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TITLE

Radioisotope investigation of iron absorption in humans, (part of a WHO/IAEA coordinated programme on iron nutrition

FINAL REPORT FOR THE PERIOD

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THE INTERNATIONAL ATOMIC ENERGY AGENCY

PROGRESS REPORT AND SUMMARY

CONTRACT NUMBER: 915/RB

TITLE OF PROJECT: "IRON ABSORPTION FROM DIFFERENT FOODSTUFFS"

INSTITUTE WHERE RESEARCH IS BEING CARRIED OUT:

Department of Medicine, University of the Witwatersrand, Medical School, Hospital Street, Johannesburg, South Africa.

PRINCIPAL SCIENTIFIC INVESTIGATOR:

Thomas Hamilton Bothwell,
Professor of Medicine.

TIME PERIOD COVERED: 1970 to July, 1975

DESCRIPTION OF RESEARCH CARRIED OUT:
See accompanying Progress Report.

Since the inception of this project, considerable insight has been gained into a number of aspects of iron nutrition. For the purposes of this review of the work conducted over the last five years, the project has been divided into four categories.

(A) Iron absorption from dietary staples

Maize, rice, wheat and soya were investigated and their potential role as a source of dietary iron was tested in a group of multiparous Indian women living under low socio-economic conditions. All the women gave their informed consent. Previous studies have shown the incidence of iron deficiency to be high in this group. This was confirmed by determining the absorption of a reference salt, consisting of 3 mg iron as ferrous sulphate and 30 mg ascorbic acid, in all volunteers.

The availability of intrinsically labelled foodstuffs made it possible to compare absorption from both native and added iron. In all the studies, the foodstuffs were prepared as meals that the subjects usually ate as part of their regular diet; maize was boiled or fried and given as porridge or patties, wheat and soya were baked and given as bread or biscuits and rice was boiled and given together with a gravy of either pea dhal or potato and onion.

These studies were planned in order to investigate the effects of both added ascorbic acid and added iron at various concentrations. In all the studies the additives were introduced into the meals prior to cooking.

The results are shown in the following table:

2/...

Staple	No. Subjects	Meal	Added Iron (mg)	Added Ascorbic Acid (mg)	Mean % Absorption
Maize	3	Patties	-	-	7.9
	7	Porridge	-	-	3.8
	9	Porridge	2.5 as Ferric Am. Citrate	50	12.1
	9	Porridge	2.5 as Ferric Am. Citrate	100	22.6
	5	Porridge	2.5 as Ferric Orthophosphate	-	1.2
	11	Porridge	2.5 Ferric Orthophosphate	100	15.7
Soya	5	Biscuit	2.0 as Ferric Am. Citrate	-	14.6
	5	Biscuit	2.0 as Ferric Am. Citrate	100	19.8
Wheat	5	Bread	-	-	7.9
	5	Bread	2.5 as Ferric Am. Citrate	50	5.3
	7	Bread	-	50	6.6
Rice	10	With vegetable gravy	4.0 as Ferrous Sulphate	-	4.6
	7	With vegetable gravy	2.5 as Ferric Orthophosphate	-	3.2
	10	With vegetable gravy	4.0 as Ferrous Sulphate	35	6.0
	8	With vegetable gravy	4.0 as Ferrous Sulphate	60	11.8
	12	With vegetable gravy	4.0 as Ferrous Sulphate	100	12.2
	7	With vegetable gravy	2.5 as Ferric Orthophosphate	100	10.4

These studies were valuable for a number of reasons:

(1) It was possible to confirm the fact that dietary non-haem iron is absorbed from a common pool. This held true despite varying amounts of added ascorbic acid and varying amounts and chemical forms of added iron. This highlighted the fact that the inhibitory substances in staples with poor iron availability have a similar effect on added iron.

(2) The addition of ascorbic acid during the preparation of maize porridge and rice was shown to be particularly effective in enhancing iron absorption. The reason why this supplement was less effective in the soya and wheat meals was investigated and found to be due to the extensive denaturation of the vitamin by the high temperatures required in baking. By comparison, only 30% of the added ascorbic acid was lost during boiling.

The investigations described in this section were published in two articles:

(a) The effects of ascorbic acid supplementation on the absorption of iron in maize, wheat and soya. British Journal of Haematology (1973), 24, 209-218.

(b) Iron absorption from rice meals cooked with fortified salt containing ferrous sulphate and ascorbic acid. British Journal of Nutrition (1974), 31, 367-375.

(B) The feasibility of using common salt as a carrier for added iron and ascorbic acid

Because the *in vivo* studies showed that iron balance could almost certainly be improved by fortification of standard meals with added iron and ascorbic acid, it was decided to look at common salt (NaCl) as a carrier for these supplements. NaCl was chosen because it is an inexpensive ingredient that is regularly consumed by all economic groups. It has the additional advantages

of a colour and taste that would not preclude the additon of ascorbic acid and white or off white iron compounds.

A number of factors were, however, found to militate against the successful fortification of NaCl. The NaCl used by the community where the in vivo studies were conducted is of a coarse variety with an high water content. Discoloration of this type of NaCl by the additives was often dramatic and occurred quicker when the fortified NaCl was stored under humid conditions. The more soluble iron preparations discolored particularly rapidly. While the addition of desiccants, such as starch, and the use of less soluble forms of iron, such as ferric orthophosphate, retarded the process, no combination of iron, ascorbic acid, and desiccant could be found that would provide a coarse, fortified salt, still acceptable to the consumer after prolonged storage under tropical conditions.

