CHAPTER - I

INTRODUCTION

i. The Problem

(a) Genesis, rationale and scope of the proposed project

Several nutritional problems are known to be of common occurrence in the vast populations of the developing countries. Iron deficiency, amongst these, is by far the commonest lack of an essential element, which mankind in general, and womankind in particular, experiences (1). Although its occurrence is decidedly world-wide, its severity has been felt more in the developing countries of South East Asia, and in particular in the teeming millions of our own country. This widespread occurrence of iron deficiency has stimulated the study of iron balance as well as of the possible remedial measures against it. Its causes are mainly nutritional deficiency on the one hand, and blood loss on the other, besides the rare malabsorption syndromes. It has been fully realised that the predominant causes of iron deficiency in a community could surely differ considerably, depending upon its socio-economic and cultural pattern, so that both the rich as well as the poor sections are affected widely (1).

The lack of iron in living organisms is in fact a paradox of poverty in the midst of plenty (1). Iron is one of the most common elements in the lithosphere, and yet only some 1-2 gm of iron required from this vast store, to provide for those in the biosphere who have developed a need for it, has become a problem of first magnitude, both to understand as well as to solve.
Investigations of public health measures to remedy this state of iron deficiency by fortifying foods with iron are in progress, though so far no tangible solution has emerged, and together with the numerous causes of blood loss beyond the capacity of dietary iron to correct it, it remains a very widespread clinical and technological problem. In its milder manifestations it affects the well being and the quality of life, but in its more severe forms seen today in the developing countries, it surely is life threatening. Its devastating effect has been felt more in the vulnerable groups like the infants and the pre-school going children, and the pregnant and lactating mothers, because of their enhanced demand. The situation is precarious in the former group because these have yet to grow to adulthood and their impeded growth amounts to a permanent loss to the nation; and in the latter group it stands accentuated because of their repeated pregnancies, identified as a social problem in their poverty stricken world. While the hematologists and the nutritionists continue to confer about the advisability of fortifying foods with iron, the pediatric hematologists are convinced of its unquestionable value for infants (2, 3), and the neonatologists even for the pre-natal life, because iron overload rarely occurs in this age group (2). With the vast annual increase in the infant group in spite of all the official population control measures, the importance, rationale and scope of the food fortification measures are beyond any iota of doubt, more so in respect of infant foods. Iron deficiency anemia has thus assumed the position of one of the most pressing national problems for us to tackle it as seriously and as urgently as a war effort.
(b) Aims of the present study

1. Preventive fortification of infant foods

It is emphasized that the primary purpose of any iron fortification programme is not to treat but to prevent iron deficiency. The main aim of the present study therefore was to devise ways and means of fortifying with iron those foodstuffs which are primarily consumed by infants, though, secondarily, these could certainly also be of equal benefit for other vulnerable target groups. The level of fortification to be selected for this purpose therefore should aim at preventing rather than treating iron deficiency.

2. Milk as carrier food for fortification

This thinking led the present author to select milk as the foodstuff of choice for the proposed fortification studies. The other foodstuffs which have been experimented upon by other investigators as vehicles for the fortification of adult foods include: (i) wheat flour, bread, and rice, (ii) salt, sugar and coffee, and (iii) soft drinks, beverages and sauces. Some of these could certainly be employed as alternate mediums, though, in comparison with these, milk definitely stands out, at least to the present author, as a more appropriate choice, because infants are wholly dependent on milk as their sole dietary regimen, and also that it is easily and cheaply available everywhere in abundance, its acceptability is rated high by consumers of all other age groups as well, and, still further, it readily lends itself to a centrally processed and controlled fortification technology.

The fortification of common salt as a vehicle has recently been successfully experimented upon and advocated by the National
Institute of Nutrition, Hyderabad. It could certainly be quite a suitable vehicle aimed at the adult population as the target group, but, surely, it leaves the infant group uncovered, besides the group consisting of those who have been advised salt restriction, howsoever small their number at present might be. And this number is likely to increase with an increase in the "mechanized" way of life so full of tension, as seen in the modern highly industrialized and affluent communities and societies. Likewise, cane sugar also might not be able to prove itself as the most suitable vehicle for all age groups, under all types of metabolic conditions, and for all countries. The reality of this situation therefore accentuates the need for the trial of an alternate or additional carrier foods for fortification programmes.

