THE EFFECT OF ASCORBIC ACID SUPPLEMENTATION ON THE ABSORPTION OF IRON FROM SOME VEGETABLE STAPLES

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This thesis is my own work. No part of it has been presented at any other University. I obtained the information used in this thesis while employed by the University of the Witwatersrand.

M.H. Sayers. May 1977

M.H. Sayers

Abstract

The studies described in this thesis measured iron absorption from four staples; maize, wheat, soya and rice, and investigated how they could be improved as sources of available iron. The staples were fed as maize porridge, whole wheat bread, soya biscuits or cooked rice with either pea dhal or potato and onion soup.

Absorption was measured in 198 human volunteers. All but 4 were Indian housewives living in Chatsworth, a lower socio-economic housing scheme on the outskirts of Durban. This group was chosen because iron deficiency has been shown to occur commonly amongst them, an observation which was confirmed when marrow aspirates in 61 of the first 82 volunteers failed to reveal stainable iron. Haemoglobin, serum iron and percentage saturation of total iron binding capacity determinations were made on all of the subjects. In addition, absorption from 3 mg of iron as ferrous ascorbate was measured in the fasting state.

The contribution to iron absorption from both the intrinsic iron present in each staple, which had been labelled with ^{55}Fe during hydroponic cultivation, and various added inorganic iron salts, labelled with ^{59}Fe, was measured. In this way it was possible to confirm the fact that the ratio of absorption of the added iron (extrinsic tag) to that of the intrinsic iron was close to unity over a wide range of absorptions. The implication was that dietary non-haem iron absorption takes place from a common pool.

In some studies, absorption from this pool was measured with an extrinsic tag only. By using ^{55}Fe or ^{59}Fe as the radio-iron component of this tag on different occasions, it was possible to measure iron absorption in the same individuals from two separate meals that differed in only one constituent.

Mean iron absorption from the staples was as follows :- soya 19.8%, bread 7.9%, maize 6.8% and rice 3.5%.

When ascorbic acid was added, before cooking, to maize porridge fortified with iron as ferric ammonium citrate, there was a significant enhancement in absorption of both the intrinsic and added iron. No effect was noted with soya biscuits or whole wheat bread. Evidence was obtained that this was due to the destruction of the added ascorbic acid during the high temperatures achieved in baking.

The addition of ascorbic acid, before cooking, also produced a significant enhancement in the absorption of both the intrinsic iron in rice and added iron in the form of ferrous sulphate.

Because a successful fortification programme must establish a suitable vehicle, the iron and ascorbic acid in the rice studies was added to the salt used during cooking. Although chemical grade sodium chloride tolerated these additives, the coarse, undesiccated salt used by the volunteers became discoloured. Heat, humidity and the presence of more soluble iron salts accelerated discoloration in the fortified salt, while the addition of starch and iron as ferric orthophosphate satisfactorily retarded any change in appearance.

When coarse cooking salt, containing added starch, ascorbic acid (50 mg) and iron as ferric orthophosphate (4 mg) was tested, there was a threefold enhancement in the absorption of the intrinsic and added iron from a rice meal and an eightfold enhancement in absorption from a maize porridge meal. The replacement of ferric orthophosphate in the fortified cooking salt with ferrous sulphate did not increase iron availability.

It was concluded that crude common salt could be fortified with 0.1% iron as ferric orthophosphate and 1.25% ascorbic acid and stored under all but the most extreme tropical conditions, provided that 2.5% starch was added, and that such fortification would significantly improve the iron nutrition in countries where the staple food is rice or maize.

Acknowledgments

I am indebted to Professor T.H. Bothwell. The opportunity to work under his supervision and guidance was a singularly rewarding and indelible experience. Indelible by virtue of the fact that I am very aware that many of my attitudes and approaches, even to matters outside the immediate concern of academic medicine, are a reflection of what I learned from my supervisor.

Professor R.W. Charlton first introduced me to scientific method when I worked as an undergraduate in his department. My decision to apply for a post as a Research Fellow, after graduating, is directly attributable to the enthusiasm that he engendered in me for a disciplined, purposeful way in which to set about problems. As it happened frequently that enthusiasm was not matched by skill, I relied heavily on his expertise.

Throughout the course of the studies to be described in this thesis Professor J. Torrance provided invaluable biochemical insight. No one individual could be more illustrative of the value of collaboration between disciplines.

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The effects of ascorbic acid supplementation on the absorption of iron in maize, wheat and soya.
Sayers, M.H., Lynch, S.R., Jacobs, P., Charlton, R.W., Bothwell, T.H., Walker, R.B. and Mayet, F.
British Journal of Haematology (1973) 24, 209

Iron absorption from rice meals cooked with fortified salt containing ferrous sulphate and ascorbic acid.
Sayers, M.H., Lynch, S.R., Charlton, R.W., Bothwell, T.H., Walker, R.B. and Mayet, F.
British Journal of Nutrition (1974) 31, 367

The fortification of common salt with ascorbic acid and iron.
Sayers, M.H., Lynch, S.R., Charlton, R.W., Bothwell, T.H., Walker, R.B. and Mayet, F.
British Journal of Haematology (1974) 28, 483

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Chapter One

NUTRITIONAL ASPECTS OF IRON DEFICIENCY

1.1 Iron Deficiency Anaemia

Iron deficiency is probably the commonest cause of anaemia in the world (Bothwell and Finch, 1962) and, while the disease is more prevalent in economically undeveloped countries, an estimated eighteen million people in the United States alone are iron deficient (Fairbanks and Beutler, 1972).

An accurate assessment of the prevalence of the condition is, however, made difficult by the fact that there are no hard and fast criteria for what constitutes iron deficiency. While changes in biochemical and haematological parameters can be measured in individuals who are iron deficient, these alterations are evident before clinical signs and symptoms can be elicited. On this basis, some authors feel that it is unrealistic to diagnose iron deficiency in asymptomatic subjects. This problem has been partly resolved by the introduction of the terms "iron depletion" and "iron deficiency with and without anaemia". Iron depletion is assessed on the state of marrow iron stores. Iron deficiency without anaemia implies absent stores and a transferrin saturation of 15% or less and iron deficiency with anaemia is present in men or women with the above features and haemoglobin and haematocrit values of less than 14 g/100 ml and 42% and 12 g/100 ml and 36% respectively (Committee on Iron Deficiency, 1968). More recently an immunoradiometric assay for serum ferritin (Addison et al., 1972; Miles et al., 1974) has been used to provide further insight into an individual's iron status.

Adoption of criteria as suggested by the Committee on Iron Deficiency, however, ignores the overlap between normal and anaemic

populations. This has been demonstrated by Garby and co-workers (1969), who gave medicinal iron to a random sample of Swedish women. Some of the women responded with a rise in their haematocrit and the assumption was made that these women must have been iron deficient. When the initial haematocrits of responders and non-responders were compared, however, they were seen to overlap, suggesting that it is not possible to separate the two populations by adopting any particular value.

The state of iron depletion or iron deficiency reflects a disparity between iron loss and iron absorption. Iron loss due to abnormal bleeding occurs more commonly and places iron balance in jeopardy when the amount of blood exceeds a few millilitres daily. Pathological iron loss in the female occurs most commonly as a result of bleeding from the genital tract. In both sexes, the gastro-intestinal tract is another important source of blood loss. In Western countries, bleeding is usually accounted for by peptic ulcers, haemorrhoids, hiatus herniae, gastritis, diverticular disease or neoplasms. By comparison, the single most important cause of iron deficiency on a world-wide basis is hookworm infestation, which may affect 450 million people (Farid et al., 1956).

At the absorption level, although steatorrhoea or gastric surgery may occasionally be incriminated as producing iron deficiency or iron depletion, the commonest problem is nutritional in that the individual is unable to obtain sufficient iron from the diet to remain in iron balance. As could be expected, nutritional iron deficiency is most prevalent when physiological demands are greatest, as occurs during growth, pregnancy and lactation.

The risk of nutritional iron deficiency is highest in countries where vegetables provide the bulk of the dietary composition (W.H.O., 1970). The limited availability of iron from these diets has been demonstrated in a number of studies which have shown poor absorption from such staples as wheat, maize and rice (Hussain et al., 1965; Layrisse et al., 1969).

By comparison, iron balance is more often positive in higher socio-economic communities. This is because the presence of meat in a meal not only enhances iron absorption from vegetables Layrisse et al., 1968), but also provides a more available source of iron (Moore, 1961).

1.2 Food Iron Fortification

The question as to whether individuals who are prone to nutritional iron deficiency should have their diets fortified has recently aroused considerable debate.

In an article which focussed attention on some of the controversies concerning iron deficiency, Walker (1969) raised a number of issues that are pertinent to the work covered by this thesis. He pointed out that information is lacking about the level of haemoglobin below which ill health is apparent, or above which clinical benefit can be anticipated by iron supplementation. He also highlighted the gross difference of opinion over the daily iron requirements as laid down by various dietary-standard bodies (W.H.O., Expert Committee Report, 1965; Council on Foods and Nutrition, 1968; Department of Health and Social Security, 1969) and questioned the extent to which an anaemic individual is at a health disadvantage when compared with haematologically "normal" subjects.

Similar queries have been raised by other workers. In Great Britain, where iron deficiency anaemia is considered sufficiently deleterious t public health to provoke substantial effort towards its prevention, Elwood (1970) has stated, "if anaemia matters, it should be possible to detect associations between circulating haemoglobin level and realistic indices of morbidity or function". It is perhaps ironical that the same author (Elwood, 1968) has demonstrated that the powde ed iron used to fortify flour in Great Britain is almost completely ineffective.

Evidence suggesting that mild anaemia is not as important as is generally believed has come from a number of authors. Cotes and co-workers (1969) were unable to demonstrate clinically or statistically important effects on cardiac function during exercise in women before and after correction of anaemia. Elwood (1970) did not show any beneficial effect from iron in the amelioration of fatigue, headache, palpitations, dizziness, irritability and breathlessness in a group of anaemic women whose haemoglobin levels were below 10.5 g/100 ml. In another study (Elwood and Hughes, 1970), psychomotor function was not influenced by iron deficiency.

The controversy is heightened by the postulate that iron deficiency anaemia is actually advantageous under some circumstances. The mean cholesterol level was significantly lower in a group of women with haemoglobin levels below 10.5 g/100 ml than in a group with higher levels (Elwood et al., 1970). A post mortem study (Zoll et al., 1951) showed a greater incidence of interarterial coronary anastomoses in the hearts of anaemic subjects. It is suggested that these observations may in part account for the fact

that arteriosclerotic vascular disease is rare in developing communities. Alternatively, diets which do not provide enough iron are also not atherogenic. In other areas there is less dissension about the deleterious effects of iron deficiency, especially with regard to its consequences for pregnancy, physical performance and resistance to infection.

Severe anaemia during pregnancy increases maternal morbidity and mortality (Llewellyn-Jones, 1965). There is also some evidence pointing to a greater incidence of stillbirths, neonatal deaths and foetal malformations in the presence of maternal iron deficiency (Roszkowski et al., 1966). As an indication of the number of mothers "at risk" in this context, a recent World Health Organization Scientific Group report on nutritional anaemia provides some disconcerting statistics. At Hadassah University Hospital, Jerusalem, 35% of pregnant women had haemoglobin values less than 11 g/100 ml during their last trimester. Comparable figures in other parts of the world were: Warsaw, 21.8%; New Delhi, 69%; Vellore, 56% and Caracas, 37%. In the Vellore group of women, 10% had haemoglobin values less than 6 g/100 ml.

With regard to growth, there is evidence to suggest that the body weight in infants is affected by iron deficiency. Judisch et al. found two patterns of response where weight measurements were available before and after treating iron deficiency (1966). One group showed a rapid weight gain in early infancy, followed by a fall off when iron deficiency developed. The second group had a normal weight gain which was accelerated after giving iron. In another study, weight gain from birth was consistently higher in infants given iron than in controls, but was only statistically

significant at the age of two (Burman, 1971). While accepting these observations, Garrow (1967) has nonetheless questioned the assumption that children who are small are necessarily malnourished.

The effects of iron deficiency on children may, however, be more subtle. Howell suggests a defect in attentiveness in mildly iron deficient children (Ross Conference, 1970). She described anorexic, irritable and inactive children whose behaviour improved following iron therapy. She found that tests of sustained interest in a learning task were significantly impaired in iron deficient five year olds and these were improved by iron treatment, unrelated to change in haemoglobin.

Considerable interest has recently been shown in defining the circumstances under which iron malnutrition adversely affects physical performance. This was in part prompted by an understanding of the adaptive mechanism that accounts for relatively slight increases in cardiac output and decreases in mixed venous oxygen tension observed in anaemic patients. As early as 1938, Isac et al. described a rightward displacement of the oxygen dissociation curve in anaemia and while this shift was originally ascribed to intracellular acidosis, it is now known to reflect the concentration of intracellular 2,3 - diphosphoglycerate. In man, this compound accounts for three-quarters of the organic phosphate in the erythrocyte and exerts its effect on the oxygen affinity of haemoglobin by its ability to stabilize the haemoglobin in the deoxy configuration (Benesch et al., 1968). The red cell 2,3 - diphosphoglycerate level increases in proportion to the degree of anaemia and this decreases the affinity of the blood for oxygen, thereby allowing greater extraction of oxygen from the blood at any given partial pressure

of oxygen (Torrance et al., 1970). The minimal symptomatology of mild anaemia reflects the efficiency of this adaptive mechanism.

Animal studies that have explored the physical performance aspects of iron malnutrition include those carried out by Edgerton and co-workers (1972). An investigation of the ability of iron deficient rats to run a treadmill in order to avoid an electric shock revealed that running times decreased in parallel with haemoglobin, myoglobin and cytochrome c levels. Other workers have measured the physical activity in normal rats and compared it with that of iron deficient animals (Glover and Jacobs, 1972). The points that emerged were that there was a reduction in basal activity in the anaemic animals, which was equally marked in both mildly and severely anaemic animals, and that the normal pattern of increased nocturnal activity was reversed in the anaemic rats. Both of these alterations in the behaviour of anaemic animals were corrected by iron repletion.

In studies on manual labourers, Viteri and Cifuentes (1972) have shown a highly significant correlation between haematocrit values and physical fitness as measured by the Harvard Step Test. Their results suggested an increase in physical fitness of not less than 4% per percentage unit of haematocrit change in the near normal range of the haemotocrit. Other workers (Grimby et al., 1973), who measured the maximal oxygen consumption during physical work before and after phlebotomy in healthy males, found a highly significant decrease of about 5% per g/100 ml of haemoglobin in the near-normal range of haemoglobin level.

Results such as these have been used to estimate the maximum workload that any individual can maintain at different haemoglobin

levels (W.H.O., 1975). This is illustrated in Fig. 1. While it is tempting to translate these data in terms of the effect that anaemia has on the efficiency of labour forces, it should be borne in mind that the relationship illustrated relates to states of maximum cardiac output. This does not mirror the physical effort expended in the majority of occupations and it is not known to what extent the capacity for non-maximum effort is reduced by anaemia.

With regard to a role for iron in influencing susceptibility to infection, a number of workers has noted that the incidence of infection in anaemic children from very low socio-economic groups is reduced by iron administration (Mackay, 1928; Andelman and Sered, 1966). The reasons why there should be a relationship between iron deficiency anaemia and infection remai[illegible]e elaborated. Part of the answer may lie in the work of Joynson and colleagues (1972). They investigated cell mediated immunity in iron deficient subjects and showed a significant impairment in lymphocyte transformation when purified protein derivative was used as an antigen. Further evidence of a defect in cell mediated immunity was noted when the negative skin hypersensitivity reactions to purified protein derivative or monilia in some patients became positive after iron therapy.

Other workers have extended these observations (Chandra, 1973) and shown a decrease in the bactericidal capacity of polymorphs in people with iron deficiency. It is possible that this effect is related to the granulocytes' iron - containing enzyme myeloperoxidase. This enzyme, which is in part responsible for the bactericidal properties of the cells, is present in lower concentrations in

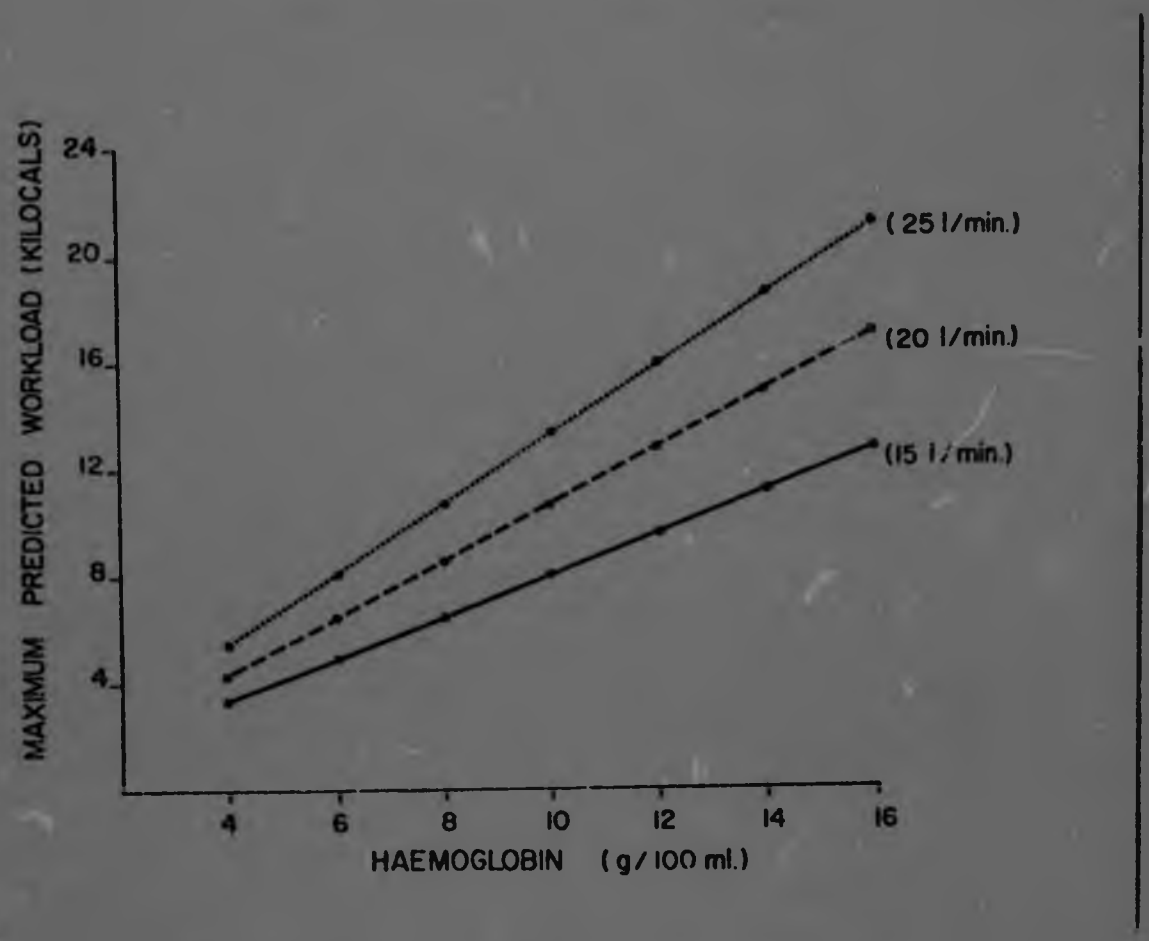

Figure 1. Effect of haemoglobin on predicted workloac' at three different maximum cardiac outputs (Data from W.H.O., 1975).

iron deficiency anaemia (Higashi et al., 1967) and has been incriminated in the defective resistance to Salmonella typhimurium that occurs in iron deficient rats (Baggs and Sandford, 1974).

If iron deficiency anaemia is indeed a risk factor for infection, it seems pertinent to make the point that in studies carried out in different parts of India (Nutrition Research Laboratories, 1966-1967), 50% of infants and young children were anaemic by the standards prescribed by the World Health Organization Study Group on Iron Deficiency Anaemia (W.H.O., Technical Report Series, 1959). A similar incidence of anaemia has been found among Indian women during the last trimester of pregnancy (Venkatchalam, 1962; Sood, 1967). Venkatchalam (1968) pointed out that most of the women and children were untreated and felt that "their anaemic state, undoubtedly, is responsible for the high morbidity and mortality". The view that iron lack places mother and child in jeopardy is shared by others (Menon 1958; W.H.O., 1959; Anaemia in Africa, 1962; Heilmeyer and Harweth, 1970).

Even in the United States, an estimate by the Department of Agriculture (Agricultural Research Service, 1969) suggested that, on the average, infants under 3 years of age and women under 55 years of age consume only half of the recommended dietary allowance (National Academy of Sciences, 1968).

Recognition of the fact that there are groups who are "at risk" of iron deficiency resulted in the adoption of fortification programmes by a number of countries. Bearing in mind the cost of

such programmes, it is understandable that such schemes have been given priority only in countries with robust economics. Nevertheless, frequent suggestions have been made that similar programmes be introduced elsewhere, particularly in India and South America (W.H.O., 1970; W.H.O., 1972; P.A.H.O., 1972).

The choice of a vehicle for iron fortification obviously demands consideration of a host of factors, but, essentially, the vehicle should reach the target group in adequate amounts, which do not vary widely from one person to the next, and fortification should not alter the acceptability of the diet. Most experience has been gained with the fortification of cereals, particularly in the United Kingdom, Sweden and the United States of America, where wheat flour is used as a vehicle. Infant foods also lend themselves to fortification and most cereals and milk powders contain added iron.

In the United States of America, legislation setting iron fortification levels for flour and bread was introduced as early as 1942. More recently, however, the Food and Drug Administration proposed a further improvement in the nutritional quality of wheat, flour, farina, bread, buns and rolls (1971). Their new standards suggested an increase in iron supplementation of enriched flour from 13.0 to 40.0 mg/lb.

This recommendation provoked considerable debate. The Food and Drug Administration's proposal was in part motivated by a survey which demonstrated an incidence of iron deficiency anaemia of 22% among American males from a low socio-economic group (Ten-State Nutrition Survey in the United States, 1968-1970). While interpretations of results of the survey, which encompassed 30,000 people,

were not without criticism (Newman and Martin, 1971; Crosby, 1974, 1975), the protagonists of the more aggressive policy pointed out that there have been declines in dietary iron intake in recent decades. They attributed this to a reduction in calory intake from grain sources (U.S. Department of Agriculture, 1968, 1971), iron loss during refinement and less iron contamination of foods during their preparation (Natvig, 1969; Senti, 1971). Their argument was bolstered by the observation that the amount of iron supplementation of flour and cereals as practised in several countries has had little impact on the prevalence of iron deficiency (Hoglund and Reizenstein, 1968).

Those who were critical of the new proposal were particularly concerned about safety. Crosby (1970) asked if the addition of 60 mg of iron per pound of flour would pose any risk to people normally prone to accumulate unneeded iron. He did not share the American Medical Association's Council on Foods and Nutrition opinion that increased fortification was a logical step and suggested that, in the presence of a "vacuum of information", implementation would be tantamount to research on the entire American population.

