Iron in flour.

Contributors

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IRON IN FLOUR

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CONTENTS

PART I

PART II

2. Materials

3. Tests at St. Albans

4. Tests at Deptford

PART III

PREFACE

These papers cover a long and complex series of investigations into the addition of iron to flour of lower extraction in order to replace the fraction of iron which, while naturally present in flour of higher extraction, is lost when white flour is milled.

The investigations, which are still in progress, were made under the aegis of the Committee on Medical and Nutritional Aspects of Food Policy which formed an expert Panel on Iron in Flour for the purpose. The work involved a wide range of skills and disciplines and a number of interesting findings have so far emerged. In addition to the three main papers, others deal with the work which was necessary to provide suitably labelled bread for the investigations. These, by Dr. James and Mr. Bullock, and other work, notably by the Laboratory of the Government Chemist (which is not included) and by the Cereals Research Station at St. Albans, were essential to the investigation.

This is an example of government, industry and research establishments all working together on a plan of research for the good of the community.

Finally, there must not be forgotten the 75 volunteers who were the subject of this study, and we are all indebted to them for the trust and assistance they gave to the scientists.

G. E. Godber,

Chief Medical Officer.

PANEL ON IRON IN FLOUR

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INTRODUCTION

Iron and other nutrients have been added to flour for a number of years in the U.K. and in many other countries. In 1960, a Joint Nutrition Panel on Bread and Flour, composed of members of the Committee on Medical and Nutritional Aspects of Food Policy and the Food Standards Committee recommended that the requirement to add iron be continued. Their report was included in the Food Standards Committee Report on Bread and Flour (Ministry of Agriculture, Fisheries and Food, 1960). The current Regulations (Statutory Instruments, The Bread and Flour Regulations, 1963), which control the composition of flour, require all flour to contain—*inter alia*—not less than 1·65 mg of iron per 100 g, the added iron to be in the form of reduced iron or ferric ammonium citrate.

In 1963, Dr. P. C. Elwood, working from Professor J. Pemberton's Department of Social and Preventive Medicine at Queen's University, Belfast, reported the results of a preliminary investigation of the availability of various forms of iron which might be added to the human diet. The Panel on Iron in Flour was formed, under the auspices of the Committee on Medical and Nutritional Aspects of Food Policy. The Panel had to consider the forms of iron which were haemopoietically potent when added to flour, and which were also suitable in commercial practice.

Dr. Elwood continued his work at the Medical Research Council's Epidemiological Research Unit, Cardiff, under Professor A. L. Cochrane, and on the Panel's behalf, he undertook the long series of double isotope studies with human volunteers described in Part I of this report. These studies enabled the availability of one form of iron to be compared with another of recognized availability, e.g. ferrous sulphate. Information was thus obtained even from those subjects in whom only a little iron was absorbed, and in whom it would otherwise have been doubtful whether this was because the iron was not readily available, or because the individual was either already fully stocked with iron, or for some reason unable to absorb it.

Part II describes a trial of the effects upon flour, stored under circumstances similar to those which prevail in commercial practice, of different sorts of iron at statutory amounts. Such tests were necessary in order to ensure that any change in the form of added iron would not have adverse effects on the bread and flour. This work was done in collaboration at the Cereals Research Station, St. Albans, by Dr. Pace and Mr. Stewart, and at the Laboratories of Messrs. Rank, Hovis and McDougall by Mr. Farrand. Previously published additional studies made at the Cereals Research Station are also included for completeness at the end of the report.

The part played by Messrs. Newton, Eakins and Brown of the Atomic Energy Establishment, Harwell, is set out in Part III. The work is a striking example of one way in which the Atomic Energy Research Establishment provides information for the benefit of medical research.

Some of the techniques employed in the investigations were far more sensitive and informative than those which were formerly available. In addition, the number of volunteers participating (75 in all) was much greater than is usually assembled for research of this sort. Even so, because of the extent to which one individual differs from another physiologically, many of the inferences drawn have had to be based on data which would not be regarded by statisticians as fully acceptable. Moreover, as is not uncommon in nutritional research, many questions were raised which still need to be investigated.

PART I

Radio-active studies of the absorption by human subjects of various iron preparations from bread

by P. C. Elwood, M.R.C. Epidemiological Research Unit, Cardiff

1. Review of the literature

Literature relevant to the absorption of iron from bread has been reviewed by Elwood (1965, 1966). Published work of immediate relevance to the studies described in this report is reviewed here.

