of the permissible whole-body burden for continuous exposure in the instance of ^{59}Fe and 0.2 per cent in the instance of ^{55}Fe (International Commission for Radiation Protection, 1960).

2.2 SOCIOLOGICAL AND ECONOMIC FEATURES OF THE POPULATION STUDIED

According to official estimates (Durban City Council City Engineer's Report, 1969), 157,799 people are resident in Chatsworth. The houses in which they live are semi-detached units with either three or four living rooms. Although the official census records the mean occupancy rate as 7.5 people per house, this is almost certainly an underestimate as houses are difficult to obtain and so the number of occupants increases unofficially and illegally. In addition, they adhere rigidly to the extended family system, the son bringing his wife to live in his mother's house. Overcrowding is therefore inevitable.

Earning is clearly the province of the male and only 9.4 per cent of the females are employed. The mean monthly salary earned is R60.60 (R20.00 - R150.00).

Little employment is available in the area and so an additional financial strain is placed on the people by expensive transport costs.

Houses are leased from the municipality at an average monthly rental of R18.50 (R5.00 - R30.00). Although this rental is low by general South African standards in many cases it consumes a significant part of the income. In addition, money is often spent on luxuries such as radiograms and cars which are bought on long term hire purchase schemes at high rates of interest. Possession of these is a status symbol in the community.

The community thus suffers from the problems of any low socio-economic group. Little meat is eaten and the diet consists mainly of cereals (Khan, 1974). Iron deficiency is a common finding (Mayet et al., 1972). In this study the prevalence of iron deficiency was estimated by measuring the serum ferritin concentration of each individual. Despite their random selection, 68 per cent of the women proved to have a serum ferritin concentration of less than 10 ng/ml.

2.3 TECHNIQUE OF THE STUDIES

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. Use was made of an "extrinsic tag" to label the iron compound or food iron with 2.5 μc ^{55}Fe on the one morning and with 2.5 μc ^{59}Fe on the other (Cook et al., 1972; Bjorn-Rasmussen et al., 1972). The iron absorption on each day could then be measured by differentially counting the two isotopes in blood samples taken 2 weeks later. In several experiments a slightly different approach was adopted in that use was made of food intrinsically labelled with ^{55}Fe (Hussain et al., 1965), and the absorption compared with that of an extrinsic label of ^{59}Fe. Each subject then ate a single meal only.

After all meals nothing further 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}Fe and ^{59}Fe content, haemoglobin concentration, serum iron concentration, unsaturated iron binding capacity and when the assay became available, serum ferritin concentration. Thereafter the subjects drank 50 ml tap water containing 170.4 /umol ascorbic acid and 53.7 /umol iron as $FeSO_4.7H_2O$ labelled with 2.5 /uc ^{59}Fe. No further food or drink was allowed for four hours. Measurement of the ^{59}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.

2.4 RADIOISOTOPIC, CHEMICAL AND STATISTICAL METHODS

Blood samples (10 ml) and aliquots of standard iron solutions and foods were prepared for differential radioactive counting by the method of Katz et al., (1964). The quantities of ^{55}Fe and ^{59}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 per cent for ^{55}Fe and 42 per cent for ^{59}Fe at optimal gain and window settings. The ^{59}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 per cent 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. The significance of differences between the absorptions of the two isotopes was assessed by means of the Student's 't' test for paired observations.

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 et al., (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 radio-immunoassay using the method of Miles et al., (1974). The International System of Units (Systeme Internationale or S.I.) has been used throughout this thesis (Baron et al., 1974; Young, 1975).

CHAPTER 3

THE PREPARATION OF SUGAR FORTIFIED WITH IRON AND ASCORBIC ACID

3.1 INTRODUCTION

Fortification of a staple foodstuff with iron and ascorbic acid is only likely to be effective in the prevention of iron deficiency if the product is acceptable to the consumer. It is thus important that the additives should cause no alteration in either the appearance or the taste of the foodstuff, not only immediately after mixing, but also on storage even at high temperatures and humidity.

Secondly, the distribution of iron and ascorbate within the vehicle must be uniform and the ratio of iron to ascorbate must remain constant. As the mere mixing of particles of different sizes results in sifting out and layering of the components the first task was to devise a method to suspend the iron and ascorbic acid evenly through the sugar.

3.2 MATERIALS AND METHODS

Iron was added to commercial white sugar (Huletts Sugar Refining Co.) at two levels of fortification, either 1791 or 3582 /umol/kg. in the form of a number of white or lightly coloured iron compounds. These included ferrous sulphate, ferrous ammonium sulphate, ferric ammonium sulphate, (all from British Drug House Chemicals Ltd., Poole, Dorset, United Kingdom), ferric sulphate, ferric nitrate, ferric orthophosphate (all from Riedel-de Haen, Seelze-Hanover, West Germany), ferric glycerophosphate (from Merck, Darmstadt, West Germany), ferric fructose (prepared by the method of Charley et al., 1963) and ferric sodium edetate (prepared by the method of Sawyer & McKinnie, 1960). In some experiments ascorbic acid (L (+)), analytical grade (British Drug Houses Chemicals Ltd., Poole, Dorset, United Kingdom), was also added to the sugar to produce a concentration of either 5680 or 11,360 /umol/kg. Supplementation was carried out in two different ways: either the iron, and where applicable

the ascorbic acid, was dissolved in distilled water and then sprayed onto the sugar, or the sugar was dampened with water (0.1 per cent by weight) before mixing with each of the finely ground dry supplements in turn. In each case the sugar was dried thoroughly after supplementation using warm air.

3.3 ESTIMATION OF SHELF LIFE

Samples of fortified sugar were kept for up to two years in a laboratory in which the temperature was 22-27°C and the mean relative humidity was 55 per cent. Other samples were kept in the Huletts Sugar Refinery in Durban, which lies in a subtropical zone where the temperature ranged between 6 and 35°C and the mean relative humidity was 77 per cent. The standard quality-control method employed by the Huletts Sugar Refining Co. Ltd. was used to assess consumer acceptability. The extinction at 420 nm of a 500 g/l solution in distilled water, measured in a Beckman spectrophotometer, was multiplied by 163.60163 and the sample was judged to be acceptable if the product was less than 50. The ascorbic acid content after storage was measured using the method of Roe (1954). As sucrose may lead to spuriously high readings with this method, a sucrose blank was used in all estimations.

3.4 RESULTS

Fortification by spraying a solution of any of the soluble iron compounds with or without ascorbic acid onto dry sugar was unsuccessful, as a purple colour appeared within minutes. Over a few days the colour changed to brown, and this was accompanied by oxidation of the ascorbic acid so that only 30 per cent of the initial reduced ascorbic acid content remained after 48 hours (Fig. 2-9). The alternative method proved much more successful. No colour change or loss of reduced ascorbic acid content occurred during storage for two years, even under subtropical conditions, if

the dry powdered supplements were mixed with dampened sugar crystals. Although the appearance of sugar fortified in this way with any of the iron compounds and ascorbic acid was subtly different when directly compared with unsupplemented sugar, it was nevertheless considered to be acceptable to consumers, since it passed the standard quality control test of the sugar-refining company.

3.5 DISCUSSION

These experiments established the possibility of fortifying sugar with several iron compounds and ascorbic acid without affecting its appearance. As most adults eat between 50 g and 100 g sugar daily (Walker, 1973) between 89.5 and 179.1 /umol iron would be added to the diet at the lower level of fortification and between 179.1 and 358.2 /umol at the higher level. Nevertheless the added iron is only of value if available for absorption, and thus the second stage of the experiment was commenced i.e. the measurement of absorption of iron from foods eaten with this form of fortified sugar.

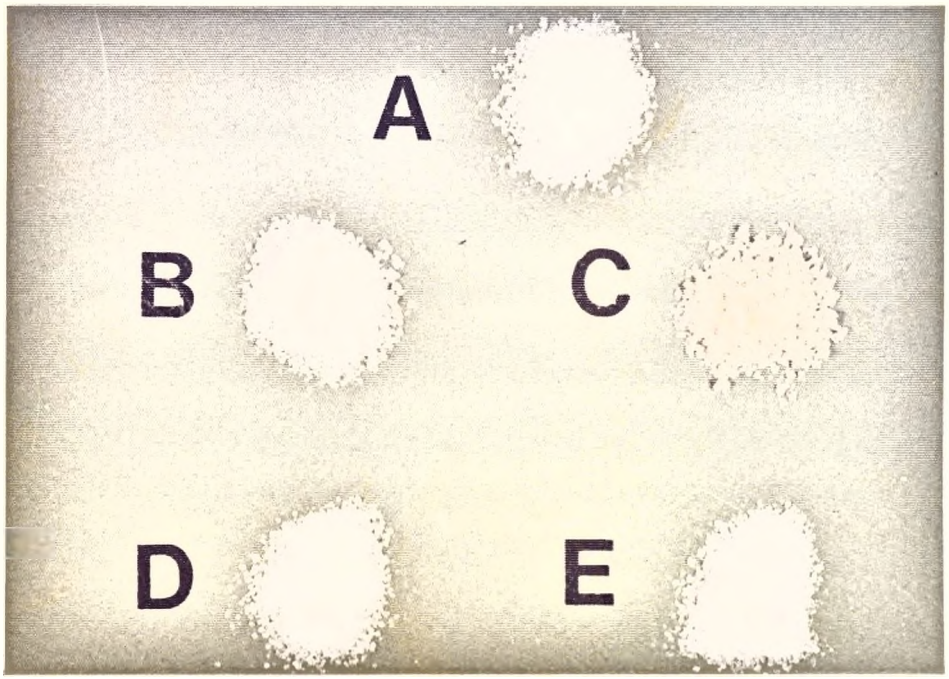

Fig. 2 A. Unfortified sugar
B. Sugar + solution of ferrous sulphate
C. Sugar + solution of ferrous sulphate and ascorbic acid
D. Sugar (dampened) + dry ferrous sulphate
E. Sugar (dampened) + dry ferrous sulphate and ascorbic acid

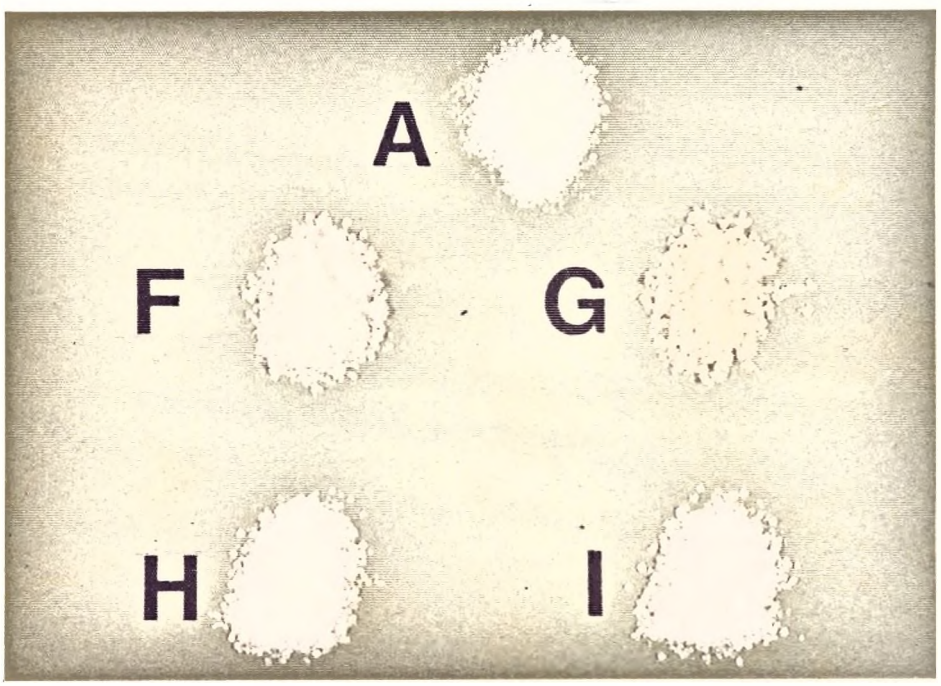

Fig. 3 A. Unfortified sugar
F. Sugar + solution of ferrous ammonium sulphate
G. Sugar + solution of ferrous ammonium sulphate and ascorbic acid
H. Sugar (dampened) + dry ferrous ammonium sulphate
I. Sugar (dampened) + dry ferrous ammonium sulphate and ascorbic acid



Fig. 4 A. Unfortified sugar
J. Sugar + solution of ferric ammonium sulphate
K. Sugar + solution of ferric ammonium sulphate and ascorbic acid
L. Sugar (dampened) + dry ferric ammonium sulphate
M. Sugar (dampened) + dry ferric ammonium sulphate and ascorbic acid

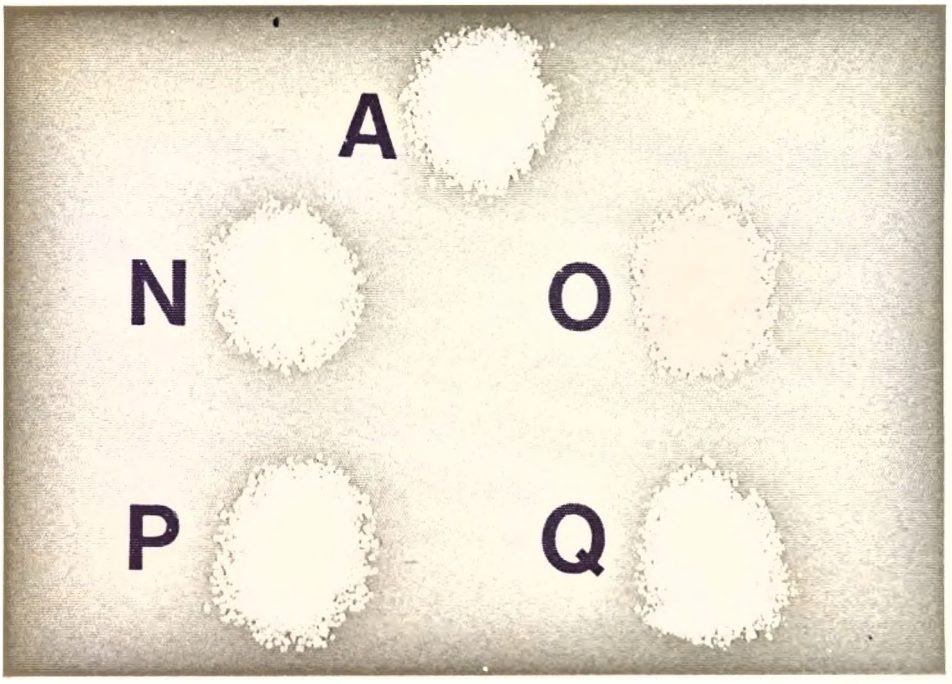

Fig. 5 A. Unfortified sugar
N. Sugar + solution of ferric orthophosphate
O. Sugar + solution of ferric orthophosphate and ascorbic acid
P. Sugar (dampened) + dry ferric orthophosphate
Q. Sugar (dampened) + dry ferric orthophosphate and ascorbic acid
(Ferric orthophosphate obtained from British Drug Houses)

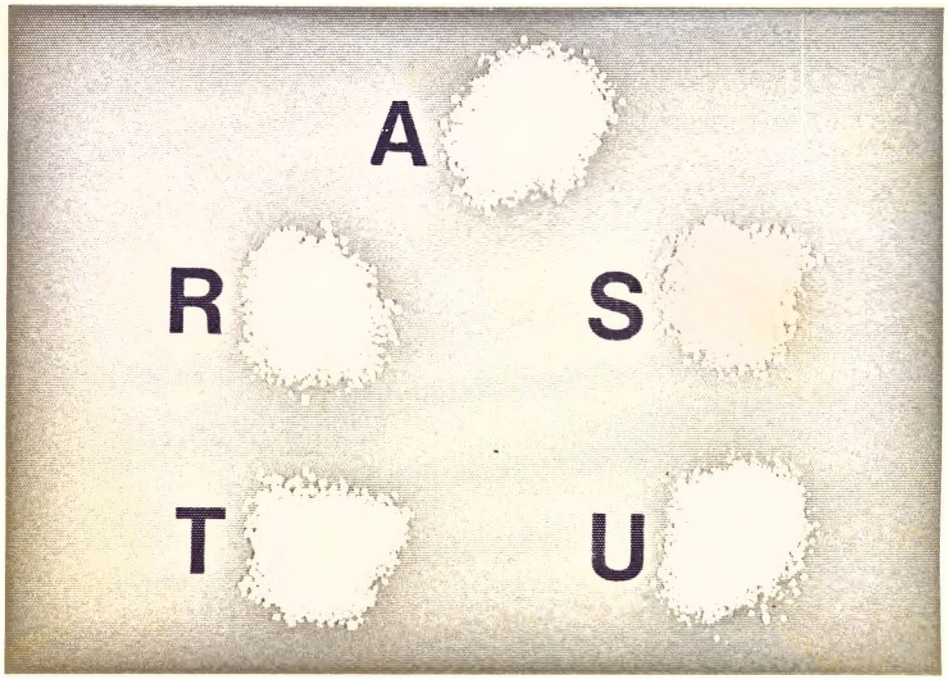

Fig. 6 A. Unfortified sugar
R. Sugar + solution of ferric orthophosphate
S. Sugar + solution of ferric orthophosphate and ascorbic acid
T. Sugar (dampened) + dry ferric orthophosphate
U. Sugar (dampened) + dry ferric orthophosphate and ascorbic acid
(Ferric orthophosphate obtained from Riedel-de-Haen)

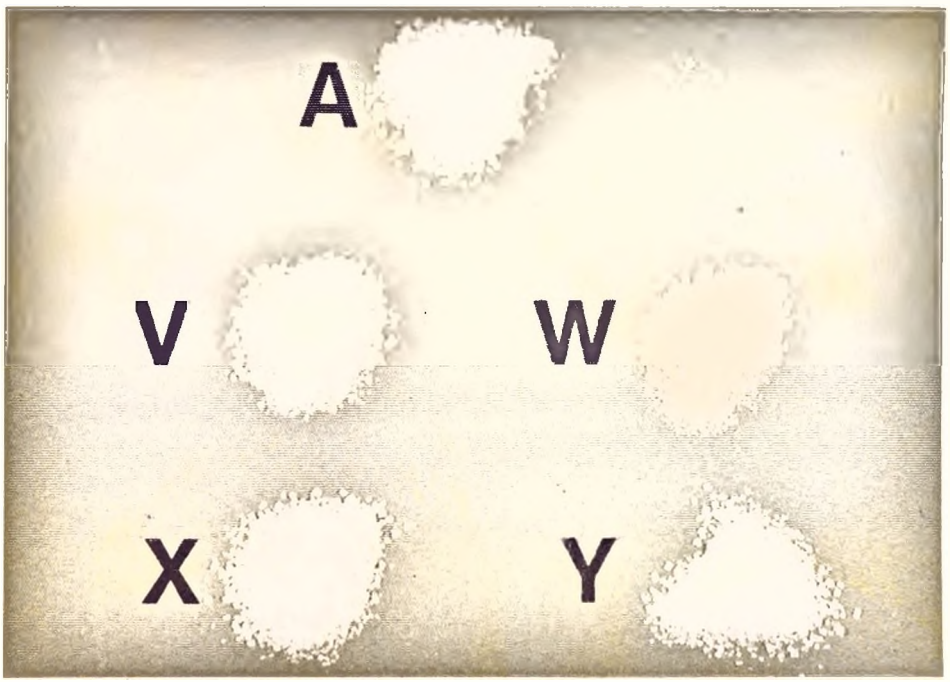

Fig. 7 A. Unfortified sugar
V. Sugar + solution of ferric nitrate
W. Sugar + solution of ferric nitrate and ascorbic acid
X. Sugar (dampened) + dry ferric nitrate
Y. Sugar (dampened) + dry ferric nitrate and ascorbic acid

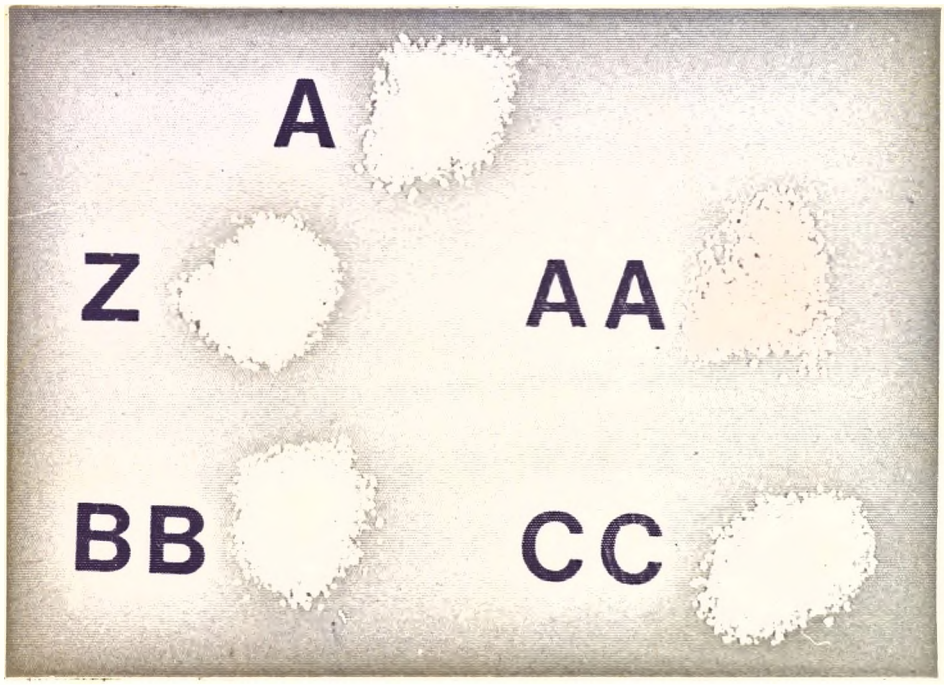

Fig. 8 A. Unfortified sugar
Z. Sugar + solution of ferric sulphate
AA. Sugar + solution of ferric sulphate and ascorbic acid
BB. Sugar (dampened) + dry ferric sulphate
CC. Sugar (dampened) + dry ferric sulphate and ascorbic acid

