A STUDY OF

DIETARY IRON COMPLEXES

AND

THEIR ABSORPTION

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SUMMARY

The world wide prevalence of iron deficiency states has led to an extensive study of iron metabolism during this century. Although much information has been amassed, the mechanism of iron absorption and factors controlling absorption, have not been clearly delineated. Furthermore, since most of the investigations have involved the use of inorganic iron salts alone, the relevance of these results to the total process of dietary iron absorption is uncertain.

The total iron content (McCance & Widdowson, 1960) and the overall iron absorption from basic foodstuffs has been quantitated by both chemical (Widdowson & McCance, 1942) and isotopic techniques (Moore & Dubach, 1951; Pirzio-Biroli, Bothwell & Finch, 1958). However, the physical and chemical nature of dietary iron remains poorly understood, and little information is currently available about the changes induced by cooking or digestion. These latter changes may cause the iron to be more or less available for absorption, and may be critical both to an understanding of deficiency states and physiological iron absorption.

This study was designed to quantitate the iron in two particular food items, namely liver and wheat; to partially

characterise the nature of the iron present, and to detect any physical or chemical changes in iron complexes induced by cooking and simulated digestion. As well, iron absorption from raw, cooked and digested foodstuffs was measured and compared between groups. Finally an attempt was made to correlate changes in absorption with observed changes in the nature of iron complexes.

Guinea pig liver was selected as a model of a dietary iron source rich in both non-haem and haem iron. Preliminary studies showed that the iron content and form of liver iron was comparable in this species with that of sheep, calf and chicken liver, which provide a rich source of dietary iron.

The guinea pig liver iron stores were labelled with Fe^{59} following depletion of body iron stores by repeated blood lettings over a period of two weeks. The iron removed was replaced, after a lapse of a further two weeks, by intra-peritoneal administration of Fe^{59} chloride together with carrier ferric chloride. This technique resulted in the localisation of sufficient radioactivity within the liver to allow detection and extraction of the various iron fractions.

Estimations of total iron in guinea pig liver showed that the concentration of iron was approximately 150 µg per g of tissue wet weight, and that this iron was uniformly

distributed throughout the organ. After the specimens had been washed repeatedly and blotted to dryness, 60 per cent of the total liver iron was found in the non-haem iron fraction, mostly as ferritin, and the remainder was haem iron. The techniques of "cooking" and digestion used in this study did not alter the distribution of iron between the haem and non-haem iron fractions.

Further characterisation of the liver iron complexes was attempted using centrifugation to measure the soluble iron fraction, and dialysis and sephadex gel filtration to allow some estimation of the physical size of the iron complexes. These estimations were done on raw liver and were repeated following simulated digestion and cooking.

Rapid, prolonged centrifugation of liver homogenate showed that two-thirds of the iron in raw liver was present in the supernatant soluble fraction, and the remainder was precipitated. Both cooking and in vitro tryptic digestion reduced the soluble iron fraction by about one-half. By contrast with a previously published study (Jacobs, Path & Greenman, 1969), this study showed that acid digestion, acid-peptic digestion, and peptic hydrolysis plus cooking did not release iron from insoluble complexes and did not increase the soluble iron component.

The results of dialysis and molecular sieving experiments indicated that various digestive procedures conducted in vitro produced a profound alteration in the size of the liver iron complexes. There was no small molecular weight dialysable fraction detected in raw and cooked liver. However following enzymatic digestion, and cooking plus acid-peptic digestion of liver, the small molecular weight dialysable iron accounted for up to 67 per cent of the total liver iron. Maximal release of small molecular weight dialysable iron complexes was achieved when liver was cooked and then acid-peptic digested. Furthermore, the increase in the dialysable iron fraction was shown to result from breakdown of the ferritin molecule rather than from disintegration of the porphyrin haem molecule.