*Milk vs other food materials*: Although milk is usually considered to be a complete food, it is known to be deficient both in iron as well as in ascorbic acid (vitamin C). In contrast, most of the common consumable food materials taken in adequate amount are known to contain sufficient iron to supply the daily human requirements as discussed by Bowering et al. (1976) (4) and Leveille (1977) (5). The limiting factor in the utilisation of this dietary iron in the human system has been considered as occurring at the level of the intestinal absorption, though recently failure or hinderance in the mobilization of iron from its storage forms, and the incorporation of iron into hemoglobin have also been considered as equally important causative factors involved in iron deficiency. In this context, the significant role played by copper, particularly by the ferroxidase activity of ceruloplasmin, has been amply appreciated. The intestinal
absorption of iron from milk, or in the presence of milk and milk products, in comparison with that from cereal foods and eggs, has convincingly been shown to be normal (6), irrespective of the presence in milk of the phospho-protein casein. Some investigators have put the absorption of iron from milk at 7-8 per cent, in contrast with the low absorption (3-6 per cent) from the phytate-rich cereal foods, and the still lower absorption of 2-3 per cent from eggs due to the phosphoprotein phosvitin (6, 47, 164, 165).

(c) Isolation of the central problem to be tackled in the proposed investigation

1. Peculiarities inherent in carrying through ironization of milk

The fortification of whole milk with suitable amounts of iron has, however, not proved very easy, as it is beset with its own peculiar and complex problems not encountered in the fortification programmes based on other mediums (vehicles) enumerated above. The main difficulty arises from the destructive action of iron on milk lipids, and also on native and added vitamins. The milk fortification programme has therefore to be considered in its own proper perspective.

2. Attributes of an ideal milk fortificant

In actual practice, therefore, the central problem to be tackled in the proposed investigation is to develop a suitable form of iron which could serve as an ideal fortificant of whole milk. The selected form of iron, in turn, needs conforming to the following characteristics and attributes:

(1) Miscibility and stability

(i) It should be completely miscible with milk in the required concentration, without leading to any perceptible change
in colour, taste or flavour of milk.

(ii) It should maintain the stability of milk which in its native state is a remarkably stabilized emulsion, i.e., it should not induce any destabilization of the micellar and other milk proteins and enzymes or of the fat globule membrane (FGM).

(iii) It should itself also remain stable in the presence of milk constituents.

(iv) It should maintain these stabilities even on repeated heating and cooling of milk as practised by the consumer, particularly in our country.

(2) Off-flavours and rancidity

(i) It should maintain the natural flavour of milk, i.e., it should not induce the development of oxidised- or any other off-flavour in milk, which simply means that its addition should not lead to rancidity of any type in milk, neither oxidative nor ketonic, nor hydrolytic. Because of its powerful catalytic effect, iron is known to induce autoxidation of milk lipids by singlet oxygen, particularly in the presence of light, via the free radical mechanism, leading to the formation of strong unpleasant, oxidized-flavours in milk. Likewise, the known affinity of iron for enzymes should not affect the milk lipase leading to lipase flavour, nor the known growth-promoting effect of iron should cause acidity in milk induced by the hydrolytic enzymes of exogenous (bacterial) origin. All these render milk unacceptable to the consumer.

(ii) It should also not induce off-flavours or rancidity in the milk products obtained after subjecting milk to various dairy technological processes.
(3) Bioavailability of iron

(i) It should itself possess complete nutritional availability so as to provide the daily intended requirement of iron based on its absorption, distribution and storage, and hemoglobin synthesis.

(ii) It should also not induce any hinderance in the metabolic utilization of other milk constituents, nor of any other food constituent consumed at the same time.

(4) Adverse effect and toxicity

It should not lead to any untowards or adverse effect, as nausea or GIT reaction, nor any acute or chronic toxicity.

(5) Keeping quality and the processibility

(i) It should maintain the keeping quality of milk, its enzymic make up, and thence its processibility in dairy technology, such as pasteurization, homogenization, conversion to curd (yoghurt), cream, butter and butter milk, cheese, condensed milk, powder milk, ice cream powder, etc.

(ii) It should maintain an adequate shelf life for storage and distribution programmes.

(6) Cost

It should be amenable to easy preparation from readily and abundantly available starting materials, so that it could be produced at a low cost for large scale use in fortification programmes to be operated on a nation-wide or global scale.
Resume of existing literature on the subject as a guide to the evaluation of the current status of the problem

(a) Review of literature

A review of attempts at fortification of milk with iron is presented here. Investigations on the fortification of other foodstuffs with iron have also been summarised for the sake of comparison. The literature has been surveyed upto the end of 1978.

Fortification of foodstuffs with iron as a remedial measure against iron deficiency has recently been reviewed (7-16). It is pointed out that the information concerning fortification of foods with iron in reality overlaps with that which has been reported on the interrelated aspects of the problem like food iron absorption (17-59), bioavailability of food iron (60-69), and iron deficiency anemia (70-75). Therefore some recently reported pertinent reviews and findings in these areas have also been listed here for reference for the sake of presenting a wider view and a more complete opinion on the current status of the problem.