In reply, the Federation of American Societies for Experimental Biology published a majority opinion (1972) that the proposed increase in iron enrichment would not jeopardize healthy, American males with normal iron metabolism, although it could accelerate the course of iron storage diseases.

While there may be room for debate as to the pros and cons of food iron fortification among more affluent societies, where the size of the target group may be small, the same cannot be said of

lower socio-economic groups, where the incidence of iron deficiency is of a completely different order.

The point to be made is that iron deficiency is a national problem in many developing countries. While no one doubts that severe iron deficiency is harmful, the recent understanding of the deleterious effects that milder degrees of anaemia may have on children, pregnant women and manual labourers adds to the argument that this deficiency should be corrected.

1.3 The Availability of Nutritional Iron

Where discussion about the American proposal to increase fortification was of particular value was in highlighting the limited information concerning nutritional iron, a fact which the Council on Foods and Nutrition (1972) had conceded when stating "attempts to define 'the availability' of iron from various foodstuffs have generally been unsatisfactory". Although the comment exaggerates the deficiencies in current understanding of food iron absorption, it did focus attention on an issue which is central to successful fortification.

The many investigations into food iron absorption have relied, essentially, on only two different techniques. In the early studies, workers used chemical methods, which involved the measurement of both the dietary iron intake and the stool iron loss. The amount of iron absorbed was then estimated as the net difference between the two determinations. This "chemical balance" method has a number of advantages; iron absorption from a complete diet may be estimated and any day to day variation in individual absorption tends to be compensated for by conducting the balance over a few days.

On the debit side, this technique does not distinguish between unabsorbed dietary iron and endogenous iron excreted through the bowel lumen and meticulous attention must be paid to the complete collection of stools and to ensuring that neither the meals nor the excreta become contaminated with iron. The demands of such methodology can be put into perspective when one considers how close the average daily dietary iron absorption of 0.7 to 1.4 mg is to the daily physiological gastro-intestinal tract losses of 0.5 mg (Green et al., 1968).

Because of the pitfalls inherent in the chemical balance method, current practice favours the use of radio-iron to measure dietary iron absorption. This technique, which was first described by Moore and Dubach (1951), was originally used to examine absorption from single foodstuffs of either vegetable or animal origin. The method depends on the labelling of iron that is intrinsically present in the food. This is achieved by growing vegetables in hydroponic media, to which radio-iron has been added, and by injecting suitable animals intravenously with radio-iron. The animals are subsequently slaughtered to provide a source of labelled meat or liver or, alternatively, bled of their labelled haemoglobin. Retention of this radio-iron after feeding is then assessed by measuring the unabsorbed radio-activity recovered in the faeces, the amount utilized for haemoglobin production or, in the case of ^{59}Fe labelled foods, by whole body counting.

Although hydroponic cultivation is tedious, costly and demands special techniques that restrict its use, the ease with which labelled haemoglobin is prepared from laboratory animals has resulted in the widespread use of this particular method of

biosynthetic labelling.

When Moore (1964) reviewed the results obtained by a number of different workers using radio-iron methods to investigate dietary iron absorption, two important observations were made: in general, iron from animal sources is more available than iron from vegetable sources (see Fig. 2), and iron deficient subjects absorb food iron better than do iron replete individuals.

More recent studies have not only confirmed these findings, but also provided considerable insight into the epidemiology of iron deficiency among populations largely dependant on vegetables as dietary staples. In some communities, vegetables account for 80 to 90% of the total dietary iron intake (Layrisse, 1975) and in this regard information concerning the limited iron availability from three major staples, wheat, maize (corn) and rice is particularly relevant, as is the iron concentration of each staple. The amount of iron present in wheat depends on the extent to which the grain is processed; whole meal wheat contains 2.5 to 4.0 mg iron per 100 g whereas white flour contains less than 2.0 mg per 100 g. Dried maize provides up to 3 mg iron per 100 g, as do some strains of rice. However, those varieties of rice which are most often consumed contain only about 0.5 mg iron per 100 g.

Wheat is usually prepared for eating either as small pancakes, 'chapattis', or as bread, and radio-iron techniques have been used to investigate absorption from both. Hussain and his colleagues (1965) found a mean absorption of 4.5% in normal subjects and 7.8% in iron deficient subjects who had been given wheat pancakes.

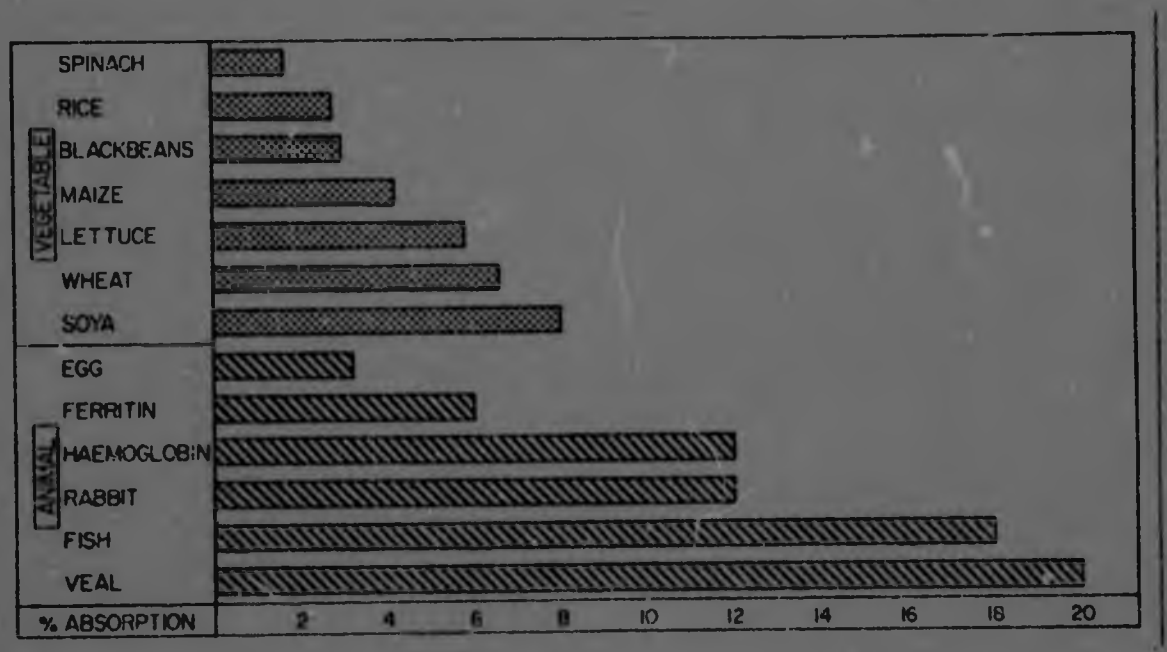

Figure 2. Iron absorption from animal and vegetable foods (Data sources are quoted in the text).

In another study on a group which included normal and iron deficient subjects, Layrisse and his co-workers demonstrated a mean absorption of 7.9% from pancakes (1969). In one of the studies which heralded a closer look at the effect of the interaction of foods on iron absorption, Elwood pointed out that the mean absorption from wheat pancakes was only 1.7% if they were fed together with a meal (1971).

Earlier work by Elwood's group (1968) showed a mean iron absorption of 19.2% from bread, taken with fruit juice, that was fed to a group which included some iron deficient subjects. In the course of this investigation, the workers also measured the effect of added bran on iron absorption from bread. In their study, the bran-containing bread was taken with a normal breakfast which included fruit juice. Mean absorption was 1.4% when fed to a group which was, however, less iron deficient than the group in whom the mean 19.2% absorption from bran-free bread had been recorded. Callender and Warner looked at the effect of bran in a slightly different way. Four volunteers each used a brown loaf, made from labelled bran mixed with white flour, as their sole source of bread for three to five days. Mean iron absorption was 6% in three normal subjects, but an absorption of 18.6% was found in one individual with a low serum iron.

The role of bran in iron absorption was more formally investigated by Björn-Rasmussen (1974), whose studies on normal subjects showed a mean absorption of 1.8% and 3.7% from rolls prepared with 40% and 10% bran respectively. This author also compared iron absorption from rolls baked without added bran with absorption

from rolls containing between 0.3% and 10% added bran and showed that inhibition of iron absorption began at a level of 3.3% added bran. In the presence of 6.7% bran, absorption was reduced from a mean of 14.9% to 6.3% in a group of healthy, student volunteers.

The percentage extraction of flour used in baking is then important in determining iron availability from bread. While bran is obviously inhibitory, it should be pointed out that host factors may have an overriding influence. Mameesh and colleagues (1970) found a mean absorption of 15% from an Arabic bread containing bran that was fed together with cheese. Their subjects were, however, a selected group of anaemic blood donors.

Maize iron is of intermediate availability when compared with wheat and rice. When maize was fed as pancakes to groups of both normal and iron deficient subjects the mean iron absorption varied from 3.2 to 4.2% (Layrisse et al., 1968, 1969). Björn-Rasmussen (1972, 1974) found a mean absorption of 1.8% from pancakes fed to normal subjects and a mean range of 2.6% to 8.5% when the maize was fed as porridge to groups of normal subjects.

The first study investigating iron availability from rice revealed a mean absorption of only 0.9% (Layrisse and Martinez-Torres, 1971). More recently, test meals given as rice porridge showed a range of absorptions which apparently depended on the manner of preparation of the rice (Björn-Rasmussen et al., 1973). When the porridge was made from raw rice, that is rice which had been subjected to dehusking alone, mean absorption was 2.7%,

this rose to 4.5% when the rice was polished, and rose further to 7% when the porridge was prepared from a flour of ground, polished rice.

Vegetable foods, which are not regarded as staples, have also been investigated. The mean iron absorption from cooked spinach and lettuce, fed with tomato juice, has been shown to be 1.7% and 5.8% respectively (Layrisse et al., 1969). Legumes that have been studied appear to differ widely in their value as sources of available iron. While the mean absorption of iron from black beans (Layrisse et al., 1968; Martinez-Torres and Layrisse, 1970) showed a narrow range between 2.3% and 3.2%, absorption from soya beans was far less predictable, even when the same workers (Layrisse et al., 1971) examined similar subjects. In 3 studies, each using a different soya bean harvest, geometric mean absorptions ranged from 3 to 11%. Björn-Rasmussen and his colleagues have also studied soya as a source of available iron (Björn-Rasmussen et al., 1973; Björn-Rasmussen, 1973). Their normal subjects absorbed a mean of 2.6% from this vegetable, while in iron deficient subjects the mean absorption was 5.7%. Mameesh and co-workers fed two other legumes, chick pea and broad bean, to anaemic blood donors and found mean absorptions of 37% and 27% respectively (1970). The absorption of iron from these latter two legumes has yet to be tested in normal individuals.

As has been pointed out earlier, iron from animal sources is generally better absorbed than vegetable iron. The iron present in meat, liver or fish is found mainly as ferritin, haemosiderin

and haem. A study by Hussain and co-workers (1965) established a mean absorption of 6% and 11% from ferritin iron in normal and iron deficient subjects. However, more recently, Layrisse and colleagues (1975) found mean ferritin iron absorption in both groups of only 3.2%. They attributed this disparity to the presence of ascorbic acid in the tomato juice that the workers in the earlier study had used to disguise the appearance of the ferritin. During the course of their study they were able to show that iron absorption from ferritin was doubled when it was given with meat or liver.

Iron absorption from haem has been found to have a similar relationship to the amount of protein present. Conrad and co-workers (1967) found a mean absorption of only 4% from the iron in purified haem by comparison, mean absorption was 12% from iron given as haemoglobin, rising to 22% when given as haemoglobin and myoglobin in meat.

A mean iron absorption of 18.3% from fish was found by Layrisse et al. (1969). Rabbit muscle has also been investigated and Heinrich and colleagues (1969, 1971) demonstrated a mean absorption of iron of 12% in normal subjects and 23% in subjects who were iron deficient. The mean iron absorption from veal was 20.2% in a group of normal volunteers (Layrisse et al., 1968).

Egg is the exception to the generalisation that iron from animal sources is more available. Mean iron absorptions of 3% and 8% have been found in normal and iron deficient subjects (Moore and Dubach, 1956; Chodos et al., 1957). Björn-Rasmussen

(1972) found an even lower mean absorption of only 1.5% when normal subjects were fed egg as an omelet.

While studies measuring absorption from single foodstuffs are certainly important in providing some idea as to the relative merits and demerits of various foods as sources of iron, it is obviously not possible to extrapolate from these results to get information about absorption from complete meals. This limitation in methodology was highlighted when more sophisticated studies on food iron absorption employed different radio-iron isotopes to tag different foods in the same meal. In this way it was possible to demonstrate that a number of interactions between foods have considerable importance in determining iron availability from mixed meals. Layrisse and colleagues (1968) found that iron absorption from vegetable meals was doubled when the meals also contained veal or fish. Subsequent studies that appeared to contribute to the understanding of this enhancement looked at the effect of various amino acids from fish on the absorption of iron from black beans. Martinez-Torres and Layrisse (1970) showed that the cysteine promoted iron absorption from the beans. This issue has, however, yet to be resolved since Björn-Rasmussen (1974) was unable to show that this particular amino acid influenced iron absorption from maize porridge.

Martinez-Torres and Layrisse (1971) also studied interactions between foods of animal origin by feeding meals containing differentially tagged veal muscle and haemoglobin. They found that haemoglobin iron was less available than veal iron when each was

fed alone, but when both were fed together, iron absorptions were similar. They concluded that during digestion the haem iron from both the veal muscle and the haemoglobin had entered a common pool, enabling the promotors of iron absorption from veal muscle to have a similar effect on the haemoglobin.

These studies were extended when two different vegetables, black bean and soya, each biosynthetically labelled with a different radio-iron isotope were fed in the same meal. Despite dissimilar iron absorption from the vegetables when each was fed alone, absorption was similar when the two were fed together (Martinez-Torres and Layrisse, 1973). The implication here was that the dietary non-haem iron had also entered a common pool which was, however, distinct from the haem iron pool where iron absorption was of a different order. The finding of separate pools for non-haem and haem iron was not surprising as a number of investigators have shown that the two are absorbed differently. Ascorbic acid does not enhance absorption of haem iron (Kuhn et al., 1968) and neither phytate nor desferrioxamine impair its absorption (Turnbull et al., 1962; Hwang and Brown, 1965).

The evidence that dietary iron absorption could be measured by simply assessing absorption from two separate pools, rather than by assessing absorption from each dietary constituent individually, encouraged a number of workers to re-investigate absorption from whole meals. Their intention was to identify what labelled compounds could be used to provide a measure of iron absorption from each pool.

Earlier work suggested that a labelled inorganic iron salt, or extrinsic tag, could be added to a meal to reflect absorption from the non-haem iron pool. Workers studying the effect of phytates on iron absorption found that food markedly inhibited the absorption of labelled inorganic iron (Sharpe et al., 1948). Pirzio-Biroli and colleagues (1958) also added tracer doses of inorganic iron to a meal and found absorption to be reduced. Although the addition of an extrinsic tag was promoted as a means of measuring iron absorption from a meal, the validity of this method depended on the demonstration that intrinsic food iron and extrinsic tag were absorbed to the same degree. A study by Schulz and Smith (1958) suggested that this might be the case, since they found similar absorption from an extrinsic tag and intrinsically tagged milk or eggs fed to children. Their studies did not, however, measure absorption of the two tags in the same individuals.

More recently, a number of investigators has made direct comparisons, in the same individuals, of absorption from extrinsic and intrinsic tags. Cook and colleagues (1972) fed complete meals and compared absorption of an extrinsic tag of inorganic iron with absorption of an intrinsic tag in a wide variety of biosynthetically labelled vegetables. They found a consistent extrinsic : intrinsic radio-iron absorption ratio of 1.10. This ratio remained the same irrespective of the valence of the added inorganic iron and was unaltered when the dose of the added iron ranged from 0.001 mg to 0.5 mg. The investigators were also able

to show that the ratio was not disturbed when a wide range of absorptions was achieved by the addition of desferrioxamine or ascorbic acid to the meals. They concluded that the extrinsic tag of inorganic radio-iron was a valid measurement of iron absorption from a common non-haem iron pool. These findings were confirmed by Layrisse and Martinez-Torres (1972) and Björn-Rasmussen et al. (1972, 1973). A model for measuring haem iron absorption from a meal has also been developed. Layrisse and Martinez-Torres (1972) used labelled haemoglobin as their extrinsic tag, added it to ground veal that had been biosynthetically labelled and fed the mixture as a hamburger. Once again, the ratio of extrinsic to intrinsic iron absorption was close to unity, despite a wide range of individual absorptions, and was maintained in the face of a meal containing vegetables that could be expected to inhibit iron absorption. These workers concluded that an extrinsic tag of labelled haemoglobin could be used to calculate total haem iron absorption from a meal.

The simultaneous use of two extrinsic tags to label the haem and non-haem iron pools in a complete meal has been used by a number of investigators (Layrisse and Martinez-Torres 1972; Hallberg and Björn-Rasmussen, 1972; Layrisse et al., 1974; Björn-Rasmussen et al., 1974) and the measurements of dietary iron absorption that these studies have yielded correlate closely with estimates of normal body iron loss. More recently, however, an investigation by Layrisse and his colleagues produced evidence that a two pool concept for dietary iron absorption could be an oversimplification (Layrisse et al., 1975). They measured iron

absorption from ferritin and found that while its absorption was subject to the same enhancing and inhibiting influences as non-haem iron in vegetable, ferritin iron was less available than vegetable iron. The authors felt that further studies were necessary to decide if these differences were sufficient to warrant the introduction of a third pool to accommodate ferritin.

An understanding of what determines the availability of dietary iron from different sources is indispensable to the planning of a fortification programme. As has already been pointed out earlier in this chapter, Elwood (1968) has argued convincingly that the mere addition of iron to a diet does not constitute a solution to the problem of iron deficiency anaemia. Although his remarks were particularly relevable to the iron powder used to restore the iron content of flour in Great Britain, other common forms of fortification iron, namely sodium iron pyrophosphate (Cook et al., 1973) and ferric orthophosphate and pyrophosphate (Rao et al., 1972) have also been shown to be negligibly available.

While it would appear obvious that iron poor diets could profitably be fortified with an available iron salt, this approach might not be justified if the diet is replete with iron that is not available. Under these circumstances, a more enterprising plan would be to enrich the diet with an agent that promotes iron absorption.

By way of a background to this type of approach to the correction of iron deficiency, the next chapter reviews the factors regulating

iron absorption and, in addition, the mechanisms underlying 'availability' are considered in more detail.

Chapter Two

FACTORS REGULATING IRON ABSORPTION

2.1 Iron Balance

The total body iron content in the adult normally amounts to between 3 and 4 g (Granick, 1958). The major part of this iron is functional in that it is incorporated into haemoglobin, which contains 0.34% iron by weight, myoglobin or certain tissue enzymes. A smaller storage component includes that iron found as either ferritin or haemosiderin. Recently, a sensitive immunoradiometric assay for circulating ferritin has been described (Addison et al., 1972; Miles et al., 1975) that has enabled a number of workers to demonstrate a close corralation between the concentration of serum ferritin and iron stores, both in normal subjects (Walters et al., 1974; Cook et al., 1974) and in individuals with iron deficiency and overload (Jacobs et al., 1972).

Ferritin is water soluble and consists of a protein, apoferritin, forming a shell around ferric ions in a hydrous ferric oxide-phosphate core (Granick, 1946). The molecular weight of apoferritin is about 480,000 (Harrison, 1963). In contrast to ferritin, which is not normally visible by conventional histological techniques, haemosiderin is visible under light microscopy. This form of storage iron may represent partially denatured and deproteinized ferritin (Sturgeon and Shoden, 1964). In the normal adult, the storage compartment contains up to 1 g of iron and the metal can be mobilized from either haemosiderin or ferritin as needed (Shoden et al., 1953).

In the healthy individual, the amount of body iron

is maintained chiefly through variations in the amounts of iron absorbed, since man has only a very limited capacity to excrete iron (McCance and Widdowson, 1937). In the normal adult male, total daily iron excretion is between 0.5 and 1.0 mg (Dubach et al., 1955; Moore, 1965). While most of this iron is derived from desquamation of epithelial cells and exudation of erythrocytes in the gastro-intestinal tract, some iron is also lost in bile and with desquamation of cells from the genito-urinary tract and the epidermis (Bagg et al., 1966; Green et al., 1968). Depending on climate, sweating may also contribute to iron loss (Moore 1965; Green et al., 1968).

In the adult female, menstruation, childbearing and lactation contribute to additional losses of iron. On the average, menstrual bleeding accounts for 20 mg of iron a month and an average of 800 mg of iron is lost with each pregnancy (Millis, 1951; Hallberg and Nilsson, 1964; Newton et al., 1961). While the amount of iron lost to the foetus during gestation, parturition and lactation is offset to a variable extent by amenorrhoea, in terms of daily iron balance, mean iron excretion in adult women is 1.3 mg as compared with 0.6 mg in adult males (Finch, 1959).

For any individual to remain in iron balance, losses have to be made good by absorption of iron from the diet. In the adult male this is seldom a problem,unless abnormal bleeding increases iron requirements. With regard to the adult female, however, excessive menstruation or the demands of many pregnancies may result in a negative iron balance.

Investigations by Hallberg and colleagues (1966) showed that Swedish women with monthly menstrual blood losses of 60 to 80 ml, equivalent to a total iron loss of 1.5 to 2.0 mg daily, experienced an iron requirement that could not be met by their diet. As many as 18% of Swedish women fall into this category and during pregnancy, when the daily iron requirement in the second and third trimester is between 3 and 7.5 mg, the proportion of women whose diets do not meet requirements is even higher. This holds true despite an increase in the rate of iron absorption that reaches a peak in the third trimester (Heinrich et al., 1968; Apte and Iyengar, 1970). The smaller size of the storage iron compartment in females further compounds their risk of iron deficiency. The average stores in menstruating women are 3000 mg of iron, which is less than half the requirements of a normal pregnancy.

2.2 Food Iron Absorption

The iron in food exists in a number of forms. While some is ionized (Ascham et al., 1938) most is complexed, such as haemoglobin myoglobin, ferritin and haemosiderin. This also holds true for dietary iron from vegetable sources, but, apart from phytoferritin (Hyde et al., 1963) and ferridoxin (Mortenson et al., 1962), most of the complexes are poorly defined.

Dietary iron that is bound in organic complexes only becomes available for absorption after digestion of the food in the gut lumen. After it has been released, iron is taken up by intestinal mucosal cells of the proximal part of the small intestine.

Once in the mucosal cell, ingested iron may follow one of two pathways. While some iron is r pidly directed to the serosal pole of the cell and then delivered to the circulation, the rest is incorporated into ferritin (Charlton et al., 1965; Huebers et al., 1971). Most of the iron that is incorporated into ferritin is subsequently lost when the mucosal cells are exfoliated. Iron that reaches the circulation becomes attached to a specific iron-binding plasma protein, transferrin. This protein in turn delivers the iron to different parts of the body, the most important of which is the erythroid marrow.