Mackay, Dobbs and Bingham (1945) seem to have conducted the first study of the absorption of iron from bread in this country. They gave over 50 nursery children, in three groups 14–20 mg iron daily as ferrous carbonate baked into bread. After five months there was no evidence of a rise in the mean haemoglobin levels of any of the groups, compared with those in comparable groups given bread normally available at that time (made from 85 per cent extraction flour). However, losses from these groups during the trial were large (about one-quarter from each group) and no allowances for these appear to have been made. A series of similar studies was conducted by Widdowson and McCance (1954) in under-nourished children in Germany. They reported that there were no differences in the growth of groups of children given bread made from wholemeal flour, unenriched 70 per cent extraction flour, 70 per cent extraction flour enriched to 85 per cent extraction, and 70 per cent enriched to whole meal levels with iron (thought to have been powdered iron). Furthermore, after one year on these diets there were no significant differences (at $P < 0{\cdot}05$) in the mean haemoglobin or haematocrit levels of the groups, despite the fact that the iron intake of the children in some of the groups was more than twice that of some of the others. On the basis of careful diet balance studies in nine young women, Harrill, Hoene and Johnstone (1957) concluded that, although differences in the availabilities from bread of ferrous sulphate, ferric orthophosphate and reduced iron were unimportant, none was well absorbed. They obtained a mean retention of 3 per cent in nine subjects each given just over 7 mg of iron daily baked into bread for 28 day periods; they point out that this figure is unrealistic as it is almost entirely due to only two subjects, one of whom absorbed between 10 and 18 per cent, the other of whom absorbed between 21 and 34 per cent of the added iron.

In a prolonged therapeutic trial, Elwood (1963) gave large groups of hospital patients about 80 mg iron per day as powdered iron baked into bread. There was no evidence of a significant effect (at $P < 0{\cdot}05$) on haemoglobin levels after three or six months, though a similar amount of iron as ferrous gluconate in tablets caused a reasonable mean rise in the haemoglobin levels of a similar group of patients. Unfortunately it was not possible to incorporate the ferrous gluconate in bread in this study and so the difference in response between it and the powdered iron could have been a consequence of the incorporation of the powdered iron in bread rather than because of a lack of its availability. To examine this point further the therapeutic effect of ferrous gluconate was compared with that of reduced iron, both given as tablets to female subjects with low haemoglobin levels. As the relevant regulations in force at that time (Statutory Instruments, The Bread and Flour Regulations, 1963) permitted the use of ferric ammonium citrate as an alternative to reduced iron, this preparation was also tested. The results of this trial, which have been reported by Elwood (1965), are not conclusive, but suggest that both reduced iron and ferric ammonium citrate are available if given in tablet form.

It is not easy to resolve the conflict between these two studies of Elwood (1963, 1965), in one of which iron appeared not to be available when baked into bread, and in the other appeared to be available if given in tablet form. There were certain unsatisfactory aspects of both trials—the use of mental hospital patients (in fact the trial of Elwood, 1963, had not been organised as an '*ad hoc*' feeding study, but had followed a screening survey in which an unexpectedly large number of women with low haemoglobin levels had been identified in a mental hospital), and the randomization of the different types of bread to wards of patients rather than, as was done with the tablets, randomisation to individual subjects. However, since these studies were reported it has become apparent that the iron which was baked into the bread was probably a powdered metallic iron, while that incorporated into the tablets was true reduced iron B.P. (1949). The results which will be presented later in this report indicate that this difference between the iron preparations is the most likely reason for the conflict between Elwood (1963) and Elwood (1965), but this did not become apparent until some time after these results were published. In any case this is unlikely to resolve all the problems raised by these two studies as the responses shown by one of the control groups in each trial to ferrous gluconate were very different.