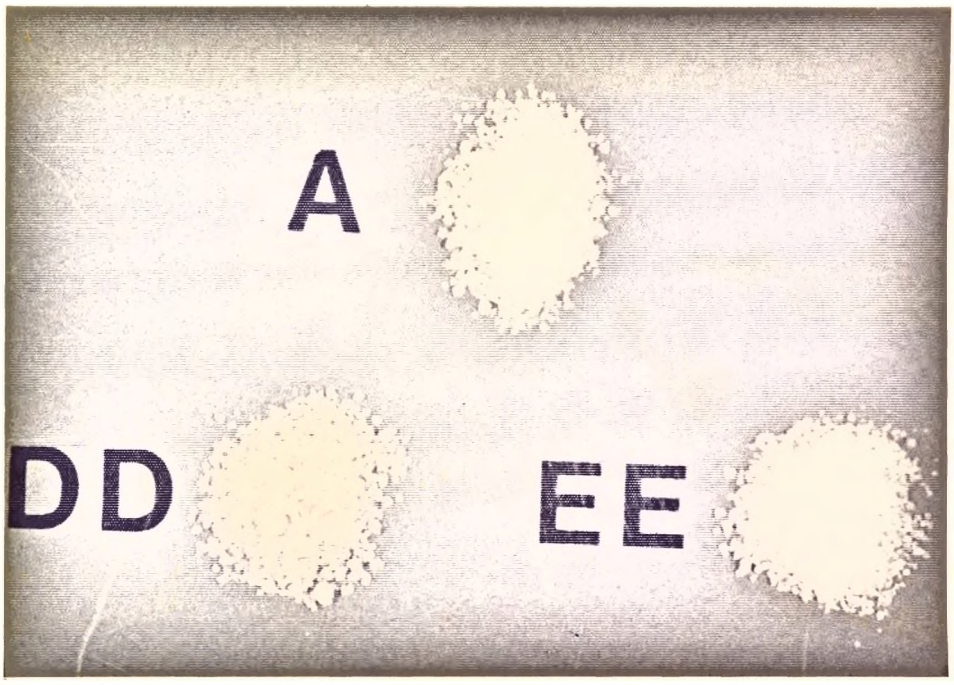

Fig. 9 A. Unfortified sugar
DD. Sugar + solution of ascorbic acid
EE. Sugar (dampened) + dry ascorbic acid

CHAPTER 4

IRON ABSORPTION FROM SUGAR FORTIFIED WITH FERROUS SULPHATE AND ASCORBIC ACID

4.1 INTRODUCTION

Ferrous sulphate is known to be well absorbed (Brise and Hallberg, 1962). Furthermore, ascorbic acid has been shown to enhance the absorption from iron in this form when eaten with cereal meals (Sayers et al., 1973; Sayers et al., 1974a). Since there did not appear to be any obvious differences between the appearances and shelf life of sugar samples fortified with the various iron compounds, it was initially decided to restrict the absorption studies to sugar fortified with ferrous sulphate with or without ascorbic acid.

4.2 PREPARATION OF MEALS

4.2.1 Maize Meal Porridge

Sufficient maize meal was weighed out to provide 30 g dry maize per person. It was cooked in four times its weight of water for 20 - 25 minutes at 90 - 95°C. The final weight of porridge eaten by each subject was approximately 100 g (plus 20 g fortified sugar). In one experiment the fortified sugar was added before cooking, but in the others it was sprinkled on top of the porridge.

4.2.2 Tea

A commercial brand 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. Forty millilitres of pastuerised cow's milk was added and fortified sugar stirred into the mixture.

4.3 RESULTS

In the first experiment ten subjects ate maize-meal porridge intrinsically labelled with ^{55}Fe. Sugar (20 g fortified with 35.8 µmol iron as $^{59}FeSO_4.7H_2O$ and 113.6 µmol ascorbic acid) was sprinkled over the

porridge and consumed at the same time (Table 5). There was no significant difference ($t = 1.01$, $P = > 0.40$) between the absorption of the intrinsic iron (mean 10.5% SD ± 6.0) and that of the extrinsic iron (mean 10.1% SD ± 6.7). This finding reinforced a considerable body of evidence indicating that the non-haem iron in a meal is absorbed from a common pool (Bjorn-Rasmussen and Hallberg, 1972; Cook *et al.*, 1972; Sayers *et al.*, 1973), and established the validity of using only an extrinsic label to assess iron absorption in subsequent studies.

In the second experiment maize-meal porridge was eaten on two successive mornings by ten subjects (Table 6). On the one morning fortified sugar containing 35.8 µmol iron as $FeSO_4.7H_2O$ was added to the porridge and on the other the sugar also contained 113.6 µmol ascorbic acid; the mean absorption values were 3.8% (SD ± 2.5) and 6.9% (SD ± 4.3) respectively; the difference was significant ($t = 2.33$, $P = < 0.05$). A similar experiment was then done in eleven subjects in which the doses of iron and of ascorbic acid were increased to 71.6 µmol and 227.2 µmol respectively. Again there was evidence that absorption of iron was increased in the presence of ascorbic acid (Table 7). The mean percentage absorption when the sugar was supplemented with both ascorbic acid and iron was 10.3% (SD ± 6.9) compared with 6.1% (SD ± 4.8) when it contained only iron; the difference was significant ($t = 2.34$, $P = < 0.05$).

While it was clear from these experiments that ascorbic acid was enhancing the absorption of iron, the degree of enhancement was less than that which had previously been noted in studies using fortified salt (Sayers *et al.*, 1974b). A further study was therefore done in which the molar ration of ascorbic acid to iron was increased from 3.1:1 to 6.3:1. Nine subjects (mean haemoglobin 11.6 g/dl (SD ± 1.0); mean serum iron 17.19 µmol/l (SD ± 6.40) and mean percentage saturation of total iron binding capacity

27.5% (SD ± 12.4) were given the standard maize porridge meal; on one morning the sugar contained 35.8 /umol iron as $FeSO_4.7H_2O$ and 113.6 /umol ascorbic acid, while on the other the sugar contained the same iron dose and 227.2 /umol ascorbic acid. The larger dose of ascorbic acid was associated with an increase in the mean absorption of iron from 5.7% (SD ± 5.5) to 19.8% (SD ± 13.4); this difference was highly significant ($t = 3.45$, $P = < 0.01$). The mean absorption of the reference iron salt was 35.3% (SD ± 17.8) (Table 8).

TABLE 5

Absorption of intrinsic iron and extrinsic iron from maize meal porridge served with 20 g sugar fortified with 35.8 µmol iron as $FeSO_4.7H_2O$ and 113.6 µmol ascorbic acid (Final iron content 53.7 µmol)

Haemoglobin (g/dl)	Serum iron (µmol/l)	Unsaturated iron binding capacity (µmol/l)	% Saturation of transferrin	% Iron absorption			
				Intrinsic label	Extrinsic label	Ratio Extrinsic/ Intrinsic	Reference Salt
13.6	17.73	51.22	25.6	4.9	4.2	0.86	87.9
8.4	16.66	53.19	23.8	4.1	4.7	0.92	33.6
12.1	20.78	68.08	23.4	7.3	5.0	0.68	34.0
11.2	7.16	64.83	10.0	6.5	5.4	0.83	48.3
9.2	6.45	80.42	7.5	6.7	6.0	0.90	91.7
18.0	12.36	65.01	16.0	9.5	9.6	1.01	99.7
14.4	13.61	61.43	18.1	11.0	10.0	0.95	44.0
13.2	16.66	58.92	22.0	11.6	12.2	1.05	100.0
6.5	5.01	84.71	5.5	19.4	21.6	1.11	32.0
9.0	6.27	70.57	8.1	22.5	22.0	0.98	90.5
Mean 11.6	12.36	65.91	15.8	10.5 (SD ± 6.0)	10.1 (SD ± 6.7)	0.96	66.2 (SD ± 29.9)

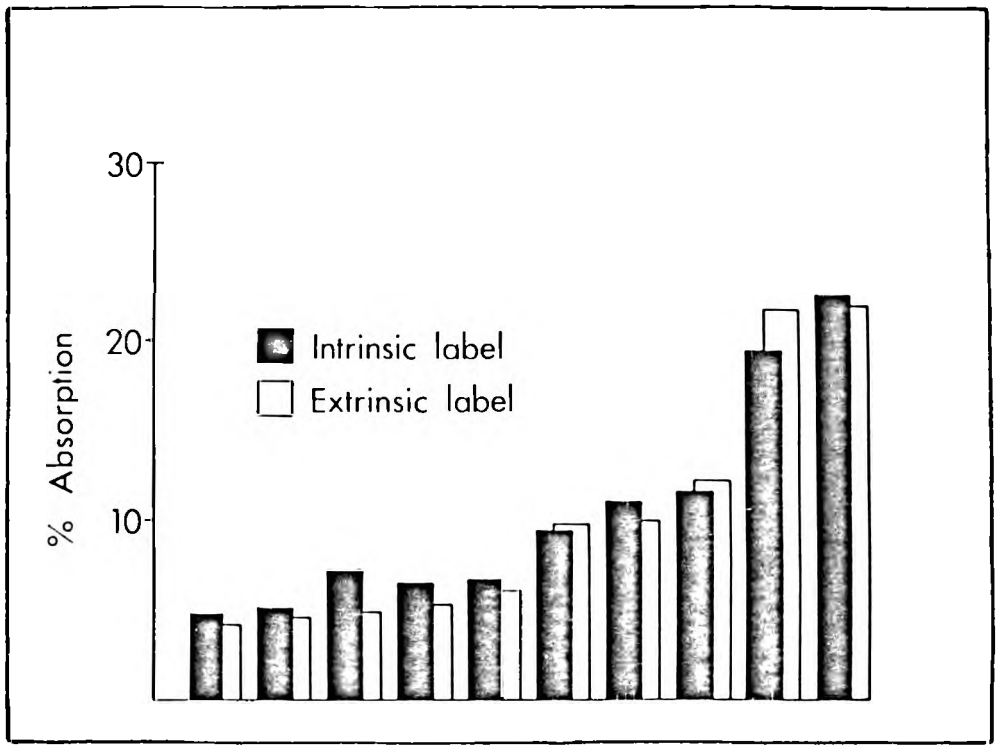

Fig. 10 Absorption of iron from maize meal porridge and fortified sugar containing 35.8 µmol iron as $FeSO_4.7H_2O$ and 113.6 µmol ascorbic acid

TABLE 6

Absorption of iron from maize meal porridge served with 20 g sugar fortified with 35.8 µmol iron as $FeSO_4.7H_2O$ and eaten with and without 113.6 µmol ascorbic acid (Final iron content 65.6 µmol)

Haemoglobin (g/dl)	Serum iron (µmol/l)	Unsaturated iron binding capacity (µmol/l)	% Saturation of transferrin	% Iron absorption		
				Without ascorbic acid	With ascorbic acid	Reference salt
12.3	19.52	52.83	27.1	1.1	10.7	16.7
10.8	18.98	75.58	20.0	1.5	11.4	92.8
14.6	30.63	45.49	40.3	2.3	2.9	35.4
6.8	6.09	74.33	7.6	2.3	3.3	73.0
6.8	6.98	77.37	8.3	2.5	2.7	31.2
7.0	6.81	74.33	8.4	3.9	5.5	84.6
9.8	13.61	76.83	15.1	3.7	2.1	37.1
14.0	27.22	60.54	31.1	6.9	7.7	21.8
12.4	14.50	57.31	20.2	7.3	8.0	24.5
12.4	8.60	77.37	9.9	7.7	14.3	35.2
Mean 10.7	15.40	67.16	18.8	3.8 (SD ± 2.5)	6.9 (SD ± 4.3)	45.2 (SD ± 27.6)

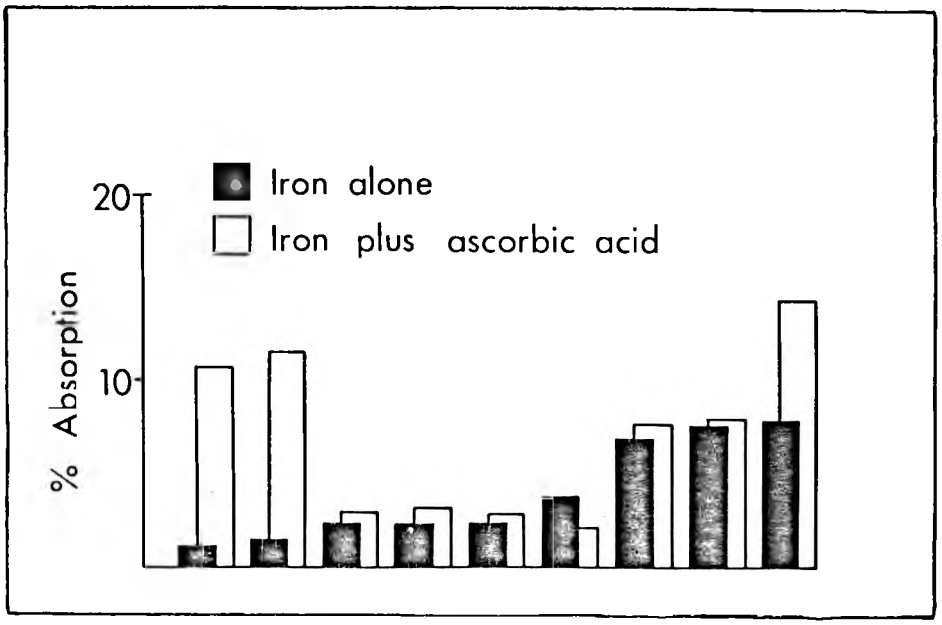

Fig. 11 Absorption of iron from maize meal porridge and fortified sugar containing 35.8 µmol iron as $FeSO_4.7H_2O$ and 113.6 µmol ascorbic acid

TABLE 7

Absorption of iron from maize meal porridge served with 20 g sugar fortified with 71.6 µmol iron as $FeSO_4.7H_2O$ and eaten with or without 227.2 µmol ascorbic acid (Final iron content 95.0 µmol)

Haemoglobin (g/dl)	Serum iron (µmol/l)	Unsaturated iron binding capacity (µmol/l)	% Saturation of transferrin	% Iron absorption		
				Without ascorbic acid	With ascorbic acid	Reference salt
13.8	20.06	52.30	27.7	0.4	0.8	8.2
11.8	17.19	88.48	16.3	0.4	4.6	1.5
13.2	19.88	58.74	25.3	1.5	2.6	14.5
13.6	24.00	50.15	32.4	4.4	8.7	12.8
13.6	15.58	65.73	19.2	5.2	12.0	29.2
12.1	9.13	68.95	11.7	5.9	18.3	65.1
13.8	12.18	71.46	14.6	6.6	7.0	59.4
13.6	8.96	72.71	10.9	7.3	23.5	58.9
11.5	9.13	89.19	9.3	7.8	11.3	99.7
12.8	20.78	64.30	24.4	12.2	8.6	31.4
13.2	25.25	56.77	30.8	16.0	15.6	43.7
Mean 11.8	16.57	67.16	20.2	6.1 (SD ± 4.8)	10.3 (SD ± 6.9)	39.5 (SD ± 30.0

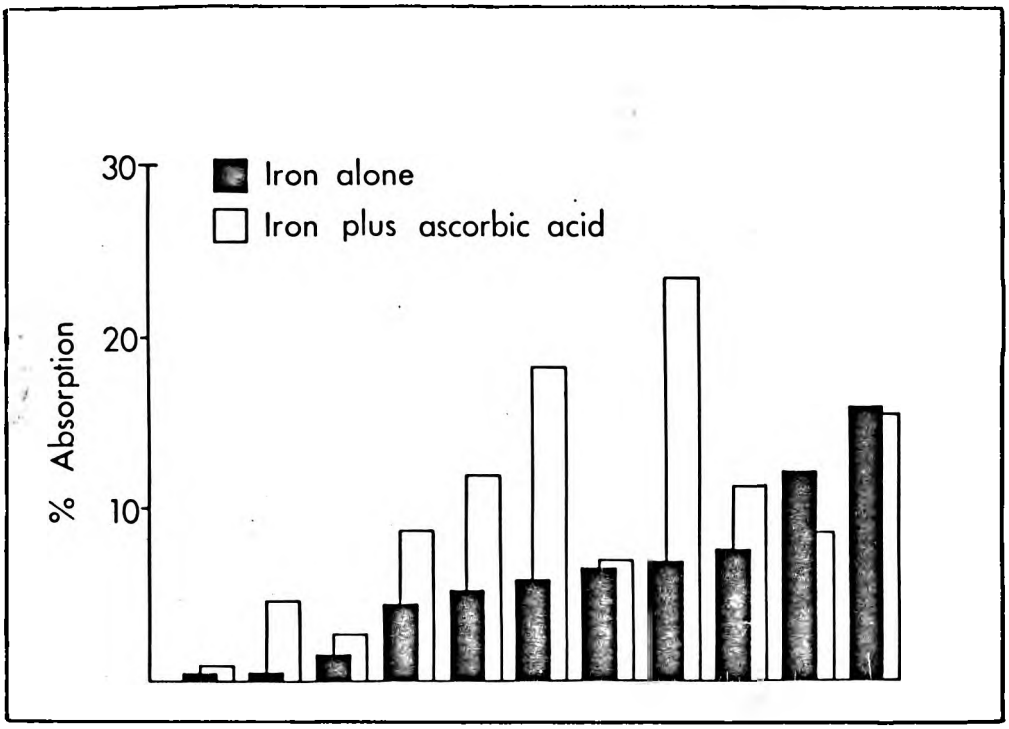

Fig. 12 Absorption of iron from maize meal porridge and fortified sugar containing 71.6 µmol iron as $FeSO_4.7H_2O$ and 227.2 µmol ascorbic acid

TABLE 8

Absorption of iron from maize meal porridge served with 20 g sugar fortified with 35.8 µmol iron as $FeSO_4.7H_2O$ and eaten with either 113.6 µmol or 227.2 µmol ascorbic acid (Final iron content 58.5 µmol)

Haemoglobin (g/dl)	Serum iron (µmol/l)	Unsaturated iron binding capacity (µmol/l)	% Saturation of transferrin	% Iron absorption		
				With 113.6 µmol ascorbic acid	With 227.2 µmol ascorbic acid	Reference salt
11.9	16.12	44.24	26.7	0.1	1.1	8.7
12.2	23.10	46.75	33.1	1.9	17.7	15.2
12.5	12.72	44.42	22.3	2.8	6.0	30.5
12.5	12.54	70.57	15.1	3.5	12.3	31.3
10.6	6.81	68.77	9.0	4.6	32.3	40.1
10.3	6.81	78.45	8.0	5.0	18.1	40.7
11.8	35.82	68.95	34.2	6.6	35.9	55.3
10.3	5.91	61.43	8.8	7.7	35.4	60.5
12.5	34.92	50.15	41.1	18.9	17.3	68.2
Mean 11.6	17.19	59.28	27.5	5.7 (SD ± 5.5)	19.8 (SD ± 13.4)	35.3 (SD ± 17.8)

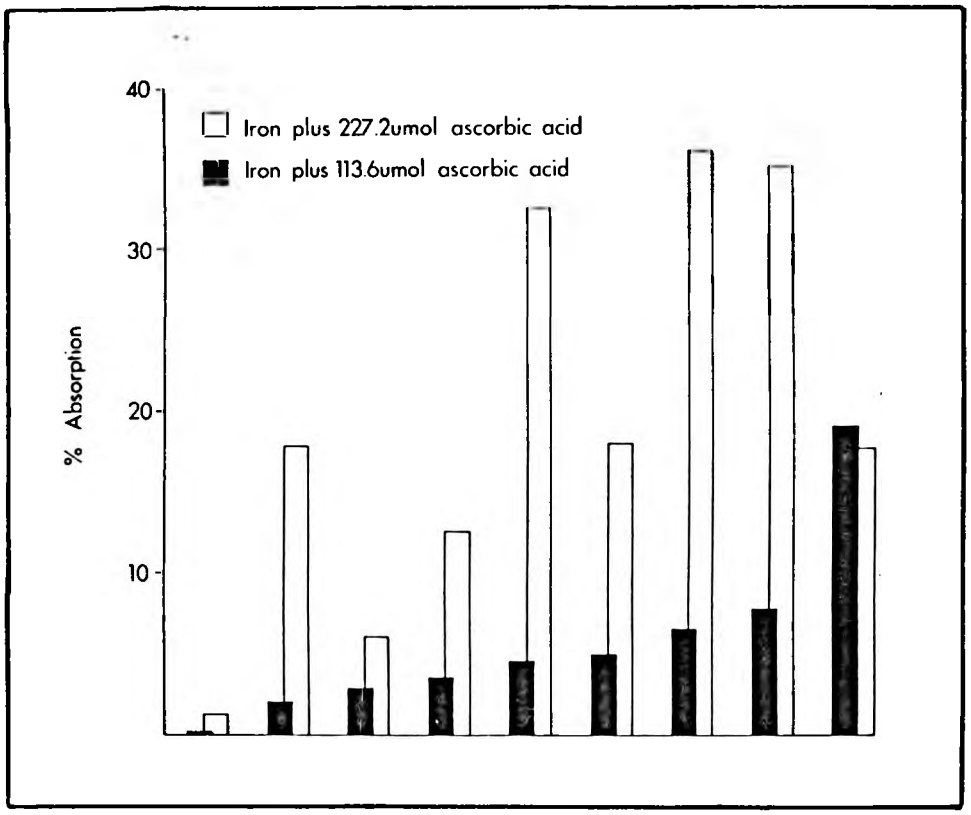

Fig. 13 Absorption of iron from maize meal porridge and fortified sugar containing 35.8 µmol iron as $FeSO_4.7H_2O$ and either 113.6 µmol or 227.2 µmol ascorbic acid

4.4 EFFECT OF FERROUS SULPHATE FORTIFIED SUGAR ON TEA AND COFFEE

Encouraged by these results, a series of studies were planned using iron-fortified sugar in tea and coffee. However, it was discovered that a marked discolouration developed almost immediately after the addition of fortified sugar to tea. The appearance was quite unacceptable whether or not the tea contained milk (Figures 14 - 15) and was similar whatever iron compound was used to fortify the sugar, with the single exception of the insoluble ferric orthophosphate (Figures 18 - 21). Moreover the effect was found with each of several brands of tea tested. The addition of sugar fortified with ascorbic acid alone produced some lightening of the colour of tea without milk, reminiscent of lemon tea (Figure 16). When added to tea with milk, however, no effect was obvious (Figure 17). The same pattern of findings was noted with various brands of instant and ground coffee, (Figure 23) but because of its initial dark colour, no change in the appearance of coffee without milk could be detected (Figure 22).