The extent to which food iron is absorbed can then be affected by events occurring at two sites; the lumen and the mucosal cell. Each site will be dealt with separately.

2.2.1 The Lumen

Much information relating to iron absorption at this level has been obtained using simple iron salts, a model which does not mimic dietary iron absorption where, as has been pointed out, the bulk of the iron is complexed. Where these studies are important, however, is in providing insight into what happens to the iron once it has been freed of its bonds and rendered soluble. Factors that are important at this level include the following:

a) The physicochemical form of iron

In contrast to the alkali metals, such as sodium and potassium, iron and other heavy metals are not freely soluble in the ionic state under biological conditions. This fundamental difference relates to the ability of heavy metals to form

co-ordination compounds at physiological hydrogen ion concentrations, that is, compounds where both electrons in the electron pair forming a bond are donated by the same atom.

In aqueous solutions, the co-ordination tendencies of iron are satisfied by water molecules (Bailar, 1956; Chaberek and Martell, 1959; Martell, 1960) and, since there are six possible co-ordination sites on iron, the metal may adopt a polymeric configuration. The term "polymer" is used to accentuate the fact that iron is bound in a structural unit (Fig. 3) which is repeated many times. With a rise in pH, more hydroxyl ions become available and polymerization is increased until a precipitate of metallic hydroxide is formed.

While the above holds true for both ferric and ferrous ions, ferrous ions do not undergo as marked polymerization as their ferric counterparts and their solubility is greater at any given pH. At pH 8, for example, the maximum concentration of ferrous ions is 1.6×10^{-2} M, whereas that of ferric ions is only 10^{-18} M (Charley et al., 1963).

Although unpolymerized ferrous and ferric ions are found at the gastric pH of 1 to 2, exposure to the higher pH in the duodenum renders them liable to co-ordinate with electron donating groups, or ligands, to form metal complexes and metal chelates. These ligands may occur naturally in foodstuffs, for example carboxylic acids, hydroxic acids, proteins, amino acids and inorganic or organic phosphates (Chenoweth, 1956), or they may be administered therapeutically, for example ascorbic acid or ethylene diamine tetra-acetic acid. The difference between

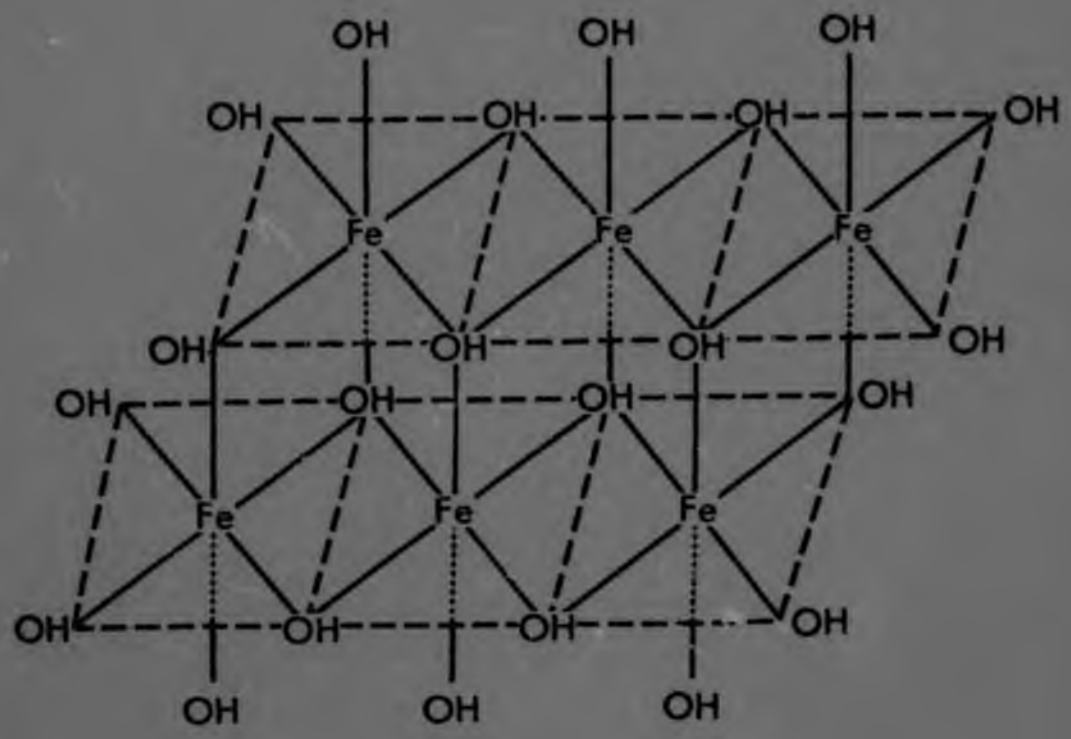

Figure 3. Schematic representation of an ionic iron polymer (After Conrad, 1970).

iron complexes and chelates resides in the nature of the bonds present: a metal complex is a co-ordination compound of iron in which one or more of the water molecules has been displaced by a different electron donor, while in chelation, two or more of the donor groups co-ordinated to the metal are themselves bound together by a chemical linkage of some sort.

It is important to note that the iron found in complexes or chelates may or may not be available for absorption. If the stability of the complex or chelate is low, iron may well be released and taken up by the mucosal cell. If however, the effective stability is high, utilization of the iron depends on the extent to which the intact complex or chelate is absorbed.

Complexed iron may also be present in the diet in the form of porphyrins, such as haemoglobin and myoglobin, and as such is not only better absorbed than inorganic iron (Walsh et al., 1955; Callender et al., 1957), but is also independant of the various intraluminal factors that affect ionizable iron (Turnbull et al., 1962). Haem iron differs from inorganic iron in that it is soluble at an alkaline pH and not at an acid pH; there is, however, a tendency for haem molecules to polymerize and become less available in the alkaline contents of the duodenum.

This polymerization may be reduced by the digestion products of globin and other proteins when the amino acids that are released form available, low molecular weight, haem co-ordination complexes (Conrad et al., 1966 a). This explains the enhancement of haem iron absorption that is found in the

presence of protein (Turnbull et al., 1962; Conrad et al., 1966 b).

This brief account of the chemical forms in which intraluminal iron may be found gives some insight into a number of absorption phenomena. Iron from ferrous salts is better absorbed than iron from ferric salts (Moore et al., 1944; Brise and Hallberg 1962). Carbonates (Benjamin et al., 1967), phosphates and oxalates (Hegsted et al., 1949; Crosby, 1968) and phytates (McCance et al., 1943; Sharp et al., 1948) depress iron absorption by forming insoluble complexes. On the other hand, sugars, particularly fructose, enhance iron absorption (Charley et al., 1963; Davis and Deller, 1966), either directly, by forming unstable, soluble, metal chelates, or indirectly, via their metabolites, lactic and pyruvic acid, which also have chelation potential. The fact that sugars act at a luminal site is further supported by the observation that even poorly absorbed varieties, such as mannitol and sorbitol, enhance absorption (Herndon et al., 1958; Loria et al., 1962).

Ascorbic acid has a dual effect. The vitamin is not only a reducing agent, which increases the solubility of iron by favouring the ferrous state, but it also produces chelates with the metal which are still soluble in the duodenum (Conrad and Schade, 1968).

Although the effect of dietary proteins, peptides and amino acids is generally to promote iron absorption, egg protein is an important exception. The limited availability of iron from egg (Pye and MacLeod, 1946; Moore and Dubach, 1951) has been

attributed to the presence of stable iron chelates with either conalbumin (Rummel, 1965) or phosphoproteins (Greengard et al., 1964; Peters et al., 1971). Yolk phosphoproteins have also been incriminated in the inhibition of bread iron absorption by egg (Elwood et al., 1968; Callender et al., 1970). In contrast, the iron absorption enhancing effect of other proteins has been well documented (Klavins et al., 1962; Layrisse et al., 1968; Layrisse et al., 1969). Of the amino acids, histidine (Kroe et al., 1963 a and b) and sulphur containing varieties (Layrisse et al., 1968) appear particularly important in ensuring the formation of available metal chelates.

b) Gastric secretions

Both gastric acid and mucus influence the availability of iron. The absorption of ferric iron is impaired in patients with achlorhydria (Goldberg et al., 1963). This deficiency may, however, be corrected by the simultaneous administration of hydrochloric acid, which increases the solubility of ferric iron (Jacobs et al., 1964); as would be expected, this effect is not as marked for ferrous iron, whose solubility is not as critically pH dependant. Furthermore, inasmuch as chelation only occurs between substances in solution, the effect of hydrochloric acid in rendering iron soluble is also important in facilitating chelation with ascorbic acid and other endogenous or exogenous agents (Conrad and Schade, 1968; Schade et al., 1968).

Jacobs and Miles (1968, 1969) have given closer attention to iron chelation by endogenous gastric juice constituents. They found that gastric juice retarded the precipitation of

simple iron salts, by forming complexes that remain soluble at a pH greater than 5, and presented evidence suggesting that this iron-binding fraction was probably a mucopolysaccharide. The concentration of this factor was unaltered in patients with iron deficiency anaemia and haemochromatosis.

There is conflicting evidence that other gastric factors may modify iron absorption. Work by Murray and Stein (1968 a and b) has shown that the *in vitro* uptake of iron by everted loops of rat small intestine and the *in vivo* absorption of iron by gastrectomized iron deficient rats is enhanced by the gastric juice of iron deficient patients. Forth and Rummel also investigated this phenomenon (1968). They showed that gastric juice from anaemic rats did not potentiate iron absorption in normal animals and gastric juice from normal animals was not inhibitory to iron absorption in anaemic animals. In studies on humans, Turnberg (1968) found that gastric juice from iron deficient subjects promoted iron absorption in normals. These findings have not, however, been confirmed by other investigators (Jacobs *et al.*, 1968; Smith *et al.*, 1969).

The work of Davis and associates (1967) adds to the controversy. They demonstrated an iron-binding substance in gastric juice, which they called gastroferrin, and postulated that the substance is a physiological inhibitor of iron absorption, which is found in reduced amounts in both iron deficiency anaemia (Luke *et al.*, 1967) and haemochromatosis (Luke *et al.*, 1968). By comparison, Wynter and Williams (1968) found that not only was there no difference in gastric iron

binding between normal individuals and haemochromatotics, but also there was no relation between iron binding and the level of absorption in haemochromatotics. Other workers (Jacobs and Miles, 1968) extended these observations and found no difference in iron binding in normal subjects and individuals with iron deficiency anaemia.

A majority opinion about the role of gastric juice in this regard admits the presence of an iron-binding mucoprotein, but does not confer on the substance any physiological role in the regulation of iron absorption (Turnbull 1973).

c) Pancreatic secretions

Interest in the role of the pancreas in influencing iron absorption was aroused when a number of investigators found an increase in the carcass iron of experimental animals that had been subjected to pancreatectomy (Taylor et al., 1931), pancreatic duct ligation (Taylor et al., 1935) or pancreatic necrosis induced by ethionine (Kinney et al., 1955). In human studies, iron absorption was reported to be enhanced in a proportion of patients with fibrocystic disease of the pancreas (Tonz et al., 1965) and chronic pancreatitis (Davis, 1961; Deller, 1965). While some authors (Balcerzak et al., 1967) attribute these results to a co-existant storage iron deficiency, there is also evidence to suggest that pancreatic secretions play a role. Benjamin and colleagues have shown that the addition of sodium bicarbonate to iron solutions produces both soluble macromolecular iron chelates and precipitates and causes a decrease in the absorption of test dose of iron by normal guinea pigs (1967). It is not

surprising, then, that injection of secretin, which in normal animals leads to an increase in pancreatic bicarbonate secretion, impairs iron absorption (Benjamin, 1967). While the decrease in pancreatic bicarbonate may then be important in producing enhanced iron absorption in pancreatic insufficiency syndromes, it is difficult to view this effect in isolation. Other workers place more importance on the role of pancreatic enzymes in providing peptide and amino acid ligands from dietary protein. These ligands may have an effect on both haem and non-haem iron in that they reduce the polymerization of haem and encourage the formation of available complexes with ionizable iron (Jacobs and Miles, 1969; Martinez-Torres and Layrisse, 1970).

d) Bile

Investigations by Hafkesbring and Freeman (1952) indicate that bile contains significant quantities of ascorbic acid. The physiological relevance of this observation has been investigated by Conrad and Schade (1968) and Conrad (1967), who showed not only that the in vitro addition of bile to ferric chloride decreased precipitation of iron by sodium hydroxide, but also that bile duct ligation in normal and iron deficient rats significantly diminished iron absorption from ferric chloride.

e) Intestinal motility

Badendoch and Callender (1954) have demonstrated that patients with chronic diarrhoea develop iron deficiency. Supportive experimental evidence that their observation may relate to the length of time that luminal contents are exposed to the mucosa was provided by Schade and co-workers (1967), who

showed an increased iron absorption in rats whose gastro-intestinal emptying time had been prolonged with atropine.

2.2.2 The Mucosa

Although events in the lumen of the stomach and first part of the small intestine can modify iron availability, the balance of current evidence indicates that the physiological regulation of iron absorption is achieved by alterations in the way mucosal cells from the proximal part of the small intestine handle absorbed iron.

The two most important factors concerned in this regulation are the size of the body iron stores and the prevailing rate of erythropoiesis (Bothwell and Finch, 1962; Beutler et al., 1963). A decrease in the size of the iron stores provokes enhanced absorption of iron salts (Hahn et al., 1943) and food iron (Pirzio-Biroli et al., 1958) and the converse is also applicable in that with increased iron stores there is a corresponding decrease in absorption (Bothwell et al., 1958 a). The enhanced absorption associated with deficiency persists until the stores are replete (Conrad and Crosby, 1962).

A number of workers has investigated how the mucosal cell handles iron. Crosby (1963) has demonstrated that the mechanisms excluding excess iron are situated intracellularly, rather than at the luminal surface of the mucosal epithelium, in that only a portion of the intracellular iron is transported into the plasma. The remaining iron becomes temporarily sequestered within the mucosal cells as ferritin (Charlton et al., 1965) before being lost when the cells are exfoliated into the intestinal

lumen (Conrad and Crosby, 1963; Charlton et al., 1965).

Other studies have suggested mechanisms whereby the mucosal cells remain attuned to body iron needs. In this regard, the amount of iron entering developing mucosal cells from the plasma is apparently important in determing their subsequent behaviour. Conrad and his colleagues (1964) showed that parenteral iron enters mucosal cells from the serosal pole at the time when the cells emerge from the crypts of Lieberkühn and suggested that this was the signal to the mucosa providing information about body iron requirements. A low iron content in mucosal cells in individuals with iron deficiency would favour the transcellular passage of increased amounts of absorbed dietary iron to the circulation, while the converse would apply in iron replete individuals.

This hypothesis was supported by the observation in rats that the non-haem iron concentration of the proximal quarter of the small intestine usually bears an inverse relationship to the percentage absorption of a test dose of iron (Weintraub et al., 1964). The same workers also demonstrated a similar relationship between iron absorption and the proportion of transferrin bound radio-iron which is deposited in duodenal epithelium (Weintraub et al., 1965).

The presence of an inverse relationship has not, however, been confirmed in studies where there has been meticulous separation of mucosal cells from iron containing reticulo-endothelial cells in the underlying lamina propria. Balcerzak

and Greenberger (1968) were unable to correlate changes in iron absorption with differing concentrations of non-haem iron in mucosal cells. Other workers, however, found differences in the iron concentration of different fractions from mucosal cell homogenates (Richmond et al., 1972) : mitochondrial and soluble fractions from mucosal cells of iron deficient animals contained less iron than did corresponding fractions in normal animals. The fact that iron in these fractions is related to both plasma and dietary iron was shown by Worwood and Jacobs (1971, 1972), who observed an increase in the affinity of mitochondria for transferrin-bound iron and oral iron in iron deficient animals.

The link between duodenal mucosa, the rate of erythropoiesis and the state of the iron stores may then be the plasma iron. In the face of active erythropoiesis, plasma iron is diverted preferentially to marrow and only small amounts are incorporated into developing duodenal mucosal cells. These relatively iron free cells, after reaching their functional position on the tips of the villi, do not sequester absorbed dietary iron, but rather permit its passage to the plasma. The same sequence of events could occur when body iron stores are depleted, while the opposite would occur with marrow depression or excessive iron stores.

While it is easy to appreciate that the iron content of newly forming mucosal cells will reflect the low plasma iron of iron deficiency anemia, other conditions, also marked by enhanced iron absorption, have a normal or increased serum iron (Bothwell

and Finch, 1962). This is exemplified by haemolytic anaemia. However, in both iron deficiency anaemia and haemolytic anaemia, clearance of plasma iron is accelerated. For this reason, Wheby (1968) has suggested that the rate of iron clearance determines how much iron is incorporated into developing mucosal cells.

The central role of transferrin in this process has been investigated by Fletcher and Huehns (1967, 1968), who place considerable importance on the nature of the bonds between transferrin and iron. Their studies pointed to a difference between the two binding sites for iron on the transferrin molecule, in that iron is preferentially removed by red cell precursors from transferrin molecules which have both their iron binding sites occupied. Since the number of molecules with two atoms of iron will reflect the rate of erythropoiesis and the body iron stores, this may represent a mechanism regulating iron transfer from mucosa to plasma. This postulate has yet to receive *in vivo* confirmation.

Humoral factors other than transferrin have been invoked as possible messengers to the iron absorbing gut epithelium. One of them is ascorbic acid, which Jacobs and his colleagues have shown to be present in amounts that are inversely related to the size of body iron stores (Jacobs *et al.*, 1971). Another is plasma ferritin, but, attractive as this compound may be by virtue of the fact that its circulating concentration reflects body iron stores, iron absorption experiments in animals given

intravenous ferritin infusions have not demonstrated any messenger function (Bothwell and Charlton, unpublished observations).

While some light has been thrown on many of the intricasies concerning iron absorption, there are still areas which are poorly understood, despite a good deal of experimental effort. At a cellular level, the handling of iron at the brush border of the duodenal mucosa is incompletely characterised, as is the passage of iron across the mucosal cell. What is more, there is still no unifying hypothesis to explain, on the one hand, the normal regulation of iron transfer from cell to plasma, and, on the other, how transfer may, under some circumstances, be inappropriate to body needs.

2.3 The Aims of this Thesis

Although this review gives some insight into what determines the choice of specific iron salts for fortification and why enhancers, such as ascorbic acid, are effective, the success of a fortification programme must also hinge on establishing a suitable vehicle for the additives. Ideally, ascorbic acid, alone or together with iron, would have to be present in some standard dietary ingredient, consumed by all members of the target population in similar and predictable quantities. In addition, a vehicle that is centrally processed would have a number of advantages in a fortification programme, not the least of which would be the relative ease with which quality

could be controlled. However, in undeveloped countries, home grown foods make a major contribution to the daily fare and the opportunities to fortify a dietary constituent that has only a few major outlets are limited.

With these considerations in mind, common salt emerges as a possible vehicle, and, indeed, the success of iodized salt in the prevention of endemic goitre has led to attempts at fortification with many other nutrients, including iron (Rao et al., 1972, 1975; Foy, 1976) and ascorbic acid (Berezovskaya et al., 1958). As will be mentioned later, the experience of Rao and his colleagues was disappointing by virtue of the limited availability of the added iron. The fortification programme discussed by Foy suffered similarly, but also brought to light a number of unanticipated difficulties. The scheme involved the fortification with iron of locally made sea salt on the island of Mauritius, where the predominant anaemia is iron deficient due to blood loss from hookworm infestation. Three years after the scheme had been in operation, an island-wide survey failed to show a significant improvement in haemoglobin levels. What is more, the added iron tended to settle out, in potentially toxic quantities, at the bottom of the storage barrels, and consumer enthusiasm was blunted by the discolouration of vegetables cooked with fortified salt.

As far as the supplementation of common salt with ascorbic acid is concerned, it should be borne in mind that most of the daily salt intake may be accounted for by seasoning during cooking, rather than at the table. In the studies which have

demonstrated the efficacy of ascorbic acid, the vitamin was either added to the food after cooking, or drunk during the meal, dissolved in water or in the form of orange juice. The extent to which any promotion of iron absorption could be negated by exposing the vitamin to cooking was not established, but obviously of paramount importance in evaluating the role of common salt as a carrier for iron and ascorbic acid.

The purpose of the investigations that follow was, then, to examine the effect on iron absorption of ascorbic acid added to staple foods prior to cooking. In this context, the absorption of both the intrinsic food iron present in maize, wheat, soya and rice and an iron salt added to the meal was measured. In addition, the suitability of common salt as a carrier for iron and ascorbic acid was tested. In order to give the studies as much relevance as possible, all the *in vivo* work was conducted among volunteers drawn from a group with a high incidence of iron deficiency.

Chapter Three

THE TEST SUBJECTS

All the iron absorption studies to be described in this thesis involved human volunteers. In every case, the nature of the investigation was explained in detail and written consent was obtained.

Sixty-six volunteers took part in the first investigation. Sixty-four were women. Sixty-two were Indian two were Bantu and two were Caucasian. All the Indians were housewives living on the outskirts of Durban in Chatsworth, a lower socio-economic group housing scheme. The mean age was 32 years (range 15 to 71). With the exception of the Caucasians, the bulk of the dietary calories of the subjects is derived from vegetables. The deleterious effect this has on iron balance has been demonstrated among the Indians, in whom iron deficiency anaemia is common (Mayet et al., 1973; Adams, 1973). This observation was confirmed when stainable iron was found in only one marrow aspirate in the Indian group. Stainable iron was also absent from the marrow of the other volunteers and on this basis the group was considered iron deficient (Rath and Finch, 1948). In addition, the majority of the subjects were anaemic, had low plasma iron levels and a low percentage saturation of circulating transferrin. However, the degree of abnormality varied from subject to subject, and it was for this reason that "reference" absorption studies were carried out on all subjects two weeks after the first investigation. In these studies a small dose of ferrous iron was fed in the fasting state; absorption of this inorganic iron was used, not only as a reference with which to compare food iron absorption, but also as a useful index of each individual's iron balance (Cooke et al., 1969; Layrisse et al., 1969).

Because stainable marrow iron was so frequently absent in the Indian subjects, this investigation was dispensed with in the subsequent studies. Haemoglobin, serum iron, unsaturated iron-binding capacity and "reference" absorption estimations were again obtained. In addition, in order to increase the possibility of examining an iron deficient group, only volunteers who had had three or more children were selected. On this basis, 65 housewives took part in the second study and 67 in the third. The mean age of the women in the second study was 38 years (range 28 to 68) and 40 years (range 26 to 70) in the third study.

With regard to the volunteers' exposure to radio-iron, it was calculated that if all the isotope administered had been absorbed, then the total radiation dose average over a period of 13 weeks would have been approximately 20% of the permissible whole body burden for continuous exposure in the case of ^{59}Fe and 0.5% for ^{55}Fe (International Commission for Radiation Protection, 1960).