The most commonly tried fortificants for milk include ferrous sulphate, ferric gluconate, lactate and fumarate (76, 77, 79), ferric polyphosphate, sodium ferric pyrophosphate, ferric ammonium citrate (83), and more recently ferric nitrilotriacetate (6), ferric fructose (6, 85) and ferric lactose (85). Amongst those that have been tried for various other foodstuffs, the three phosphates, namely the ortho-, pyro- and poly-phosphates in the ferric form, ferric glycerophosphate, ferric-EDTA (57), and, more recently, cattle hemoglobin (86), and elemental (reduced) iron (68, 138-140) deserve more detailed information. The effect of fructose
on ferrous and ferric iron absorption in man has been studied by Heinrich et al. (1975) (87).

1. Fortification of milk and milk products

The iron content of milk is very small. Ling et al. (1961) (88), in their treatise on milk, have reported its amount in cow's milk to be in the range of 0.15-0.37 ppm (89) and 0.67 ppm (90). More recently, Murthy et al. (1972) (91) have reported this amount to be 0.5-1.2 ppm with an average of 0.63 ppm. The iron content of Indian cow, buffalo and goat milks have been reported differently: by Handa and Johri (1972) (92) as 0.36, 0.30 and 0.37 ppm; and by Suresh Chandra et al. (1975) (93), using colorimetry (1:10-phenanthroline), as 173.7+63.0 ug/litre (cow) and 207.2±

µg/litre (buffalo). Mathur and Roy (94), using atomic absorption technique, quote a value of 2.4±0.2 mg/litre in Murrah buffalo milk. In comparison, the iron content of dried skim milk is 6 mg/kg and that of dried whey 14 mg/kg (95). Picciano and Guthrie (1976) (96) have reported the iron content of mature human milk as in the range of<0.1-0.6 µg/ml, with an average of ca. 0.3 µg/ml, depending on personal factors, lactating history of the donor women and the time of withdrawal of milk, etc. They quote other reported values (97) to be 0.44 to 5.0 µg/ml, with an average value of 0.84 µg/ml. The iron content of breast milk taken from ten Indian village women averaged 0.15 mg/100 ml (98). The average iron output in mature human milk is between 1.0 to 1.5 mg daily (99). It has been confirmed that the iron content of several species cannot be raised above normal levels by dietary excesses of iron salts (88). McMillan et al. (1976) (100) have reported on iron availability from human milk in infants. In contrast to an iron content of 0.5-1.0 ppm in the breast milk and cow milk, iron-fortified milks usually contain
10-12 ppm iron as FeSO₄. It is interesting to note that whereas on the average only 4 per cent of iron of fortified milk is absorbed, 49 per cent of breast milk iron is absorbed (37).

Rat's milk is exceptional in comparison to the milks of other mammals, as it contains about 10 times more iron (101), and is devoid of lactoferrin. Loh and Kaldor (1976) (102) have reported on the passage of plasma iron to milk iron. Loh and Kaldor had also previously, in 1974, reported their comparative studies on rat, rabbit, bovine and human milk iron content as 15, 0.3 and 0.6 μg/ml. This was in contrast to a plasma iron content of 0.5-3.0 μg/ml in rat, rabbit and quokka. Most of the iron in rat milk went to the casein fraction (103).

In view of the fact that the iron content of milk cannot be significantly increased by oral administration of iron salts to lactating humans or animals (104, 105), direct fortification of milk with iron emerges as the only effective means of increasing the dietary intake of iron in infants, and of improving that in the adult population. Addition of iron drops to milk at the time of feeding the human infant is rather cumbersome, and other infants impracticable. The wide consumption of milk by infants, children and adults makes it an attractive vehicle for iron fortification schemes. Several attempts have been made to fortify dry milk (vide infra) with iron and vitamins, but trials to fortify whole (liquid) milk with iron and with vitamin C have presented several problems, because of the detrimental effect on the quality and acceptability of milk, caused by the oxidation of the milk lipids, particularly its polyunsaturated fatty acids, resulting in oxidized flavours, and the oxidative loss of the native and
the added vitamins, particularly vitamin E, vitamin A and carotene, and vitamin C (83).

As early as 1955, Reisfeld et al. (106) had reported that the addition of ferric ammonium citrate to milk protected milk lipase against heat inactivation at the normal pasteurization temperature of 73.3°C for 16 sec. Scanlan and Shipe (1962) (107) studied factors affecting the susceptibility of multivitamin mineral milk to oxidation. Added iron was in the form of ferric pyrophosphate soluble (containing 40 per cent citrate). Fortification of homogenized and homogenized-treated milks with ferric and ferrous compounds showed that both milks developed oxidized flavours when fortified with ferrous iron. However, the homogenized-treated milk was more susceptible than the homogenized milk when ferric iron was added. It was suggested that the difference in susceptibility between homogenized and homogenized-treated milk was a difference in the capacity of these milks to reduce ferric iron to the stronger pro-oxidant ferrous form.

Panero et al. (1968) (108) enriched milk with 1.25 mg ferrous sulphate per 100 ml (12.5 ppm) and tried on 20 immature neonates. Good tolerance and favourable action on growth were accompanied by higher mean total hemoglobin iron levels than those observed in controls fed with non-iron supplemented foods. This result was confirmed in 3 pairs of immature twins in which one of the two severed as control.