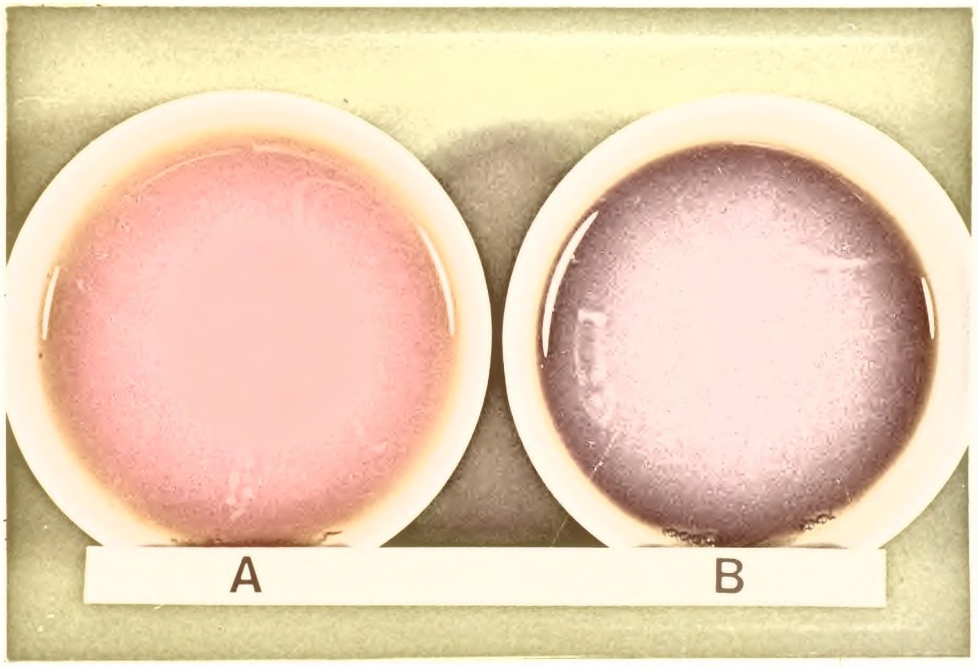

Fig. 14 A. Black tea
B. Black tea + 35.8 µmol iron as ferrous sulphate

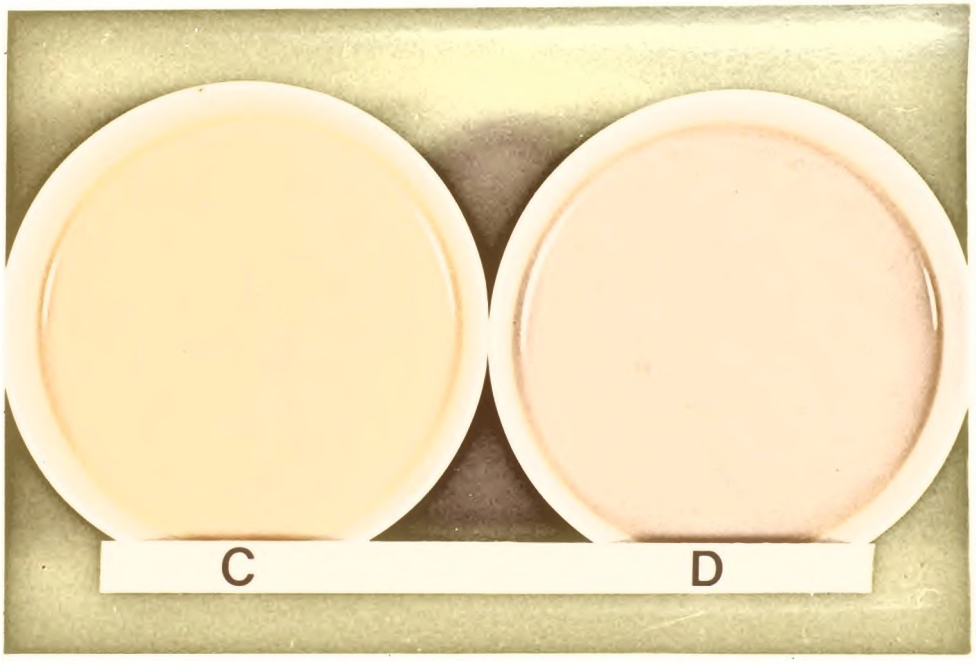

Fig. 15 C. White tea
D. White tea + 35.8 µmol iron as ferrous sulphate

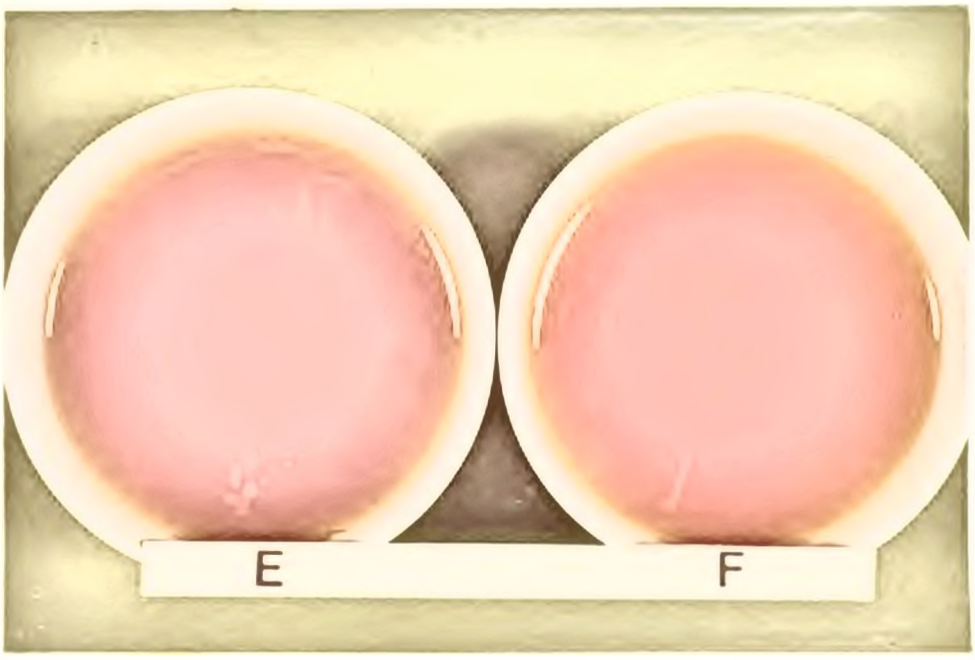

Fig. 16 E. Black tea
F. Black tea + 113.6 µmol ascorbic acid

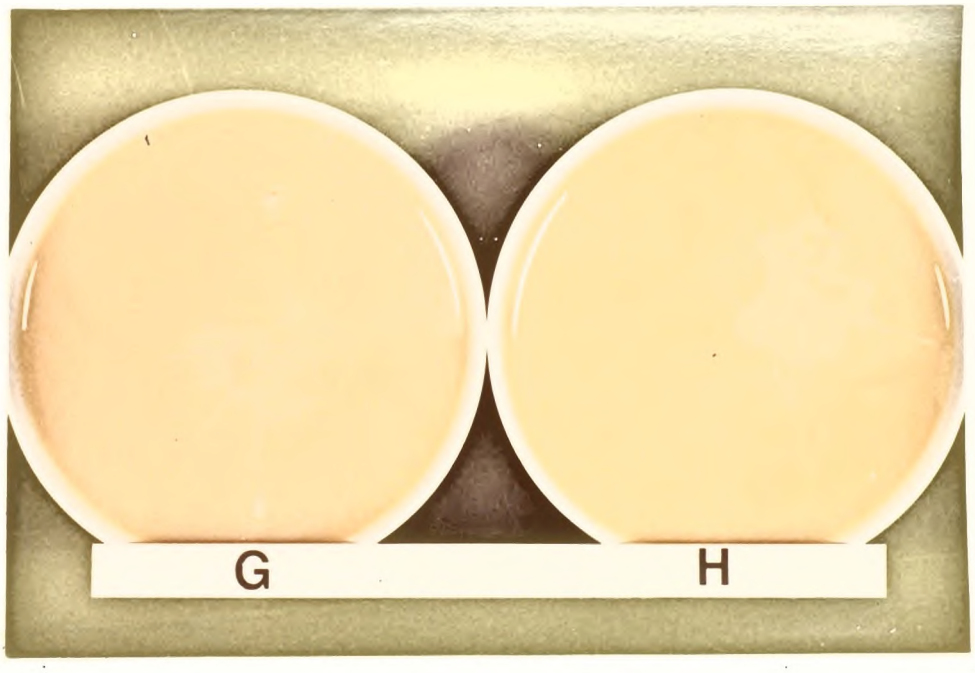

Fig. 17 G. White tea
H. White tea + 113.6 µmol ascorbic acid

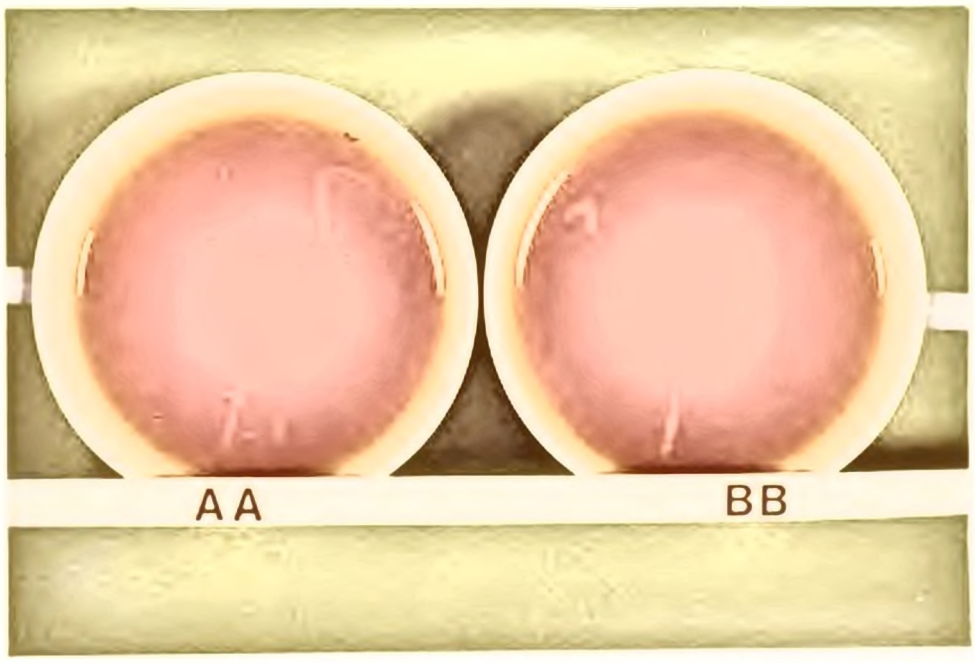

Fig. 18 AA Black tea
BB Black tea + 35.8 µmol iron as ferric orthophosphate

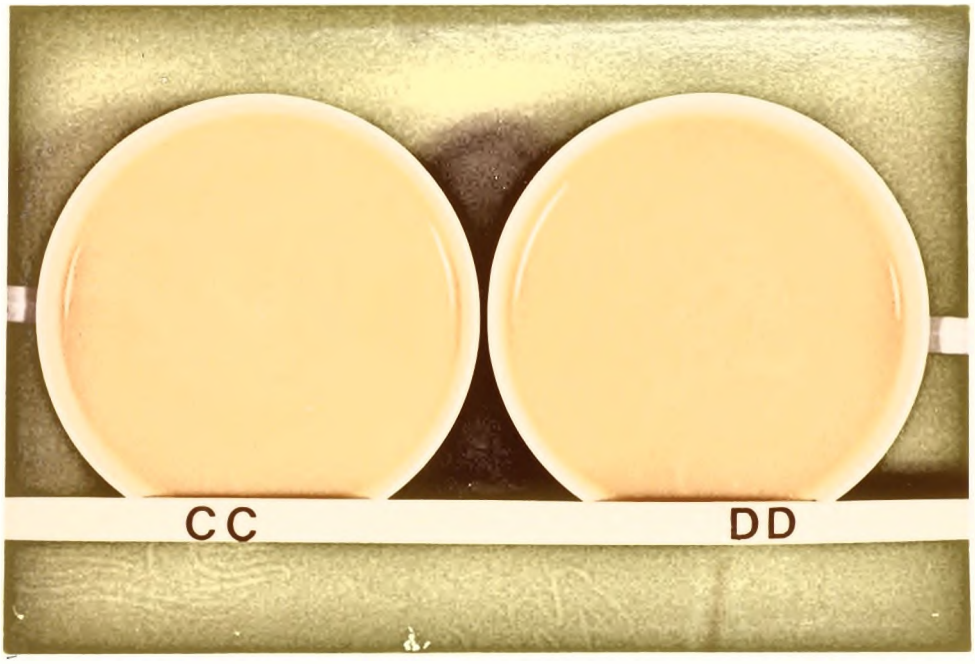

Fig. 19 CC White tea
DD White tea + 35.8 µmol iron as ferric orthophosphate



Fig. 20 EE Black tea
FF Black tea + 35.8 µmol iron as ferric orthophosphate and 113.6 µmol ascorbic acid

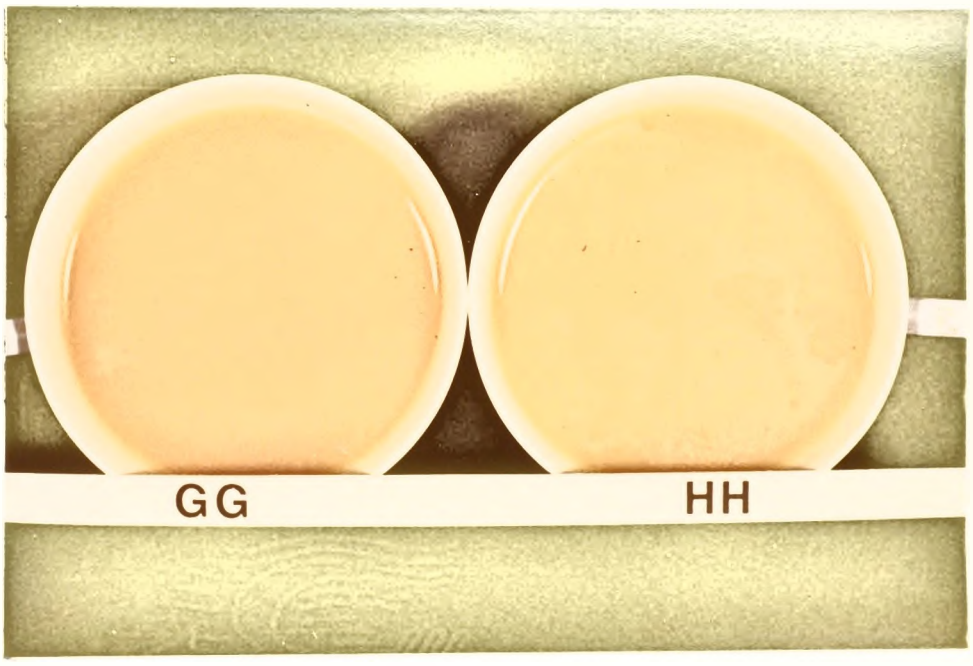

Fig. 21 GG White tea
HH White tea + 35.8 µmol iron as ferric orthophosphate and 113.6 µmol ascorbic acid