Chapter Four

THE EFFECTS OF ASCORBIC ACID SUPPLEMENTATION ON THE ABSORPTION OF IRON IN MAIZE, WHEAT AND SOYA

4.1 Introduction

A number of workers has demonstrated the poor availability of iron from vegetable staples. Mean iron absorption from wheat prepared as pancakes or whole wheat bread fed to normal and iron deficient subjects ranged from 0.5% (Elwood et al., 1968) to 5.1% (Hussain et al., 1965). Absorption from maize is of a similar order. 4% (Layrisse et al., 1969; Layrisse, 1970). By comparison, a staple with a high protein content, soya, appears to be a better source of dietary iron with mean absorptions of up to 11% being reported (Layrisse and Martinez-Torres, 1971).

The precarious iron balance of individuals subsisting on wheat and maize is compounded by the low iron content of the staples. While the iron content of wheat varies with extraction, 100% extraction flour contains only 4 mg of iron per 100 gm. Maize contains up to 3 mg of iron per 100 gm. These concentrations compare poorly with soya, which contains up to 13 mg of iron per 100 gm.

Attempts to improve iron nutrition by adding iron salts to cereals have proved disappointing (Elwood, 1968; Layrisse et al., 1973, Cook et al., 1973), suggesting that the factors present in cereals, which inhibit the absorption of intrinsic iron, also affect the supplemental iron. In addition, some iron salts interfere with the storage properties of flour (Farrand et al., 1968).

The investigation described in this chapter was done to find out if a different approach might be more rewarding Chemical

analyses suggest that the quantity of iron present in many cereal diets is adequate (Ramalingaswami and Patwardhan, 1949); it is its availability for absorption that is poor. If such iron could be rendered more absorbable, a significant improvement in iron nutrition would be anticipated. There is previous evidence that ascorbic acid can enhance the absorption of iron in certain foods, including eggs (Moore and Dubach, 1951; Callender et al., 1970) and cereals (Steinkamp et al., 1955; Callender and Warner, 1968; Elwood et al., 1968; Kuhn et al., 1968). However in these studies, the ascorbic acid was taken as orange juice or added after cooking, and it is not known whether it would be effective if introduced before the preparation of the food. Since a successful dietary supplement would have to be present in some standard dietary ingredient, such as salt, the present investigation was designed to examine the effect of ascorbic acid added to staple foods prior to cooking. The staples tested were maize, soya and wheat, and they were fed as porridge, biscuits and bread respectively. In these studies use was made of different radio-iron isotopes, one labelling the intrinsic vegetable iron and the other labelling an added iron salt. In this way it was possible to monitor the contribution of each to iron absorption.

4.2 Methods

4.2.1 Preparation of Test Meals

The vegetables that were used in this study had been hydroponically labelled with iron (^{55}Fe) in the Department of Botany, University of Washington, by methods previously described (Hussain et al., 1965), and 5 μc was administered to each subject.

In addition to the intrinsic ^{55}Fe label, 1.5 µc extrinsic ^{59}Fe tracer was added to the water prior to cooking in all studies; in some experiments it was given together with small amounts of iron (2 to 5 mg) as ferric ammonium citrate. Ascorbic acid in doses varying between 50 and 250 mg was added prior to cooking in certain studies. Aliquots of porridge, biscuits or bread were taken for the preparation of isotopic standards and for determining the total iron and ascorbic acid contents.

The meals themselves were prepared as follows :

a) Maize Porridge

^{55}Fe-labelled maize was thoroughly blended with carrier maize to provide 50 g dry maize per subject and cooked with four times its weight of water to make a porridge. The final weight of porridge eaten by each subject was approximately 200 g.

b) Soya Biscuits

^{55}Fe-labelled soya was thoroughly blended with carrier soya to provide 20 g dry soya per subject and water was added to form a thick paste. This was separated into approximately equal parts and baked into biscuits in an oven for 45 minutes at 400°F.

c) Whole Wheat Bread

Because there were no facilities for milling the radio-active wheat, it was necessary to use 100% extraction flour. The labelled wheat was thoroughly blended with carrier whole wheat

to provide a final weight of 450 g 100% extraction flour. The baked loaf weighed 550-600 g and each subject ate approximately 80 g.

4.2.2 Administration of Test Meals

After fasting overnight, the volunteers were weighed and blood samples were taken for serum iron, unsaturated iron-binding capacity and haemoglobin estimations. Precisely weighed quantities of maize porridge, soya biscuits or whole wheat bread were then eaten. Each subject was given 250 ml water to drink during the meal and, in addition, sugar was allowed with the porridge. No further food or drink was permitted for 4 hours. Fourteen days later the volunteers were again assembled after an overnight fast and blood samples were taken for the measurement of ^{55}Fe and ^{59}Fe activities. Immediately thereafter, a freshly prepared solution containing 3 mg iron as ferrous sulphate, 30 mg ascorbic acid and 3 μc ^{59}Fe tracer in 150 ml distilled water was given to each subject. The absorption of the iron in this 'reference' salt was calculated from the corrected radio-activity present in a blood sample taken after a further 14 days.

4.2.3 Isotopic and Chemical Methods

Aliquots of food (1 g) and blood samples (10 ml) were prepared for differential radio-active counting using the method of Katz et al. (1964). The quantities of ^{55}Fe and ^{59}Fe in the processed samples were determined using a liquid scintillation spectrometer (Packard Tri-Carb Model 3002) with Insta-Gel (Packard Instrument Co., Donners Graz, Illinois) as the scintillant.

The counting efficiency was 3-4% for ^{55}Fe and approximately 50% for ^{59}Fe at optimal gain and window settings. The radio-activity present in the 4 ml blood samples collected immediately before the administration of the 'reference' iron salt, and again 14 days later, was determined by means of a scintillation spectrometer (Packard Auto-Gamma Tri-Carb Model 3001) against suitable standards. The counting efficiency was approximately 20%. Percentage absorption was calculated on the assumption that 100% of the absorbed radio-activity was in circulation and that the blood volume of each subject was 65 ml/kg body weight.

Plasma iron concentrations were measured by a modification (Bothwell & Finch, 1962) of the method of Bothwell & 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 & Finch, 1962) of the method of Lorber (1927).

Measurement of the reduced ascorbic acid content of the meals was performed using the modified method of Tillmans et al. (1932) as described by Roe (1967). The content of total ascorbic acid (including dehydroascorbic and diketogulonic acids) was estimated by the method of Roe & Kuether (1943) as described by Roe (1967).

4.3 Results

4.3.1 Comparison between Absorption of Extrinsically and Intrinsically Labelled Iron.

The availability of staples which had been labelled hydroponically with ^{55}Fe made it possible to find out the degree to which the absorption of ^{59}Fe labelled extrinsic iron added during cooking reflected the absorption of the iron contained within the foodstuff. If the ^{55}Fe labelled intrinsic iron and the ^{59}Fe labelled extrinsic iron formed a single pool, then it would be anticipated that the ratio of the absorptions of each isotope would be unity. The chronological order of the studies was not the same as the order in which they are set out in the Tables. This was due to the fact that use had to be made of hydroponically labelled material as it became available. The first three studies were A and B in Table I and J in Table IV. In each of these studies, difficulty was experienced in preparing the blood samples for measurement of the ^{55}Fe content and experiments indicated that not much more than 50% was being recovered. This is reflected in the mean ratios of extrinsic ^{59}Fe to intrinsic ^{55}Fe labels, which were 1.85, 2.42 and 1.85 respectively. Minor modifications in the methods of sample preparation were then instituted and no further difficulties were encountered. The mean ratios of extrinsic to intrinsic labels in the remaining studies were 1.15, 1.13, 1.12, 1.03, 1.08, 1.11, 1.14 and 1.15. The mean ratio for the 42 individuals in whom the absorption was greater than 1% was 1.12 (S.D. $\pm$ 0.08).

4.3.2 Effect of Ascorbic Acid on the Absorption of Iron in Maize Porridge (Table 1 and 11).

The mean absorption of the reference salt was 55.8% in the control group (A), and this was higher than it was in all the other groups with the exception of E (65.2%). Furthermore, the mean haemoglobin concentration of 7.3 g/100 ml in group A was more than 2 g/100 ml lower than that of any other group. On this basis it was felt that the increased iron absorption in the groups given ascorbic acid was not ascribable to a greater degree of iron deficiency.

There are several points of interest in the results. Firstly, it is apparent that ascorbic acid in a dosage of 50 mg increased the mean absorption by between two and four times (compare studies B and C with study A). Comparable mean absorptions were obtained with a 100 mg does of ascorbic acid (studies D and E). The addition of between 2.5 and 5.0 mg iron to the porridge appeared to have little, if any, effect on the percentage absorption. Further insight into the potential benefit to iron nutrition which might derive from ascorbic acid supplementation was obtained by calculating the actual quantities of iron absorbed in the various experiments. These quantities were derived from direct measurements of the iron contents of aliquots of the fed cereal. (The quantities obtained were invariably higher than expected, probably because of variable contamination of the carrier maize and/or iron contamination during cooking). The mean figures for absorption were as follows: maize porridge alone - 0.12 mg; supplement 50 mg ascorbic acid - 0.31 mg;

Table I. Absorption of iron in maize porridge with special reference to the effects of varying doses of ascorbic acid added prior to cooking.

TABLE I. Absorption of iron in maize porridge with special reference to the effects of varying doses of ascorbic acid added prior to cooking.

	Hb (g/100 ml)	Plasma iron (ug/100 ml)	% Saturation	% Iron absorption			Ratio Extrinsic / Intrinsic
				Reference Salt	^{55}Fe (Intrinsic)	^{59}Fe (Extrinsic)	
Study A	4.6	28	4.8	18.2	0.8	0.5	0.63*
	4.1	53	9.5	29.9	0.9	0.01	0.01*
No added ascorbic acid	9.0	47	9.9	76.6	2.3	3.9	1.70
	7.8	42	7.6	56.1	4.2	7.0	1.67
(final iron content 1.8 mg)	9.8	32	5.5	47.0	5.0	9.8	1.96
	8.2	29	6.0	65.1	6.4	13.4	2.09
	7.4	37	6.6	97.6	7.0	12.8	1.83
Mean	7.3	38	7.1	55.8	3.8	6.8	1.85
Study B	12.8	72	9.1	15.2	1.0	6.5	6.50
	10.0	58	33.8	46.2	7.2	21.2	2.94
50 mg ascorbic acid	9.0	41	8.8	42.6	13.6	26.0	1.91
	7.4	47	8.3	50.4	14.0	24.0	1.71
(final iron content 1.3 mg)	9.6	54	16.2	24.2	18.3	24.0	1.31
	10.6	75	14.3	88.4	23.7	28.1	1.19
	7.2	33	5.9	75.3	26.6	36.3	1.37
Mean	9.5	54	13.8	48.9	14.9	23.7	2.42
Study C	12.0	43	12.4	13.4	4.1	5.4	1.32
	13.6	152	38.7	14.8	8.8	9.2	1.05
50 mg ascorbic acid + 2.5 mg added iron	10.0	43	7.4	50.7	9.8	11.2	1.14
	9.4	29	5.1	36.4	10.5	12.6	1.20
(final iron content 7.4 mg)	12.4	91	25.4	34.9	12.6	14.2	1.13
	11.8	112	18.9	47.5	12.9	14.4	1.12
	11.8	102	21.8	13.6	14.3	16.6	1.16
	8.2	39	8.6	65.0	15.3	17.5	1.14
	12.2	70	15.3	20.8	20.4	22.6	1.11
Mean	11.3	76	17.1	33.4	12.1	13.7	1.15

*Results in which the absorption of food iron was less than 1.0% were excluded from calculations of the mean.

Table II. Absorption of iron in maize porridge with special reference to the effects of varying doses of ascorbic acid added prior to cooking.

	Hb (g/100 ml)	Plasma iron (µg/100 ml)	% Saturation	% Iron Absorption			Ratio Extrinsic / Intrinsic
				Reference salt	^{55}Fe (Intrinsic)	^{59}Fe (Extrinsic)	
Study D	12.1	86	17.7	66.6	17.9	20.0	1.12
100 mg ascorbic acid + 2.5 mg added iron	8.6	37	6.5	39.7	17.9	23.1	1.29
	10.8	44	8.5	35.6	23.6	25.6	1.09
(final iron content 5.5 mg)	11.5	35	6.0	24.7	25.1	27.9	1.11
	11.8	141	32.5	21.8	28.5	30.1	1.06
Mean	11.0	69	14.2	37.7	22.6	25.3	1.13
Study E	7.2	24	4.7	59.6	9.8	11.1	1.13
100 mg ascorbic acid + 5 mg added iron	10.8	87	17.6	78.4	11.6	13.4	1.16
	12.4	117	21.5	72.5	14.8	16.3	1.10
(final iron content 9.4 mg)	13.2	148	35.4	52.0	20.0	22.6	1.13
	7.8	32	6.2	53.5	21.4	23.6	1.10
Mean	10.3	82	17.1	65.2	15.5	17.4	1.12
Study F	10.6	67	22.6	9.6	2.4	0.1	0.04*
250 mg ascorbic acid + 2.5 mg added iron + 1 egg	6.4	21	3.2	122.6	21.0	20.9	0.99
	12.5	140	33.7	27.6	23.8	24.4	1.03
(final iron content 6.1 mg)	10.4	42	8.9	33.4	35.5	37.2	1.05
	[illegible]	37	7.2	55.6	41.3	43.4	1.05
Mean	10.0	61	15.1	49.8	24.8	25.2	1.03

*Results in which the absorption of food iron was less than 1.0% were excluded from calculations of the mean.

supplements 50 mg ascorbic acid and 2.5 mg iron - 0.96 mg; supplements 100 mg ascorbic acid and 2.5 mg iron - 1.36 mg; supplements 100 mg ascorbic acid and 5 mg iron - 1.60 mg. In a final experiment, porridge containing a large amount of ascorbic acid (250 mg) and 2.5 mg iron was fed together with a soft boiled egg. The mean absorption remained high (25%).

4.3.3 Effect of Ascorbic Acid on the Absorption of Iron in Soya Biscuits (Table 111).

The mean absorption of iron in soya biscuits containing 2 mg supplemental iron was good, being approximately 20% in five individuals. In a further five subjects, supplementation with 100 mg ascorbic acid had no effect. Indeed, the mean absorption of 15% was somewhat lower than in the basal study. This may have been due to the fact that mean reference absorption in the second group was less than in the first, the figures being 47.1% and 72.8% respectively.

4.3.4 Effect of Ascorbic Acid on the Absorption of Iron in Bread (Table 1V).

The three groups of subjects which were studied were reasonably well matched in terms of the degree of iron deficiency. The mean percentage absorption in the five subjects given unsupplemented bread was similar to that noted for maize porridge (9.0% as compared with 6.8%). In the second experiment, enough ascorbic acid was added to the dough to provide 50 mg per person, but the mean absorption of the bread iron (10.0%) was not enhanced. In a final experiment, a 2.5 mg iron supplement was present in addition to the ascorbic acid.

Table III. Absorption of iron in soya biscuits with special reference to the effects of ascorbic acid added prior to baking.

	Hb (g/100 ml)	Plasma iron (µg/100 ml)	% Saturation	% Iron absorption			Ratio Extrinsic / Intrinsic
				Reference salt	^{55}Fe (Intrinsic)	^{59}Fe (Extrinsic)	
Study G	11.6	64	18.5	93.4	7.1	7.9	1.11
2 mg added iron but no ascorbic acid	10.0	31	6.8	57.6	8.1	8.7	1.07
	10.3	80	14.2	102.0	16.3	17.7	1.09
(final iron content 4.6 mg)	10.4	38	8.7	11.5	19.6	21.5	1.10
	11.5	56	11.2	99.7	48.0	49.9	1.04
Mean	10.8	54	11.9	72.8	19.8	21.1	1.08
Study H	12.6	142	45.5	8.7	1.0	1.3	1.30
2 mg added iron plus 100 mg ascorbic acid	10.7	68	16.6	38.1	11.9	12.6	1.06
	11.4	75	18.4	63.5	15.6	16.5	1.06
(final iron content 4.6 mg)	8.0	113	21.2	51.6	20.5	20.4	0.99
	8.0	71	13.9	73.6	23.9	26.7	1.12
Mean	10.1	94	23.1	47.1	14.6	15.5	1.11

Table IV. Iron absorption from wheat bread (100% extraction) with special reference to the effects of ascorbic acid added prior to baking.

	Hb (g/100 ml)	Plasma iron (µg/100 ml)	% Saturation	% Iron absorption			Ratio Extrinsic / Intrinsic
				Reference salt	^{55}Fe (Intrinsic)	^{59}Fe (Extrinsic)	
Study I	8.4	18	3.7	69.7	0.6	1.2	2.00*
No added ascorbic acid	6.0	41	9.0	115.9	5.2	6.4	1.23
(final iron content	10.3	66	10.2	58.8	7.0	7.8	1.11
3.7 mg)	6.6	26	5.5	13.6	10.9	12.8	1.17
	9.0	18	3.8	112.8	15.9	16.9	1.06
Mean	8.1	34	6.4	74.2	7.9	9.0	1.14
Study J	8.0	37	8.7	90.0	0.8	2.1	2.63
50 mg ascorbic acid	11.9	72	25.7	2.7	1.7	3.5	2.06
(final iron content	9.1	23	4.2	13.2	2.2	4.3	1.96
3.1 mg)	8.4	25	4.8	11.7	2.7	5.5	2.19
	7.4	28	6.6	43.4	9.4	12.4	1.32
	8.4	19	4.9	101.0	13.4	18.2	1.36
	9.0	34	6.7	112.0	16.0	23.4	1.46
Mean	8.9	34	8.8	53.4	6.6	10.0	1.85
Study K	9.0	20	3.2	63.6	2.9	3.5	1.21
50 mg ascorbic acid	10.0	25	4.9	36.9	3.3	4.1	1.24
plus 2.5 mg added iron	8.4	15	2.7	8.5	3.4	3.8	1.12
(final iron content	8.4	59	10.7	78.5	4.3	4.6	1.07
4.7 mg)	9.3	32	5.6	56.3	12.4	13.9	1.12
Mean	8.6	30	5.4	48.8	5.3	5.9	1.15

*Results in which the absorption of food iron was less than 1% were excluded from calculations of the mean.

The mean percentage absorption (5.9%) was somewhat lower than in the two previous studies, although the amount absorbed was similar.

4.3.5 *In vitro* Tests on the Stability of Ascorbic Acid during Cooking

The fact that ascorbic acid enhanced the absorption of iron in corn porridge, but not in soya biscuits or wheat bread, suggested that it might have been oxidised to different degrees by the various cooking procedures. This was tested in several *in vitro* experiments. The concentrations of total and of reduced ascorbic acid were measured before and after preparation. Most of the ascorbic acid added to maize porridge was still in the reduced form after cooking, namely 68% of 50 mg and 71% of 100 mg. The levels of 'total' ascorbic acid, which include dehydroascorbic acid and diketogulonic acid, were virtually unchanged by cooking.

By comparison, only 15% of 100 mg of added ascorbic acid was present in the reduced form in soya biscuits after baking for 45 minutes. The level of "total" ascorbic acid at this time was 37%.

In the case of bread, losses in reduced ascorbic acid were revealed, not only after, but also prior to baking (Table V). For example, at the concentration of ascorbic acid used in the *in vivo* studies, 35% of the reduced ascorbic acid had disappeared even before baking. After baking there was virtually no reduced ascorbic acid left in either the white (72% extraction) or brown bread (80% extraction) and the amount of 'total' ascorbic acid was about half the original figure.

Sample	Ascorbic acid added (mg)	% Loss of ascorbic acid	
		"Total" **	Reduced
White dough	250*	12	4
	1000	8	37
Brown dough	250*	10	35
	1000	10	63
White bread	250*	52	84
	1000	34	89
Brown bread	250*	54	86
	1000	45	86

* Indicates level of ascorbic acid added in in vivo bread studies.

** "Total" ascorbic acid refers to ascorbic acid, dehydroascorbic acid and diketogulonic acid.

Table V. Recovery of ascorbic acid added to white and brown dough (600 g) before and after baking into loaves (540 g)

4.4 Discussion

Some of the findings in these studies confirm the observations of Cook et al. (1972) and of Björn-Rasmussen and Hallberg (1972) that when inorganic iron and non-haem food iron are eaten together, they are absorbed from a common pool. With the exception of the initial experiments, in which there were technical difficulties, the ratio of the absorption of the extrinsic to intrinsic radio-active tags was close to unity (1.12,S.D. ± 0.03). These figures were obtained in a consecutive series of 42 tests in which the absorption varied between 1% and 50% and the quantity of extrinsic iron ranged from tracer amounts up to 5 mg (Fig.4).

All the subjects studied were sufficiently iron deficient to absorb large percentages of the 3 mg reference dose of ferrous iron, and yet the absorption of similar amounts of food iron was poor. Comparable mean figures were as follows : maize porridge 6.8% as compared with 55.8%, soya biscuit 19.8% as compared with 72.8%, and wheat bread 7.9% as compared with 74.2%. The iron in soya beans was the best absorbed of the three vegetables. This is in keeping with the earlier observations made by Layrisse et al. (1969). It should be noted, however, that in later investigations by Layrisse & Martinez-Torres (1971), the percentage absorption of iron from soya beans was considerably lower. This discrepancy may be the result of different cooking methods or differences in the cultivation of the soya beans.

The most striking observation in the study was the pronounced enhancement of iron absorption on adding ascorbic acid to maize

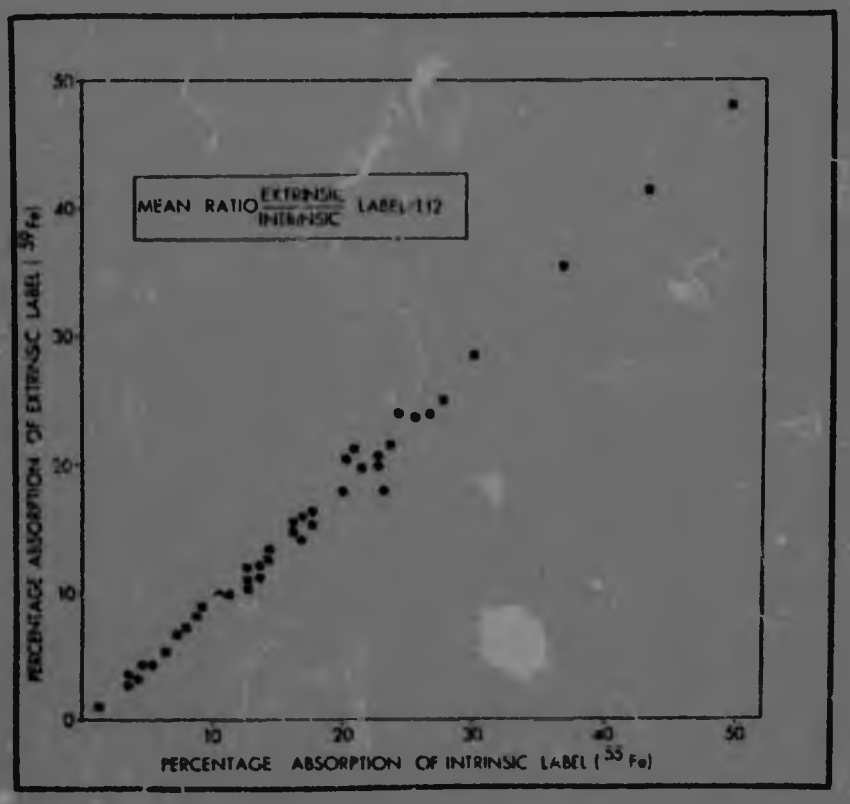

Figure 4. Plot of extrinsic iron (as ferric ammonium citrate) absorption against intrinsic iron (wheat, maize and soya) absorption.

porridge prior to cooking. When the iron content of the meal was increased by supplementation with ferric ammonium citrate, the quantity of iron absorbed was greater than with ascorbic acid alone, although the percentage absorption fell. Doubling the ascorbic acid supplement led to a further significant increase in the actual amount of iron absorption. The nutritional advantage in quantitative terms was considerable: the mean calculated absorption from unsupplemented porridge was 0.12 mg, whereas it rose to 1.60 mg when 100 mg ascorbic acid and 5 mg inorganic iron had been added to the porridge (Fig.5). It was also possible to show that the consumption of an egg, which is known to inhibit iron absorption (Chodos et al., 1957; Elwood et al., 1968), failed to reduce absorption of iron in porridge to which a large amount of ascorbic acid had been added prior to cooking.