What was nonetheless reassuring was the observation that ferric orthophosphate, a compound regarded as a poor source of iron, is as available as ferrous sulphate when both forms of iron are taken with sufficient ascorbic acid.

These investigations were published in a paper "The fortification of common salt with ascorbic acid and iron" in the British Journal of Haematology (1974), 28, 483-495.

(C) The feasibility of using sugar as a carrier for added iron and ascorbic acid

When preliminary in vitro studies showed that ferrous sulphate and ascorbic acid also discolored sugar, attention was again directed to using ferric orthophosphate as an alternative. Experiments were conducted to find a suitable method for binding the additivies to sugar to prevent the sifting out that occurs in

5/...

a dry mixture of iron, ascorbic acid and sugar. The fortified sugar that was finally used in the *in vivo* studies was prepared by just moistening the carrier with 0.1% by weight of water and then adding the iron and ascorbic acid as a powder. After this the mixture was dried in warm air. When this fortified sugar was used in *in vivo* absorption studies, however, results were disappointing when compared with absorptions obtained when the ferric orthophosphate was replaced by ferrous sulphate. This is shown in the following table:

Staple	No. of subjects	Meal	Added iron(mg)	Added ascorbic	Mean % absorption
Maize	10	Porridge	4 mg as Ferrous Sulphate	-	6.2
	10	Porridge	4 mg as Ferrous Sulphate	40	10.3
	21	Porridge	2 mg as Ferric Orthophosphate	-	1.3
	21	Porridge	2 mg as Ferric Orthophosphate	40	2.9

The disparity between these results and results using similarly fortified salt was explained when it was established that the added ferric orthophosphate in the sugar studies had not entered a common non-haem iron pool. In contrast, addition of fortified salt *prior* to cooking had ensured the solubility of the added ferric orthophosphate. The fact that ferric orthophosphate was only available if added before cooking was confirmed when a mean absorption of 12.7% was obtained from a porridge meal prepared by boiling the fortified sugar together with the maize.

This work appeared in an article "Studies on the fortification of sugar with iron and ascorbic acid" in the British Journal of Nutrition (1975), 34, 141-152.

6/...

(D) The effect of tea on food iron absorption

Because a sugar fortification programme must take into consideration the fact that sweetened tea accounts for a considerable portion of the per capita daily sugar intake, it was decided to investigate iron absorption from this beverage.

Preliminary studies revealed that soluble iron salts discolored tea. While this was not the case when sugar fortified with 2 mg iron as ferric orthophosphate and 20 mg ascorbic acid was used, the mean iron absorption from this source was only 1.3%.

Absorptions of this order suggested that tea drinking might have wider implications for iron metabolism than within the strict confines of sugar fortification. The effect of tea on iron absorption from a number of sources was investigated. Results are shown in the following table:-

Source	No. of Subjects	Mean absorption (%)	
		Without tea	With tea
Ferrous ascorbate	10	30.9	11.2
Ferric chloride	10	21.7	6.2
Bread	8	10.4	3.3
Rice with 100 mg ascorbic acid	7	12.4	2.2
Haemoglobin iron (uncooked)	12	14.7	6.0
Haemoglobin iron (cooked)	10	14.3	13.5

Studies were then conducted to elucidate further the mechanism of inhibition of iron absorption by tea. In experiments conducted on rats, tannin free tea did not inhibit iron absorption while both tea tannins and tannic acid inhibited absorption to much the same extent as did the original tea. For these reasons it was

7/...

concluded that the deleterious effect of tea on iron absorption was due to the tannin content of the beverage. Confirmatory evidence for the role of an iron and tannins interaction was found when the optical spectra and molecular weights of iron and tea and iron and tannin complexes were found to be similar.

These studies appeared in two publications:-

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

(b) The mechanism of the inhibition of iron absorption by tea. South African Journal of Medical Sciences (In press).

Conclusions

While it may be possible to fortify refined salt with ascorbic acid and iron, the problem remains that those individuals whose diets are most in need of fortification use a crude salt that currently defies successful fortification. In this regard it will obviously be necessary to investigate other iron compounds and other promotors.

The use of sugar as a carrier is still, however, a possibility, provided the added iron is insoluble. As a corollary the added iron would only be of nutritional benefit if its solubility were ensured by cooking. Since these circumstances are satisfied only infrequently, as with jams and boiled puddings, an argument could be made in favour of fortification with ascorbic acid alone.

With regard to the effect of tea on iron absorption it seems relevant to make the point that tannins, by virtue of their presence in many vegetables, may be important in determining the availability of iron from staples.

8/...

Final acknowledgement.

The joint programme undertaken by the International Atomic Energy Agency and World Health Organization to study the fortification of food with iron has, I believe, been a model of collaborative endeavour and has advanced scientific knowledge in this field very significantly. It was a privilege and a pleasure to participate in the programme and I should like to record my sincere personal thanks to the International Atomic Energy Agency and to Dr. R.A. Dudley in particular for all the generous help that I have received.

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