Studies of Fritz et al. (1970) (61) showed that dissolving ferrous sulphate in either evaporated or skim milk did not influence its availability to anemic chicks.
Takahashi (1970) observed that immediate spray drying of concentrated whole or skimmed milk after mixing with a soluble starch hydrolyzate and ferric pyrophosphate gave good quality products. The products had good appearance and taste and kept well for a week at 40°C with no loss in iron content.

Edmondson et al. (1971) found that ferric iron compounds uniformly resulted in lipolytic rancidity when milk was pasteurized at minimum to moderate temperature (below 79°C). This off-flavour was reduced acceptably or was completely eliminated simply by pasteurization at 81°C. Their data consistently showed that milk lipase was made more heat resistant by ferric forms of iron. Ferrous salts normally caused definite oxidized flavour when added to raw whole milk before pasteurization. However, this off-flavour was markedly reduced by de-aerating the milk before adding the iron compounds. Ferrous compounds caused oxidized flavour but not rancid flavour (lipase flavour).

Demott (1971) fortified milk with several iron salts, all of which caused off-flavours, but ferrous salts were the most detrimental in comparison to ferric salts.

Significant results on the administration of iron fortified milk were observed by Demott (1972). Weanling rats were fed only homogenized vitamin D containing milk for 3 weeks, and body weights and hematocrit values for males and females were noted. They were then fed milk and 10 ppm Fe in the form of ferric pyrophosphate, milk and 10 per cent added non-fat dry milk solids, or milk or 10 per cent dried milk solids and 10 ppm added
Fe. According to Demott (1975) (78), raw milk containing 20 ppm iron as ferrous sulphate had an oxidized flavour after pasteurization.

A study by Horst (1971) (80) in South Africa showed that even ideal powder milks like Brenil, Klim, and Nespray contained no iron. Lactogen, Nan and Pelargon contained iron below 10 mg/qt. Only Similac contained 12 mg/qt iron and hematocrit values of children given Similac were found to be higher than those of controls (Kattamis et al., 1972) (81).

Wang (1972) (82) made a chemical-organoleptic study of iron-fortified milk and the biological availability of the iron. Wang and King (1973) (83) studied assimilation of iron from iron-fortified milk by pigs and got a good response. Wang and King (1973) (83) studied chemical and sensory evaluation of iron-fortified milk. In this study ferric ammonium sulphate, ferric ammonium citrate, ferric choline citrate and ferrous sulphate were tried for milk fortification. Ferric ammonium citrate was found most satisfactory as regards its acceptability and its effect on vitamin content. It had a utilization of about 30 per cent as indicated by animal experiments. Addition of 100 ppm ferric ammonium citrate to milk before pasteurization or homogenization did not affect the acceptability and the vitamins E and A, and carotene content even after storage for 7 days, whereas 7 other iron salts had detrimental effect on oxidative stability and vitamin contents.

Kurtz et al. (1973) (110) studied the effect of fortification with FeCl₃ or ferric ammonium citrate on susceptibility of skim milk and non-fat dry milk with no adverse affects upto
24 ppm iron. Effect of processing on the iron availability in milk-based infant formula products with ferrous sulphate, ferric pyrophosphate and sodium iron pyrophosphate was studied by Richard et al. (1973) (111). Byrissé et al. (1973) (112) studied iron absorption from skim milk enriched with iron glycerophosphate and found it was well absorbed.

A milk carbohydrate formula containing iron in the form of ferrous gluconate had shown anti-anemic effect. After 28 days, iron content in liver and spleen was significantly higher and level of Hb, which had been reduced by bleeding, could be brought to normal (Wysokinska et al., 1974) (113). Dried products enriched with iron have also been reported by Kubat et al. (1974) (114), while Schoppet et al. (1974) (115) have tried iron enrichment of vacuum foam drying milk.

A contradictory data was reported by Heinrich et al. (1975) (38), who reported that absorption of ferrous iron in normal and iron deficient infants was reduced after mixing it with milk, whereas Hb iron absorption was not affected. Thein-Than et al. (1975) (116) have reported inhibition of iron absorption by coconut milk. The controversy regarding the inhibition of iron absorption by milk was finally solved by Carmichael et al. (1975) (6), who studied the absorption of labelled iron in mice and chicks as FeCl₃ complexed with nitrilo-triacetate and as ferric fructose, alone and in the presence of milk, and showed no significant differences in the absorption of these compounds with either milk or casein. Effect of an iron-fortified milk formula on iron nutrition in infancy was reported by Læmmi et al. (1975) (117), and that of iron enrichment of infant milk on iron metabolism was elaborated by Rauhat et al. (1975) (118).
Davis et al. (1976) (119) in Australia studied a milk powder fortified with iron. Still more recently, effect of iron enriched milk on blood crisis in low-birth-weight newborn infants was reported by Pieterangeli et al. (1977) (120).