In contrast to its effect on the absorption of iron from maize porridge, the addition of ascorbic acid to soya biscuits and to bread made no difference. This appeared to be due to the fact that ascorbic acid was destroyed during the baking process, whereas only minimal oxidative degradation occurred during the preparation of the porridge. Presumably this can be ascribed to the much higher temperatures reached during baking, which are several fold greater than during boiling. Any potential value that ascorbic acid supplementation might have would therefore appear to be confined to those foodstuffs that are normally prepared by boiling. This would not necessarily exclude its use, since staples such as potato, rice and maize are usually cooked in this way. In addition,

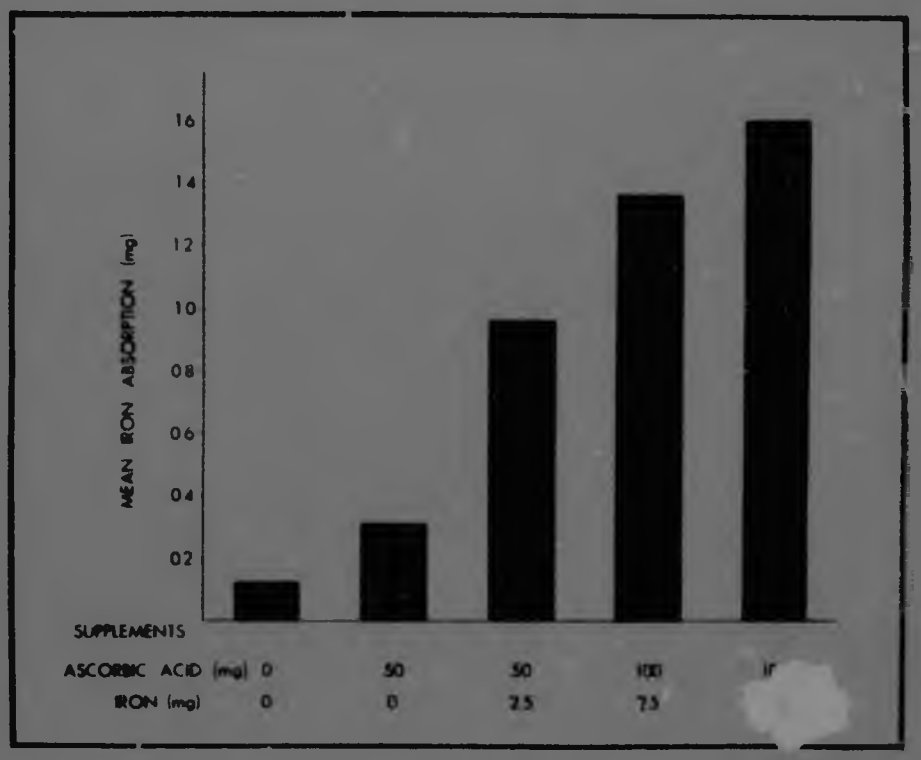

Figure 5. Iron absorption from a maize porridge meal showing the effect of various amounts of supplementary ascorbic acid and iron (as ferric ammonium citrate).

there is only 13.4% loss of reduced ascorbic acid during 2 hour cooking of soup (Sayers, 1972). Of more practical importance is the cost of supplementing diets with large quantities of ascorbic acid. It remains, however, possible that small amounts together with iron supplements may improve iron nutrition to a sufficient degree; alternatively, there may be cheaper substances that are as effective.

One final point deserves comment. In the present study the correlation between the percentage absorption of the iron in the reference salt and the iron in the vegetable was not statistically significant. Relevant figures were as follows: maize without ascorbic acid supplementation, $r = +0.71$, $P > 0.05$; maize with ascorbic acid supplementation, $r = +0.26$, $P > 0.1$; soya bean, $r = +0.45$, $P > 0.1$; wheat, $r = +0.44$, $P > 0.05$. These results are different from those reported by Layrisse et al. (1969), who noted a significant correlation between the reference salt absorption and that of the iron in a variety of vegetable foodstuffs. There are several possible reasons for this discrepancy. The numbers in the present study were small, while the degree of ascorbic acid and of iron supplementation varied. In addition, all the subjects were iron deficient, and the groups were therefore more homogeneous in terms of their absorptive behaviour than were the heterogeneous subjects studied by Layrisse et al.(1969).

Chapter Five

THE EFFECTS OF ASCORBIC ACID SUPPLEMENTATION ON THE ABSORPTION OF IRON IN RICE

5.1 Introduction

The study described in the preceding chapter, while confirming the beneficial effect of ascorbic acid on iron absorption from vegetables, was also of value in delineating the circumstances under which enhancement could be anticipated. The fact that ascorbic acid added during the preparation of a meal is advantageous only if the vitamin is not denatured by high temperatures imposes obvious limitations on its value to iron nutrition. What was of importance, then, was to establish whether the experience with maize could be extended to include other staples whose cooking did not jeopardize the efficacy of an ascorbic acid supplement. Rice was the obvious choice, not only because the staple is prepared by boiling, but also because it is a notoriously poor source of available iron that none the less provides the bulk of the dietary calories for many millions of people. The limited absorption of iron from rice, implying the presence of powerful inhibitory factors, seemed to provide a particularly relevant set of circumstances under which to test the role of a promoter. In this regard, it was decided to pay particular attention to the level of ascorbic acid supplementation required before iron nutrition was favourably influenced. The reasons for this were twofold. If a high level of vitamin supplementation was required, then, in the first place, the addition of a relatively expensive nutrient could impose economic restrictions on a fortification programme, and, secondly, there was the possibility that ascorbic acid in high concentration could alter the taste of the test rice meal.

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In keeping with the original intention that salt be investigated as a possible carrier for iron and ascorbic acid, these two supplements were added to the salt that was used in cooking the test meals. Although the major concern of the study was not the consumer acceptability of the fortified salt, it was nevertheless decided to enrich with ferrous sulphate rather than use ferric ammonium citrate, which was the form in which iron had been added to the staples tested in the first study. This step was taken because ferric ammonium citrate was immediately apparent to the naked eye when added to the cooking salt in the concentrations used in the study. The salt that was used was reagent grade sodium chloride (B.D.H. Chemicals Ltd., Poole, England). The feasibility of using salt in this way was tested by adding $FeSO_4.7H_2O$ and ascorbic acid in the concentrations used in the absorption experiments and leaving the mixture at room temperature in the laboratory. There was no discoloration or change in taste in a period of two months and the ascorbic acid content was unchanged.

5.2 Methods

5.2.1 Preparation of Test Meals

The absorption of iron from a traditional meal of rice with dhal (split pulses) sauce, or rice with potato and onion soup, was studied. The decision to give the meal in this form was taken after consultation with the field workers, who interviewed the Indian housewives volunteering for the absorption studies. It was their opinion that rice with either dhal or potato and onion was the most common main meal of the day that the housewives themselves prepared

for their families.

In some experiments, rice intrinsically labelled with ^{55}Fe by hydroponic culture (Hussain et al., 1965; Layrisse et al., 1969) was mixed with carrier rice to provide 5.0 µc per person, and the absorption of the ^{55}Fe was compared with that of 1.5 µc ^{59}Fe added as $FeSO_4.7H_2O$ during cooking. In other studies two meals were eaten on consecutive days, the $FeSO_4.7H_2O$ added during cooking being labelled with 5.0 µc ^{55}Fe on the one morning and 1.5 µc ^{59}Fe on the other morning.

a) Rice

Sufficient rice was weighed out to provide 100 g dry rice per individual. It was washed in running tap water and then boiled until it was soft in two and a half times its weight of tap water containing 4 g salt per individual. This usually occurred after about 15 minutes. In some studies the added salt contained 4 mg iron as $FeSO_4.7H_2O$ labelled with ^{59}Fe or ^{55}Fe. Various amounts of ascorbic acid were also present in the salt in certain experiments. After the grains were soft, the water was decanted and the rice was allowed to steam for a further 20 minutes. The cooked rice was divided into equal portions by weight and either pea dhal or potato and onion soup was added. An aliquot was retained for determining the contents of chemical iron and of radioisotopes.

b) Dhal

Sufficient dhal was weighed out to provide 50 g dry weight for each volunteer. It was boiled in four times its weight of water for 30 minutes together with small quantities of tomato, onion and seasoning in the form of tumeric powder and caraway seeds. Curry

leaves, garlic and jeera were fried and then homogenized with the pea dhal. The final volume eaten by each individual was approximately 200 ml.

c) Potato and Onion Soup

Sufficient onions and potatoes were weighed out to provide 50 g dry weight of each vegetable per subject. The onions were fried in oil and then added, together with the potatoes, to twice their combined weight of water. The mixture was boiled for 20 minutes, pepper and curry leaves being added as seasoning. The approximate volume of soup consumed by each individual was 175 ml.

5.2.2 Administration of Test Meals

Before each study the subjects were weighed and blood was taken for serum iron, unsaturated iron-binding capacity and haemoglobin estimations. The meals were eaten after an overnight fast. Water was allowed ad libitum during the meal and for 4 hours afterward, but no other food or drink was consumed during this time. Fourteen days later a blood sample was collected, after an overnight fast, for determination of its ^{59}Fe and ^{55}Fe content. Immediately afterwards 150 ml of a freshly prepared solution containing 30 mg ascorbic acid and 3 mg iron as $FeSO_4.7H_2O$ labelled with 3.5 μc ^{59}Fe was drunk. After a further 14 days a second blood sample was collected and its ^{59}Fe activity measured. The absorption of this 'reference' iron salt, which gave a measure of the absorbing capacity of each individual, was then determined by difference.

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5.2.3 Isotopic and Chemical Methods

The same methods as described in the preceding chapter were used for preparation of food and blood samples for differential radioactive counting and for measurement of serum iron concentrations, iron-binding capacities and iron contents of digested samples of food.

5.3 Results

5.3.1 Comparison between Absorption of Extrinsically and Intrinsically Labelled Iron (Table VI).

It was first considered necessary to establish whether an iron salt added to rice during cooking was absorbed to the same degree as was the intrinsic rice iron. This has been shown to occur with several other staple vegetable foods (Cook et al., 1972; Björn-Rasmussen & Hallberg, 1972) and was confirmed in the first study as occurring with maize, wheat and soya.

When six subjects consumed a single meal of rice intrinsically labelled with ^{55}Fe, which had been cooked with salt containing 4 mg iron as $^{59}FeSO_4$ and the rice was fed together with potato and onion soup, the absorption of each isotope was similar; the mean figure for the intrinsic label being 2.0% (SD $\pm$ 1.6) and for the extrinsic label 2.6% (SD $\pm$ 1.8) (Table VI). The poor absorption of the food iron was in striking contrast to the absorption of a reference iron salt, the mean figure for which was 49.0% (SD $\pm$ 18.5).

A second, similar experiment was performed in 9 subjects; the only difference was the presence in the salt of 100 mg ascorbic acid together with the 4 mg iron as $^{59}FeSO_4.7H_2O$. The mean absorption of the rice iron was 11.8% (SD $\pm$ 11.5) and

Table VI. Absorption of intrinsic iron (^{55}Fe) and extrinsic iron (^{59}Fe) from rice and vegetable soup meals fed with and without 100 mg ascorbic acid.

	Hb (g/100 ml)	Plasma iron (µg/100 ml)	% Saturation	% Iron absorption			Ratio
				Reference s.t	^{55}Fe (Intrinsic)	^{59}Fe (Extrinsic)	Extrinsic / Intrinsic
4 mg added iron as $FeSO_4.7H_2O$	13.4	123	33.2	29.2	0	0.6	-*
No added ascorbic acid.	14.0	61	16.8	29.4	0.7	1.2	1.7
(Final iron content 5.5 mg)	14.0	93	31.7	50.6	1.3	1.4	1.1
	9.0	44	9.7	64.3	2.5	2.9	1.2
	9.2	53	10.5	45.3	3.5	5.1	1.5
	12.8	36	9.4	75.2	4.1	4.3	1.1
Mean	12.1	68	18.6	49.0	2.0	2.6	1.3
4 mg added iron as $FeSO_4.7H_2O$ 100 mg added ascorbic acid. (Final iron content 6.6 mg)	10.7	142	50.2	10.8	0.9	1.2	1.3
	11.5	59	14.0	102.4	1.8	4.3	2.4
	14.8	150	-	40.9	5.5	7.5	1.4
	10.2	37	8.2	108.0	6.5	6.7	1.0
	13.2	98	30.2	34.8	8.9	10.2	1.2
	12.8	104	-	53.9	12.9	12.5	1.0
	13.4	73	17.5	43.7	15.5	18.2	1.2
	12.8	176	40.9	89.7	15.5	20.5	1.3
	13.4	96	20.3	65.9	38.8	34.0	0.9
Mean	12.5	104	25.9	61.1	11.8	12.8	1.3

*Results in which the absorption of food iron was less than 1.0% were excluded from calculations of the mean.

Table VI. Absorption of intrinsic iron (^{55}Fe) and extrinsic iron (^{59}Fe) from rice and vegetable soup meals fed with and without 100 mg ascorbic acid.

	Hb (g/100 ml.)	Plasma iron (µg/100 ml)	% Saturation	% Iron absorption			Ratio Extrinsic / Intrinsic
				Reference salt	^{55}Fe (Intrinsic)	^{59}Fe (Extrinsic)	
4 mg added iron as $FeSO_4.7H_2O$	13.4	123	33.2	29.2	0	0.6	-*
	14.0	61	16.8	29.4	0.7	1.2	1.7
No added ascorbic acid.	14.0	93	31.7	50.6	1.3	1.4	1.1
	9.0	44	9.7	64.3	2.5	2.9	1.2
(Final iron content 5.5 mg)	9.2	53	10.5	45.3	3.5	5.1	1.5
	12.8	36	9.4	75.2	4.1	4.3	1.1
Mean	12.1	68	18.6	49.0	2.0	2.6	1.3
4 mg added iron as $FeSO_4.7H_2O$ 100 mg added ascorbic acid. (Final iron content 6.6 mg)	10.7	42	50.2	10.8	0.9	1.2	1.3
	11.5	59	14.0	102.4	1.8	4.3	2.4
	14.8	150	-	40.9	5.5	7.5	1.4
	10.2	37	8.2	108.0	6.5	6.7	1.0
	13.2	98	30.2	34.8	8.9	10.2	1.2
	12.8	104	-	53.9	12.9	12.9	1.0
	13.4	73	17.5	43.7	15.5	18.2	1.2
	12.8	176	40.9	89.7	15.5	20.5	1.3
	13.4	96	20.3	65.9	38.8	34.0	0.9
Mean	12.5	104	25.9	61.1	11.8	12.8	1.3

*Results in which the absorption of food iron was less than 1.0% were excluded from calculations of the mean.

of the extrinsic label 12.8% (SD ± 10.1); the figure for the reference salt was 61.1% (SD ± 33.0) (Table VI). It was concluded from these two experiments that supplemental $FeSO_4.7H_2O$ with or without added ascorbic acid was absorbed to the same degree as was the intrinsic rice iron. In addition, it seemed clear that 100 mg ascorbic acid enhanced the absorption of both the added iron and the intrinsic iron.

An important point to emerge during this study was the fact that these conclusions only hold if the intrinsically labelled rice is properly cooked. In a preliminary study on 9 subjects, iron absorption was measured from rice prepared with salt containing 35 mg added ascorbic acid. The mean absorption of the intrinsic label was significantly less than that of the extrinsic label; the figures were 3.0% (SD ± 2.4) and 7.4% (SD ± 4.9) respectively ($t = 4.24$, $p < 0.005$). On inspection, some grains of rice were obviously incompletely cooked and when separated were found to contain the ^{55}Fe. It was thus clear that the labelled rice required longer cooking than the carrier rice. Thereafter, care was taken to ensure that the labelled rice was cooked until soft.

5.3.2 Effect of 35 mg Ascorbic Acid on the Absorption of Iron in a Rice Meal (Table VII).

Since the results of the previous study strongly suggested that 100 mg ascorbic acid enhanced the absorption of iron in a rice meal, further studies were done to find out whether smaller doses had a similar effect.

In the first experiment, 10 volunteers ate a meal of rice and dhal sauce. On the one morning, the meal was cooked with salt

Table VII. Absorption of iron from rice meals fed with 4 mg supplementary iron as $FeSO_4.7H_2O$ with and without 35 mg ascorbic acid.

	Hb (g/100 ml)	Plasma iron (µg/100 ml)	% Saturation	% Iron absorption		
				Reference Salt	Without ascorbic acid	With ascorbic acid
Final iron content	12.8	130	28.5	7.4	0.7	0.1
	12.8	155	29.9	29.4	1.1	2.6
	11.6	103	29.6	74.5	2.0	7.9
a) Meal without ascorbic acid 7.8mg.	12.8	93	20.9	10.6	2.1	0.4
	12.0	93	20.8	43.0	2.8	6.6
	13.6	105	21.4	70.7	3.4	4.8
	12.8	70	23.5	10.9	5.3	1.6
	11.2	31	6.7	102.0	6.3	13.4
b) Meal with ascorbic acid 7.0mg.	12.2	62	17.4	61.2	8.1	7.4
	8.8	54	10.4	101.3	11.7	15.3
Mean	12.1	90	20.9	51.1	4.4	6.0

containing 4 mg iron as $FeSO_4.7H_2O$ and on the other with the same dose of iron plus 35 mg ascorbic acid. The mean absorption without ascorbic acid was 4.4% (SD ± 3.5), while the mean absorption of the iron in the meal with added ascorbic acid was 6.0% (SD ± 5.2) (Table VII). The difference between the two means was not significant ($t = 1.52$, $P > 0.1$).

The failure to demonstrate significant enhancement of iron absorption at this level of fortification could have been for one of two reasons. While it seemed probable that the dose of ascorbic acid was too low, the possibility that the dhal sauce contained some inhibitory factor not present in the potato and onion soup, could not be excluded. A formal comparison was therefore made between the two meals. As before, 4 mg iron as $FeSO_4.7H_2O$ and 35 mg ascorbic acid were present in the salt. Twelve subjects consumed the rice with dhal sauce on the first morning, the $FeSO_4.7H_2O$ being labelled with ^{55}Fe, and the rice with soup meal on the second morning. The isotope label on the second occasion was ^{59}Fe.

While the mean absorption from the meal containing dhal sauce was somewhat less (3.2%, SD ± 1.9) than that from the soup meal (6.6%, SD ± 6.3), the difference was not significant ($t = 2.15$, $P > 0.05$). Mean absorption from the reference salt was 44.7% (SD ± 24.5) (Table VIII).

5.3.3 Effect of 60 mg Ascorbic Acid on the Absorption of Iron in a Rice Meal (Table IX).

Since it appeared that the presence of dhal sauce was not enough to account for the relatively low absorption obtained

Table VIII. Absorption of iron from rice and soup compared with absorption from rice and dhal, both meals fed with 4 mg supplementary iron as $FeSO_4.7H_2O$ and 35 mg ascorbic acid.

	Hb (g/100 ml)	Plasma iron (µg/100 ml)	% Saturation	% Iron absorption		
				Reference salt	Dhal meal	Soup meal
Final iron content	11.5	118	39.8	12.1	0.2	0.5
	12.1	-	-	18.9	1.0	0.3
a) Meal fed with dhal	13.2	168	44.1	31.5	1.4	3.2
	13.2	184	28.2	38.9	1.7	1.6
6.2 mg	9.4	45	9.9	39.6	2.2	4.4
	11.8	47	9.6	34.6	3.1	7.3
b) Meal fed with soup	9.7	54	11.5	60.9	3.3	4.4
	10.1	38	8.6	103.8	3.3	23.4
5.1 mg	7.0	43	9.4	50.9	5.0	7.1
	9.4	34	8.9	60.7	5.0	8.4
	11.2	62	15.9	58.1	5.4	11.2
	12.0	72	14.8	26.7	6.3	7.4
Mean	10.9	79	18.2	44.7	3.2	6.6

with 35 mg of added ascorbic acid, a study was done in which the effect of increasing the amount to 60 mg was assessed. This was carried out in 8 volunteers given a rice and soup meal. The presence of 60 mg significantly increased the percentage absorption from 3.2% (SD ± 2.4) to 11.9% (SD ± 9.7) ($t = 3.25$, $P < 0.02$). The mean absorption of the reference salt in the group was 41.6% (SD ± 29.6) (Table IX).

5.3.4 Effect of 100 mg Ascorbic Acid on the Absorption of Iron in a Rice Meal (Table X).

In a final study the level of ascorbic acid supplementation was increased to 100 mg to find out whether further enhancement of iron absorption could be achieved. Eleven volunteers consumed rice meals with potato and onion soup on two successive mornings; on the one occasion the added $^{55}Fe\ SO_4.7H_2O$ was included without ascorbic acid while on the other the label was $^{59}FeSO_4.7H_2O$ and 100 mg ascorbic acid was also present. The mean absorption without ascorbic acid was 4.2% (SD ± 4.2), while with ascorbic acid it was 12.2% (SD ± 10.2); the difference was significant ($t = 4.42$ $P < 0.005$). The mean absorption of the reference iron salt was 44.5% (SD ± 27.0) (Table X). It was concluded that 100 mg ascorbic acid was no more effective than was 60 mg in enhancing iron absorption from a rice meal.

5.4 Discussion

The results of this study indicate that the intrinsic iron present in rice is absorbed to the same degree as is a small quantity of an inorganic iron salt fed at the same time, provided the rice is well cooked (Fig.6). The findings are in agreement with those of

Table IX. Absorption of iron from rice meals fed with 4 mg supplementary iron as $FeSO_4.7H_2O$ with and without 60 mg ascorbic acid.