Fig. 22 I Black coffee
J Black coffee + 35.8 µmol iron as ferrous sulphate



Fig. 23 K White coffee
L White coffee + 35.8 µmol iron as ferrous sulphate

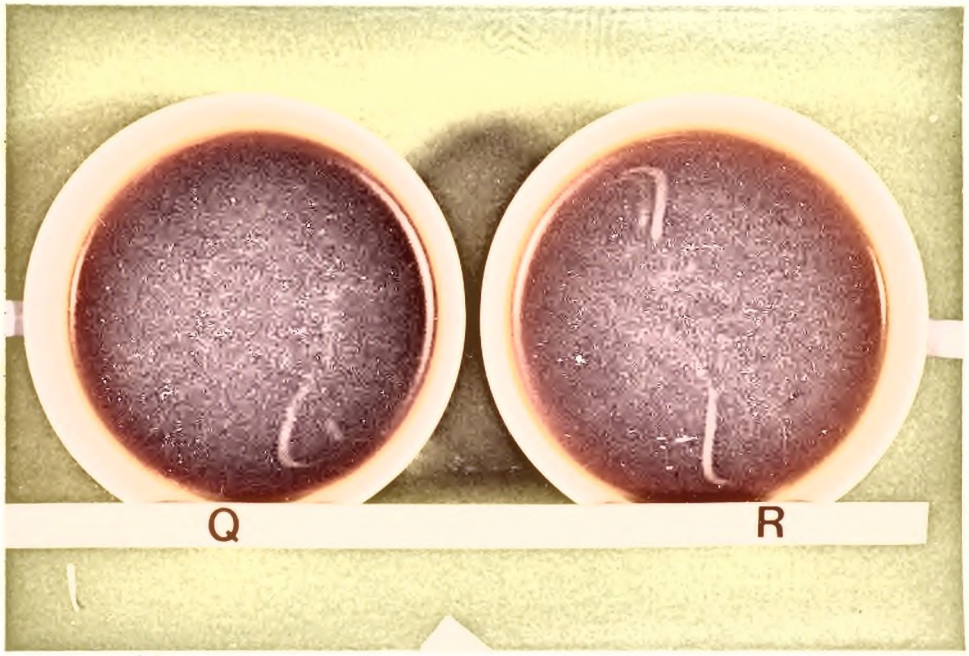

Fig. 24 Q Black coffee
R Black coffee + 113.6 µmol ascorbic acid

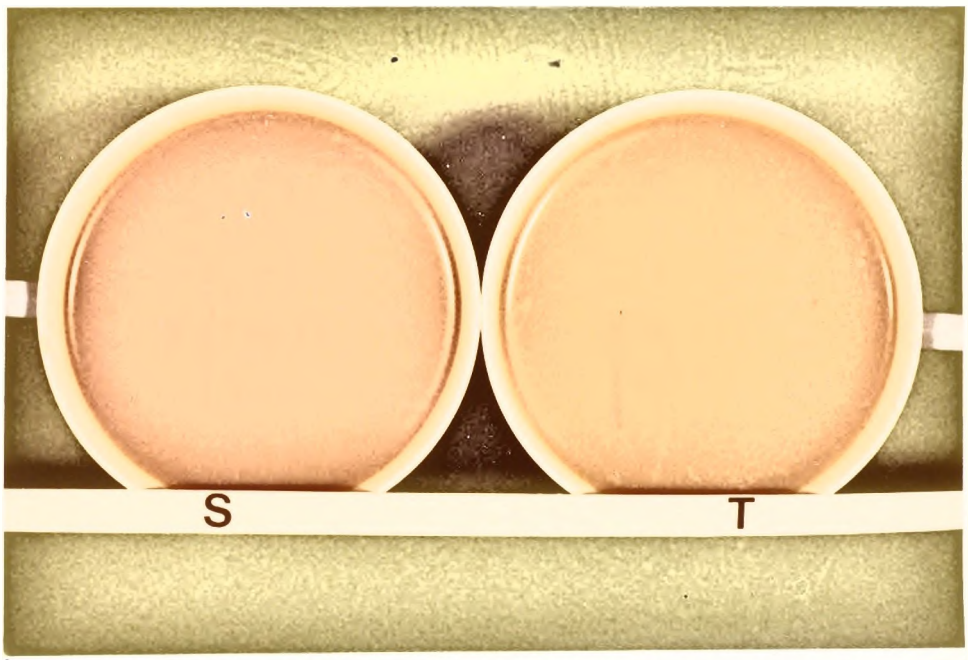

Fig. 25 S White coffee
T White coffee + 113.6 µmol ascorbic acid

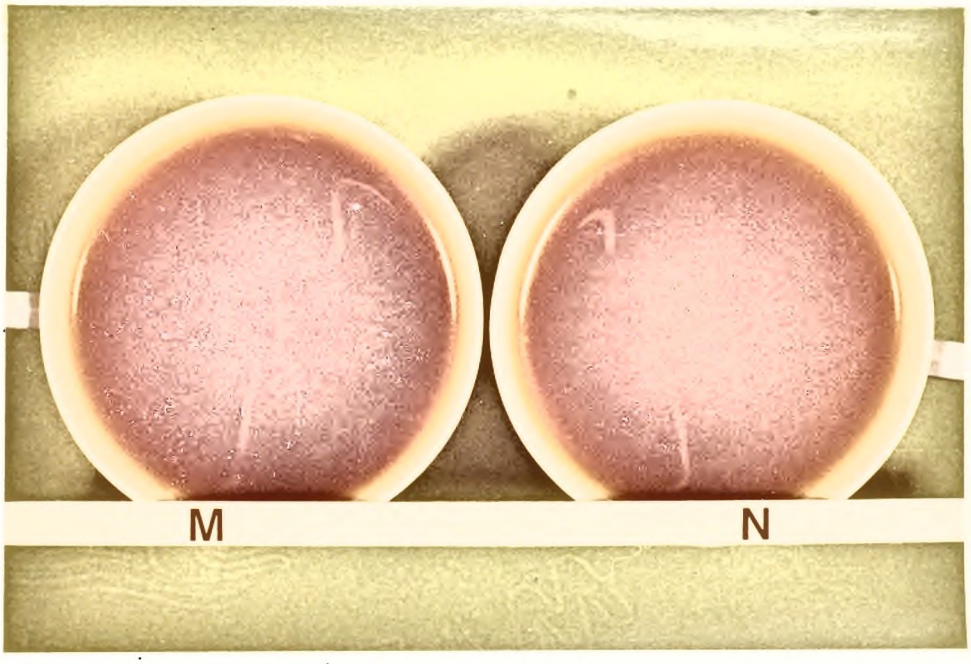

Fig. 26 M Black coffee
N Black coffee + 35.8 µmol iron as ferric orthophosphate

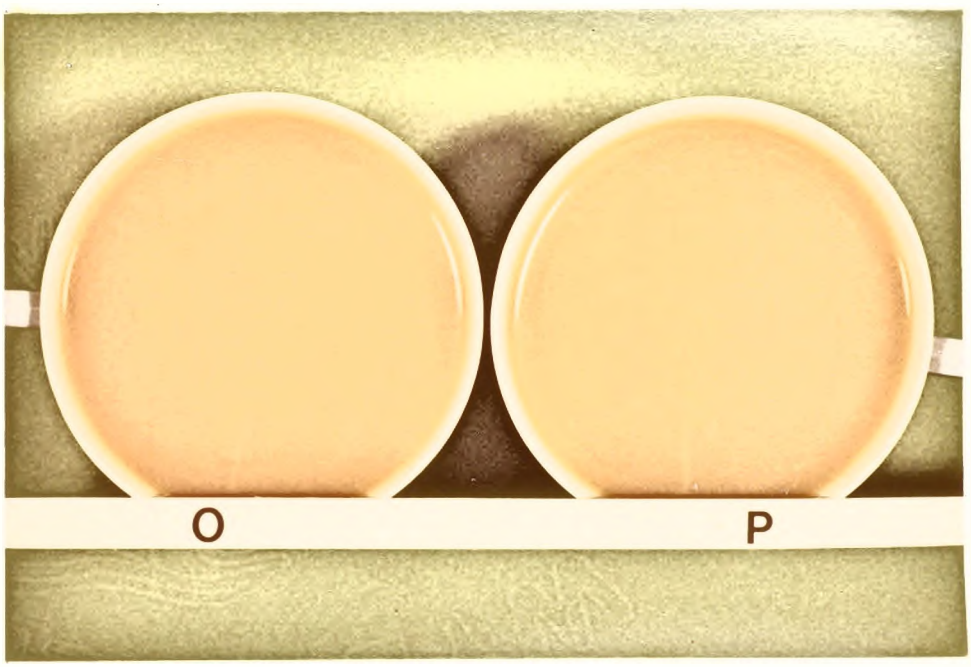

Fig. 27 O White coffee
P White coffee + 35.8 µmol iron as ferric orthophosphate

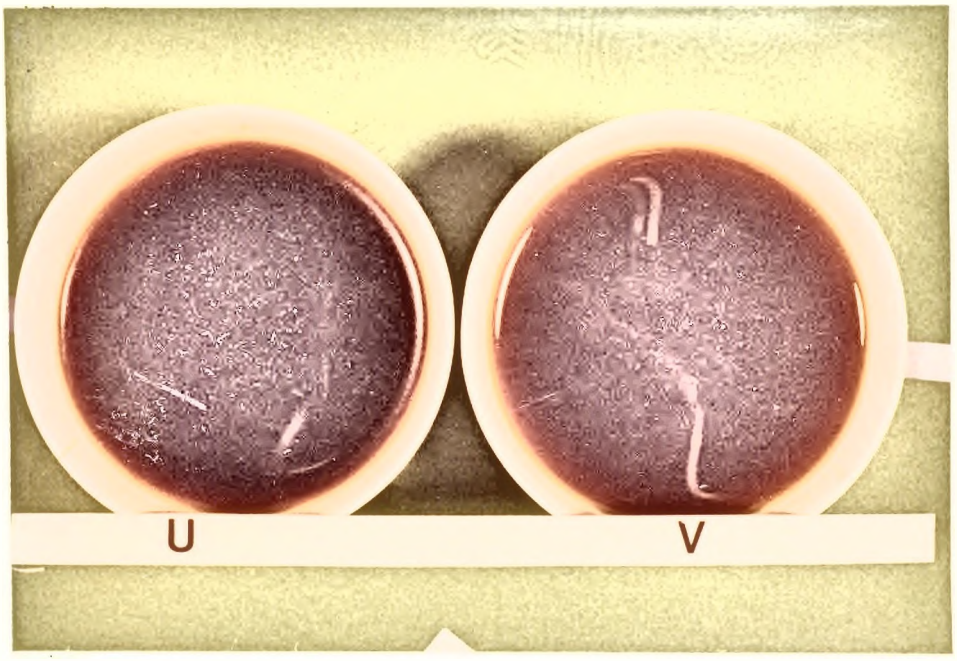

Fig. 28 U Black coffee
V Black coffee + 35.8 µmol iron as ferric orthophosphate + 113.6 µmol ascorbic acid

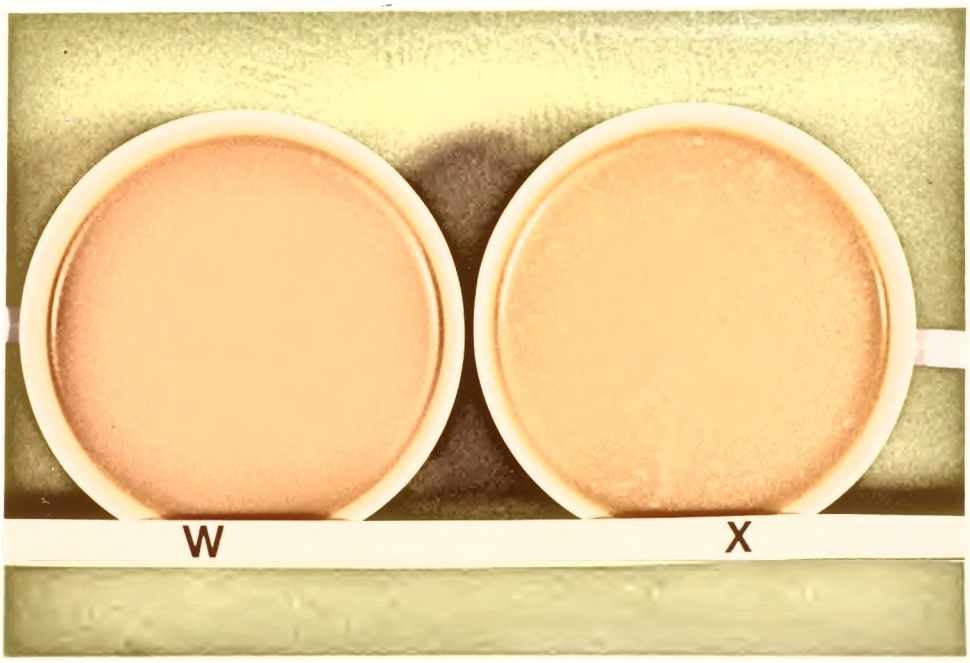

Fig. 29 W White coffee
X White coffee + 35.8 µmol iron as ferric orthophosphate + 113.6 µmol ascorbic acid

4.5 DISCUSSION

These studies established that the iron in sugar fortified with ferrous sulphate and ascorbic acid was well absorbed. even in the presence of a cereal, provided enough ascorbic acid was present. Nevertheless, as up to 60% of the daily sugar intake is consumed with beverages (Walker et al., 1971) the effect of fortified sugar on tea and coffee appeared to rule out sugar as a vehicle for dietary supplementation with soluble salts of iron. However, the possibility remained that ferric orthophosphate might still prove suitable. Although very poorly absorbed under most circumstances, it has been found that absorption of iron from meals cooked with household salt fortified with both ferric orthophosphate and ascorbic acid, was as good as when ferrous sulphate was substituted for the ferric orthophosphate (Sayers et al., 1974b). A further series of experiments was therefore planned to investigate the feasibility of fortifying sugar with ferric orthophosphate and ascorbic acid.

CHAPTER 5

IRON ABSORPTION FROM SUGAR FORTIFIED WITH FERRIC ORTHOPHOSPHATE AND ASCORBIC ACID

5.1 INTRODUCTION

Iron absorption was measured from sugar fortified with iron as ferric orthophosphate with and without ascorbic acid. The sugar was eaten with maize meal porridge, apple jam and bread, biscuits or tea.

5.2 PREPARATION OF MEALS

5.2.1 Maize Meal Porridge

was made as previously described (Page 39).

5.2.2 Apple Jam

Apples were peeled and cored and then homogenized in a Waring blender. The homogenate was boiled for 20 minutes, then the fortified sugar (670g/kg) was added and the mixture was boiled for a further 20 minutes. The jam was eaten with a slice of commercially available white bread (made from flour of 70-80% extraction and weighing approximately 90g).

5.2.3 Biscuits

A biscuit was made for each subject by mixing 60g white (70-80% extraction) flour with baking powder, salt, 20g butter and 20g fortified sugar. After thorough kneading to ensure even distribution of the isotope, the biscuits were baked for 40 minutes at 190°C.

5.2.4 Labelled Ferric Orthophosphate

was made from ferric chloride by the method of Steinkamp et al., 1955).

5.3 RESULTS

5.3.1 Absorption of Iron from Maize Meal Porridge and Sugar Fortified with Ferric Orthophosphate or Ferric Orthophosphate and Ascorbic acid

On successive mornings 12 subjects were given a meal of maize porridge with 20g iron fortified sugar. On the one morning the sugar contained 35.8 µmol iron as ferric orthophosphate and on the other, 227.2 µmol ascorbic acid was also present.

The mean percentage absorption rates were low on both occasions, being 2.1% and 4.1% respectively. Since these figures were lower than had been obtained in any of the previous studies the experiment was repeated in a further nine subjects with essentially similar results. When the twenty-one results were pooled together, the mean absorption without ascorbic acid was 1.3% (SD $\pm$ 1.7) as compared with 2.8% (SD $\pm$ 2.9) in the presence of ascorbic acid (Table 9). The difference was significant ($t = 3.59$, $P = < 0.01$). These low figures contrasted with the mean absorption of the reference iron salt, which was 31.9% (SD $\pm$ 25.5).

The question then arose as to whether the low absorption figures obtained in these experiments also reflected the absorption of the intrinsic iron present in the maize. A further study was therefore done in which seven subjects ate porridge made from ^{55}Fe-labelled maize onto which sugar containing 35.8 /umol iron as $^{59}FePO_4+H_2O$ and 227.2 /umol ascorbic acid had been sprinkled. There was a significant difference ($t = 5.08$, $P = < 0.01$) between the mean absorption of the intrinsic iron (9.4%; SD $\pm$ 9.5) and that of the added iron (1.4%; SD $\pm$ 1.5) (Table 10).

In seeking a reason for the marked discrepancies between the present results and those reported previously using salt fortified with ferric orthophosphate and ascorbic acid (Figure 30, Sayers et al., 1974b), one major difference was noted. In the previous studies the salt was added prior to the cooking of maize porridge or rice, while in the present studies the sugar was added only after cooking. This raised the possibility that heating was necessary for the formation of an absorbable iron complex between the insoluble ferric orthophosphate and the ascorbic acid. Support was given to this hypothesis by an in vitro experiment in which the solubility of ferric orthophosphate in water was measured in the presence of ascorbic acid. In cold water ferric orthophosphate is almost completely insoluble while if it is boiled for only fifteen minutes the iron becomes completely soluble. (Figure 31).

An experiment was then performed to test this hypothesis in man.

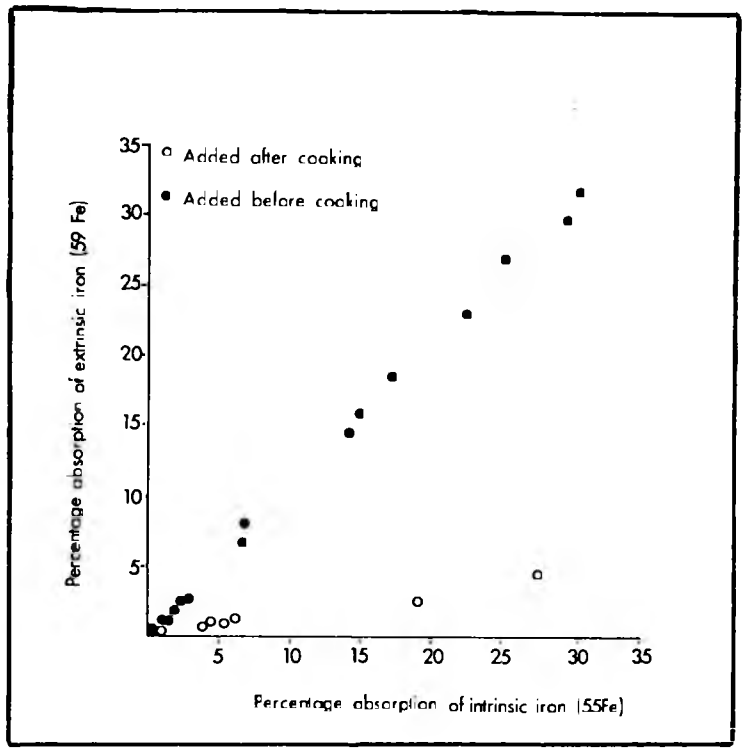

Fig. 30 Extrinsic ferric orthophosphate and intrinsic iron absorption from maize meal porridge in the presence of ascorbic acid (data compared with that of Sayers et al., 1974b)

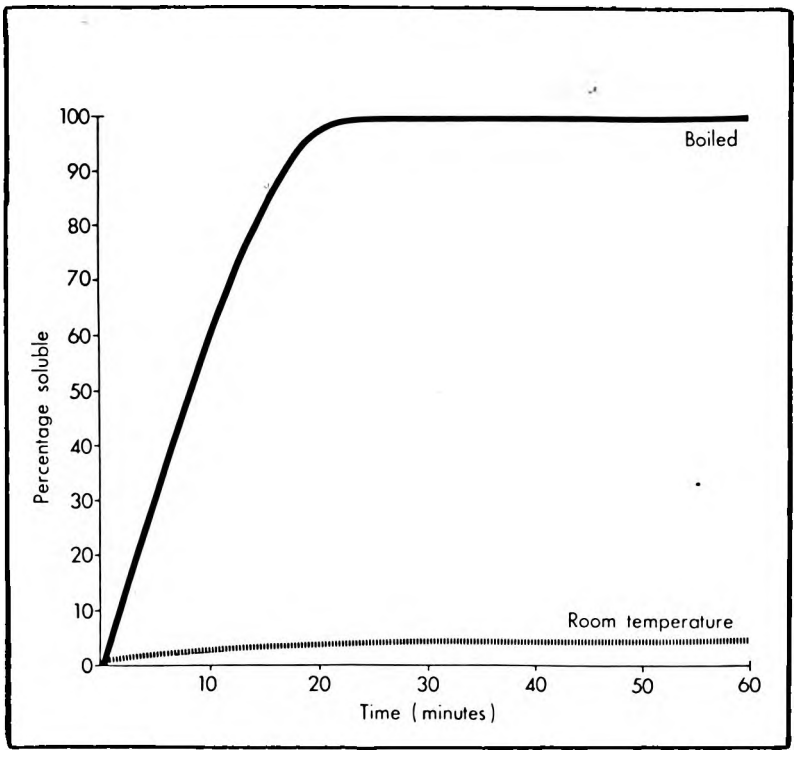

Fig. 31 Effect of boiling on the solubility of ferric orthophosphate in the presence of ascorbic acid

5.3.2 Effect of Cooking on Absorption of Iron in Sugar Fortified with Ferric Orthophosphate and Ascorbic Acid

A direct comparison between the effects of adding fortified sugar before and after the cooking of maize porridge was then made in ten subjects (mean haemoglobin 13.0 g/dl; mean serum iron 12.72 /umol/l; mean percentage saturation of total iron binding capacity 22.7). On the one morning sugar containing 35.8 /umol iron as ferric orthophosphate and 227.2 /umol ascorbic acid was sprinkled on the cooked porridge in the usual manner, while on the other the sugar was added before the porridge was cooked. The mean absorption rates were 1.8% (SD ± 1.1) and 12.7% (SD ± 4.2) respectively, a difference that was highly significant ($t = 3.79$, $P = < 0.01$). The mean absorption of the reference iron salt was 44.1% (SD ± 22.4) (Table 11).

The effects of cooking on the absorbability of 35.8 /umol iron when given as ferric orthophosphate with 227.2 /umol ascorbic acid was assessed with two other foods, namely jam and a slice of bread on two consecutive mornings. On the one the jam had been prepared from fortified sugar containing ferric orthophosphate alone and on the other, both ferric orthophosphate and ascorbic acid. The presence of ascorbic acid was associated with a highly significant ($t = 3.91$, $P = < 0.01$) increase in iron absorption, from a mean 2.3% (SD ± 1.5) to 13.8% (SD ± 8.5) (Table 12).

The effects of higher temperatures were assessed in a final experiment in which biscuits were baked using sugar fortified to the same degree as in the previous study. The mean absorption of iron in eight subjects was 1.5% (SD ± 2.0) in the absence of ascorbic acid and 2.6% (SD ± 1.4) when ascorbic acid was present during baking; the difference was not significant ($t = 1.30$, $P = > 0.05$) (Table 13). The negative results were presumably ascribable to the destruction of ascorbic acid by the high temperatures that are necessary for baking (Sayers et al., 1973).

5.3.3 Absorption of Iron from Tea Drunk with Fortified Sugar

The addition to tea of sugar fortified with iron as ferric orthophosphate, did not alter the acceptability of the tea. Nevertheless, when iron absorption was measured from a cup of such tea, the results were disappointing. Six subjects drank tea to which sugar fortified with iron only was added on one day, and the mean absorption was 2.4% (SD ± 3.1%). When ascorbic acid was added together with the iron a mere 1.4% (SD ± 1.5%) was absorbed (Table 14). There was no significant difference between the two absorption values ($t = 1.21$, $P = > 0.05$). Thus even though the iron and ascorbic acid had been added to extremely hot tea, and some solubilisation of iron would be expected (Figure 31), the absorption of iron in the presence of ascorbic acid was even lower than the absorption of iron from maize porridge.