Distribution pattern of added iron in milk: Herald et al. (1957) (121) observed that ash from the fat globule membrane protein material of milk contained ca. 325 ppm iron, which was higher than the 20 to 50 ppm iron in the ash of the whole milk (88), indicating a preferential adsorption of iron on to the fat globule membrane. According to Ling et al. (1961) (88), the native trace elements in milk were usually attached to the proteins of the fat globule membrane (FGM); iron and copper were thus fixed to the FGM as iron or copper proteinates, and therefore the FGM was liable to stimulate fat oxidation.

As regards the distribution of exogenously added iron in the various cow milk compartments is concerned, King et al. (1959) (122) reported that when iron was added as $^{59}\text{Fe-FeCl}_3$, almost the whole of it was taken up by casein. Iron added to milk binds to protein molecules and is only slightly dialyzable (123). Vaughan and Knauff (1961) (60) showed that most of the ferrous iron added by them to an infant formula existed as a soluble iron protein complex.

Iron distribution in rat milk and its fractions has been reported by Loh and Kaldar (1973, 1974) (103, 124).

Basch et al. (1974) (125) studied the distribution of iron and polyphosphate phosphoros added to cow's milk by both analytical and radiochemical techniques. Whole milk was separated
isoelectrically and also centrifugally into three major fractions, cream, casein and whey, after the addition of $^{59}$Fe-ferripolyphosphate. Casein, which as phosphoprotein had a greater affinity for binding iron than for phosphorus, was found to bind 85-95 per cent of iron and 50-55 per cent of phosphorus when it was an acid-precipitated casein, and when the casein was obtained by centrifugation, 60-70 per cent of the iron and 50-55 per cent of the phosphorus were found bound by micelles.

Demott and Park (1974) (126) have investigated the effect of processing upon the extent of association of added iron with various milk protein fractions. The order of association of iron with milk proteins from raw and pasteurized milk was fat globule membrane protein, rennin-coagulated casein, proteose-peptone, acid-precipitated casein and whey proteins, and in the case of homogenized milk, rennin-coagulated casein, fat globule membrane protein, acid-precipitated casein, proteose-peptone and whey proteins.

According to Demott and Dincer (1976) (127) labelled $^{59}$Fe-FeCl$_3$ was added directly to raw whole milk at 0.02 and 10 ppm isotope and carrier concentration, respectively, and held overnight at 4°C. 5 ml of the skim milk was chromatographed on a Sephadex G-150 column and fractionated into casein, whey protein, and non-protein materials. The casein fraction was chromatographed on a DEAE-cellulose column and separated into its components, $\kappa$-, $\beta$-, and $\lambda$-caseins. Casein bound about 85 per cent of the added iron in skim milk; of this amount, 72 per cent and 4 per cent were associated with the $\kappa$, $\beta$-, and $\lambda$-caseins respectively.
Milk is an oil in water type emulsion with a 0/W ratio of 0.02/1.00, and with a redox potential of +0.13 volts under anaerobic conditions, which rises to 0.2 to 0.3 volts in the commercial commodity (88). Recently, milk proteins (128), whey proteins (129-131), folate-binding proteins (132), and milk fat (133) have been reviewed. Iron complexes of whey proteins as potential nutritional supplements have been reported (65). Milk substitutes based on oilseeds and nuts have been reported (134).

2. Fortification of other foods

The ideal material for iron fortification of cereal foods is still being sought. It has to be stable enough to withstand the baking and other processing conditions. Ferrous sulphate has high bioavailability, but it reacts with flour and other cereals during prolonged storage. Iron phosphates have been in use since long, but their very poor bioavailability has now been convincingly proved, and this renders them totally useless for iron fortification.

Layrisse et al. (1976) (135) and Cook and Monsen (1976) (46) reported their results on the use of Fe(III)-EDTA as an iron fortificant for foods. Their investigations showed this complex to be fairly stable and useful, but the idea still persisted that it could hinder iron absorption, as well as cause other side effects. In a latter work, Layrisse et al. (1977) (136) conclusively showed that in the 1:1 molar ratio this complex did not hinder iron absorption, as against the 2:1 molar ratio employed by Cook and Monsen (1976) (46) when their results on iron absorption had shown almost 50 per cent inhibition. According to this work, Fe(III)-EDTA presents several advantages over other compounds including ferrous sulphate. The exchange with vegetable food iron in the lumen of
the gut was complete, and its absorption was twice that of ferrous sulphate. Even ferric ammonium citrate was shown by them to be less well absorbed. Rao et al. (1978) (137) have advocated the use of monoferrous acid citrate (Fe C₆H₆O₇·H₂O) as an iron fortificant suitable for cereals, salt, sugar, etc. Using this iron complex containing labelled iron, they proved that the bioavailability of this form of iron was comparable to that of ferrous sulphate, and that it was a stable salt in comparison with the latter.