The elution pattern of raw liver homogenate following filtration through sephadex G200 revealed two protein fractions which contained Fe^{59} activity. These were a high molecular weight fraction, which was subsequently identified as ferritin, and a fraction of intermediate molecular weight identified as haemoglobin. When liver was cooked and digested these two peaks were replaced by a single low molecular weight fraction which contained the whole of the radioactivity.

Furthermore the pattern of the dialysis and the filtration

experiments indicated that the digestion of liver homogenate with acid alone, in the absence of pepsin, was sufficient to release low molecular weight iron components.

The in vitro studies demonstrated that a reduction in the size of the liver iron complexes occurred when liver was subjected to procedures similar to those encountered during food preparation, mastication and gastric digestion. Although the physical and chemical changes induced in the iron complexes were of interest alone, their physiological importance could only be demonstrated if they produced changes in the degree of iron absorption. Accordingly the absorption of iron from these various fractions was tested in rats and compared with iron absorption from an inorganic source, ferric chloride. Iron retention was estimated using a small animal counter by determining the whole body radioactivity eight days after administration of the test dose.

The absorption of selected liver fractions was also tested in six normal male volunteers. Each subject received both cooked, and cooked and then acid-peptic digested liver. Iron^{59} retention was measured in a whole body liquid scintillation counter. In this case the iron retention was calculated from the radioactivity remaining in the body after fourteen days.

In the rat the efficiency of iron absorption from raw, cooked and enzymatically digested liver did not correlate absolutely with the size of the iron complexes. Although both peptic digestion, and cooking followed by peptic digestion produced an increase in small molecular weight iron components, retention of iron was increased only when liver was pretreated by cooking and acid-peptic digestion. Following these combined procedures liver iron was absorbed equally efficiently as the iron from inorganic ferric chloride.

It is true that differences in iron absorption from raw liver and from liver which had been peptic digested in vitro, may have been masked, to a degree, by the processes of in vivo digestion which occurred following administration of the test dose. However absorption from cooked liver remained low despite subsequent exposure to gastric juice. By contrast in vitro cooked and digested liver iron was avidly absorbed. In addition raw liver was readily absorbed even when the stomach was by-passed and the liver preparation was injected directly into the duodenum following pyloric ligation.

Although the correlation was not absolute, the study did strongly suggest that the size of the iron complexes was an important determining factor in iron absorption. Thus,

cooking combined with acid-peptic digestion released the largest percentage of low molecular weight liver iron complexes, as demonstrated by dialysis, and produced an almost two-fold increase in liver iron absorption. By contrast, iron was retained poorly from cooked liver in which all the iron was shown to occur as high molecular weight complexes. The relationship between molecular size of the iron complexes and efficiency of absorption was further suggested by the observed increase in iron absorption from the dialysable fraction when compared with both the non-dialysable fraction and the total liver iron.

In man there was no statistically significant difference between the absorption of iron from cooked liver and from liver which had been both cooked and acid-peptic digested. However, a similar trend of greater iron absorption from in vitro cooked and peptic hydrolysed liver, than from liver which had been cooked but not digested, emerged. Paired studies showed that five of the six test subjects absorbed a higher percentage of iron from cooked and digested liver than from cooked liver. The less striking difference in absorption of iron from these two preparations in man, compared with the rat, may reflect a more efficient mechanism of in vivo peptic hydrolysis in the human subject.

Although the results of this study are consistent overall in demonstrating an effect of cooking and peptic hydrolysis to reduce the size of iron complexes and so increase iron absorption, other factors cannot be excluded. Thus, cooking and peptic hydrolysis result in protein degradation and release a variety of peptides and physiologically active amino acids, as well as reducing the size of the iron complexes. Earlier investigators (Kroe, Kaufman, Klavins & Kinney, 1966; Layrisse, Martinez-Torres & Roche, 1968) have previously shown that certain amino acids do enhance iron absorption; such a stimulating effect cannot be excluded in the present study.