TABLE 9

Absorption of iron from maize meal porridge served with 20g sugar fortified with 35.8 µmol iron as ferric orthophosphate and eaten with or without 227.2 µmol ascorbic acid (Final iron content 60.8 µmol)

Haemoglobin (g/dl)	Serum iron (µmol/l)	Unsaturated iron binding capacity (µmol/l)	% Saturation of transferrin	% Iron absorption		
				Without ascorbic acid	With ascorbic acid	Reference salt
13.2	31.52	54.80	36.5	0	3.0	60.3
12.8	20.95	65.19	24.3	0.1	0.4	20.0
10.8	18.27	59.10	23.6	0.2	0.7	2.7
12.8	16.66	51.94	24.3	0.3	0.1	15.0
9.8	7.88	73.43	9.7	0.4	0.7	36.6
13.2	26.15	62.69	29.4	0.5	0.4	21.9
12.8	14.87	66.27	18.3	0.6	0.5	12.5
12.8	18.63	63.04	22.8	0.7	2.6	2.4
11.1	23.82	52.48	31.2	0.7	0.2	8.6
11.0	50.69	36.54	58.1	0.7	4.1	42.2
13.2	13.43	68.60	16.4	1.1	4.3	3.5
12.9	21.67	52.48	29.2	1.1	6.1	53.4
12.7	14.87	69.85	17.5	1.2	2.8	57.2
10.6	7.88	80.24	8.9	1.3	7.1	16.2
10.6	9.85	72.89	11.9	1.3	1.2	37.4
11.6	19.34	58.39	24.9	1.6	4.1	30.7
14.2	26.51	54.09	32.9	1.6	3.8	60.3
9.0	8.78	90.09	8.9	1.8	1.7	69.6
13.4	13.79	48.89	22.0	2.1	0.1	2.7
10.2	6.45	68.77	8.6	2.6	5.4	24.9
10.0	6.09	80.60	7.0	8.2	11.7	91.6
Mean 11.8	18.09	63.40	22.2	1.3 (SD ± 1.7)	2.8 (SD ± 2.9)	31.9 (SD ± 25.5)

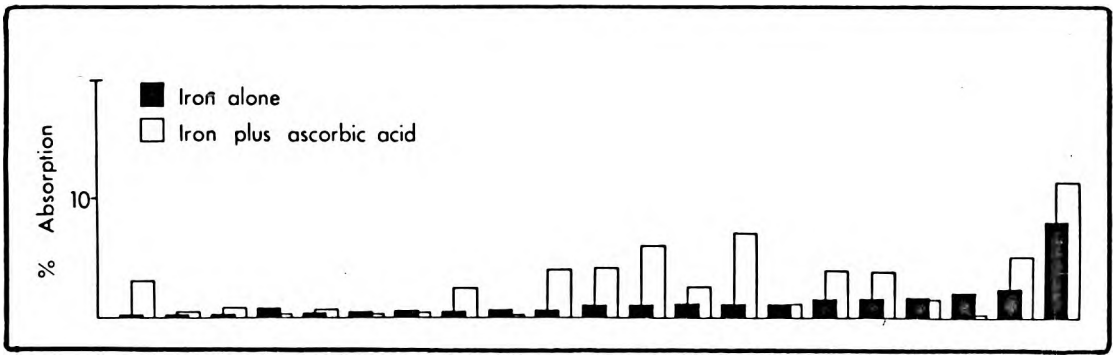

Fig. 32 Absorption of iron from maize meal porridge and fortified sugar containing 35.8 µmol iron as ferric orthophosphate with or without 227.2 µmol ascorbic acid

TABLE 10

Absorption of intrinsic iron and extrinsic iron (35.8 /umol iron as ferric orthophosphate) from maize meal porridge served with 227.2 /umol ascorbic acid (Final iron content 57.3 /umol)

Haemoglobin (g/dl)	Serum iron (/umol/l)	Unsaturated iron binding capacity (/umol/l)	% Saturation of transferrin	% Iron absorption			
				Intrinsic label	Extrinsic label	Ratio Extrinsic/ Intrinsic	Reference salt
12.9	14.33	35.46	31.0	0.1	0.5	5.0	11.7
11.4	18.81	56.95	24.8	0.5	5.6	11.2	21.7
14.4	34.03	32.42	51.2	0.6	5.8	9.7	36.4
12.2	26.87	45.85	36.9	0.8	4.4	5.5	29.8
13.7	23.28	32.24	31.7	0.9	4.8	5.3	40.2
14.8	14.33	60.89	19.0	2.7	17.2	6.4	41.7
11.2	9.85	84.18	8.8	4.1	27.5	6.7	51.3
Mean 12.9	20.24	49.79	28.9	1.4 (SD ± 1.5)	9.4 (SD ± 9.5)	7.1 (SD ± 2.4)	34.0 (SD±14.0)

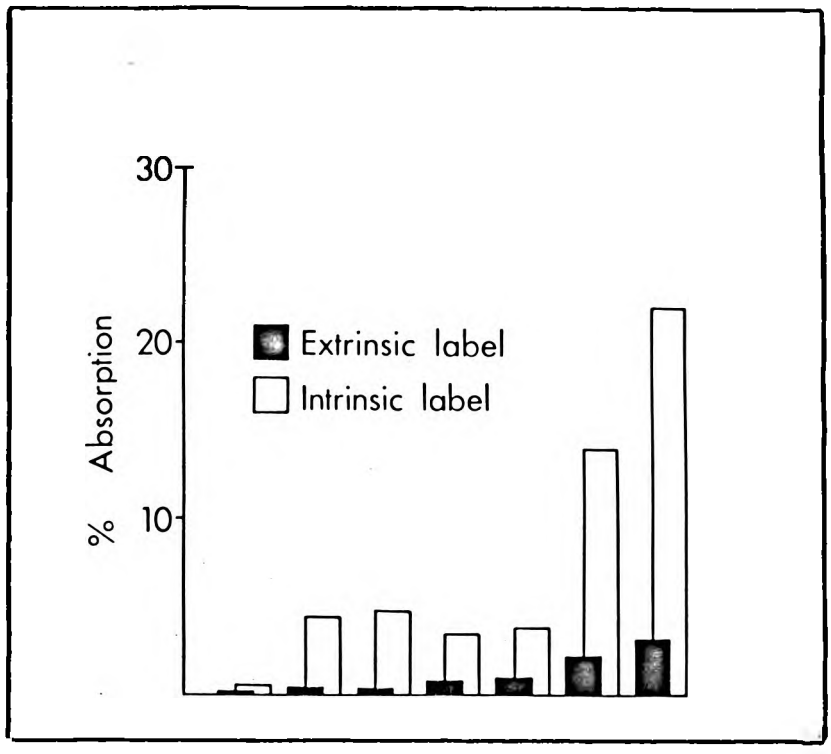

Fig. 33 Absorption of iron from maize meal porridge and fortified sugar containing 35.8 µmol iron as ferric orthophosphate and 227.2 µmol ascorbic acid

TABLE 11

Absorption of iron from maize meal porridge served with 20 g sugar fortified with 35.8 /umol iron as ferric orthophosphate and 227.2 /umol ascorbic acid. Fortified sugar either added dry on top of porridge or added to cooking water before preparation (Final iron content 55.5 /umol)

Haemoglobin (g/dl)	Serum iron (/umol/l)	Unsaturated iron binding capacity (/umol/l)	% Saturation of transferrin	% Iron absorption		
				Sugar added dry	Sugar added before cooking	Reference salt
12.2	17.01	83.10	17.0	0.7	2.7	25.8
14.1	9.67	51.58	15.8	0.8	2.7	10.3
14.3	13.07	68.06	16.1	1.2	6.4	30.6
12.5	8.96	76.12	10.5	1.2	26.3	68.3
10.6	5.37	70.74	7.1	1.3	12.5	54.2
13.3	16.12	62.51	20.5	1.4	12.9	48.2
13.5	19.34	65.91	22.7	1.8	23.5	57.9
11.8	12.54	66.80	15.8	2.5	22.6	72.1
13.1	16.12	31.34	34.0	2.9	1.4	13.8
14.1	8.96	78.62	10.2	4.1	16.4	60.0
Mean 13.0	12.72	65.37	22.7	1.8 (SD ± 1.1)	12.7 (SD ± 4.2)	44.1 (SD ± 22.4)

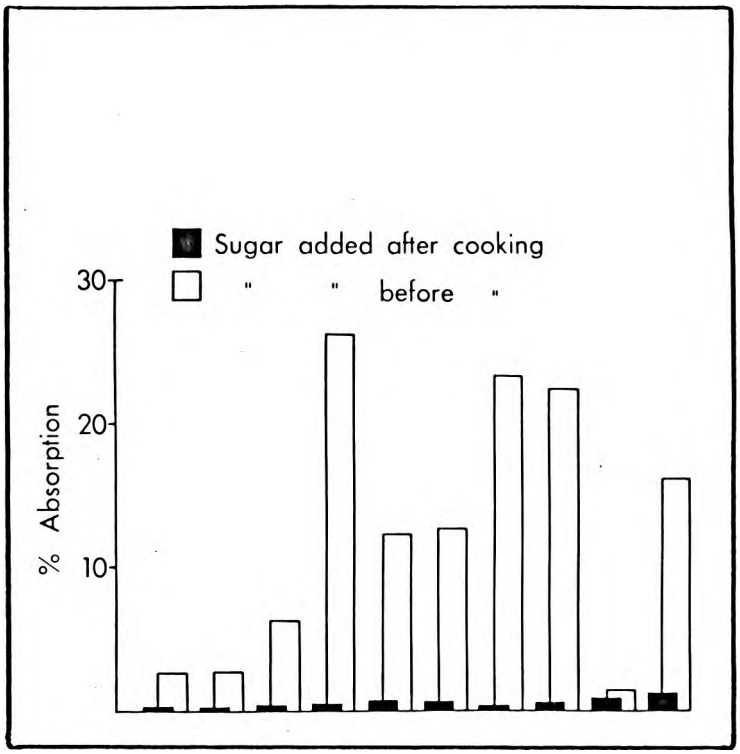

Fig. 34 Absorption of iron from maize meal porridge and fortified sugar containing 35.8 µmol iron as ferric orthophosphate and 227.2 µmol ascorbic acid - sugar added either before or after cooking the porridge

TABLE 12

Absorption of iron from apple jam cooked with 20 g sugar fortified with 35.8 µmol iron as ferric orthophosphate with or without 227.2 µmol ascorbic acid (Final iron content 51.9 µmol)

Haemoglobin (g/dl)	Serum iron (µmol/l)	Unsaturated iron binding capacity (µmol/l)	% Saturation of transferrin	% Iron absorption		
				Without ascorbic acid	With ascorbic acid	Reference salt
14.4	20.95	45.85	31.4	0.1	3.6	15.7
14.1	17.55	58.74	23.0	0.5	11.9	25.1
11.8	12.36	69.31	15.1	1.6	20.2	28.7
13.7	15.76	86.33	15.4	2.2	19.1	43.1
12.9	20.95	53.01	28.3	2.8	4.7	57.1
14.1	24.18	38.33	38.7	3.2	28.5	65.2
11.4	6.98	72.00	8.8	3.4	13.9	68.9
12.5	24.54	60.71	28.8	4.5	8.4	73.4
Mean 13.1	17.91	60.54	23.7	2.3 (SD ± 1.5)	13.8 (SD ± 8.5)	47.2 (SD ± 22.1)

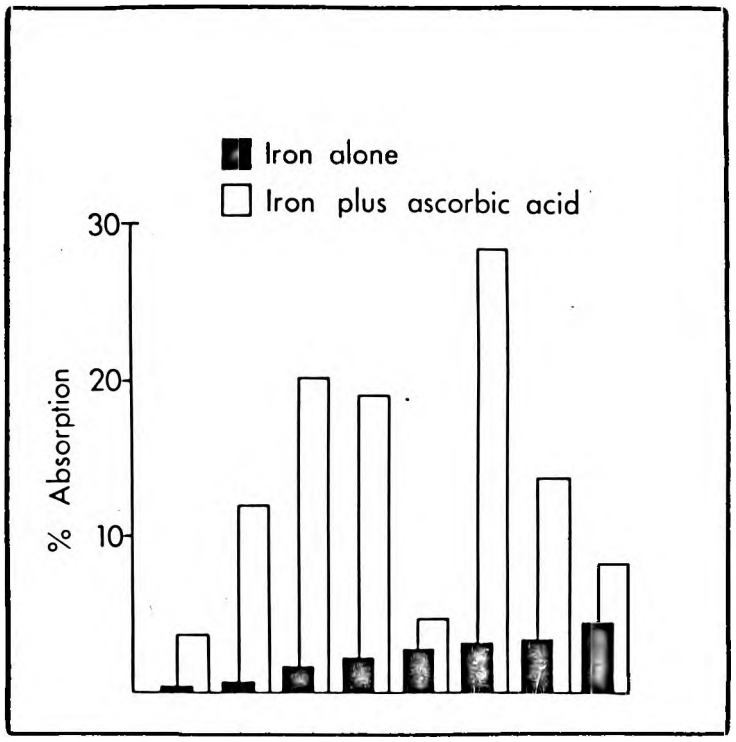

Fig. 35 Absorption of iron from apple jam and fortified sugar containing 35.8 µmol iron as ferric orthophosphate with or without 227.2 µmol ascorbic acid

TABLE 13

Absorption of iron from a biscuit baked with 20 g sugar fortified with 35.8 µmol iron as ferric orthophosphate and with or without 227.2 µmol ascorbic acid (Final iron content 57.3 µmol)

Haemoglobin (g/dl)	Serum iron (µmol/l)	Unsaturated iron binding capacity (µmol/l)	% Saturation of transferrin	% Iron absorption		
				Without ascorbic acid	With ascorbic acid	Reference salt
11.4	12.72	63.58	16.7	0.1	3.5	18.3
12.9	16.66	56.77	27.7	0.1	1.5	45.8
15.8	25.61	55.16	31.7	0.2	4.7	79.7
13.6	20.95	36.89	36.2	0.6	0.3	33.0
11.8	11.28	52.48	17.7	0.8	2.9	75.3
14.8	19.34	36.72	34.5	1.6	1.7	32.2
12.5	12.18	57.31	17.5	3.6	2.4	88.4
11.2	5.91	67.34	8.1	5.6	3.8	85.8
Mean 13.0	1 5.58	53.37	23.1	1.5 (SD ± 2.0)	2.6 (SD ± 1.4)	57.3 (SD ± 28.0)

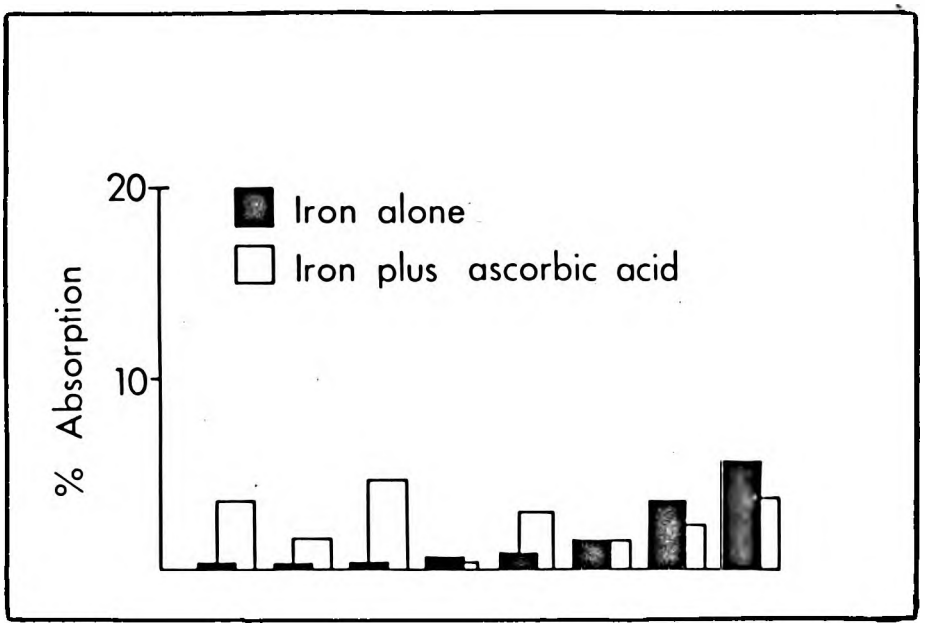

Fig. 36 Absorption of iron from biscuits and fortified sugar containing 35.8 µmol iron as ferric orthophosphate and with or without 227.2 µmol ascorbic acid

TABLE 14

Absorption of iron from a cup of tea drunk together with 20 g sugar fortified with 35.8 µmol iron as ferric orthophosphate and with or without 227.2 µmol ascorbic acid
(Final iron content 41.2 µmol)

Haemoglobin (g/dl)	Serum iron (µmol/l)	Unsaturated iron binding capacity (µmol/l)	% Saturation of transferrin	% Iron absorption		
				Without ascorbic acid	With ascorbic acid	Reference salt
12.1	22.92	64.48	26.2	0.3	0.4	6.7
11.5	9.31	79.16	10.5	0.4	0.7	94.1
12.4	17.01	63.76	21.1	0.7	0.5	20.9
12.4	13.07	44.24	22.8	0.9	0.3	7.4
13.8	23.82	69.85	25.5	4.4	3.9	100.0
13.2	10.75	69.85	13.3	7.8	2.3	39.7
Mean 12.6	16.15	65.22	19.9	2.4 (SD ± 3.1)	1.4 (SD ± 1.5)	44.8 (SD ± 24.1)

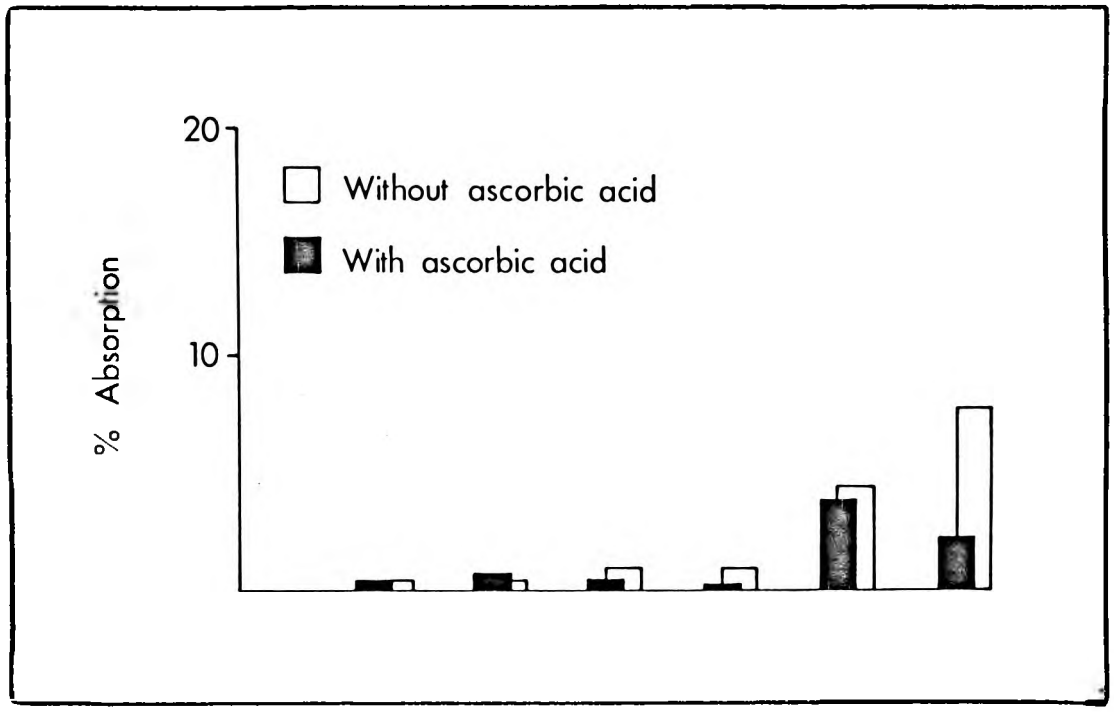

Fig. 37 Absorption of iron from tea and fortified sugar containing 35.8 µmol iron as ferric orthophosphate and with or without 227.2 µmol ascorbic acid

5.4 DISCUSSION

The great advantage of common salt as a vehicle for dietary iron supplements is that it is consumed by all members of the population in relatively constant amounts which are related to the individual's total food intake (Sayers et al., 1974a). The consumption of sugar is certainly more variable, but a recent study revealed that most South Africans eat between 50 and 100 g daily, figures similar to those reported from other countries (Walker et al., 1971). In the present investigation sugar was shown to be superior to salt as a vehicle for supplementation with iron and ascorbic acid in one important respect, namely its consumer acceptability after storage in the hot, humid conditions which pertain in many countries where nutritional iron deficiency is a major problem. No colour changes developed under these conditions, whereas coarse, unrefined salt fortified with ascorbic acid and any soluble iron compound became rapidly discoloured. Even when the supplemental iron was in the form of the insoluble ferric orthophosphate, discolouration remained a problem which was only partially solved by mixing the fortified salt with 25 g starch/kg (Sayers et al., 1974b).