Another notable advance that has been made in this direction is the use of reduced iron, which recently has become available commercially in any desired amount from iron carbonyl (68, 138-140). Very finely divided iron powders had been produced by the H-reduction and electrolytic processes, to which now this iron powder obtained from the decomposition of iron carbonyl has been added. Carbonyl iron is 4-8 mμ in size, in comparison to 5-10 mμ of that produced by H-reduction or electrolytic iron. It is cheap, non-toxic, stable, compatible with baking processes, and has excellent absorption when compared to ferrous sulphate. Carbonyl iron thus answers most of the requirements expected from food-fortification iron.

Likewise, the iron fortification of common salt with iron in the presence of stabilizers and promoters like NaH₂O₄ have also gained ground, because of the universal use of salt and its cheap and ready availability, though in the case of this vehicle up to now only phosphates of iron had been tried instead of some other more useful ligand.

Recently, because of the far superior (almost seven time) absorption of heme iron, from its own separate pool, cattle
Hemoglobin has been proposed as a possible dietary iron supplement (86). Heme iron absorption is not affected by ascorbic acid or other absorption enhancers.

Recently another interesting vehicle has been introduced by Garby (1974) (141). It is fish sauce, and has been fortified with Fe(III)-EDTA.

The role of ascorbic acid as a promoter of iron absorption from foods has been reported by several workers (Sayers et al., 1973) (142); Cook and Monsen (1977) (143), though it did not prove useful for iron-fortified common salt (Narasinga Rao and Vijaya Sarthi, 1975) (144).

Detailed information on the iron fortification of various food materials vehicle-wise has been discussed below.

2.1 Fortification of wheat flour, bread and rice

A brief history of enrichment of flour and bread was reported in 1956 by Wilder (145). Swiss and Beaton (1974) (8) reported their prediction of the effects of iron fortification of foods, and selected bread as the most efficient choice.

The use of wheat flour and bread as vehicle for iron fortification has been reported by several investigators (27, 30, 62, 63, 146-152). Amounts of iron ranging from 10-70 ppm per 100 gm flour have been used in different countries. Bread fortification with iron has usually been done by using various iron phosphates, though it has not elicited a good response due to the low biological availability of this form of added iron. Other iron-organic acid complexes are not stable under baking conditions and give rise to visible colour.
A study of the availability of wheat iron from chapati and of an iron salt added to flour given to 21 Indian women as part of their usual diet has been reported by Elwood et al. (1970) (146). The wheat iron appears to have been better absorbed than the iron salt, but the availability of both was very low. The mean absorption of wheat iron was 4.0 and 2.2 per cent from wheat and whole meal chapati respectively, but only 2.1 and 1.8 per cent from the iron salt baked in similar chapati. Hagarty et al. (1970) (147) have also concluded that iron added to bread was of low biological availability to the subjects given fortified wheat bread. Similarly Mameesh et al. (1970) (62) have also proved by isotopic studies that whole wheat iron was significantly less available than iron in other vegetable sources examined.

Whole body counting studies of Natvig and Vellor (1973) (148) showed that the absorption of iron from FeSO₄-enriched bread was much higher than when reduced iron was used for fortification. Bioavailability of iron from FeSO₄-enriched bread also indicates that FeSO₄ has a higher relative biological value compared with other iron compounds. It was also found that FeSO₄ added to a biscuit mix prior to baking was utilised nearly as well as the same quantity of FeSO₄ added directly to the test diet.

Binding of iron and zinc to wheat bread, wheat bran and their components, mostly to cellulosae, hemicellulosae and dextrans has recently been reported from Iran (Ismail-Beigi et al., 1977) (52).
Direct fortification of rice with iron alone does not seem to have been reported. However, Sayers et al. (1974) (33) reported that, irrespective of the fact that ascorbic acid increases iron absorption, iron metabolism of rice-eating communities could be improved significantly by the addition of ascorbic acid to the diet. On the other hand, Hallberg et al. (1978) (59) reported that fish meat addition to rice meal doubled iron absorption from added ferrous sulphate. Their results indicated that iron fortification programme may be effective in countries with rice-based diets provided that there was a suitable vehicle for fortification.

Recently Gershoff et al. (1977) (153) reported their investigations on the effect of fortification of rice with lysine, threonine, vitamins A, B\textsubscript{1}, and B\textsubscript{2}, and iron on pre-school going children; they concluded that in the presence of these co-fortificants the availability of iron from rice meal was not much affected.