All these studies suggest that iron, baked into bread, is at best poorly available to man. The only long-term study for which an unequivocally favourable result is claimed seems to be that of Stott (1960). In this, an additional 10 mg of iron per day was given as ferrous sulphate baked into bread to a group of 124 previously untreated male prisoners in Mauritius. The mean haemoglobin level in the group was 14·70 ± 0·14 g at the commencement of the trial, and this is said to have risen by 0·65 ± 0·20, 1·15 ± 0·18, and 1·33 ± 0·18 g after one, two and four months of therapy respectively, and to have fallen by 0·94 ± 0·16, 1·15 ± 0·17, and 2·13 ± 0·18 g two, four and six months respectively after iron enrichment of the bread had been discontinued. All these changes are significant at $P < 0{\cdot}05$. Unfortunately, however, it is difficult to accept the conclusions Stott bases on these findings without reservations. No control group seems to have been included in the study, there is no evidence that it was conducted 'blind', and possible sources of bias such as a seasonal variation in haemoglobin level, or changes in the mean haemoglobin level of the group due to selective discharge of subjects, seem to have been ignored. Furthermore, re-examination of the data presented by Stott suggests that if the rise in haemoglobin level is to be attributed to the additional iron supplied in the bread alone, then the percentage utilization of this iron must have been on average in the group, at least, about 35 per cent during the first month and about 27 per cent during the second month of enrichment. Such levels of utilization in a group of male subjects, very few of whom appear to have had haemoglobin levels consistent with iron deficiency, are surprising, and are not in keeping with expectations based on most published work in this field. Furthermore, these estimates of 35 and 27 per cent utilization are based on the assumption that the subjects of the study were not in negative iron balance. The reported fact that their mean haemoglobin level fell substantially immediately following cessation of enrichment of the bread would suggest that the proportionate utilization of the added iron was considerably higher than these estimates.

Two studies based on the absorption from bread of a single dose of iron labelled with Fe^{59} have been reported. Steinkamp, Dubach and Moore (1955) studied the absorptions by 'healthy' male and female subjects, of Fe^{59} incorporated into about 2–4 mg of iron in four different iron preparations baked into bread. Absorption was estimated to be between 1 and 12 per cent in 28 of their 32 subjects, and differences between the four preparations were not significant (at $P < 0{\cdot}05$). In the other four subjects between 26 and 28 per cent of the oral dose was absorbed, but the authors subsequently found reasons to suspect that these had had sub-optimal iron stores. Choudhury and Williams (1959) also baked iron labelled with Fe^{59} into bread in an investigation of the absorption of iron by patients with gastric achlorhydria. They included 'normal subjects with and without anaemia', and gave them either 3 mg or 0·6 mg approximately of iron as ferric chloride in bread. The mean absorption in four of these subjects with haemoglobin levels above 12·5 g was 1·9 per cent when the dose of iron was 3 mg, and in one such subject given 0·6 mg of iron it was 8·4 per cent. In subjects with haemoglobin levels below 12·5 g the mean absorption in five given 3 mg of iron was 10·9 per cent and in three given 0·7 mg it was 5·7 per cent. Choudhury and Williams comment on this apparent reduction in absorption when the amount of iron in the bread was reduced and they suggest that it may have been due to an increased phosphate/iron ratio with the smaller dose of iron. However, the range of absorptions of their subjects was very great and consequently the use of mean values may be misleading. Furthermore, such a conclusion is not in keeping with the more usual finding that, when given alone, the smaller the oral dose the greater the proportionate absorption of iron (Brise and Hallberg, 1962, and others). If true, however, this finding of Choudhury and Williams (1959) and the explanation they advance are of considerable relevance to the problem of the availability of iron from bread as considered in this report. At present the amount of iron in a 30 g slice of bread is only about 0·5 mg, and one-half to two-thirds of this is naturally present in the flour. The addition of large amounts of an iron preparation to flour in an '*ad hoc*' study of the absorption of iron from bread may therefore give misleading results.

The effects on iron absorption of factors relating to the subjects have been studied by many workers. Of importance is an increased absorption of iron salts in subjects with iron deficiency. A decreased absorption of iron salts in subjects with gastric achlorhydria has been detected by Cook, Brown and Valberg (1964), Goldberg, Lockhead and Dagg (1963) and others. This is of particular relevance to studies of the availability of iron from bread, as the iron used in most countries for addition to flour appears to be finely divided metallic iron (reduced iron, or powdered iron), the absorption of which probably has to be preceded by solution in gastric acid.

Other factors of relevance to the absorption of iron from bread relate to the bread itself and to the meal in which the bread is given. The effect of the baking process on the absorption of iron from bread was examined by Steinkamp, Dubach and Moore (1955). They compared the absorption in very small numbers of subjects of small doses of labelled iron baked into bread and the same amount of iron given with bread. They found no evidence of an important difference, and this has led most recent workers to assume that the baking process has no effect on availability. The effect of excess calcium on iron absorption is controversial, but, while most of the work on which the controversy is based was done on rats, Widdowson and McCance (1942) reported no adverse effect in eight human subjects of calcium carbonate added to the flour used to bake bread.