	Hb (g/100 ml)	Plasma iron (µg/100 ml)	% Saturation	% Iron absorption		
				Reference salt	Without ascorbic acid	With ascorbic acid
Final iron content	14.8	84	25.2	4.9	0.2	1.0
	10.8	51	11.9	51.1	0.8	3.6
a) Meal without ascorbic acid 5.4 mg	12.8	50	10.7	8.6	1.9	6.1
	13.4	82	19.6	35.6	2.0	7.7
	12.1	76	23.2	51.1	3.6	16.9
b) Meal with ascorbic acid 5.7 mg	8.7	35	5.9	52.9	4.7	15.3
	10.8	53	21.7	30.4	5.4	12.8
	12.1	46	10.5	98.5	7.2	31.4
Mean	11.9	60	16.1	41.6	3.2	11.9

Table X. Absorption of iron from rice meals fed with 4 mg supplementary iron as $FeSO_4.7H_2O$ with and without 100 mg ascorbic acid.

	Hb (g/100 ml)	Plasma iron (µg/100 ml)	% Saturation	% Iron absorption		
				Reference salt	Without ascorbic acid	With ascorbic acid
Final iron content	14.8	161	43.2	29.5	0.2	0.2
	13.2	70	22.7	12.7	1.5	6.4
a) Meal without ascorbic acid 7.6 mg	12.0	124	31.2	6.2	1.6	7.7
	11.8	156	45.4	52.8	1.7	8.5
	8.7	26	4.3	46.2	2.5	7.6
b) Meal with ascorbic acid 6.3 mg	10.8	63	14.0	37.9	2.7	6.3
	11.2	100	24.0	13.7	3.2	6.4
	11.8	48	14.3	52.7	3.4	12.7
	11.2	78	13.5	60.3	3.7	12.3
	9.4	65	17.0	102.3	4.2	11.3
	11.0	43	6.8	52.6	12.8	31.8
	12.3	73	15.6	67.2	13.1	34.4
Mean	11.5	84	21.0	44.5	4.2	12.2

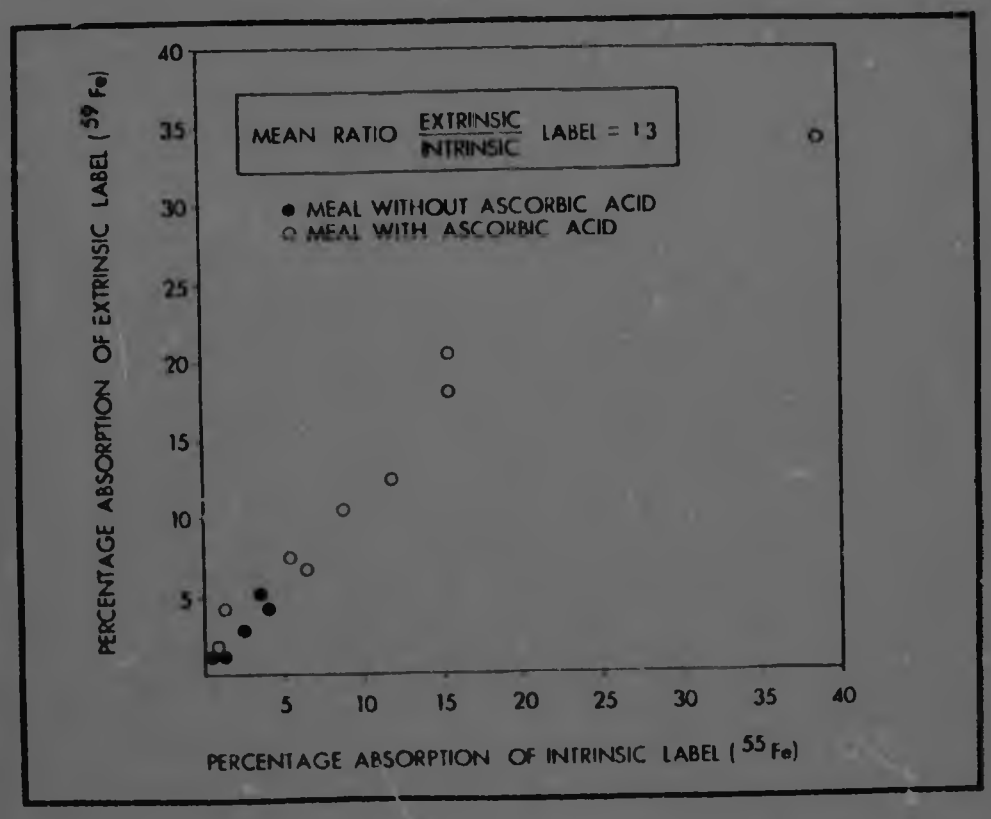

Figure 6. Plot of extrinsic iron (as ferrous sulphate) absorption against intrinsic iron absorption from rice.

other workers, who have noted the same phenomenon with a number of other vegetables (Björn-Rasmussen and Hallberg, 1972; Cook et al., 1972) and are in keeping with the observations that were made in Chapter Four. These various results are of both theoretical and practical importance. On the one hand, they suggest that the two forms of iron form a common pool, in so far as absorption is concerned, and, on the other hand, they indicate that there is little purpose in fortifying with iron a foodstuff that contains strong inhibitors of iron absorption. For example, the mean absorption of rice iron in a recent study was reported as less than 1% (Layrisse & Martinez-Torres, 1971); it would be anticipated that any iron added to such rice would be similarly absorbed.

The position can be put into even better perspective by the present results. The subjects studied were multiparous Indian women living in a community where iron deficiency anaemia is common. Further confirmation of the presence of iron deficiency in the group was obtained by feeding each of them a small dose of a ferrous salt; the majority absorbed the iron avidly. However, even in this group of women, absorption of food iron was low, with a mean figure of less than 4% for both intrinsic rice iron and added iron. Calculations from these data indicate a mean absorption of less than 0.3 mg from a supplemented meal containing a total of 7 mg iron. The addition of an adequate amount of ascorbic acid prior to boiling exerted a marked effect, with a threefold increase in total iron absorption (Fig.7). By varying the amount of added ascorbic acid it was possible to show that 60 mg was a satisfactory

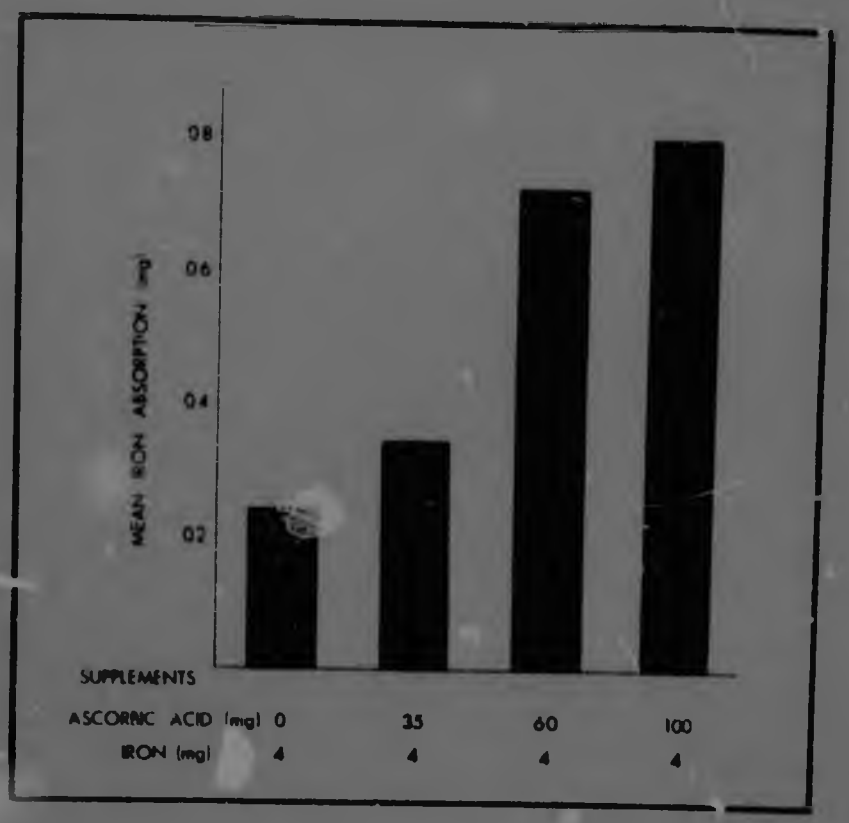

Figure 7. Iron absorption from a rice meal showing the effect of various amounts of supplementary ascorbic acid and iron (as ferrous sulphate).

dose, while 35 mg was insufficient.

These findings, together with those described in the preceding chapter, suggest that the iron nutrition of individuals subsisting on vegetable diets could be improved significantly if ascorbic acid could be added to the diet.

In the studies described in this chapter, the salt that was used in the cooking processes acted as the carrier for both the ascorbic acid and ferrous sulphate. Although these studies were not primarily concerned with the storage properties of supplemented salt, it was possible to show that both supplements could be added to reagent grade sodium chloride without producing discoloration or change in taste. However, the environment was temperate and it is possible that salt would prove a less satisfactory carrier under hot, humid conditions.

One sidelight of the study merits comment. A reference absorption was carried out on each subject using a small dose of a ferrous salt. This was done in order to have some idea of the iron status of individual subjects. The majority had high percentage absorptions thus confirming the presence of iron deficiency. Previous workers have noted a correlation between such reference absorptions and the absorption of food iron (Cook et al., 1972; Layrisse et al., 1973). In the present study, separate correlation coefficients were calculated for subjects receiving no ascorbic acid and for those receiving 35, 60 and 100 mg respectively. The figures were as follows:- no ascorbic acid, $r = +0.53$, $P < 0.001$; 35 mg, $r = +0.77$, $P < 0.001$, 60 mg, $r = +0.86$, $P < 0.01$; 100 mg, $r = +0.29$, $P > 0.1$.

In general, these results confirm the value of the reference salt absorption as a means of defining the absorbing capacity for iron of individual subjects.

Also important was the finding that the enhancement in iron absorption produced by a given dose of ascorbic acid was predictable on the basis of the absorption from an unsupplemented meal. The correlations between unsupplemented meals and meals supplemented with different amounts of ascorbic acid were as follows:- 35 mg, $r = +0.76$, $P < 0.01$; 60 mg, $r = +0.93$, $P < 0.001$; 100 mg, $r = +0.98$, $P < 0.001$.

Chapter Six

THE ROLE OF COMMON SALT AS A CARRIER FOR ASCORBIC ACID AND IRON

Chapter Six

THE ROLE OF COMMON SALT AS A CARRIER FOR ASCORBIC ACID AND IRON

6.1 Introduction

At this stage of the investigations, considerable headway had been made toward an appreciation of what role added ascorbic acid and iron could play in the enhancement of iron absorption from vegetable staples. It had been shown that the absorption of both supplemental ferric ammonium citrate and the intrinsic iron in maize is significantly improved if ascorbic acid is added before boiling. This improvement had also been demonstrated with supplemental ferrous sulphate and the intrinsic iron in rice. The observation that this did not occur with wheat bread and soya biscuits was ascribed to the high temperatures that are present during baking. What had also been established was the optimal quantity of ascorbic acid required for a standard rice meal and the efficacy of ascorbic acid when mixed together with the supplemental ferrous sulphate in the salt used for cooking. However, as only a cursory glance had been paid to the consumer acceptability of the fortified salt, it was decided to study this aspect more closely.

Fortification of common salt with ascorbic acid or iron (or both) would be practicable only if the appearance and taste of the salt does not alter, not only immediately after mixing, but also after storage, and if the appearance and taste of meals prepared with the salt are similiarly unaffected. With reference to these stipulations, it was pointed out in the last chapter that there were no objections from the Indian housewives when fortified, reagent grade sodium chloride was used in the preparation of the test meals.

Furthermore, this fortified sodium chloride stored well in the temperate conditions that prevailed in the laboratory.

While these observations were reassuring and provided some justification for the use of salt as a carrier for the two additives, it was obviously necessary to test this carrier under conditions that prevail in the home. In the first place, greater relevance to the domestic scene could be ensured by looking rather at the types of salt that are in common use. While the variety that is available is sufficiently impressive to exclude an individual assessment of the carrier potential of each brand, all the preparations that are sold for use in the kitchen or at the table can be grouped according to whether they are 'coarse' or 'fine' (Fig.8). Coarse salts are unrefined and, in contrast to the fine varieties, they have a larger particle size and contain no desiccants. The greater cost of the refined salts restricts their use to the more affluent consumer, a point which was underlined in the community where the absorption studies were being performed. This lower socio-economic group uses coarse salt exclusively. For this reason, particular attention was paid to the feasibility of fortifying the two brands of coarse salt that are most commonly used by the group.

6.2 Materials

6.2.1 Iron Preparations

The white or off-white iron compounds examined were ferrous sulphate $7H_2O$, ferrous ammonium sulphate, ferric ammonium sulphate, ferric orthophosphate xH_2O (all from B.D.H. Chemicals Ltd., Poole), ferric sulphate, ferric nitrate and ferric orthophosphate $4H_2O$ (all from Riedel-de Haen A.G., Seelze-Hannover).

Certain coloured iron compounds were also examined. These were ferric citrate, ferric ammonium citrate, ferric oxide and ferric

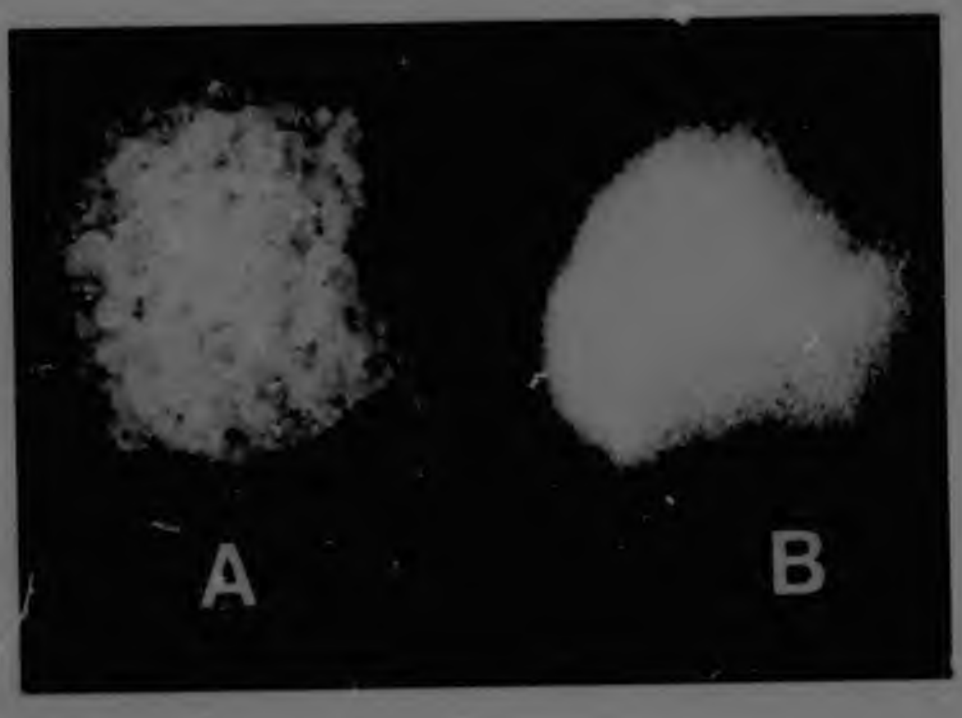

Figure 8. A 'Coarse' salt

B 'Fine' salt

chloride anhydrous (all from B.D.H. Chemicals Ltd., Poole) and ferric chloride $6H_2O$ (E. Merck A.G., Darmstadt). In addition, there were 3 iron powders; iron reduced fine powder (Malinckrodt Chemical Works, St. Louis) and two ferrum reductum samples with Fisher numbers 12.5 and 4.36 microns (Gliddon-Durkee, Cleveland).

6.2.2 Ascorbic Acid Preparations

The following types of ascorbic acid were used: L (+) ascorbic acid, analytical grade (B.D.H. Chemicals Ltd., Poole), L (+) ascorbic acid crystals, extra pure coated special grade S, sodium ascorbate (both E. Merck A.G., Darmstadt), ascorbic acid-2-sulphate dipotassium salt and coated ascorbic acid type EC (both F. Hoffmann-La Roche and Co. Ltd., Basle). An acetylated ascorbic acid prepared by the method of Creighton et al. (1948) was also tried.

6.2.3 Salt Preparations

Two grades of chemical NaCl were tested, namely 'laboratory' and 'analytical' (B.D.H. Chemicals Ltd., Poole) as well as a number of samples of commercial common salt. They included 'coarse', 'fine' and 'fine iodinated' salt from the Buffalo Salt Works and Packing Company, Johannesburg, and 'coarse' salt from Protea Provisions, Clairwood, Durban; Saxa Table Salt (Saxa Salt Company, Willesden, London) and Fine Fare Cooking Salt (Fine Fare Limited, Welwyn Garden City); Feinstes Tafel Saltz (Osterr. Salinen); and Dos Anclas Sal Fina (Compania Introductora de Beunos Aires S.A., Buenos Aires). The two 'coarse' salt samples from the South African suppliers differed from the other commercial varieties in that the salt had not been dried, washed or ground, and did not

contain desiccants.

6.2.4 Desiccants

The nature and concentration of desiccants present in the commercial salt preparations could be established only in certain instances. The two Buffalo 'fine' salts contained aluminium trisilicate. Saxa Fine Table Salt and Fine Fare Cooking Salt contained magnesium carbonate, while Dos Anclas salt contained both magnesium carbonate and sodium alumino-silicate. The concentration of all these desiccants was approximately 1%. In some experiments 1% magnesium carbonate, light (Hopkin and Williams Ltd., Chadwell Heath), 1% calcium chloride, dried (B.D.H. Chemicals Ltd., Poole), and 2.5% or 5% starch, dried (May and Baker Ltd., Dagenham) were added to the 'coarse' salts as desiccants.

6.2.5 Miscellaneous Additives

Sodium thiosulphate (B.D.H. Chemicals Ltd., Poole) was tried as an antioxidant at concentrations of 1 mg and 10 mg per 100 g, and ethylene diamine tetra-acetic acid (EDTA) (Miles-Seravac, Cape Town) 100 mg per 100 g as a chelating agent. Metaphosphoric acid 5% solution was made up from metaphosphoric acid sticks (B.D.H. Chemicals Ltd., Poole) and 0.15 ml was added to 100 g coarse salt in one experiment as an acidifier.

6.2.6 Storage Conditions

Samples were kept for up to 2 years at room temperature in Johannesburg under a calcium chloride desiccator, and also exposed on the laboratory shelf. The temperature range in the heated building was 22 to 27°C, and the mean relative humidity was 55%. Other

samples were kept in Durban, which enjoys a subtropical climate. The temperatures ranged from 5.5°C to 34.7°C, and the mean relative humidity was 77%. Extreme tropical conditions were simulated by storing further samples in an incubator at 40°C and 100% humidity. In addition, the effect of sealing some samples in plastic bags was evaluated.

6.3 Results

Whereas the white colour of ascorbic acid permitted its unobtrusive addition to common salt, this was not so in the case of many of the iron compounds. At a concentration of 100 mg iron per 100 g salt, which was calculated to be necessary for effective fortification, all but the white or off-white preparations produced an obvious change in the appearance of the salt. All the coloured iron compounds were therefore excluded from further study. An exception was, however, made in the case of the iron powders, since it was felt that the pale grey appearance of the fortified salt might still be acceptable to consumers.

Although initially acceptable, however, samples of salt fortified with powdered iron or with the white or off-white iron compounds frequently became grossly discoloured on storage, in some instances after only a few days. The colour varied with both the salt sample and the iron preparation, and ranged from yellow to black. Certain samples of salt fortified with ascorbic acid also became grossly discoloured on storage. Salt fortified with both ascorbic acid and iron became discoloured more rapidly than comparable samples fortified with either nutrient alone. Attempts were made to determine the factors influencing the development of colour in the

hope of discovering ways of preventing it.

6.3.1 Grades of Common Salt

Neither grade of chemical sodium chloride was discoloured by ascorbic acid or iron preparations, separately or in combination, except that a brown colour appeared if the fortified samples were kept in the incubator at 40°C and 100% relative humidity. This is illustrated in Fig.9. All the samples shown are the analytical grade of sodium chloride described earlier. Sample 'A' is without additives. 'B' and 'C' contain 100 mg of iron as ferrous sulphate and 1 g ascorbic acid respectively, per 100 g salt. Sample 'D' demonstrates the effect of incubation on sodium chloride supplemented with both iron and ascorbic acid.

In contrast to the way in which fortified, chemical grade sodium chloride behaved on storage, the two commercial brands of coarse salt became discoloured very rapidly. Within one week a yellow colour became obvious in coarse salt mixed with ascorbic acid (1 g ascorbic acid per 100 g salt) and stored in the Johannesburg laboratory. Samples 'A' and 'B' in Fig. 10 illustrate this point; while both samples were fortified with ascorbic acid at the same time, the change in appearance of the coarse salt when compared with that of the chemical sodium chloride is immediately apparent. When coarse salt was mixed with iron as ferrous sulphate (100 mg iron per 100 g salt) there was an even more rapid change in colour. Sample 'C' in Fig. 10 shows the appearance of the salt after only 48 hours. If both nutrients were present together, the discoloration was dramatic and the salt locked black after only 24 hours. (Sample 'D', Fig. 10).

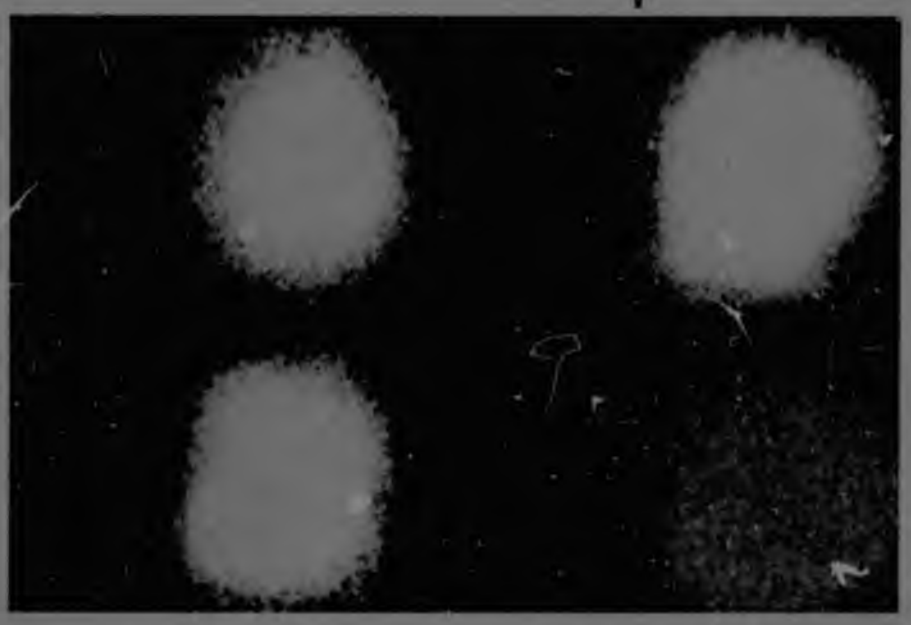

Figure 9. A Chemical grade sodium chloride

B Sodium chloride with iron as ferrous sulphate (100 mg/100 g NaCl)

C Sodium chloride with ascorbic acid (1 g/100 g sodium chloride)

D Effect of incubation on sodium chloride with iron and ascorbic acid

Figure 10. A Sodium chloride with ascorbic acid (1 g/100 g sodium chloride)

B Coarse salt with ascorbic acid (1 g/100 g coarse salt)

C Coarse salt with iron as ferrous sulphate (100 mg/100 g coarse salt)

D Coarse salt with iron and ascorbic acid

Fine commercial salt was much more resistant to discoloration than the coarse grades. Only one of the six brands became discoloured when fortified with either ascorbic acid or ferrous sulphate alone and stored in Johannesburg. The brand in question (Saxa Table Salt; Sample 'A', Fig.11) developed a tan colour when mixed with ascorbic acid (Sample 'B', Fig.11), and an orange speckled appearance when mixed with ferrous sulphate (Sample 'C', Fig.11). If both ascorbic acid and ferrous sulphate were present, the salt took on the appearance of Sample 'D' in Fig.11. The other five brands remained white after fortification with ascorbic acid, even when they were stored under subtropical conditions in Durban, but became discoloured when fortified with ferrous sulphate. When both ascorbic acid and ferrous sulphate were present, a pale, greyish-brown colour gradually appeared in every sample, even under the temperate climatic conditions of Johannesburg.