2.2 Fortification of salt, sugar and coffee

Fortification of common salt with iron has been suggested as a practical and an effective method for preventing iron-deficiency anemia. Iron salt selected for this purpose should satisfy the following criteria: (1) it should be stable, (2) it should be colourless by itself and should not impart colour to common salt after mixing and after storage, (3) bioavailability of iron compounds contained in it should be satisfactory, particularly when ingested with food (144).
According to Sayers et al. (1974) (33) common salt fortified with vitamin C and iron compounds became discolored on storage, the reaction being accelerated by heat and humidity. Soluble iron compounds such as FeSO$_4$ caused discoloration more readily than insoluble compounds such as ferric orthophosphate (FePO$_4$). Fortification with both ascorbic acid and iron led to more rapid discoloration than fortification with either alone. Color developed more rapidly in coarse salt than in refined commercial salt, while sodium chloride itself developed a faint discoloration only under very hot and humid conditions. Discoloration was inhibited if starch was mixed with the salt. Coarse salt mixed with 2.5 per cent starch, 1.25 per cent ascorbic acid and 0.1 per cent iron as FePO$_4$ retained an acceptable appearance during storage under sub-tropical conditions for more than one year. A double isotope red cell utilization method was employed to assess the absorption of iron from maize-porridge meal and from a standard rice meal cooked with fortified salt. The absorption of both the intrinsic food iron and the iron in the fortified salt was increased three fold if the salt contained 50 mg ascorbic acid as well as FePO$_4$. Such fortification could thus significantly improve the iron nutrition in countries where staple food was rice or maize.

In an effort to identify a suitable method for fortifying common salt with iron two approaches were investigated by Narasingha Rao et al. (1972, 1975) (154, 144). In one the possibility of fortifying salt with FeSO$_4$ and a stabilising agent, H$_3$PO$_4$, was found to be the most promising one. Salt fortified with FeSO$_4$ and H$_3$PO$_4$ was found to keep well on storage without colour
Bioavailability of iron from this fortified salt, although satisfactory at the beginning, deteriorated on storage. An alternate approach in which salt was fortified with FePO₄, a stable iron compound, and an absorption promoter like NaHSO₄ was found to be more satisfactory. Salt fortified with FePO₄ (3500 ppm) and NaHSO₄ (5000 ppm) kept well without any color development for several months. The bioavailability of iron from this formula was comparable to that from FeSO₄, and bioavailability did not decrease even after storage for 4-5 months. This fortified salt was also acceptable. This formula was considered as offering a practical solution to fortification of salt with iron.

In a more recent communication, Narasingha Rao and Sarthi (1978) have reported that fortification of common salt with ferrous orthophosphate and NaHSO₄ was good from the point of view of stability, bioavailability and acceptability, but its cost was high. The bioavailability of the combination FeSO₄ + orthophosphoric acid decreased on storage because of the oxidation and formation of ferric phosphate. So an alternate combination FeSO₄ + orthophosphoric acid + NaHSO₄ was tried by them in four combinations with different amounts of iron and the stabilizer (NaHSO₄). One of these combinations proved as good for absorption as ferrous sulphate, without the decomposition defect of the latter.

Foy (1976) (156) reported his views on the fortification of common salt with iron.

Sugar

Sugar as a vehicle for iron fortification presents several advantages over the other vehicles used in the last three
decades. In vitro studies demonstrated that FeSO₄ added to sugar in proportion of 1 mg to 1 gm was maintained in the ferrous form for a period of at least one year, and did not induce adverse changes in the vehicle. Sugar, by itself, carries practically no inhibitors for the absorption of iron.

Disler et al. (1975) (157) reported their data on the fortification of cane sugar with ascorbic acid and iron. They tried a large number of iron salts as fortificants; these were ferrous sulphate, ferrous ammonium sulphate, ferric nitrate, ferric sulphate, ferric ammonium sulphate, ferric orthophosphate, ferric glycerophosphate, ferric fructose and ferric sodium EDTA complex. It was found possible to add a number of iron salts together with ascorbic acid to cane sugar without affecting its appearance or storage properties. Ascorbic acid was found to increase the iron absorption 2-fold with an ascorbic acid:Fe ratio of 10:1 and 3-fold with the ratio 20:1. Iron from FePO₄·H₂O was poorly absorbed when added with sugar to maize-meal porridge, and also when added with adequate quantities of ascorbic acid. In fact, this form of iron was much less well absorbed than was the intrinsic iron (native iron) present in maize. When sugar fortified with FePO₄·H₂O and ascorbic acid was added to maize-meal porridge before cooking, or was made into jam, there was seven-fold increase in the amount of iron absorbed. Sugar fortified with soluble iron salts like FeSO₄ discoloured tea and coffee, but that with FePO₄·H₂O did not have this effect.

