Genesis of the present study

The present study originated from a programme of research which had been undertaken in our research group in connection with iron deficiency anemia during infancy, which is of fairly common occurrence in our region. As a matter of fact, iron deficiency anemia has persisted in several age groups all over the world; it is quite common even in developed nations. Basically, it is not the lack of iron in the food but its assimilation and proper utilization which ultimately leads to iron deficiency; blood loss is another main cause; it includes the periodic loss in the female, loss due to intestinal worms, and that due to hemorrhage etc.

The organism's need at the crucial periods of life has to be met. One such period is infancy, when the increase in blood volume requires its full quota of iron for adequate formation of Hb, and for the proper development of the body mass, and the brain. The infant had low iron stores at birth, because the mother during pregnancy had comparatively poor iron stores. The deficiency in this age group results in devastating effects, which persist during the later periods of life.

Strangely, the data available at present in respect of the iron requirements and the iron nutrition of infants is still relatively meagre, or at least is not available to the desired extent. The WHO Report of 1975 (70) on this subject also had stressed this point. The use of ferrous sulphate, hitherto
claimed to be the best, the cheapest and the safest of the hematinics, is now known to cause several adverse effects, particularly those from its chronic use. Several other bound forms of iron heme also been tried, but they too have not yielded wholly satisfactory results for this age group.

Milk as vehicle for iron fortification

The superiority of milk as a vehicle for the iron nutrition of infants is unquestionable, because of the fact that milk is their natural and main diet. It is rather disappointing that so far no method has been found which was able to increase the iron content of the human or cattle milk; neither any form of exogenous iron is known to be capable of doing the needful, nor the mechanism(s) of the uptake of iron by the mammary gland during the biosynthesis of milk are clear unto now. Therefore, under these circumstances, iron fortification of milk remains, for the present, the only rational course to adopt. The practice of fortifying the feed just before giving to the baby has not found favour, being cumbersome and unacceptable.

Milk vs. other carrier foods

The earlier controversy that the milk phosphoprotein casein, like the egg phosvitin, hindered iron absorption, was resolved ultimately in favour of casein; it was established that casein did not hinder iron absorption on the normal milk intake of 1-2 litre per day, though it could do so (hinder) if this amount was increased to 5-10 litres a day per individual.

The merits and demerits of using milk as the vehicle for iron fortification have been discussed in detail, especially in comparison with other carrier foods, such as the cereals, the
cane sugar and the common salt. It is interesting to find that the latter is being tried on a community level in certain regions of India, and has been claimed to have given a good response in the adult population, but surely its use leaves the infant group totally uncovered.

Attributes of an ideal vehicle and an ideal fortificant, and also the peculiarities inherent in the iron fortification of milk have been explained.

**The vehicle and the fortificant**

In the light of the above facts, a programme of research was initiated to design and develop a form of iron suitable for the fortification of milk. For this purpose, buffalo milk was our target milk, because it was the most commonly available and used milk in our region. After screening a large number of iron-sugar and iron-amino acid complexes, ferric sorbitol (FS) was selected for detailed studies, on the basis of its properties explained below. It was felt that the rigid and the fairly exacting requirements fixed for a good fortificant would require the use of a cofortificant with it. Ascorbic acid (AA) was selected for this purpose; reasons for its selection have been explained. This system was studied in detail to understand its physico-chemical behaviour in aqueous solution, and in milk. In aqueous solution, its solubility, pH, dissociation characteristics, its behaviour on sedimentation, dialysis, TLC molecular sieving, electrophoresis and the UV and visible absorption were examined. These rather exhaustive studies revealed that, like all other ferric complexes at physiological
and higher pH, FS also is heterogeneous in nature and occurs in the polynuclear forms in which the larger species exist in equilibrium with the lower species. AA has a depolymerizing effect to produce lower mol. weight species. There is some free or loosely bound sorbitol, probably surrounding the inner dense core.

Fortified milk

The intended level of fortification was 10 ppm iron + 500 ppm AA. The reasons for selecting these levels have been explained; this was meant to supply 1 mg iron per day of absorbed iron. It was planned as a preventive fortification to slowly build up iron stores rather than as a therapeutic measure to treat iron deficiency.

The fortified milk was studied with respect to the four criteria fixed to evaluate its overall suitability. These were the physico-chemical stability of the fortificant system in milk, and the consumer acceptability, the bioavailability and the industrial processability of the fortified milk.