6.3.2 Storage Conditions

The rate of discoloration increased with increasing temperature and humidity, being critically dependent on the presence of water. Sealing the polythene packets containing the samples did not delay the discoloration. If the unsealed packets were kept in a calcium chloride desiccator in the Johannesburg laboratory, even the coarse commercial salts fortified with both ascorbic acid and ferrous sulphate were not discoloured after two years.

6.3.3 Desiccants

The rate of discoloration of coarse salt was not affected by adding 1% magnesium carbonate, one of the desiccants present in the

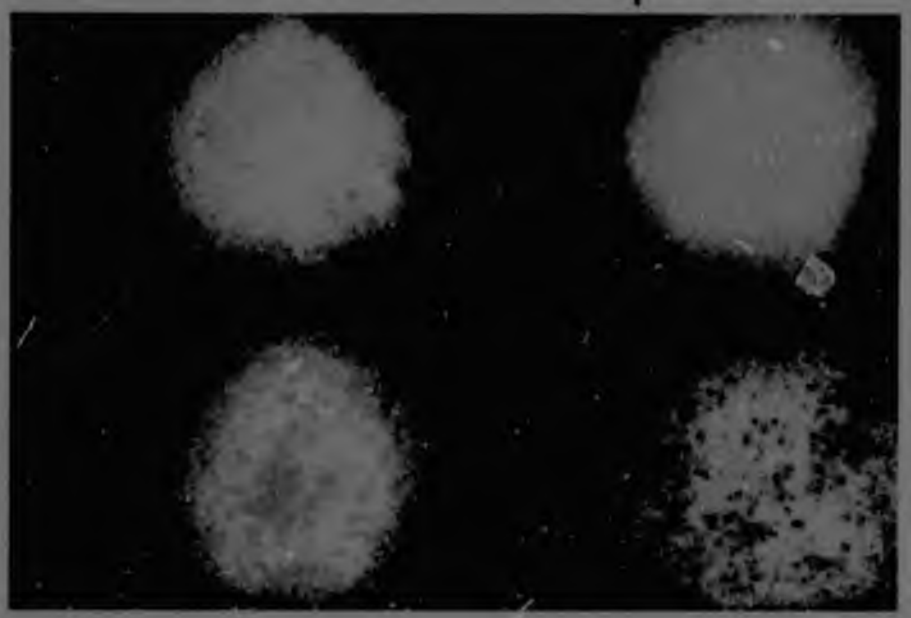

Figure 11. A Saxa Table Salt

B Saxa Table Salt with ascorbic acid (1 g/100 g salt)

C Saxa Table Salt with iron as ferrous sulphate (100 mg/100 g salt)

D Saxa Table Salt with iron as ascorbic acid

fine commercial salts, or 1% calcium chloride, either separately or in combination. The addition of 2.5% starch was more successful, and only a faint discoloration developed in coarse salt containing ascorbic acid and 2.5% starch, or in coarse salt containing ferrous sulphate and 2.5% starch, even when the samples were stored in Durban. When stored at 40°C and 100% relative humidity, however, colour did develop, and this was not prevented by increasing the concentration of starch to 5%. Coarse salt containing both ascorbic acid and ferrous sulphate together with 2.5% starch developed only a pale grey colour on storage in Durban, the best results being obtained if the starch and the ascorbic acid were thoroughly mixed with the salt before the ferrous sulphate was added.

6.3.4 Ascorbic Acid Preparations

Varying the preparation of ascorbic acid had no obvious effect on the rates of discoloration.

6.3.5 Iron Preparations

Discoloration developed more readily with some iron compounds than with others. The best results were obtained with ferric orthophosphate, which is relatively insoluble. It could be added with 2.5% starch to coarse salt and stored even at 40°C and 100% relative humidity without the development of discoloration. If ascorbic acid was also present, however, discoloration did appear under these extreme conditions, but the appearance of coarse salt containing 2.5% starch and fortified with both ascorbic acid and ferric orthophosphate remained acceptable on storage in Durban for more than one year.

The samples of coarse salt shown in Fig.12 all contain iron as ferric orthophosphate (100 mg iron per 100 g salt) and ascorbic acid (1 g per 100 g salt), however, only 'A' and 'B' contain starch as well. 'A' and 'C' were stored in Johannesburg and 'B' and 'D' in Durban. The effect of the starch in preventing discoloration is particularly striking in the Durban stored samples.

6.3.6 Miscellaneous additives

Neither sodium thiosulphate nor EDTA was found to be effective in retarding discoloration in any of the fortified samples studied. Metaphosphoric acid, however, did delay the discoloration of coarse salt by ascorbic acid stored under temperature conditions. No effect was seen under hot,humid conditions.

6.4 Discussion

In those countries where improvement of iron nutrition is most needed, much of the household salt consumed by the general population is unrefined. Usually it is prepared by solar evaporation of sea water at the coast, or brine at inland salt lakes, and the crystals may vary in size from 0.5 mm to 10 mm (Valassi, 1970). The observations reported in this chapter almost certainly indicate that such salt cannot be fortified with ascorbic acid unless steps are taken to prevent the development of discoloration which would make it unacceptable to the consumers. Further refining of the evaporate might be sufficient to achieve this if the climatic conditions in the region where the salt is to be distributed are moderate, since evidence was obtained in

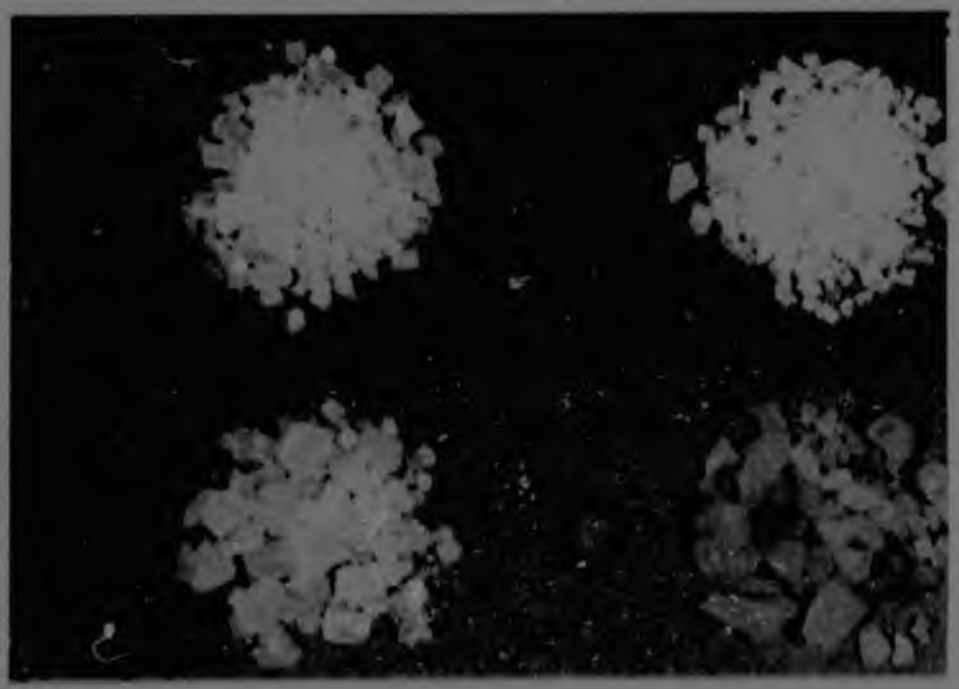

Figure 12. Coarse salt containing iron as ferric orthophosphate (100 mg/100 g salt)

A With additional 2.5% starch, stored in Johannesburg

B With additional 2.5% starch, stored in Durban

C Without starch, stored in Johannesburg

D Without starch, stored in Durban

the present investigation that impurities play a major role in the development of colour. Discoloration developed in only one of the six commercial brands of refined salt from various parts of the world when they were fortified with ascorbic acid and stored under all but the most extreme conditions, while the two chemical sodium chloride samples remained white unless they were kept in an incubator at 40°C and 100% relative humidity, when only a faint discoloration developed.

The nature of the reaction responsible for the development of colour was not investigated. One possibility is the 'browning reaction' which has been ascribed to the polymerization of unspecified lower molecular weight oxidation products of ascorbic acid (Hay et al., 1967). The discoloration did not seem to be influenced by the addition of the reducing agent, sodium thiosulphate, however, and the coloured compound or compounds may have been formed from the impurity in the salt rather than from the ascorbic acid. In favour of this suggestion is the fact that a similar discoloration developed when iron compounds were mixed with the salt and no ascorbic acid was present.

While further purification of the crude household salt might prevent discoloration, it must be doubted whether it is realistic to propose that this should be done on a large scale in order to make fortification with ascorbic acid possible. Not only would the expense be prohibitive, but the refined salt might be unacceptable to consumers accustomed to the coarse variety, unless the change were accompanied by an extensive education campaign. It would seem to be better if the salt could be fortified without its appearance or taste being altered

in any way. The present investigation indicated that it might be possible to achieve this by the addition of 2.5% starch.

Chapter Seven

IRON ABSORPTION FROM RICE AND MAIZE COOKED WITH FORTIFIED SALT

7.1 Introduction

The studies described in chapter six showed that crude common salt can be fortified with iron as ferric orthophosphate and ascorbic acid and stored under all but extreme tropical conditions, provided that starch is added to inhibit the development of discoloration. Although refined salt is more easy to fortify, its relevance to iron nutrition is limited by the fact that it is mainly the crude variety that is consumed in those countries where iron deficiency is a major problem.

While it was reassuring to find a way of getting iron and ascorbic acid into common salt, a major reservation concerned the availability of the supplementary ferric orthophosphate.

Early animal studies showed that anaemic rats utilized ferric orthophosphate less well than ferric chloride for haemoglobin regeneration (Freeman and Burrill, 1945).

Anaemic rats were also used to compare absorption of ferrous sulphate, ferrous chloride and ferric orthophosphate baked into bread (Blumberg and Arnold, 1947). Both ferrous preparations of iron were found to be four or five times as effective as ferric orthophosphate. Steinkamp and her colleagues subsequently employed radio-iron techniques in human volunteers to measure the availability of various iron compounds baked into bread. The compounds, which included ferric orthophosphate, were labelled with ^{59}Fe and absorption in healthy and anaemic volunteers was assessed by measuring both unabsorbed radio-activity in the stools and ^{59}Fe activity in the

circulating red cells. The iron preparations which they studied, namely ferric orthophosphate, reduced iron powder, ferrous sulphate and sodium ferric pyrophosphate were chosen because they were the iron salts then in vogue for the enrichment of baked foods. They found the four compounds to be equally effective, an unexpected result bearing in mind that the valence and solubility of the iron phosphate compounds detract from their availability.

This issue was reinvestigated by Cook and his colleagues, who also used radio-isotopic methods in healthy human volunteers (1973). They were concerned that the sodium iron pyrophosphate used by Steinkamp and her associates differed from that in current use by the baking industry for iron fortification, a concern that was justified by their results. Absorption of the sodium iron pyrophosphate was one-tenth and ferric orthophosphate one-third that of ferrous sulphate, when these compounds were used to fortify bread rolls.

Rao and Apte (1972) have also examined absorption from a range of iron phosphate compounds. They were particularly interested in sodium iron pyrophosphate, ferric pyrophosphate and ferric orthophosphate, because a number of their properties, namely insolubility, stability and lack of colour lend themselves to the fortification of common salt. When these labelled preparations were given alone to human volunteers and their absorption measured by whole body counting, ferric pyrophosphate was found to be completely unavailable. Mean absorption from ferric orthophosphate and sodium iron pyrophosphate was 8.3% and 6.3% respectively. By comparison, mean absorption

from ferrous ascorbate was 30.6%. In order to get a better understanding of the possible role of one of their iron preparations as an agent for fortifying common salt, they fed labelled sodium iron pyrophosphate with two meals. The bulk of the calories in these meals, which were typical of those normally eaten in India, was provided by wheat, rice and potato. These investigators found a mean iron absorption of only 0.8% when the meals contained 22.5 mg iron as sodium iron pyrophosphate and they were unable to detect any absorption when the meals contained 15.0 and 7.5 mg iron, also as sodium iron pyrophosphate.

Investigations such as these underlined the importance of *in vivo* testing of the fortified common salt whose development was described in the previous chapter. In particular, the point made by Rao and Apte that one of their iron phosphate compounds was even less available in the presence of a typical meal, was well taken. They stressed that not only is the nature of the iron salt important, but also the nature of the meal. In this regard, they postulated that factors such as phytates had an inhibitory influence on the absorption of the iron preparation and also pointed out that the actual bulk of the diet could play a similar role, an observation first made by Sharpe and his colleagues (1948). Rao and Apte's study did not, however, provide any information about the intrinsic iron content of the test meal or the absorption from this source. While it seemed unlikely that a relatively insoluble form of iron, namely sodium iron pyrophosphate, could enter a "common pool" with the non-haem iron in the meal, it was decided that this avenue should be investigated in the case of the ferric orthophosphate

fortified salt. The reasons for this were twofold. In the first place, the fortified salt also contained ascorbic acid and it was not known to what extent this could influence the availability of the added iron and, secondly, if absorption did take place from a "common pool", then the design of the absorption studies would be simplified considerably In particular, by being able to use only one radio-iron isotope to measure absorption from an entire meal, there would be opportunities for the volunteers to act as their own controls in the comparison of absorption from slightly different meals labelled with the other isotope.

It was planned, then, to measure absorption of both intrinsic and extrinsic (added) iron from typical rice and maize meals which had been cooked using the fortified coarse salt. For purposes of comparing the availability of a more soluble form of added iron, batches of fortified salt were also made up with iron as ferrous sulphate and used in the preparation of some of the meals. Because it had been demonstrated that coarse salt fortified in this way became unacceptably discoloured on storage, the ascorbic acid and iron as ferrous sulphate were added immediately before use.

7.2 Methods

7.2.1 Preparation of Test Meals

The absorption of iron from a traditional dish, either maize porridge or rice with potato and onion soup, was measured. These meals were prepared as described earlier in chapters four and five.

In the first two experiments, maize meal intrinsically labelled

with ^{55}Fe by hydroponic culture (Hussain et al., 1965; Layrisse et al., 1969) was mixed with unlabelled maize meal to provide 5.0 µc per person, and the absorption of the ^{55}Fe was compared with that of 2.5 µc ^{59}Fe which was present as ferric orthophosphate in the cooking salt. The second experiment differed from the first only in that 100 mg ascorbic acid was added with the ferric orthophosphate in the cooking salt. In all the other studies, rice or porridge meals were eaten on consecutive days, the variables being the nature of the added iron in the fortified salt (ferrous sulphate or ferric orthophosphate), the amount of added iron (sufficient to provide 2.5 mg or 4.0 mg per volunteer) and the amount of added ascorbic acid (0 mg, 50 mg or 100 mg per volunteer). In these studies the added iron compound was labelled with 5.0 µc ^{55}Fe on the one day and with 2.5 µc ^{59}Fe on the next. The ferrous sulphate was labelled by the addition of a solution of radio-active ferric chloride to a solution of ferrous sulphate. Labelled ferric orthophosphate was prepared from radio-active ferric chloride by the method of Steinkamp et al. (1955). This method, which was also used by Rao and Apte (1972) and Cook and his colleagues (1973) in their studies on the absorption of iron from ferric orthophosphate, involves an initial precipitation of ferric hydroxide. The ferric hydroxide is then dissolved in ortho-phosphoric acid and ferric orthophosphate is in turn precipitated out of solution by the addition of ammonium hydroxide.

7.2.2 Administration of Test Meals

After fasting overnight, the volunteers were weighed and blood samples were taken for serum iron, unsaturated iron-binding capacity and haemoglobin estimations. The subjects were given 250 ml water to drink while the meal was eaten, after which no further food or drink was permitted for 4 hours. In all but the first two experiments the procedure was repeated on the following morning. Fourteen days later the volunteers were again assembled after an overnight fast, and blood samples were taken for the measurement of ^{55}Fe and ^{59}Fe concentrations. Immediately afterwards they were given 150 ml of a freshly prepared solution containing 30 mg ascorbic acid and 3 mg iron as $FeSO_4.7H_2O$ labelled with 3.5 μc ^{59}Fe to drink. After a further 14 days a second blood sample was collected and the ^{59}Fe concentration measured.

7.2.3 Isotopic and Chemical Methods

The same methods as described in chapter four were used in the preparation of food and blood samples for differential radio-active counting and for measurement of serum iron concentrations, iron-binding capacities and iron contents of digested samples of food.

7.3 Results

7.3.1 Comparison between Absorption of Intrinsic Maize Iron and Added Ferric Orthophosphate (Table XI).

It was first considered necessary to establish whether the labelled iron compound used to fortify the cooking salt entered a common pool with the intrinsic maize iron. As was pointed out

earlier, the absorption of dietary non-haem iron from a common pool has been demonstrated with a number of staples (Björn-Rasmussen and Hallberg, 1972; Cook et al., 1972). What is more, the investigations described in chapter four confirmed absorption from a common pool in the case of maize, wheat and soya and results from studies described in chapter five indicated that the intrinsic iron in rice also entered a common pool with added iron.

Where the current experiment was particularly different was in the addition of a relatively insoluble iron compound; in the other studies referred to above, the added iron compound was more soluble, for example ferrous sulphate, ferrous chloride or ferric ammonium citrate. For this reason some concern was expressed as to the feasibility of monitoring what was happening to iron absorption from the meal by measuring absorption of an added iron preparation of limited solubility.

Five subjects ate a single meal of porridge made from maize labelled intrinsically with ^{55}Fe. The water used in cooking the porridge contained salt fortified with $^{59}FePO_4$ (2.5 mg iron for each volunteer). The absorption of the two isotopes was similar, the mean figure for both the intrinsic and the extrinsic labels being 1.2% (S.D. $\pm$ 0.97) (Table XI). The poor absorption of the food iron and the added iron was in striking contrast to the absorption of the reference iron salt, the mean figure for which was 47.8% (S.D. $\pm$ 24.4).

7.3.2 Effect of 100 mg Ascorbic Acid on the Absorption of Intrinsic Maize Iron and Added Ferric Orthophosphate (Table XI).

In an experiment similar to the previous one, 11 subjects were given the same meal, except that the cooking salt also contained sufficient ascorbic acid to provide 100 mg per participant. The mean absorption of the intrinsic label was 15.7% (S.D. ± 10.5) and of the extrinsic label 16.3% (S.D. ± 10.8) (Table XI). The mean absorption of the reference iron salt was 61.5% (S.D. ± 37.4). It was concluded from the first two experiments that iron added to the porridge in the form of ferric orthophosphate, with or without ascorbic acid, was absorbed to the same extent as the intrinsic maize iron. It was therefore considered permissible to use only an extrinsic isotopic label in subsequent studies. This effectively "freed" one isotope for use in a different meal. This meant that iron absorption could be measured, in the same individual, from two meals which differed in only one constituent.

In addition, ascorbic acid in a dosage of 100 mg appeared to enhance the absorption of both the intrinsic maize iron and the supplemental iron, although the comparison was not a direct one (Fig. 13).

7.3.3 Effect of 100 mg Ascorbic Acid on the Absorption of Iron in a Rice Meal (Table XII).

Seven subjects ate the rice meal prepared with salt containing 2.5 mg iron as $^{55}FePO_4$. On the following day the

	Hb (g/100 ml)	Plasma iron (µg/100 ml)	% Saturation	% Iron absorption		Ratio
				Reference salt	^{55}Fe (Intrinsic)	^{59}Fe (Extrinsic)	Extrinsic / Intrinsic
2.5 mg added iron but no added ascorbic acid. Final iron content 4.1 mg	13.4	61	16.9	39.6	0.2	0.3	-
	9.4	49	9.6	75.2	0.3	0.2	-
	16.8	206	53.9	20.1	1.1	2.2	1.1
	12.8	100	27.1	43.5	1.9	1.9	1.0
	13.4	62	13.5	70.6	2.4	2.4	1.0
Mean	13.2	96	24.2	47.8	1.2	1.2	1.0
2.5 mg added iron and 100 mg added ascorbic acid. Final iron content 4.0 mg	14.0	-	-	4.2	1.4	1.2	0.9
	12.1	56	13.8	23.2	2.7	2.8	1.0
	[illegible]	72	22.0	56.8	6.6	6.6	1.0
	[illegible]	38	9.7	41.7	6.8	7.9	1.2
	13.4	104	25.2	99.9	14.3	14.5	1.0
	11.5	43	10.9	101.1	15.0	16.0	1.1
	14.0	71	15.4	85.3	17.4	18.6	1.1
	10.6	48	8.6	103.0	22.5	23.1	1.0
	14.0	-	-	19.8	25.3	26.9	1.1
	14.8	216	52.4	79.6	29.9	29.9	1.0
	10.8	-	-	-	30.8	31.7	1.0
Mean	13.5	84	19.8	61.5	15.7	16.3	1.0

Table XI. Absorption of intrinsic iron (^{55}Fe) and extrinsic iron ($^{59}FePO_4$) from maize porridge meals fed with and without 100 mg ascorbic acid.