During 1976 Layrisse et al. (1958) reported their findings on the possible use of sugar as a vehicle for iron
fortification. They argued that wheat flour, maize and milk contained an excess of absorption-hindering substances that affect the absorption of both the native food iron (intrinsic iron) as well as the fortified iron (extrinsic iron), independent of dose and valency of iron. Ingestion of meat, fish and fruits, on the other hand, increased iron absorption. Sugar was the best vehicle as it did not at all interfere with iron absorption. In accordance with this thinking, they observed that the iron absorption from fortified sugar with FeSO₄ mixed with vegetables was the same as that of the native vegetable iron. The absorption from fortified sugar was increased more than 50 per cent over that observed from native vegetables, when it was administered as a drink during the ingestion of a meal. A further increase in absorption was found when fortified sugar was administered with beverages. The mean absorption ratio of fortified sugar given with orange juice, Coca cola and Pepsicola to a reference dose of iron ascorbate was between 0.45 and 0.66, which is more than 3 times the absorption of this iron fortification mixed with vegetables. With this fortification, there was no adverse change in the vehicle, such as taste and colour, that could prevent the commercial use of the article. The only beverage so far affected by fortified sugar is tea; its colour changes rapidly to black and iron is precipitated and poorly absorbed. In a later communication (135), they observed that ferric-EDTA and FeSO₄ exhibited very high absorption, almost of the same order, while, in comparison, ferric ammonium citrate was poorly absorbed. Ferric-EDTA reacted with tea tannins very slowly, the colour changing only after 2 hours. The absorption of EDTA complex increased with vegetable meal using fortified sugar.
Dermán et al. (1977) (1959) have also recently reported on iron absorption from a cereal-based meal containing cane sugar fortified with ascorbic acid.

Coffee

Klug et al. (1976) (160) have recently tried the fortification of coffee by holding commercial coffee extract at 80°F for some time, and clarifying substances that form precipitates with iron are removed. Then iron may subsequently be added without affecting the amount of sediment remaining after the dehydrated product is rehydrated at normal coffee strength. For example, commercial coffee extract (20 per cent solids) was chilled to 55°F, held for 60 minutes to precipitate iron reactive compounds, then centrifuged. \( \text{Fe}_4(\text{P}_2\text{O}_7)_4 \cdot 9\text{H}_2\text{O} \) (at 0.1 per cent Fe) in 40 per cent citric acid complex was added to the clarified extract and the preparation spray dried and agglomerated. Upon adding water to standard coffee strength, the sediment level was 12 vs 62 for the untempered control product.

2.3 Fortification of drinks, beverages and sauces

The use of orange juice, Coca cola and Pepsi cola as vehicles has already been reported above (158). Recently, Meyer et al. (1975) (161) have observed that oral iron administration to piglets through drinking water was quite comparable with parenteral administration of iron-dextran preparations. This reminds us of using water from a blacksmith's shop in which hot iron being hammered into swords was dipped to be given to persons afflicted with iron deficiency to recoup their strength (162, 163). The use of fortified fish sauce has already been included (141).
(b) **Lacunae in our current knowledge about the problem**

A closer study of the review compiled above reveals that there still exist several lacunae in our understanding of the problems facing any programme of iron fortification of milk. The following aspects need more information.

**Form of iron:** Iron salts and complexes which have so far been tried as fortificants for milk, namely FeSO₄, ferric pyro- and poly-phosphates and ferric ammonium citrate, do not in reality fulfil the several requirements considered essential for an ideal fortificant. Their main drawbacks are the off-flavours as well as the instability in milk induced by them. A suitable form of iron still awaits development.

**Characteristics of milk:** The interaction of the fortificant with the complex matrix of the milk, i.e., with the unique physicochemical environment of the stabilized emulsion system characteristic of the whole milk, the precise binding site on the complex casein micellar structure, the disturbance, if any, caused to the organizational integrity of the fat globule membrane (FGM), and the effect on the milk enzymes present in the different compartments, all need detailed studies in order to understand how to avoid off-flavour induced by the powerful catalytic effect of iron, and how to control the factors disturbing the processibility characteristics of milk, which form the basis of various product conversions in the modern dairy technology.

**Bioavailability and side effects:** The knowledge about the factors which govern, regulate and control the tissue distribution pattern of iron in the consumer needs a clearer
picture than is available at present. The iron has to be obtained from a fortificant which, after fortification of milk, has been embedded deep in the specific microenvironment of the different compartments of milk. Its extrication from this matrix and its uptake by the body need more detailed study for its availability and usefulness. This is of particular importance in view of the fact that the fortified milk is going to be used by the consumer over life-long periods. The cumulative effects of such an iron enrichment programme, which is meant primarily to prevent and not to treat iron deficiency, in a large population with the respective biochemical individuality of its components, certainly needs a much more elaborate study in order to evolve a perfectly safe regimen for the different age groups.
iii. Specific objectives to be achieved

The specific objectives to be achieved in the present study programme could therefore be summarised as the designing of a form of iron which, as a fortificant, would not only maintain its own stability in milk, but would also maintain:

(1) the characteristic stability of the complex emulsion system of milk even on repeated heatings and coolings, particularly as practised in our country;

(2) the acceptability of the fortified milk to the consumer by not inducing any kind of off-flavour or rancidity, or any change in colour and taste;

(3) a high metabolic availability of the added iron without any side effects, or any acute or chronic toxicity, or any hypersiderosis; and

(4) the processability of fortified milk for dairy technology.