Sugar also fulfilled the second essential requirement, namely that the supplementary iron should be absorbed. When maize porridge was eaten with fortified sugar containing ferrous sulphate and ascorbic acid, both the intrinsic maize iron and the supplemental iron were absorbed to a similar degree. As has previously been shown (Sayers et al., 1973; Bjorn-Rasmussen and Hallberg, 1974; Sayers et al., 1974b), the enhancing effect of the ascorbic acid was dose-dependent; only a modest effect was seen when the molar ration of ascorbic acid: iron was 3.1:1 but absorption was increased several-fold when it was 6.3:1. When the fortified sugar

was added to tea, however, there was such a marked colour change that sugar would effectively be ruled out as a vehicle for dietary iron supplementation in any country where tea is customarily drunk with sugar. The same conclusion must probably be reached with regard to coffee, although the discolouration was less obtrusive, and it is conceivable that it might not be entirely unacceptable if the advantages of sugar as a vehicle proved compelling.

Ferric orthophosphate did not discolour the beverages, and since it had already been shown to be an effective substitute for ferrous sulphate when the vehicle was salt (provided that ascorbic acid was also present), absorption studies were undertaken with sugar containing ferric orthophosphate and ascorbic acid. The absorption was very poor, and this was in striking contrast to the results obtained when salt was the vehicle (Sayers et al., 1974b). The reason for the discrepant findings was soon discovered: heat must be applied if the iron in ferric orthophosphate is to be converted into an absorbable form by ascorbic acid. The fortified salt had been added to the porridge before it was cooked, and the fortified sugar after; when the sugar was cooked with the porridge, absorption was markedly enhanced and was comparable with that found previously with salt. Ferric orthophosphate is therefore an appropriate iron compound for dietary supplementation only when it is present, together with an adequate amount of ascorbic acid, in the food before cooking. Most of the salt which is eaten each day is added during cooking rather than at the table: on the other hand, most sugar is probably consumed uncooked, although a proportion (which will obviously vary according to local custom) is of course used for such purposes as making jam.

The present findings indicate that there are real problems in using sugar as a vehicle for fortification of the diet with iron and ascorbic acid. Soluble iron salts that are well absorbed cause discoloration of tea and coffee, while insoluble salts are poorly absorbed unless added prior to cooking. These largely negative results underline the need for fresh approaches. The iron content of some cereal diets seems adequate (Ramalingaswami & Patwardhan, 1949) but little of the iron is absorbed (Martinez-Torres & Layrisse, 1973). In such circumstances the addition of ascorbic acid alone to a vehicle such as sugar may be all that is necessary to improve iron nutrition. However, in such diets a significant proportion of the iron content probably represents contamination. The degree to which the absorption of this contaminating iron will be enhanced by ascorbic acid obviously depends on its nature. In this context the present findings may have relevance, since they indicate that a compound such as ferric orthophosphate, unlike more soluble iron salts (Bjorn-Rasmussen and Hallberg, 1972; Cook et al., 1972; Sayers et al., 1973), does not form a common pool with the intrinsic non-haem iron in food, and is not affected to any degree by enhancing agents such as ascorbic acid. There is therefore no assurance that ascorbic acid would increase the absorption of dietary iron derived from soil or other forms of contamination.

CHAPTER 6

THE EFFECT OF TEA AND COFFEE ON IRON ABSORPTION IN MAN

6.1 INTRODUCTION

In the previous study, the absorption of iron from fortified sugar in tea seemed lower than might be 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.

6.2 PREPARATION OF MEALS

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. This was followed immediately by either 200 ml of warm water, 200 ml of tea or 200 ml of coffee.

6.2.1 Iron Compounds

Every subject received 53.7 /umol iron on each of the two mornings. In different experiments the iron was administered as ferric chloride, or as ferrous sulphate together with 170.4 /umol ascorbic acid, as rabbit haemoglobin or as crystallised rabbit haem. The iron salts were freshly dissolved in 50 ml tap water, but the haemoglobin was administered in 50 ml preserved tomato juice in order to disguise its appearance. Radioactive haemoglobin was prepared by injecting male New Zealand white rabbits intramuscularly with 200 /uc of either ^{59}Fe of ^{55}Fe. After some weeks blood was obtained from a marginal ear vein. The erythocytes were separated by centrifigation and washed three times with sterile isotonic sodium chloride solution. They were suspended in distilled water, frozen to $-20^{o}C$ and thawed, and membranes were separated from the haemoglobin solution by centrifugation at 2500 x g. Radioactive haemoglobin in a dosage of 2.5 /uc was used in each study. It was either mixed with unlabelled haemoglobin or with mince to provide 53.7 /umol iron per person. Haem was extracted from the haemoglobin by the method of Labbe and Nishida (1957).

Radioactive haem was mixed with unlabelled haem to provide 53.7 /umol iron and 2.5 /uc per person.

6.2.2 Iron in Bread

Two loaves of white bread were baked, using 70-80% extraction flour. Sufficient $^{59}FeCl_3$ or $^{55}FeCl_3$ was mixed into the dough together with the yeast so as to provide 53.7 /umol and 2.5 /uc/100g bread, the quantity consumed by each subject. The bread was eaten without butter or jam.

6.2.3 Iron in Rice and Potato and Onion Soup

Sufficient rice intrinsically labelled with ^{55}Fe by hydroponic culture (Hussain et al., 1965; Layrisse et al., 1969) was mixed with carrier rice to provide 2.5 /uc ^{55}Fe and 45g 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 1kg peeled potatoes and 500g onions in 2 tablespoonfuls of sunflower seed oil. After adding 850ml water and 5g curry powder it was brought to the boil and allowed to simmer for 30 minutes. During this time 537,0 /umol iron as ferrous sulphate labelled with 25.0 /uc ^{59}Fe was added together with 5680.0 /umol 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}Fe as the label for the intrinsic rice iron and ^{59}Fe as the label for the added ferrous sulphate.

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}Fe and on the other ^{55}Fe.

6.2.4 Haemoglobin Iron in Minced Lamb

Enough minced lamb to provide a total of 53.7 /umol 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 µc 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 into the fried mince helpings.

6.2.5 Tea

The tea used has been previously described (Page 39). 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 volumesthe same.

6.2.6 Coffee

The 200 ml drunk by each individual was prepared from 5g dry instant coffee (Nescafe, Nestle, S.A. Pty., Ltd.). This brand is advertised as pure coffee and contains no additives such as chicory. The coffee was mixed with 40 ml pasteurised cow's milk before being drunk.

6.3 RESULTS

6.3.1 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 ferric chloride, ($t = 2.68$, $P = < 0.05$) and also from a solution of ferrous sulphate containing ascorbic acid ($t = 4.46$, $P = < 0.01$) (Table 15). Tea with milk produced much the same effect on the absorption of iron from a solution of ferrous sulphate 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 16). When 200 ml milk without tea was drunk after the solution of ferrous sulphate and ascorbic acid, absorption was also inhibited, ($t = 3.44$, $P = < 0.01$).

6.3.2 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 17). The absorption 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 18). 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 et al., 1972; Bjorn-Rasmussen and Hallberg, 1972; Sayers et al., 1973; Sayers et al., 1974a) 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 ^{59}Fe on the one occasion and ^{55}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 19) in spite of the presence of 568.0 /umol ascorbic acid.

6.3.3 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 20). Of greater practical importance, however, was the finding that tea had no significant effect on the absorption of haemoglobin from the fried lamb mince with rabbit haemoglobin gravy (t = 0.28, P = > 0.05). The conclusion that tea did not affect the absorption of haem iron from cooked food was checked by a comparison between the absorption of cooked and uncooked rabbit haemoglobin, administered in tomato juice and followed by a cup of tea on each occasion (Table 21). 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 cooked or uncooked ((Table 20), and this is in agreement with previous reports that cooking does not affect the absorption of haem (Callender et al., 1957; Turnbull et al., 1962). It therefore seemed justifiable to conclude that tea inhibits the absorption of haemoglobin iron only if it

had not been cooked. Finally the effect of tea on the absorption of crystallised rabbit haem was examined. No inhibition was found, the mean figures (± SD) in nine subjects being 11.8 (± 3.5)% with water and 10.6 (± 5.0)% with tea (Table 22) ($t = 0.82$, $P = > 0.05$).

6.3.4 Effect of Coffee on the Absorption of Iron from Ferrous Sulphate and Ascorbic Acid

In the single experiment performed, the drinking of coffee was found to inhibit the absorption of iron from a solution of ferrous sulphate with ascorbic acid ($t = 3.61$, $P = < 0.01$).

6.4 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 a comparison between the rate of iron absorption and the serum ferritin concentration. The percentage iron absorption from the different experiments were grouped into decades and were plotted against the mean log serum ferritin concentrations (Fig. 49). 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 ($t = 2.26$, $P = < 0.05$).

6.5 DISCUSSION

The results of the present study indicate that tea significantly inhibits the absorption of non-haem iron. 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 the same whether or not the tea contained milk.

Since meat is almost invariably cooked before it is eaten, little nutritional significance can be attached to the finding that tea interferes with the absorption of uncooked haem iron. If 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., 1974a) and if the already low percentage absorption were further reduced by tea, the nutritional problem would be aggravated. Since tea is commonly drunk with meals in many parts of the world, it may play some part in the pathogenesis of inadequate iron nutrition. Since these observations indicate that coffee has a similar effect, the nutritional implications of beverages may be even wider. It appears that all the non-haem iron in a given meal forms a common pool within the intestinal lumen (Bjorn-Rasmussen et al., 1972; Cook et al., 1972; Sayers et al., 1973), so that the presence in the meal of a promoter of absorption such as ascorbic acid, or an inhibitor such as tea, affects both the intrinsic and the supplemental iron. The maximum quantity of iron which can be absorbed is determined to a large extent by the distribution of the iron between the various competing ligands, some of which promote absorption while others render the iron unavailable (Forth and Rummel, 1973), and the success of any measures to improve iron nutrition depends as much on enhancing the availability of iron in the diet as on increasing its amount.

It was thus thought to be of some interest to investigate further the mechanism whereby tea influences iron absorption.

An interesting peripheral observation was the inverse correlation between the serum ferritin concentration and the percentage absorption of iron (Fig. 49). The relationship was similar to that found by Cook et al.,

(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 after the solution of ferrous sulphate with ascorbic acid can be ascribed to the effective sequestration of a proportion of the iron in unabsorbable complexes.

TABLE 15

Effect of tea on the absorption of iron salts

	Haemoglobin (g/dl)	Serum iron (/umol/l)	Unsaturated iron binding capacity (/umol/l)	% Saturation of transferrin	Serum ferritin (/ug/l)	% Iron absorption		
						Iron alone	Iron with 'black' tea	Reference salt
53.7 /umol iron as ferric chloride	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
	24.3	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±19.7)	6.2 (SD±3.9)	30.3 (SD±17.3)
53.7 /umol iron as ferrous sulphate + 170.3 /umol ascorbic acid	14.1	22.72	48.69	31.8	7	4.0	4.0	
	13.7	14.50	62.82	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	14.6	63.80	17.0	32.0	30.9 (SD±19.3)	11.2 (SD± 7.5)	

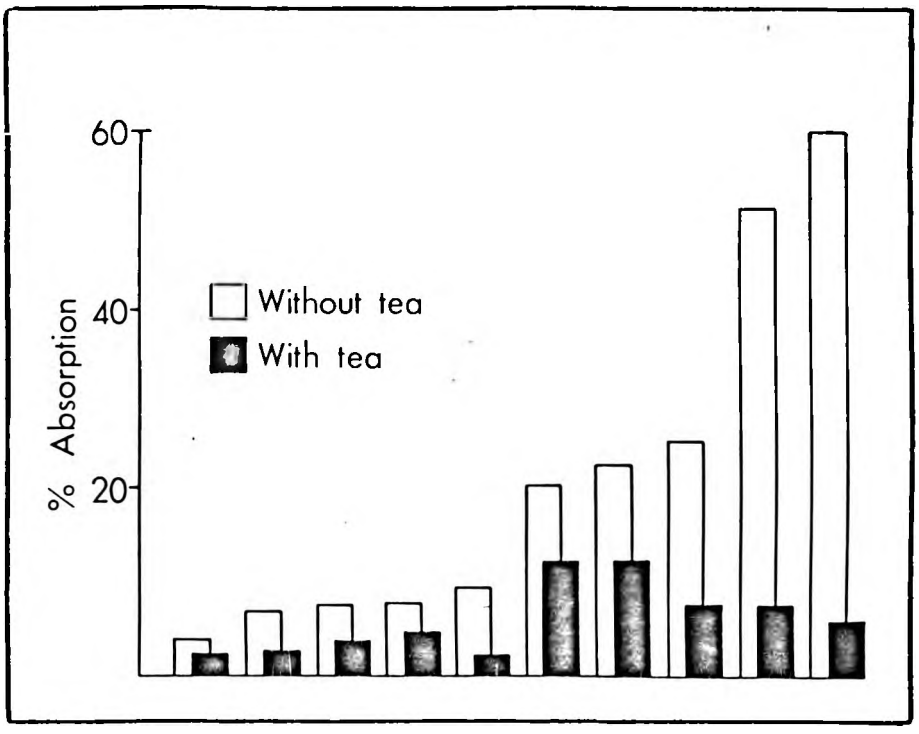

Fig. 38 Effect of tea on the absorption of 53.7 μmol iron as ferric chloride

TABLE 16

Effect of tea and milk on the absorption of 53.7 /umol iron as ferrous sulphate with 170.4 /umol ascorbic acid

Haemoglobin (g/dl)	Serum iron (/umol/l)	Unsaturated iron binding capacity (/umol/l)	% Saturation of transferrin	Serum ferritin (/ug/l)	% Iron absorption		
					Iron with 'black' tea	Iron with 'white' tea	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	21.5	13.3 (SD± 12.0)	15.9 (SD± 14.4)	57.0 (SD± 22.1)
					Iron alone	Iron with milk	
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	86.68	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	19.0	40.9 (SD± 22.2)	19.4 (SD± 10.2)	

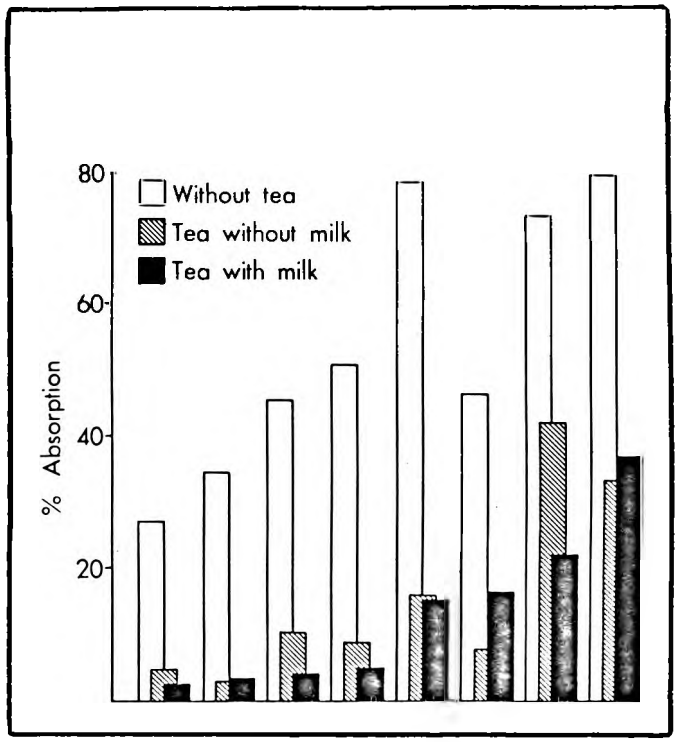

Fig. 39 Effect of tea and milk on the absorption of 53.7 μmol iron as ferrous sulphate and 170.4 μmol ascorbic acid

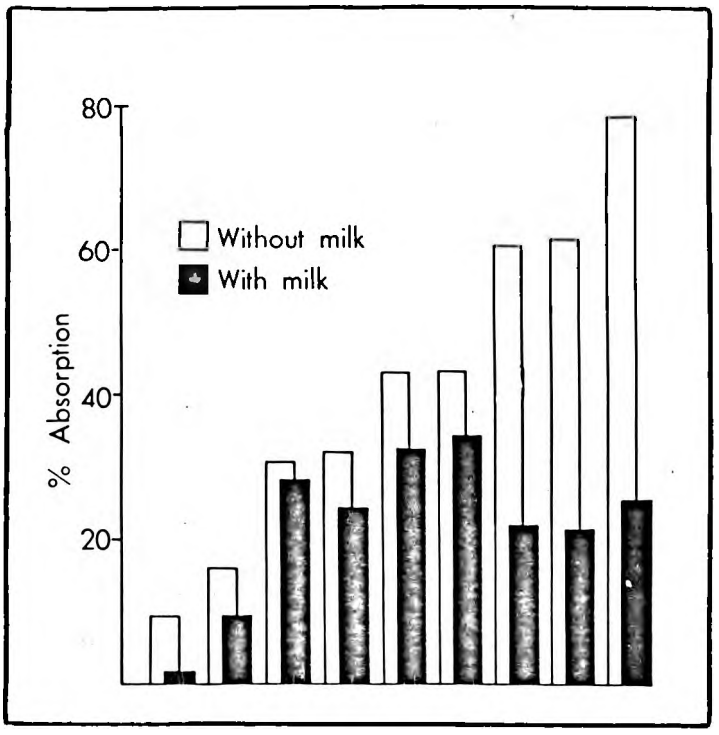

Fig. 40 Effect of milk on the absorption of 53.7 µmol iron as ferrous sulphate and 170.4 µmol ascorbic acid

TABLE 17

Effect of tea on the absorption of 53.7 /umol iron in white bread

Haemoglobin (g/dl)	Serum iron (/umol/l)	Unsaturated iron binding capacity (/umol/l)	% Saturation of transferrin	Serum ferritin (/ug/l)	% Iron absorption		
					Iron alone	Iron with 'black' tea	Reference
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	52.54	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± 4.4)	3.2 (SD± 3.0)	35.8 (SD± 25.0)

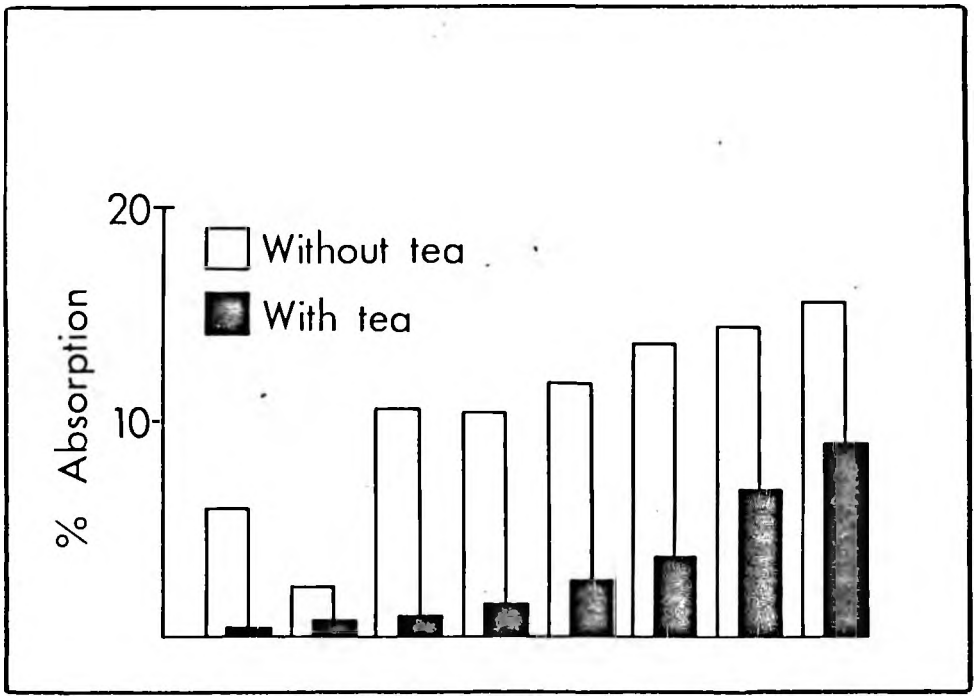

Fig. 41 Effect of tea on the absorption of 53.7 μmol iron in bread

TABLE 18

Effect of tea on the absorption of 53.7 µmol iron with 568.0 µmol ascorbic acid in intrinsically labelled rice and extrinsically labelled potato and onion soup

	Haemoglobin (g/dl)	Serum iron (µmol/l)	Unsaturated iron binding capacity (µmol/l)	% Saturation of transferrin	Serum ferritin (µg/l)	% Iron absorption			
						Intrinsic tag	Extrinsic tag	Extrinsic / Intrinsic	Reference Salt
Meal followed by 240 ml water	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± 6.1)	13.3 (SD± 6.8)	1.05 (SD± 0.17)	36.6 (SD± 11.8)
Meal followed by 240 ml tea	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± 2.0)	2.6 (SD± 2.0)	1.18 (SD± 0.27)	31.7 (SD± 12.2)