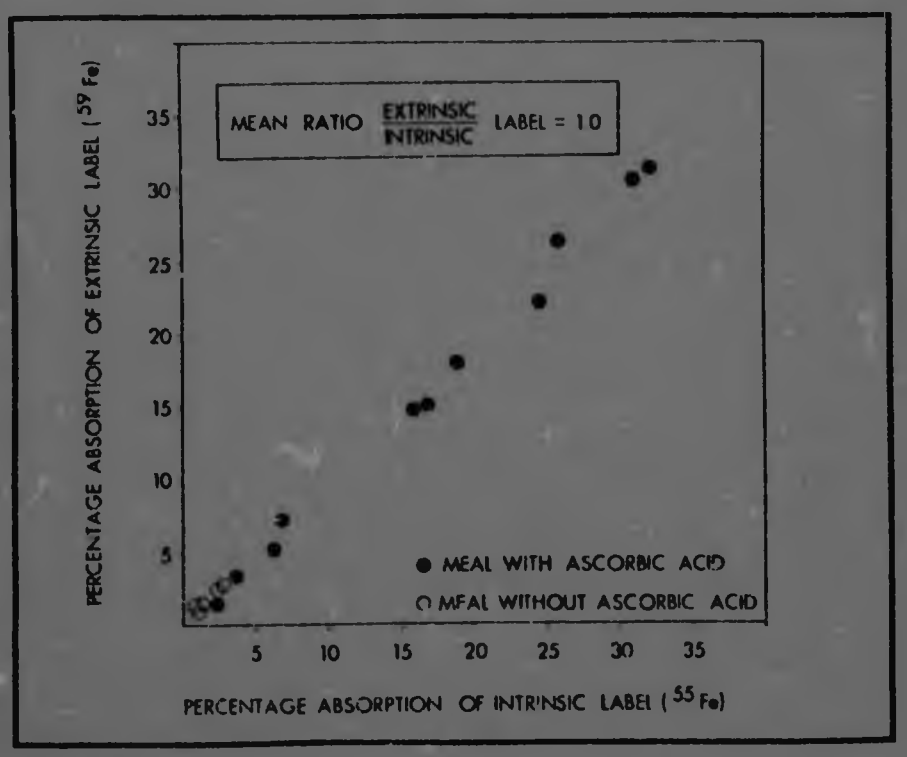

Figure 13. Plot of extrinsic iron (as ferric orthophosphate) absorption against intrinsic iron absorption from maize.

procedure was repeated, except that the iron in the salt was labelled with ^{59}Fe and 100 mg ascorbic acid was also present. There was a significant difference between the mean absorption of 3.2% (S.D. ± 1.7) from the meal fortified with iron alone and that of 10.4% (S.D. ± 8.2) from the meal fortified with both ascorbic acid and iron ($t = 2.71$, $p < 0.05$) (Table XII). The mean absorption of the reference iron salt was 53.7% (S.D. ± 24.0).

7.3.4 Effect of 50 mg Ascorbic Acid on the Absorption of Iron in Rice and Maize Meals (Tables XIII, XIV).

Similar experiments were performed using 12 volunteers in each case, the only variation from the previous experiment being the reduction of the quantity of ascorbic acid from 100 mg to 50 mg per meal. The mean absorption from a rice meal containing 2.5 mg iron as ferric orthophosphate was only 4.8% (S.D. ± 3.8), but it rose to 14.5% (S.D. ± 10.8) when 50 mg ascorbic acid was also present in the cooking salt, a significant difference ($t = 3.36$, $p < 0.01$) (Table XIII). The mean reference absorption for the group was 42.5% (S.D. ± 29.0). In the maize porridge study, the mean absorption from a meal fortified only with 2.5 mg iron as ferric orthophosphate was 0.9% (S.D. ± 0.6) compared with 7.6% (S.D. ± 4.8) when 50 mg ascorbic acid was also present in the cooking salt (Table XIV). This difference was also significant ($t = 5.32$, $p < 0.001$). The mean absorption of the reference iron salt was 33.6% (S.D. ± 25.5). The results of these two experiments indicated that iron absorption from rice and maize porridge meals was markedly increased if they were cooked with salt containing

Hb (g/100 ml)	Plasma iron (µg/100 ml)	% Saturation	% Iron absorption		
			Reference salt	Without Ascorbic Acid	With Ascorbic Acid
14.8	93	21.4	45.3	2.3	4.6
14.8	87	19.1	62.1	2.7	5.4
13.4	58	14.5	10.3	4.2	4.5
14.0	63	16.8	77.3	0.9	4.6
11.0	60	13.8	40.0	2.0	9.5
13.2	59	12.5	78.3	6.0	22.1
11.3	49	12.1	62.8	4.2	22.2
Mean 13.2	67	15.7	53.7	3.2	10.4

Table XII. Absorption of iron from rice meals fed with 2.5 mg supplementary iron as $FePO_4$ with and without 100 mg ascorbic acid (final iron contents 3.0 mg and 2.6 mg respectively).

Table XIII. Absorption of iron from rice meals fed with 2.5 mg supplementary iron as FeO_4 with and without 50 mg ascorbic acid (final iron contents 2.9 mg and 2.8 mg respectively).

Hb (g/100 ml)	Plasma iron (ug/100 ml)	% Saturation	% Iron absorption		
			Reference salt	Without Ascorbic acid	With Ascorbic Acid
13.6	42	7.6	27.7	0.8	2.6
13.4	137	35.6	69.6	0.9	1.4
13.2	126	39.5	2.7	1.8	10.6
13.6	83	22.3	9.9	2.9	25.8
7.5	45	9.9	-	3.2	7.4
10.3	38	7.3	72.0	3.4	17.4
12.4	74	16.5	74.2	3.4	2.8
5.0	25	4.9	37.9	4.2	20.2
12.8	125	38.4	44.5	5.5	27.7
10.8	45	9.8	86.7	9.1	30.6
14.4	123	34.2	27.3	11.3	4.4
12.8	50	13.5	15.3	11.6	22.5
Mean 11.7	76	20.0	42.5	4.8	14.5

Hb (g/100 ml)	Plasma iron (µg/100 ml)	% Saturation	% Iron absorption		
			Reference salt	^{55}Fe Without Ascorbic Acid	^{59}Fe With Ascorbic Acid
16.5	89	19.3	11.0	0.1	.9
13.6	78	21.3	39.1	0.2	3.3
12.1	91	24.4	33.1	0.4	2.3
12.1	52	14.7	31.4	.4	10.8
15.3	91	22.9	2.0	.6	1.2
11.5	79	22.9	45.8	0.7	10.2
11.9	80	25.9	11.9	0.7	4.7
10.0	46	13.2	38.7	0.8	13.4
9.2	42	8.5	91.0	1.2	10.5
13.6	83	21.4	22.6	1.3	5.9
13.2	61	15.8	65.8	1.7	10.4
12.3	70	16.9	11.0	2.1	15.6
Mean 12.6	72	18.9	33.6	0.9	7.6

Table XIV. Absorption of iron from maize porridge fed with 2.5 mg supplementary iron as $FePO_4$ with and without 50 mg ascorbic acid (final iron contents 3.2 mg and 3.5 mg respectively).

2.5 mg iron as ferric orthophosphate and 50 mg ascorbic acid per meal. It was not, however, known whether the enhancement would have been even greater if the salt had been fortified with an 'available' salt such as ferrous sulphate. It was therefore decided to undertake a direct comparison.

7.3.5 Iron Absorption from Maize Porridge Meals containing 50 mg Ascorbic Acid and either 2.5 mg or 4 mg Iron; Comparison between Ferrous Sulphate and Ferric Orthophosphate. (Table XV).

Eleven volunteers ate labelled porridge meals on consecutive days. In both meals the cooking salt containing 50 mg ascorbic acid and 2.5 mg iron, but on the first day the iron supplement was ferrous sulphate, and the second day ferric orthophosphate. The mean absorptions did not differ significantly, being 9.4% (S.D. $\pm$ 8.0) and 8.7% (S.D. $\pm$ 9.1) respectively. The mean reference absorption for this group was only 23.0% (S.D. $\pm$ 19.0). The experiment was repeated in 10 subjects using a 4 mg instead of a 2.5 mg iron supplement. The mean absorption from the meal fortified with ferrous sulphate was 15.5% (S.D. $\pm$ 15.9) and from that fortified with ferric orthophosphate was 19.6% (S.D. $\pm$ 17.5) (Table XV). The difference was not significant ($t = 0.95$, $p > 0.1$). The mean reference iron absorption was greater than in the previous group (55.4%, S.D. $\pm$ 35.8). It was concluded that the absorption of iron from a maize porridge meal cooked with salt fortified with ascorbic acid and ferric orthophosphate was no less than when the salt was fortified with similar amounts of ascorbic acid and ferrous sulphate.

Table XV. Comparison between absorption of iron from maize porridge supplemented with either $FeSO_4$ or $FePO_4$.

	Hb (g/100 ml)	Plasma iron (ug/100 ml)	% Saturation	% Iron absorption		
				Reference salt	With $FeSO_4$	With $FePO_4$
2.5 mg added iron and 50 mg added ascorbic acid. Final iron contents 3.6 mg ($FeSO_4$) and 4.1 mg ($FePO_4$).	12.4	112	36.7	2.5	0.5	0.2
	11.8	83	26.5	4.1	2.1	1.5
	8.7	25	5.8	32.2	2.3	1.9
	12.8	101	27.9	24.4	6.6	6.4
	12.0	129	37.8	3.2	7.9	5.7
	12.4	142	38.1	8.7	9.1	9.7
	10.8	42	11.0	21.1	11.3	6.7
	14.2	62	13.4	57.1	17.6	6.3
	14.8	96	25.8	29.5	13.1	18.1
	13.2	98	24.4	46.9	28.2	30.2
Mean	12.3	89		23.0	9.4	8.7
4.0 mg added iron and 50 mg added ascorbic acid. Final iron contents 5.3 mg in each instance.	11.2	79	16.6	15.6	2.1	3.1
	12.8	65	12.5	52.5	2.2	3.6
	12.8	57	13.8	12.1	6.7	2.3
	12.3	82	17.6	105.5	8.3	7.5
	12.4	93	21.8	41.1	9.0	20.2
	13.2	65	15.8	17.9	13.4	8.1
	11.2	80	20.0	-	15.6	49.0
	10.3	59	12.6	83.8	18.7	22.9
	16.0	91	22.3	92.8	22.0	38.4
	7.6	30	5.0	77.0	56.7	40.5
Mean	12.0	70	15.8	55.4	15.5	19.6

Table XV. Comparison between absorption of iron from maize porridge supplemented with either $FeSO_4$ or $FePO_4$.

	Hb (g/100 ml)	Plasma iron (ug/100 ml)	% Saturation	% Iron absorption		
				Reference salt	With $FeSO_4$	With $FePO_4$
2.5 mg added iron and 50 mg added ascorbic acid. Final iron contents 3.6 mg ($FeSO_4$) and 4.1 mg ($FePO_4$).	12.4	112	36.7	2.5	0.5	0.2
	11.8	83	26.5	4.1	2.1	1.5
	8.7	25	5.8	32.2	2.3	1.9
	12.8	101	27.9	24.4	6.6	6.4
	12.0	129	37.8	3.2	7.9	5.7
	12.4	142	38.1	8.7	9.1	9.7
	10.8	42	11.0	21.1	11.3	6.7
	14.2	62	13.4	57.1	12.6	6.3
	14.8	96	25.8	29.5	13.1	18.1
	13.2	98	24.4	46.9	28.2	30.2
Mean	12.3	89	24.7	23.0	9.4	8.7
4.0 mg added iron and 50 mg added ascorbic acid. Final iron contents 5.3 mg in each instance.	11.2	79	16.6	15.6	2.1	3.1
	12.8	65	12.5	52.5	2.2	3.6
	12.8	57	13.8	12.1	6.7	2.3
	12.3	82	17.6	105.5	8.3	7.5
	12.4	93	21.8	41.1	9.0	20.2
	13.2	65	15.8	17.9	13.4	8.1
	11.2	80	20.0	-	15.6	49.0
	10.3	59	12.6	83.8	18.7	22.9
	16.0	91	22.3	92.8	22.0	38.4
	7.6	30	5.0	77.0	56.7	40.5
Mean	12.0	70	15.8	55.4	15.5	19.6

7.4 Discussion

None of the subjects commented on any unusual appearance or taste of the meals which had been prepared with the fortified coarse salt containing ascorbic acid, starch and iron as ferric orthophosphate or ferrous sulphate. However, as only a limited number of dishes has been prepared with this salt, it is possible that the taste or appearance of other meals may be altered.

The measured iron contents of the meals in the present study indicated that they contained less than 2 mg intrinsic iron (Tables XI - XV), so that fortification with ascorbic acid alone would hardly be sufficient to correct the iron deficiency if these meals were representative of the diet of the population. There may, however, be more iron in these vegetable st as consumed in other countries, since both Ramalingaswami and Patwardhan (1949) and Apte and Iyengar (1970) have reported that the iron content of the average Indian diet is adequate.

Additional iron may reach the diet by a number of routes. Under some circumstances, a significant quantity is derived from cooking utensils. This point is exemplified by the observation that the iron overload that is so prevalent among South African Bantu is largely the result of a high oral intake of inorganic iron (Walker and Arvidsson, 1953). While iron cooking utensils account for a portion of the dietary iron intake, the major contribution is made by iron derived from containers such as oil drums, tins and iron pots which are used in the manufacture of home-brewed beer. The iron content of the end product may reach 15 mg/100 ml, thereby exposing many consumers to a daily

iron intake of the order of 100 mg in beer alone.

The iron skillet is another potential source of dietary iron. In a study by Moore (1965), cooking in a skillet was shown to add considerably to the iron content of a number of foods. He compared estimates of food iron content, derived from food composition tables, with chemical estimations of iron content and was able to show that in a number of United States armed forces messes, daily iron intake measured by assay was two to three times greater than the calculated intake. The author went on to speculate about the "most unfortunate effect" that the substitution of steel and aluminium cooking vessels would have on iron nutrition.

A community survey among Delhi Indians from a low socio-economic group indicated another source of iron not allowed for in food composition tables (Lal et al., 1973). The majority of housewives in the study used cast iron knives for cutting vegetables and the authors felt that this habit also contributed to daily iron intake.

Contamination with soil rich in iron may also explain the high iron content of some cereal based diets. An extreme example concerns a cereal known as "teff", which is grown in Ethiopia. Although this staple may provide adults with a daily iron intake of up to 500 mg (Blix, 1965), thoroughly cleaned teff has an iron content similar to that usually found in other cereals.

While this additional dietary iron is obviously available under some circumstances, as evidenced by the high incidence of iron overload in the South African Bantu, this is not the rule.

Other nutritional surveys, similar to those conducted by Apte and Rao, have borne this out. In Thailand, Vy found a high incidence of iron deficiency anaemia despite a high dietary iron intake (1971). Hallberg and his colleagues (1974) investigated this phenomenon in human volunteers, who ate a simple diet typical of that taken by lower socio-economic communities in South East Asia. Iron absorption from their meal of rice, vegetables and spices, containing approximately 10 mg iron, was only 0.4% in normal subjects. Absorption of this order was lower than the amount estimated to maintain iron balance and prompted the authors to examine factors that could alter the availability of the iron in their test meals. They found that the presence of meat or fruit enhanced absorption and suggested that the ascorbic acid content accounted for the effect of the fruit. They went on to postulate some unknown inhibitory compound or compounds in their meals.

Where a consideration of contaminating iron is important is in the design of a fortification programme. If it could be shown that ascorbic acid increases the total quantity of iron absorbed from diets that contain adequate amounts of iron, then it might prove unnecessary to introduce iron fortification. Under these circumstances, all that would be necessary would be a supplement of ascorbic acid. On the other hand, if there is not enough iron in the average diet, the study described in this chapter has shown that it would be possible to add ferric orthophosphate to the cooking salt, together with ascorbic acid, provided that 2.5% starch is present to inhibit discoloration. It will be recalled that coarse salt fortified in this way became

discoloured on storage under subtropical conditions no more readily than did salt with ascorbic acid alone.

Because ferric orthophosphate has been reported to be very poorly absorbed, however, it was important to demonstrate that iron absorption would be effectively enhanced if it were used. The mean absorption from the standard rice meal supplemented with 2.5 mg iron as ferric orthophosphate was only 0.14 mg, but it was 0.46 mg when 50 mg ascorbic acid was also present in the cooking salt (Table XIII). This represents a worthwhile improvement, and increasing the amount of ascorbic acid to 100 mg did not further increase the amount of iron which was absorbed. Similarly, the mean absorption from the meal of maize porridge supplemented with 2.5 mg iron as ferric orthophosphate was only 0.04 mg, but it rose to 0.24 mg with the addition of 50 mg ascorbic acid (Table XIV). When the iron supplement was increased to 4.0 mg while the ascorbic acid remained at 50 mg, the mean absorption from the maize porridge was as much as 1.04 mg (Table XV). The subjects taking part in this particular experiment absorbed a high percentage of the 'reference' iron salt (mean 55.6%), and because they were so avid for iron, effectively demonstrated the availability of the iron in the meal. The iron and ascorbic acid supplements were present in 4 g salt, and the daily consumption of salt is of the order of 15 g (Valassi, 1970). Fortification with 1.25% ascorbic acid and 0.1% iron as ferric orthophosphate might therefore be expected to permit the absorption of more than 3 mg iron per day. This should be adequate to maintain iron balance in most subjects,

in spite of repeated pregnancies, heavy menstrual losses or hookworm infestation (Bothwell & Finch, 1962).

It was noteworthy that similar amounts of iron were absorbed when the supplemental iron compound was ferric orthophosphate and when it was ferrous sulphate (Table XV). This observation is in striking contrast to those of other workers. As was pointed out in the introduction to this chapter, Cook et al. (1973) have reported that iron absorption from bread rolls was more than three times greater when the supplementary iron they contained was ferrous sulphate than when it was ferric orthophosphate. Rao et al. (1972) found that the absorption of iron from ferric orthophosphate was so poor that they recommended that a more available compound, such as ferrous sulphate, should be selected for the fortification of common salt. However, when they measured the absorption of iron from a typical Indian meal, which included rice, dhal, potato and onion, that had been fortified with ferrous sulphate, the mean figure was only 3.7%. The major point of difference between these two studies and the present investigation was the supplementation with ascorbic acid as well as iron in the latter, and this emphasizes the validity of the concept that it is the availability of the iron in the meal, both intrinsic and supplemental, which needs to be improved if dietary iron deficiency is to be corrected. When this is done, even such a relatively unavailable compound as ferric orthophosphate becomes an effective supplement. Indeed, replacing ferric orthophosphate with ferrous sulphate in the household salt used in the preparation of the meal does not increase the amount of iron which is absorbed,

provided that sufficient ascorbic acid is present. For the fortification of salt, however, ferric orthophosphate is probably preferable to ferrous sulphate because the tendency of the fortified salt to become discoloured is significantly diminished.

In another study, ferric orthophosphate was also found to be preferable to ferrous sulphate for the fortification of cane sugar (Disler et al., 1975a). The reason for this was because sugar fortified with soluble iron salts, including ferrous sulphate, discoloured both tea and coffee; sugar fortified with iron as ferric orthophosphate did not have this effect. Investigation of the colour reaction taking place pointed to the formation of iron-tannin complexes in the tea (Disler et al., 1975b).

An important point to emerge from the studies on the fortification of cane sugar was the fact that the absorption of iron from maize porridge sweetened with sugar containing iron as ferric orthophosphate and ascorbic acid was poor. The mean absorption from porridge containing 2 mg iron and 40 mg ascorbic acid was only 2.9%. These results differed remarkably from those obtained in the experiments described in this chapter, even although the only difference was the addition of the supplements prior to cooking. This raised the possibility that heating was necessary for the formation of an absorbable iron complex from the insoluble ferric orthophosphate and ascorbic acid. This was confirmed when the mean absorption from the test porridge meal rose to 12.7% when the fortified sugar was added prior to cooking, instead of being

sprinkled over the meal immediately before it was eaten (Disler et al., 1975b).

These observations about the availability of a relatively insoluble iron compound, ferric orthophosphate, may have some relevance in predicting the extent to which contaminating iron absorption can be enhanced by ascorbic acid. When this form of iron was added before cooking, it was shown to enter a common pool with the intrinsic food iron, thereby ensuring its exposure to the effects of the ascorbic acid. As has been pointed out earlier in this chapter, some contaminating iron gains access to meals either before or during cooking and, as a result, is subjected to the heating that may be necessary if relatively insoluble forms of iron are to form available complexes with ascorbic acid. What is needed here, are formal studies on the availability of different forms of contaminating iron and the effect of promotors on its absorption.

A final point for discussion is the surprising lack of correlation between the absorption of the reference iron salt and the absorption of the iron from the meals. The figures for maize porridge were as follows: no added ascorbic acid, $r = +0.26$ ($p > 0.1$); 50 mg added ascorbic acid, $r = +0.37$ ($p > 0.05$); 100 mg added ascorbic acid, $r = +0.50$ ($p > 0.1$). For the rice meal the figures were: no added ascorbic acid, $r = -0.09$; 50 mg added ascorbic acid, $r = -0.01$; 100 mg added ascorbic acid, $r = +0.45$ ($p > 0.1$).

Previous studies have demonstrated that these figures correlate closely (Cook et al., 1972; Layrisse et al., 1973)

and a significant correlation was obtained in the studies described in chapter 5 of this thesis. It would appear that the correlation is not invariable (Cook et al., 1973).

In contrast, the correlations between absorptions from meals cooked with and without ascorbic acid were significant: for maize porridge, $r = +0.66$ ($p < 0.05$), and for rice, $r = +0.46$ ($p < 0.05$). The correlation between the absorptions from meals fortified with ascorbic acid and ferric orthophosphate and with ascorbic acid and ferrous sulphate was also significant ($r = +0.75$, $p < 0.001$).

Possibly the high incidence of iron deficiency among the subjects in the present study, and thus the more uniform avidity for iron than in other groups, may have played some part. Another possibility is that some individuals in the present study may not have fasted completely for the reference iron absorption study.

Chapter Eight

CONCLUSIONS

1. Iron deficiency is common among Durban Indian women.

2. When iron absorption from four vegetable staples was measured in this group, mean values were soya 19.8%, bread 7.9%, maize 6.8% and rice 3.5%.

3. This was in striking contrast to the mean absorption from a solution of ferrous ascorbate of 49.8%.

4. Absorption from a common pool of non-haem iron was demonstrated under the following circumstances;

   (a) added ferric ammonium citrate and the intrinsic iron in maize, wheat and soya

   (b) added ferrous sulphate and the intrinsic iron in rice

   (c) added ferric orthophosphate and the intrinsic iron in maize and rice.

5. The addition of ascorbic acid to the staples, during cooking, promoted a marked enhancement of iron absorption from maize and rice, but not from bread and soya.

6. The failure of ascorbic acid to enhance absorption from bread and soya was attributed to the oxidation of the vitamin that occurred during baking.

7. Coarse cooking salt used as a carrier for added iron and ascorbic acid became unacceptably discoloured during storage. This discolouration was accelerated by heat, humidity and the presence of more soluble iron salts.

8. Coarse cooking salt can be fortified with 0.1% iron as ferric orthophosphate and 1.25% ascorbic acid and stored under all but the most extreme conditions, provided that 2.5% starch is added. The

replacement of ferric orthophosphate with ferrous sulphate does not alter iron availability.

9. Such fortification will significantly improve the iron nutrition in countries where the staple food is rice or maize.

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