These studies have been grouped as in vitro and in vivo studies.

The in vitro studies

The exact location of the fortificant in milk was investigated by studying the distribution of labelled FS in the three main compartments of milk, viz. the micellar caseins, the cream and the whey. It was found that almost ca. 95% of it was taken up by casein, some 0.1% by the cream, and the rest was with
the whey proteins. This was followed by studies on the nature of the fortificants in milk. These studies showed that ca. 95% of the iron present in milk was in the readily absorbable ferrous state, mostly (95%) remaining associated with casein; ca. 85% of the added AA was still intact, and only 15% had been lost by oxidation. Although a part of it was present as DAA, it is known that both AA and DAA were equivalent for vitamin C activity. This was a very encouraging state.

The consumer acceptability was studied by finding if the fortification had resulted in any change in the colour, the taste and the flavour of milk. The sensory evaluation test showed that there was no change at all in the colour and the taste at the intended 10 ppm iron fortification level; the slight colour obtained with higher concentration of iron also disappeared in the FS + AA system. The flavour problem, which proved more complex, was studied both by sensory as well as by chemical methods. The sensory test showed no change in favour at 10 ppm level; the chemical tests, based on the determination of lipase flavour microbial acidity (measured through TTC and DP values), the total acidity and change in pH, on the one hand for hydrolytic rancidity, and the TBA values on the other for oxidized flavour, all showed that the 10 ppm level of fortification did not in any way cause any hydrolytic or oxidative changes to invite consumer rejection. The TBA test was applied even to the isolated FGM, which was studied in detail for any effect of fortification on the FGM proteins and phospholipids, and the FGM enzymes. These also proved encouraging. But one thing that became quite clear was that the buffalo and the cow milks behave
quite differently in all these tests. Their gross similarities do not really mean anything with respect to fortification; they are two entirely different milks.

The industrial processability was examined by studying the effect of the fortificant on the milk proteins, in four different ways: effect on the amount of the total separable proteins, both by HCl as well as by TCA, the relative distribution pattern of proteins in the skin milk, the curdling behaviour of milk (both by HCl as well as by rennin), the electrophoretic behaviour, and the effect on the whole milk enzymes. These studies showed that fortification did not bring in any drastic changes in the characteristics of the milk proteins, though each milk and each process has to be studied separately in detail, as the milks were quite different in their nature.

In vivo studies

The main purpose of these studies was: (1) to ascertain if iron from the fortified milk remained biological available. This was checked by using labelled FS in comparison with two labelled reference compounds, ferrous ascorbate and ferrous sulphate, in three separate responses, viz. (a) the distribution pattern in circulation, in the tissues, and in excretion at 2 hr to 4 hr duration, (b) the hematological response based on Hb, and hematocrit, and the total WBC, and (c) the distribution pattern of the hepatic and the spleenic heme and the nonheme ferroprotein iron. All these tests showed that the absorption and the utilization characteristics of the test system were almost the same as for the reference compounds selected for this purpose. (2) The second main purpose of these animal studies was
to assess if the powerful oxidizing properties of iron could in any way elicit any adverse tissue response in the consumer, because of any possible change in the characteristics of milk. This aspect was ascertained by studying the rate of body and organ growth, the redox state in the liver and the blood and the two sensitive enzymic indicators of liver viz. the two aminotransferases (GOT and GPT) and the two phosphohydrolases (the alk and the acid Pases). The redox state was evaluated by studying the status of the GSH level, the reduced ascorbic acid level, the xanthine oxidase level, and the lipid peroxidation. It was considered that an examination of these parameters would be a fairly reasonable estimate even of an incipient damage to liver caused by the fortified milk. The milk was to be used for human consumption over life long periods, and the chronic effects could arise only after a long period of administration. All these tests revealed that the 10 ppm concentration level was as safe as possible.

These studies were then extended to find if this fortificant system could be used for the fortification of other foods and feeds. These aspects were studied in a double blind method in the volunteer consumer families only by the sensory technique. The results were encouraging. Incidentally, this study gave us quite a number of other suitable vehicles that could be employed for the adults; the drinks, the sprinkles, and the spreadings. The preliminary screening as poultry feed fortificant did not show any adverse response.
In view of the encouraging results obtained in the above studies, it is suggested that this work might be extended further on the lines envisaged under "Suggestions for further work".