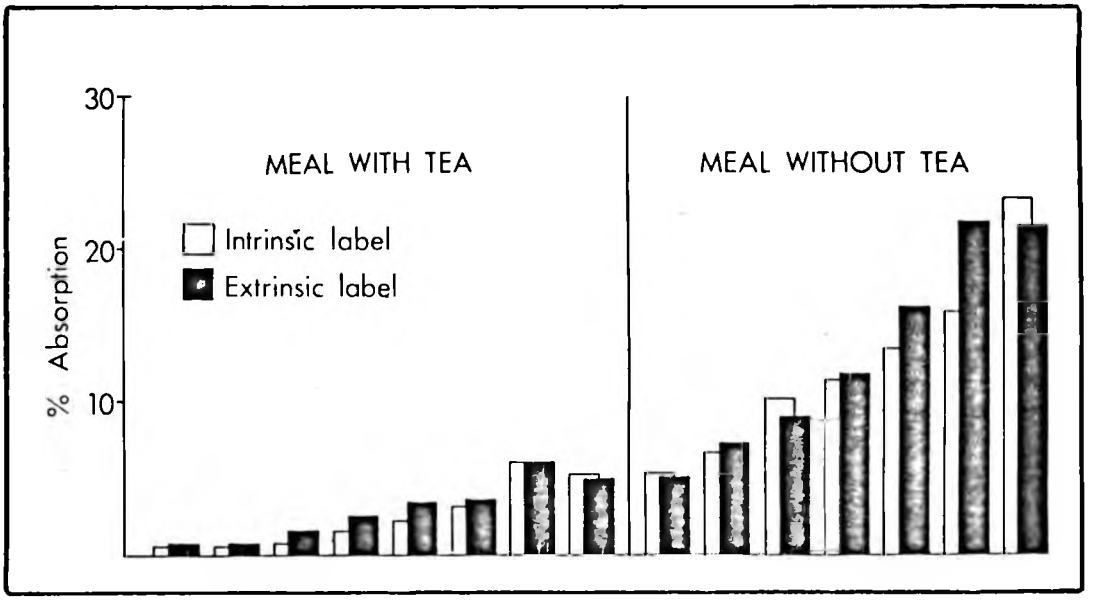

Fig. 42 Effect of tea on the absorption of 53.7 μmol iron in a rice meal containing 568.0 μmol ascorbic acid. Absorption of intrinsic iron compared with that of extrinsic tracer

TABLE 19

Effect of tea on the absorption of 53.7 µmol iron and 568.0 µmol ascorbic acid in rice and potato and onion soup

Haemoglobin (g/dl)	Serum iron (µmol/l)	Unsaturated iron binding capacity (µmol/l)	% Saturation of transferrin	Serum ferritin (µg/l)	% Iron absorption		
					Iron alone	Iron with 'black' tea	Reference 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± 4.2)	2.4 (SD± 1.7)	34.7 (SD±24.7)

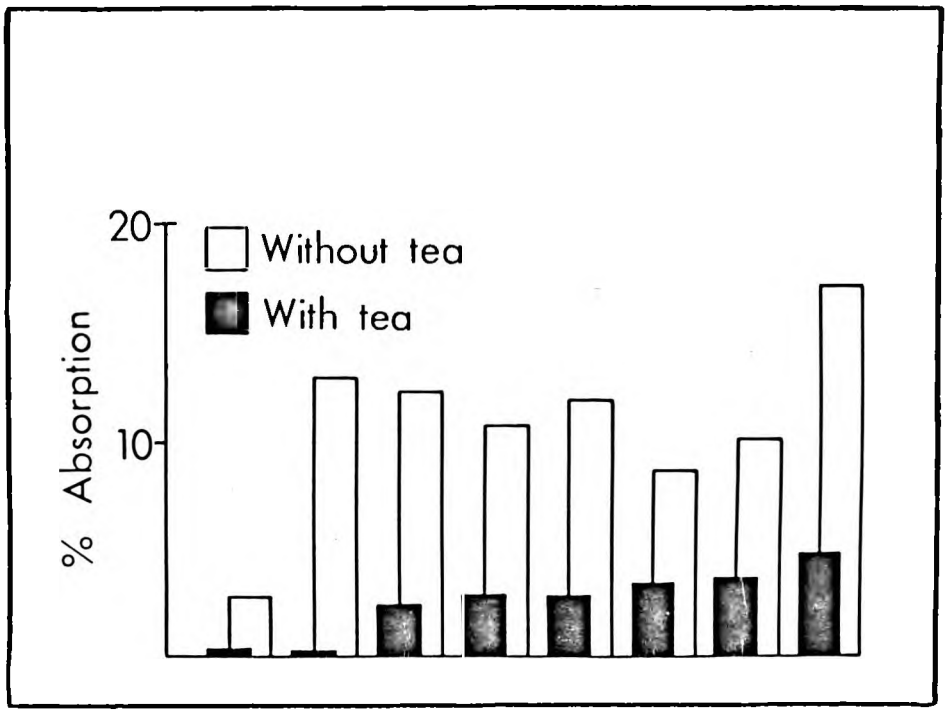

Fig. 43 Effect of tea on the absorption of 53.7 umol iron in a rice meal containing 568.0 umol ascorbic acid

TABLE 20

Effect of tea on the absorption of 53.7 /umol/1 iron in uncooked and cooked haemoglobin

	Haemo-globin (g/dl)	Serum iron (/umol/1)	Unsaturated iron binding capacity (/umol/1)	% Saturation of transferrin	Serum ferritin (/ug/1)	% Iron absorption		
						Iron alone	Iron with 'black' tea	Reference salt
Iron as uncooked haemo-globin	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	22.72	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	23.62	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
	Mean 12.5	16.75	69.60	19.7	20.7	14.7 (SD± 11.4)	6.0 (SD± 4.5)	36.6 (SD± 31.6
Iron as cooked haemo-globin	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
	Mean 12.7	14.79	51.34	22.8	27.1	14.3 (SD± 9.2)	13.5 (SD± 7.0)	30.5 (SD± 26.6)

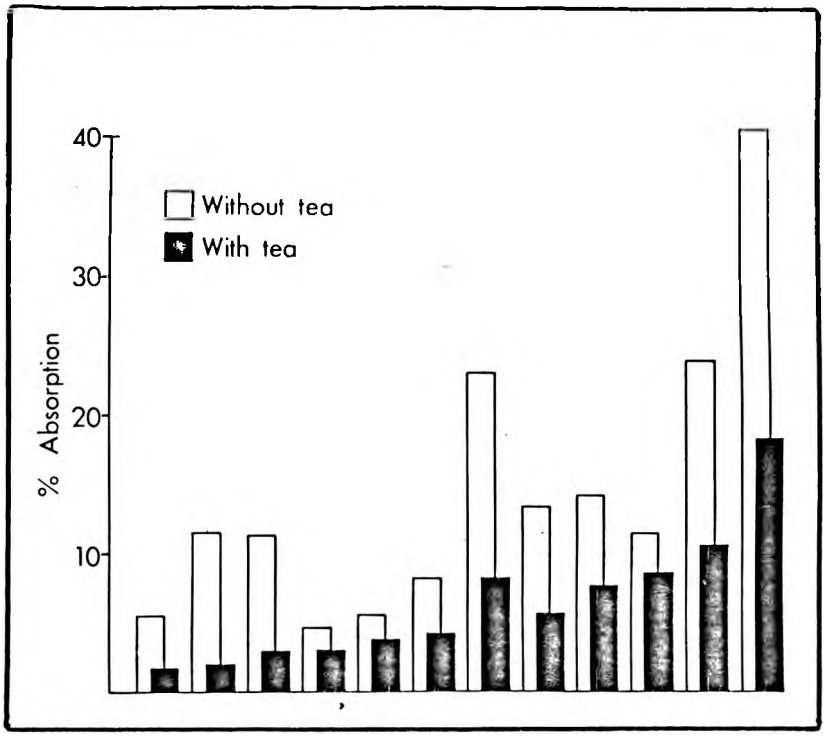

Fig. 44 Effect of tea on the absorption of 53.7 µmol iron as uncooked haemoglobin

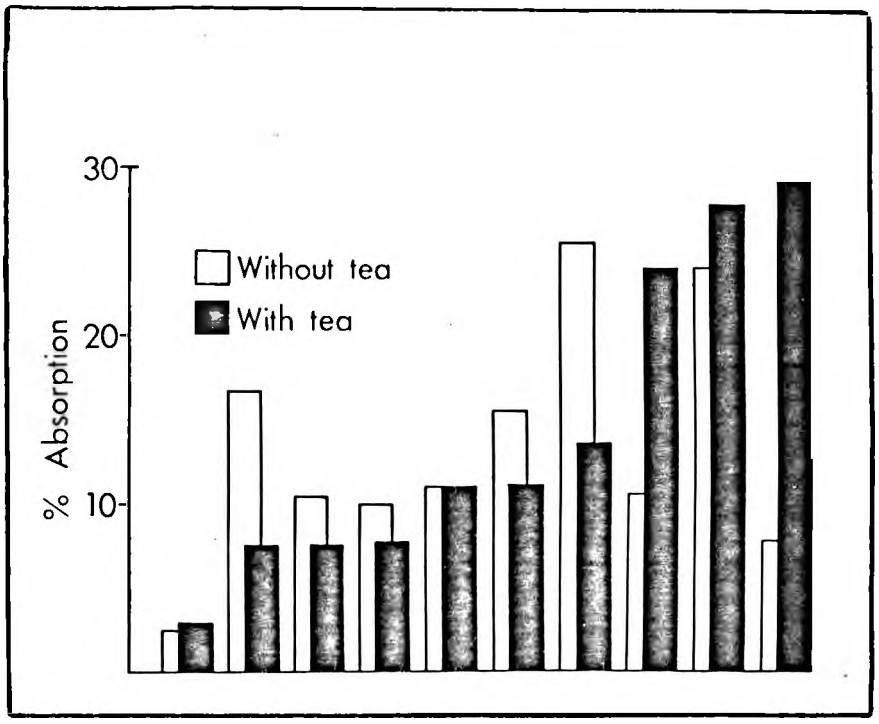

Fig. 45 Effect of tea on the absorption of 53.7 μmol iron as cooked haemoglobin

TABLE 21

Comparison of effect of tea on the absorption of 53.7 /umol iron in cooked and uncooked haemoglobin

Haemoglobin (g/dl)	Serum iron (/umol/l)	Unsaturated iron binding capacity (/umol/l)	% Saturation of transferrin	Serum ferritin (/ug/l)	% Iron absorption		
					Cooked Haemoglobin	Uncooked Haemoglobin	Reference 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± 5.5)	8.1 (SD± 5.0)	28.2 (SD± 17.1

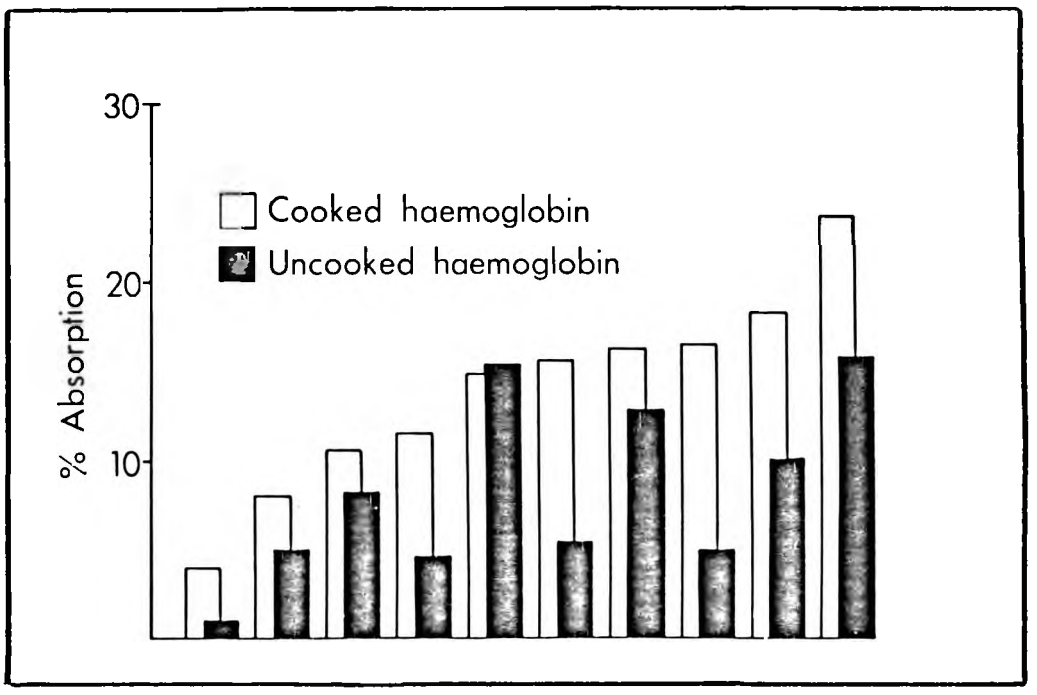

Fig. 46 Effect of tea on the absorption of 53.7 umol iron as either cooked or uncooked haemoglobin

TABLE 22

Effect of tea on the absorption of 53.7 /umol iron in haem

Haemoglobin (g/dl)	Serum iron (/umol/l)	Unsaturated iron binding capacity (/umol/l)	% Saturation of transferrin	Serum ferritin (/ug/l)	% Iron absorption		
					Iron alone	Iron with 'black' tea	Reference salt
9.9	5.19	73.25	6.6	28	14.0	5.7	34.5
11.0	20.06	54.63	26.9	3	12.5	8.6	19.7
11.4	17.91	53.73	20.0	19	6.8	9.0	18.3
13.3	11.28	45.49	19.9	16	9.9	9.2	21.8
14.1	24.54	56.42	30.3	23	10.8	11.7	21.5
11.4	5.19	62.33	7.7	7	18.8	11.8	61.9
12.5	26.15	41.55	38.6	6	13.7	12.0	50.9
11.8	9.31	64.12	12.7	3	9.0	12.1	100.0
9.1	2.69	78.62	3.3	2	10.6	15.2	100.0
Mean 11.6	13.58	58.89	18.4	11.9	11.8 (SD± 3.5)	10.6 (SD± 5.0)	47.6 (SD± 33.3

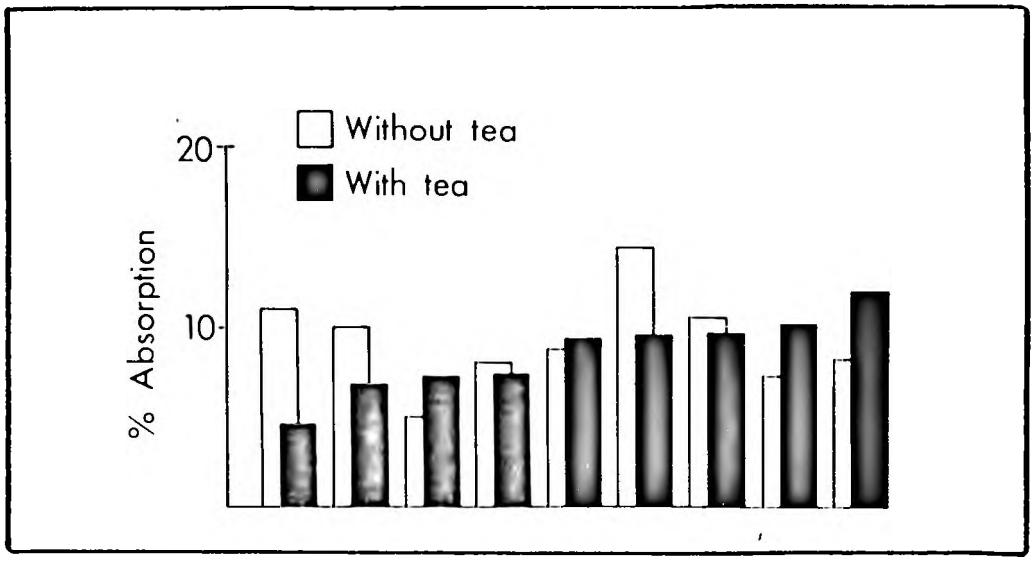

Fig. 47 Effect of tea on the absorption of 53.7 umol iron as haem crystallised from haemoglobin

TABLE 23

Effect of coffee on the absorption of 53.7 /umol iron as ferrous sulphate with 170.4 /umol ascorbic acid

Haemoglobin (g/dl)	Serum iron (/umol/l)	Unsaturated iron binding capacity (/umol/l)	% Saturation of transferrin	Serum ferritin (/ug/l)	% Iron absorption	
					Iron alone	Iron with 'black' coffee
9.9	10.03	23.82	29.6	40.0	0.7	0.5
14.1	20.24	50.69	28.5	15.1	11.4	3.7
11.8	24.18	49.61	26.4	9.3	19.9	4.8
10.6	21.49	38.15	36.0	6.0	33.5	16.0
12.2	20.95	50.15	29.5	8.0	33.5	17.0
12.5	20.24	49.43	29.1	10.0	24.0	18.0
13.7	12.90	53.55	19.4	1.0	50.8	34.7
10.3	7.70	81.67	8.6	1.0	71.3	53.2
Mean 11.9	17.19	49.61	25.9	11.4	30.1 (SD± 22.3)	18.5 (SD± 17.8

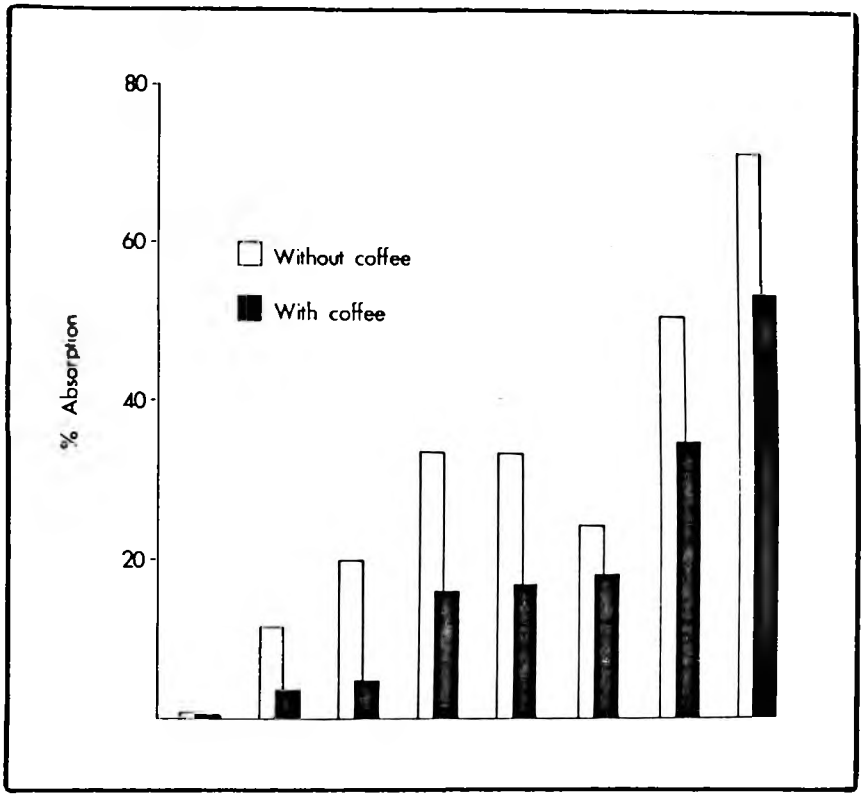

Fig. 48 Effect of coffee on the absorption of 53.7 /umol iron as ferrous sulphate and 170.4 /umol ascorbic acid

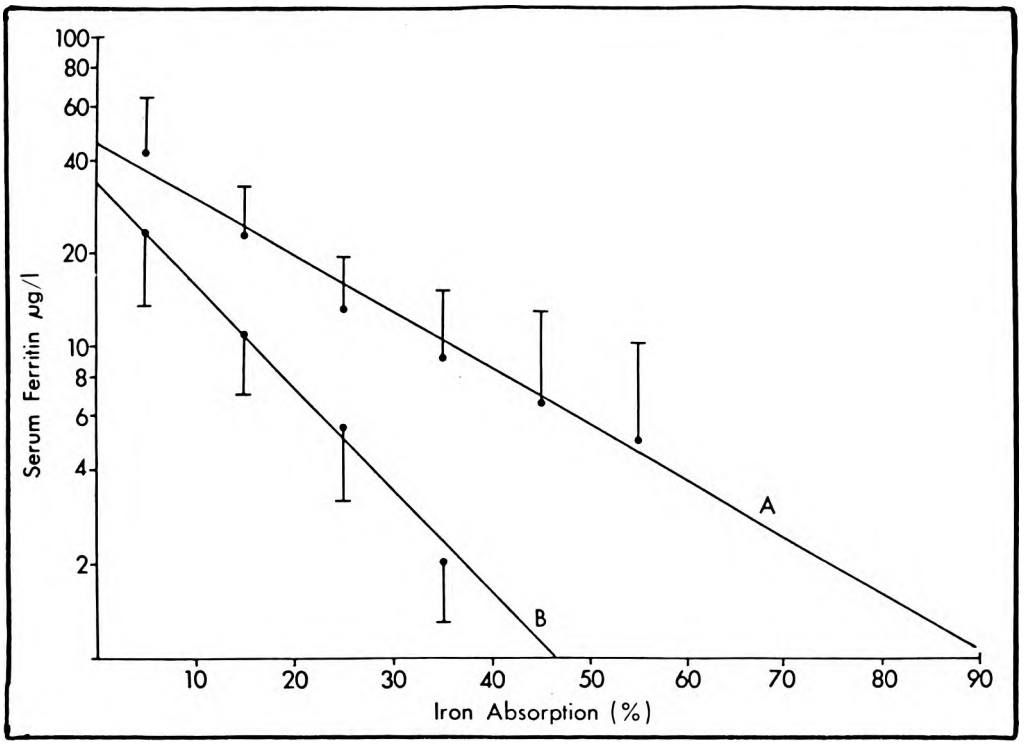

Fig. 49 Relationship between serum ferritin and absorption of ferrous sulphate and ascorbic acid

CHAPTER 7

THE MECHANISM OF THE INHIBITION OF IRON ABSORPTION BY TEA

7.1 INTRODUCTION

Drinking tea was shown to inhibit the absorption of both the non-haem iron in food and medicinal iron in the form of solutions of simple iron salts. It was thought to be of interest to investigate the mechanism involved.