The meal in which iron is given in absorption studies involving a single dose of radio-active iron is usually referred to as a 'standard' meal. The use of this term is presumably justified within each separate study, but as the 'standards' used by different workers differ greatly comparisons between different studies must be made with great caution. Many factors which are known to affect absorption could easily be standardized, such as, in addition to those mentioned above, the size of the meal (Sharp, Peacock, Cooke and Harris, 1950; Brise, 1962), the presence of other foodstuffs (Chodos *et al.*, 1957; Sorensen, 1964) and, in particular, foods containing ascorbic acid (Moore, 1955), which may have an even more marked potentiating effect on the absorption of inorganic iron salts than on food iron (Pirzio-Biroli, Bothwell and Finch, 1958). In fact, the composition of a 'standard breakfast' has varied in practice from 'bread with butter . . . (and) . . . water as desired' (Steinkamp, Dubach and Moore, 1955) to 'tomato juice, a chicken sandwich and tinned peaches!' However, in view of our present state of ignorance about many of the factors involved in iron absorption, rigid standardization of any aspect in a field of study such as iron absorption might well lead to a situation in which misleading conclusions would be drawn from unrealistic studies. In the absence of fully realistic studies based on representative samples of the community, firm conclusions should only be based on large numbers of studies conducted on a wide variety of subjects and under many different experimental conditions.

Published work therefore indicates that iron absorption in man is dependent on many factors, only one of which is the relative availability of the iron in any particular foodstuff. For this reason the absorption of a single dose of iron, such as is measured in most experiments based on a radio-active technique, may be misleading, and the results of such studies should be confirmed by long-term feeding trials which simulate more closely conditions in real life. The fact that the results of the radio-active isotope studies of the absorption of iron from bread (Steinkamp, Dubach and Moore, 1955; Choudhury and Williams, 1959) are not substantiated by the long-term studies of Mackay, Dobbs and Bingham (1945), Widdowson and McCance (1954) and Elwood (1963), clearly justify further work in this field.

2. Objectives of the present study

The basic objective of the studies presented in this section was to determine the availability to human subjects of four iron preparations when baked into bread. These were:

(a) Reduced iron or ferrum redactum;

(b) Ferric ammonium citrate, either the green or the brown form;

(c) Powdered iron, a finely divided, metallic iron preparation;

(d) Ferrous sulphate.

The methods of preparation of these substances and of their irradiation are given on pp. 31, 32.

It was also intended to determine the availability of sodium ferric pyrophosphate in the same way but for reasons given later (p. 32) it was decided not to include this preparation.

Subsidiary objectives of the studies were to assess the relative importance to the absorption of iron from bread of certain factors related to the subjects, such as iron deficiency and achlorhydria; certain factors related to the bread, such as the effect of the baking process and the presence of additives other than iron in the flour; and the presence of foods other than bread, in particular egg, in the stomach.

3. Methods

Introduction

Evidence of the absorption of iron given orally can be sought from changes in serum iron level following a single large dose, changes in circulating haemoglobin concentration following relatively large doses given for a prolonged period, or by diet balance studies. Recently the use of radio-active techniques has enabled the relatively accurate quantitative estimation of either the retention, or the utilization of a small single dose of iron.

If an oral dose of an iron preparation, labelled with Fe^{59}, is given to a subject the amount absorbed can be estimated after an interval long enough to ensure that unabsorbed Fe^{59} has been voided (usually about two weeks). Estimates of absorption are either based on measurements of the total body content of Fe^{59}, made with a whole-body counter, or on estimates of the amount of Fe^{59} utilized for haemoglobin regeneration in a sample of blood.

The absorption of iron is influenced by a very large number of factors, including factors relating to the iron preparation itself, factors relating to the meal in which it is given, and factors relating to the subject. Consequently the absorption of a standard dose of iron may vary considerably in different subjects and within any one subject at different times. While the influence of some of these factors can be controlled to some extent, comparisons of the availability of different iron preparations are difficult and usually demand the use of large groups of subjects.

A double radio-active isotope technique, however, enables the availability of two iron preparations to be estimated in a subject at the same time, thereby controlling most of the sources of variation relating to the subject, and the method of administration. In such studies two iron preparations are labelled with different radio-active isotopes (usually Fe^{59} and Fe^{55}), and a known amount of each given to a number of subjects. The proportionate utilization of each isotope for haemoglobin regeneration is then estimated later from their content in a blood sample taken about two weeks after administration of the iron preparations.

Estimations of absorption which are based on the content of an isotope in a sample of blood usually necessitate certain assumptions regarding total blood volume and the proportionate utilization of absorbed iron for haemoglobin regeneration. Estimations of absorption which are based on the whole body content of an isotope (Fe^{59}, but not Fe^{55}, can be measured in this way) do not require these assumptions. Whole body counts can be used therefore to obtain an independent estimate of the absorption of Fe^{59} in double isotope studies, and estimates so made are basically more reliable than those based on the examination of a sample of blood.