Gabo variety wheat was chosen as an example of a basic dietary iron component of vegetable origin. The physico-chemical form of the iron complexes and the effect on them of acid, acid-pepsin and cooking plus peptic hydrolysis was examined. This study thus allowed a limited comparison of the handling of dietary iron of animal and vegetable origin.

The wheat iron stores were isotopically labelled by allowing the ears of grain to mature in the laboratory in a sucrose medium with added radioactive ferric citrate. After ten days, at a stage when the wheat was judged to be mature, a maximum of 5 per cent of the added radioactivity was incorporated into the grain. The total iron content of the

matured, dried and threshed wheat averaged 27 µg Fe per g of wheat, and as such was in agreement with the figures published by McCance & Widdowson (1960).

Untreated wheat contained only a minimal soluble iron fraction, and following centrifugation over 90 per cent of the total iron was located in the precipitate. By contrast with liver, cooking, acid and enzymatic digestion all produced alterations in the soluble wheat iron fraction. Both acid digestion and to a greater degree acid-peptic hydrolysis increased the soluble iron component. Cooking alone and cooking in conjunction with peptic hydrolysis reduced the percentage of soluble iron when compared with raw wheat and peptic hydrolysed wheat respectively.

The changes in soluble iron detected following various digestive procedures were accompanied by an expected pattern of changes in the small molecular weight components detected by dialysis experiments. Raw wheat contained a very small quantity of dialysable iron complexes, and cooking reduced this to barely detectable levels. However when wheat was digested, and both cooked and digested, more low molecular weight complexes were released. Overall the maximum effect was considerably less than in liver homogenates, and the small dialysable components approached only 4 per cent of the total wheat iron.

Sephadex gel filtration of the iron compounds in the supernatant of the wheat homogenate largely substantiated the results of the dialysis experiments. An elution curve for raw wheat was unattainable due to very low concentrations of soluble iron complexes. Following acid digestion a small intermediate iron protein peak and a larger low molecular weight iron protein peak were detected. After exposure to pepsin only a single low molecular weight iron fraction was present. Following cooking and pepsin digestion both a high and a low molecular weight protein fraction were demonstrated. To this point there were certain similarities in the handling of liver and wheat in that dialysis and molecular sieving experiments confirmed release of small molecular weight iron components following digestion. The patterns of release were, however, different. In liver the maximum release of low molecular weight iron complexes was achieved by cooking combined with peptic hydrolysis, whereas with wheat acid-peptic digestion alone achieved the maximum release.

It was possible to measure wheat iron absorption only in rats and thus no comparison is possible with the liver iron absorption studies in human subjects. The absorption of iron from raw, acid digested, acid-peptic digested and cooked as well as peptic hydrolysed wheat preparations did not differ significantly. However, following cooking, iron absorption

from wheat was markedly increased. In direct contrast to the situation with liver, the wheat preparation which had the highest concentration of high molecular weight iron complexes, and the lowest proportion of soluble iron complexes, was the most efficiently absorbed.

The physico-chemical studies and absorption patterns of wheat iron, therefore, did not indicate any critical relationship between the maximal size of iron complexes and absorption. Thus, factors other than molecular size need to be considered in wheat iron absorption. Phosphorus in the form of phytate compounds occurs in wheat and is known to inhibit iron absorption (McCance & Widdowson, 1935). It may be that the processes of cooking and digestion alter the chemistry of the phytate complexes naturally occurring in the aleurone layer of the grain, or other iron-binding substances within the grain, and so affect iron absorption.

The work in this thesis has shown using a single animal source of iron, guinea pig liver, that cooking, acid and enzymatic digestion alter the physico-chemical nature of the iron complexes and also iron absorption. Increased iron absorption correlates best with decreasing size of the iron complexes; with vegetable wheat iron no such dependence on molecular size has emerged. In this case iron absorption is

probably profoundly affected by iron-binding materials within the grain.

No information has been obtained concerning interactions between various articles of diet. Extension of work in this field would be directed towards similar physico-chemical characterisation of a broad variety of foodstuffs and examination of their absorption, both singly and in combination.