7.2 MATERIALS AND METHODS

7.2.1 Tea

Tea was made by pouring 100 ml boiling water onto 2.5g dry leaves of a commercially available household tea (Pot o' Gold, O.K. Bazaars, Johannesburg), and decanting the supernatant liquid after five minutes.

7.2.2 Tea Tannin Solution

This was prepared by extracting 2.5g dry leaves with 100 ml 100% ethanol for 48 hours at room temperature (Slabbert, 1973). The supernatant was decanted and the tannins purified by the lead salt precipitation method (Roux, 1949).

Such an extract has been shown to contain about 95% of the tannins and about 5% of the sucrose and its hydrolysis products originally present in the tea (Roux, 1951). The extracted tannins were redissolved in 100 ml water. The residual tea leaves were dried in a rotary evaporator to remove the ethanol.

7.2.3 Tannin-free Tea

This was prepared from these leaves by pouring 100 ml boiling water over them and decanting the supernatant liquid after five minutes.

7.2.4 Iron Solutions

Solutions containing 100 µmol iron/100 ml distilled water were freshly prepared from ferrous sulphate ($FeSO_4.7H_2O$), ferric chloride ($FeCl_3.6H_2O$) or ferrous sulphate with L-ascorbic acid (all Analar grade from BDH, Poole, England), the molar ratio of iron : ascorbic acid being 1 : 3.18.

7.2.5 Caffeine Solution

This was prepared by dissolving 125 mg caffeine (Merck, Darmstadt), the approximate amount present in 2.5g dry tea leaves (Kaplan et al., 1974), in 100 ml distilled water.

7.2.6 Tannic Acid Solution

This was made by dissolving 400 mg tannic acid (pentadigalloyl glucose) (BDH, Poole, England), the estimated molar equivalent of the tannins in the tea, in 100 ml distilled water.

7.2.7 Optical Spectra of the Complexes Formed on Mixing Iron Solutions with Tea

One millilitre of iron solution was added to 2.0 ml aliquots of tea and the pH was adjusted with either 6N hydrochloric acid or solid sodium bicarbonate to produce a range of values between 1.0 and 9.5. The final volume of each mixture was made up to 4.0 ml with distilled water, and its optical spectrum between 200 nm and 800 nm was determined using a Unicam SP 1800 Ultra-violet Spectrophotometer.

7.2.8 Estimation of the Molar Ratio of the Complexes Formed on Mixing Iron Solution with Tea

The molar ratio of iron to ligand was estimated by the continuous variation method (Harvey and Manning, 1950). Ferrous sulphate solution (1.0 ml) was mixed with 2.0 ml of various dilutions of tea ranging from 10% up to full strength. The pH of aliquots of each mixture was adjusted to 2.0, 5.5 and 8.0 as before, and the volume was again made up to 4.0 ml with distilled water. The optical density of each sample was then measured, the wavelength for the pH 2.0 samples being 610 nm, for the pH 5.5 samples, 580 nm and for the pH 8.0 samples, 500 nm.

7.2.9 Estimation of the Molecular Size of the Iron-tea Complexes

Samples were prepared by adding 1.0 ml ferrous sulphate solution labelled with 0.5 μc $^{59}FeCl_3$ to 2.0 ml tea,

adjusting the pH to 2.0, 5.5 or 8.0 respectively and making up the volume of each sample to 4.0 ml with distilled water as before. Each sample was submitted to dialysis at 37°C for 24 hours against one litre of the appropriate buffer solution, which was repeatedly changed. The pH 2.0 buffer was KCl/HCl, and the pH 5.5 and pH 8.0 buffers were KH_2PO_4/Na_2HPO_4 (Documenta Geigy Scientific Tables,1970). The amount of iron that had crossed the membrane was determined by estimating the radio-activity remaining in the bag, using a Packard Armac small animal whole body scintillation spectrometer (Model 3003).

In addition, 2.0 ml aliquots were subjected to gel filtration at pH 2.0, 5.5 and 8.0 using columns of Sephadex G10, G15 and G25 which had been equilibrated with the appropriate buffer, and eluting with the buffer solution. The ^{59}Fe activity present in 2.0 ml serial aliquots of the eluant was determined.

7.2.10 Absorption Studies

Groups of 10 male Sprague-Dawley rats weighing between 150 g and 200 g were fasted overnight. An oesophageal cannula was used to administer 0.22 μmol iron labelled with 0.1 μc ^{59}Fe in 0.5 ml water mixed with 1.0 ml of either water, tea, tea tannin solution, tannin-free tea, tannic acid solution or caffeine solution. This dose of iron was selected in order to be able to compare the results with those obtained in human studies; the approximate molar ratio of iron : tannin was 1 : 10. In one experiment the tea was given at various intervals up to 3 hours before the iron solution. No food was given to the rats for 4 hours after the iron solution had been administered. Seven days later the percentage absorption of the iron was determined by placing each animal in a Packard Armac small animal whole body scintillation spectrometer (Model 3003), and comparing the radio-activity with that present in suitably prepared standards.

7.3 RESULTS

7.3.1 Optical Spectra of the Complexes Formed on Mixing Iron Solutions with Tea

There were two peaks in the ultraviolet spectrum of tea, at just over 200 nm and at 280 nm, and one in the visible spectrum at 420 nm (Fig. 50). The ultra-violet peaks corresponded to those described for tannins by Roux (1957) and were not altered by changing the pH or adding iron solution. The addition of any of the iron solutions produced an immediate blackish discoloration which was accompanied by a displacement of the peak in the visible spectrum, its new position being dependent on the pH. When the pH was below 3.0 the peak was at 610 nm, at pH values between 4.0 and 6.0 it was at 580 nm, while it was 500 nm if the pH was higher than 7.0 (Fig. 51). These peaks appeared to be similar to those described by Slabbert (1970) for the complexes of iron with wattle tannin.

7.3.2 Estimation of the Molar Ratio of the Complexes Formed on Mixing Iron Solution with Tea.

At pH 2.0 the optical density had almost reached a plateau in the 20% tea : iron mixture, whereas at pH 5.5 this point was only attained in the 40% tea : iron mixture, and at pH 8.0 in the 60% tea : iron mixture (Fig. 52).

7.3.3 Estimation of the Molecular Size of the Iron-tea Complexes

On dialysis almost all the ^{59}Fe crossed the membrane when the pH was 2.0, but when it was 5.5 or 8.0 almost none of it did. On gel filtration at pH 2.0 all the radioactive iron was eluted as a single peak which was in the void volume of the G10 column, but was retarded on both the G15 and the G25 columns, indicating a molecular weight between 700 and 1500 daltons. At both the other pH values the elution of the iron complex was retarded only on the G25 column, so that the molecular weight was between 1500 and 5000 daltons.

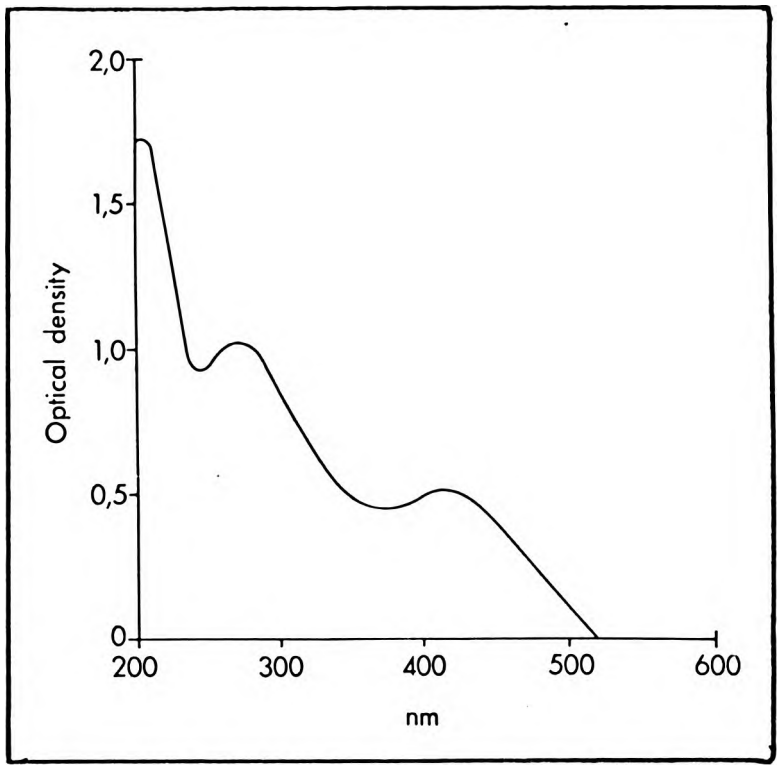

Fig. 50 Optical spectrum of tea

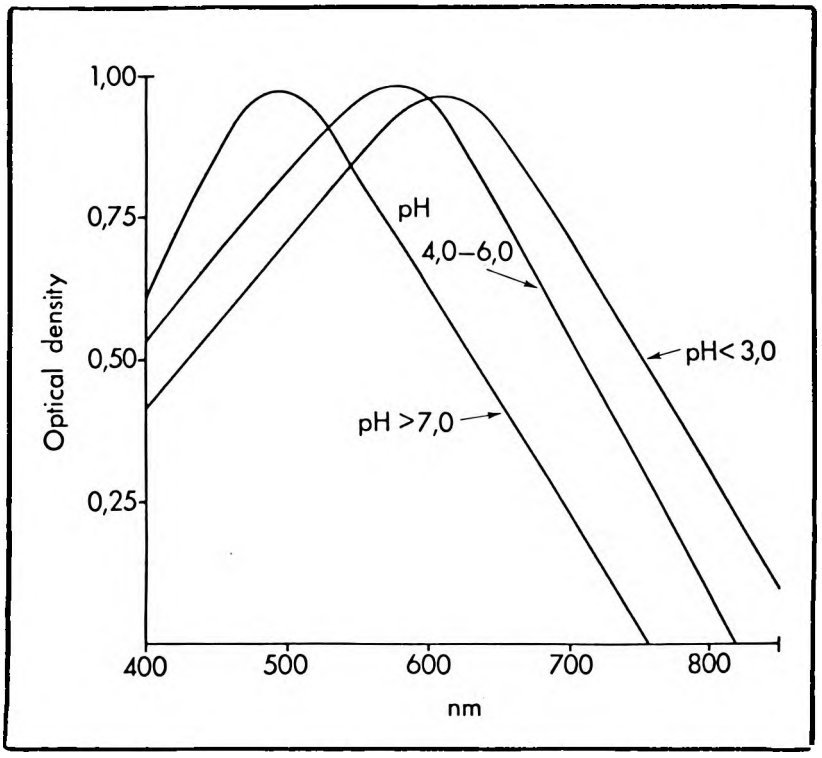

Fig. 51 Optical spectrum of iron-tea mixtures at various pH values

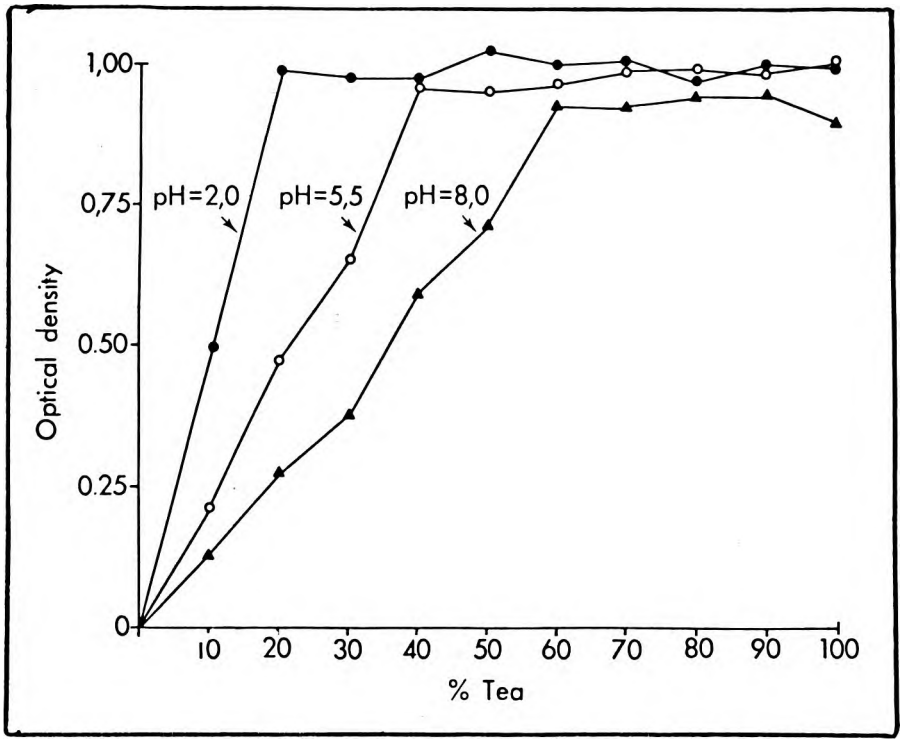

Fig. 52 Optical densities of mixtures of iron solution with increasing concentrations of tea. When the pH was 2.0 a plateau was reached at 20% tea, but at pH 5.5 the plateau was at 40% and at pH 8.0, at 60%. Since there was 1 µmol iron in every mixture and 5 µmol tannins in 100% tea, the molar ratios of the complexes formed were probably 1:1, 2:1 and 3:1 respectively

TABLE 24

Effect of tea, tannin solution, tannin free tea, tannic acid solution and caffeine solution on iron absorption in rats

Iron salt	Possible inhibitor	Absorption of iron: mean % ± SD	Significance of difference from control absorption	
			t	P
Ferrous sulphate + ascorbic acid	tea	15.0 ± 6.8	5.03	< 0.001
	control	48.9 ± 20.2		
ferric chloride	tea	21.6 ± 7.3	5.85	< 0.001
	control	42.4 ± 8.4		
ferric chloride	tea	13.8 ± 5.6	9.95	< 0.001
	tea tannin solution	16.1 ± 10.3	7.71	< 0.001
	tannin free tea	49.6 ± 7.5	0.84	> 0.05
	control	53.2 ± 11.2	-	
ferric chloride	tea	21.7 ± 8.7	5.35	< 0.001
	tannic acid solution	24.1 ± 2.6	6.83	< 0.001
	control	40.9 ± 7.3	-	
ferric chloride	caffeine	56.4 ± 8.0	1.12	> 0.05
	control	61.7 ± 12.6		

TABLE 25

Effect of tea administered at various intervals before the iron solution($^{59}FeCl_3$) on iron absorption in rats

Interval between tea and iron solution (hours)	Absorption of iron: mean % ± SD	Significance of difference from control absorption	
		t	P
0	27.6 ± 13.9	7.56	< 0.001
½	50.7 ± 14.1	3.60	< 0.005
1	56.7 ± 10.5	2.99	< 0.01
2	64.7 ± 11.3	1.38	> 0.05
3	61.2 ± 12.6	1.94	> 0.05
control	72.0 ± 12.3	-	-

7.3.4 Absorption Studies

The absorption of iron was inhibited by tea (Table 24). The tea tannin solution and the tannic acid solution had a similar effect, but tannin-free tea and the caffeine solution produced no inhibition. When the tea was administered together with the iron solution, the effect was more marked than when it was given some time before the iron, but a significant inhibition was demonstrable even if the interval between the administration of the tea and the iron solution was as long as 1 hour (Table 25).

7.4 DISCUSSION

The results of the present study indicate that tea inhibits the absorption of non-haem iron in rats as well as man. Tea from which the tannins had been extracted had no such effect, but the tea tannins alone inhibited absorption to much the same extent as did the original tea, as did tannic acid. It therefore seems reasonable to conclude that the inhibition of iron absorption by tea is due to its tannin content, and not to any other ingredient.

Tannins are polyphenols, and are very widely distributed throughout the vegetable kingdom. In tea leaves nine different tannins can be identified by paper chromatography (Haslam, 1969). The constitute about 10% by weight of the dried leaves, or up to 35% of the solids (Roberts, 1962; Bokuchava and Skobeleva, 1969). Tea made as in the present study contains about 80% of the tannins (Roberts, 1962).

Evidence has been obtained that tannins can interfere with the transport of substrates across membranes, possibly as a result of protein precipitation (Luciani, 1972) and the fact that absorption was inhibited when the tea was given as long as one hour before the iron solution (Table 25) is compatible with such an action on the duodenal mucosa. A more likely explanation for the inhibition of iron absorption, however, is that the tannins form complexes with the iron within the lumen of the gastrointestinal tract and thus render it unavailable for absorption, as do phytates. It has been known for centuries that a blue-black colour develops when iron is in contact with certain vegetable matter.

Pliny in his Naturalia Historia (79 AD) described a test for the detection of iron as an adulterant of verdigris (Lillie, 1972) in which the sample is placed in papyrus which has been soaked in oak-galls and if iron is present the papyrus turns black. During the nineteenth century botanists identified the vascular channels in leaves by placing them in ferrous sulphate solution and observing the spread of the blue-black pigment (Lillie, 1972). The colour is due to the formation of iron-tannin complexes, and the reaction is commercially important in the manufacture of many inks and dyes. Wattle tannins form co-ordination compounds with iron by displacement of protons from either the orthodihydroxybenzene (pyrocatechol) or vicinaltrihydroxybenzene (pyrogallol) nuclei (Roux, 1951; Slabbert,1970; Slabbert, 1973). Polymerisation occurs at higher pH values until a stable octahedral complex is formed with three ligands. The results obtained in the present investigation suggest that the black discoloration seen on adding iron salts to tea represents a similar reaction. Not only did the optical spectra of both the tea and the mixture of tea and iron solution resemble those described for wattle tannins and iron-tannin complexes (Roux,1958; Slabbert,1973), but the evidence from the molar ratio experiment suggested that altering the pH had a similar effect on the proportion of iron to tannin in the complex (Fig. 52). The molecular weight of tea tannins is about 800 (Roberts, 1962; Bokuchava and Skobeleva, 1969), and since there was approximately 200 mg tannin in 100 ml tea, 2 ml contained about 5 μmol. There was roughly 1 μmol iron in 1 ml of the iron solution, and the fact that the optical density did not increase further when tea stronger than 20% was mixed with it at pH 2.0 (Fig. 52) indicates that the molar ratio of the complex formed under these conditions was probably 1:1. Similar calculations suggest that the ratio was 2:1 at the intermediate pH values and 3:1 when it was above 7.0. The evidence from the gel filtration studies is consistent with these interpretations, since it suggests that a single species of iron tannin complex exists at each

pH tested and that the molecular weight of the low pH complex was 700 - 1500 daltons while that of each of the other two was between 1500 and 5000 daltons.

Haem iron is absorbed as such, and it is only within the mucosal epthelial cells that the iron is liberated from the porphyrin (Weintraub et al., 1968). Luminal chelators including ascorbic acid have been shown not to influence its absorption (Callender et al., 1957; Turnbull et al., 1962; Conrad et al., 1967), and the significant inhibition produced by tea in the present study (Chapter 6) was therefore surprising. An agent capable of chelating ionic iron is most unlikely also to be able to form complexes with haem (Conrad, 1970). These considerations suggested that tea might inhibit the absorption of haem iron by a mechanism different from that responsible for the inhibition of non-haem iron absorption. The observation that tea had no inhibitory effect if the haemoglobin were cooked strengthened this possibility, since cooking as such does not affect the absorption of haem iron (Callender, et al., 1957; Turnbull et al., 1962). 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 (Haslam, 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 thus 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). Further supportive evidence was provided too by the finding that tea had no effect on haem iron absorption per se. The effect of tea thus seems to depend on the presence of an intact globin moiety.

The finding that tannins are the constituent of tea which

inhibits iron absorption, may have much wider implications. Tannins are found in all vegetable foods, and it is reasonable to postulate that the poor availability of iron from these foods in general may be partly attributable to their tannin content.

CHAPTER 8

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