Estimations of absorption

In this report the 'absorption' of an iron preparation represents the estimation of the proportion (expressed as a percentage) of an oral dose which has been absorbed into the body. The statistical uncertainties of individual estimations of absorption cannot easily be determined, so each has been used as an original variate. Estimates of confidence, such as standard errors of mean absorptions, have been based simply on the variation in individual absorptions, and have ignored the statistical errors in the absorptions themselves. It must be appreciated that because of this the tests of statistical significance are not strictly valid.

It is unfortunate that more data are not available, but in work such as that presented here small numbers are inevitable and conclusions have often to be based on reasonable inferences without the support of tests of statistical significance. In fact, although pairs of estimations of absorptions are available for 75 subjects, certain subgroups are represented by very few subjects.

Transformation

Estimations of absorptions in different subjects showed great variation. The use of means of estimates in small subgroups of subjects is likely therefore to be misleading and tests of significance involving such means invalid. In an attempt to increase the stability of means the original data have been transformed to the angle whose sine is the square root of the percentage (Snedecor, 1956). The mean angles obtained in this way have then been transformed back and expressed as mean percentage absorptions. In almost every subgroup used in the analyses of these data a very marked reduction in the variance proportionate to the mean followed the use of this transformation. However, the original data are presented in full (Tables VI–XIII, Appendix 1) and most of the mean absorptions are also presented without transformation (Table III, p. 12).

Tests of statistical significance

The term 'significant' is only used in this section of the report in a statistical context. Tests of significance have only been done on means obtained by transformation of the original data as described above. For convenience and simplicity the conventional level of statistical significance $P < 0 \cdot 05$ is used. However, the limitations mentioned above must be appreciated throughout. Because of the transformation used, standard errors are not quoted after the mean estimates, but where appropriate the result of a test of significance on the transposed data is indicated.

General plan of the studies

Experiments were designed which were as 'realistic' as possible. Bread was prepared in a normal commercial way (p. 32), and given to subjects, many of whom were haematologically 'normal' (p. 21), as part of a breakfast which was similar to that eaten by many people in this country (p. 8).

A small pilot experiment was done followed by a series of five main experiments. In each of the main experiments the subjects were given iron baked into bread as part of the breakfast. Each subject received 0·7–1·0 mg of iron in each of two iron preparations, one of which was ferrous sulphate as a 'control'. One preparation had been labelled with 1·0–5·0 μCi of Fe^{59}, the other with 15–25 μCi of Fe^{55}. The three subjects in the pilot experiment received no ferrous sulphate.

In both the small pilot experiment and the first main experiment, estimations of absorptions were obtained which were very much lower than had been expected (around 1 per cent instead of 5–10 per cent). Therefore, in addition to examining differences between iron preparations in subsequent experiments, other factors which might have accounted for these low absorptions were examined.

Pilot experiment. Three haematologically 'normal' subjects were each given a standard breakfast which included approximately 1 oz (28 g) of bread into which had been baked about 1 mg elemental iron incorporated into brown ferric ammonium citrate and labelled with about 1 μCi of Fe^{59}.

Experiment I. Fourteen haematologically 'normal' subjects received 1 oz (28 g) of bread containing 0·7–1 mg of iron labelled with Fe^{55}. This had been incorporated into reduced iron for seven subjects and into green ferric ammonium citrate for the other seven. Each subject was also given, as part of the same meal, 1 oz (28 g) of bread containing 0·7–1 mg of iron labelled with Fe^{59} as ferrous sulphate.

Experiment II. This experiment was similar to Experiment I, except that two iron preparations were not given to any subject on the same day. Instead, standard breakfasts were given on six consecutive days and portions of bread containing the two iron preparations were given on alternate days. With each standard meal 0·7–1·0 mg of iron was given, though the total dose of Fe^{59} and Fe^{55} in all the breakfasts together was the same as that given in the single meal of other experiments.

Experiment III. Iron powder labelled with Fe^{59} and ferrous sulphate labelled with Fe^{55} were baked into different loaves. A 1 oz (28 g) portion of each of these, containing 0·7–1 mg of iron, was given to each subject in a single standard breakfast. Bread had been made for half the subjects from normal flour, and for half from flour to which no calcium carbonate etc. had been added (p. 33). In addition to calcium carbonate a small amount of iron is normally added to flour, so that the total iron content of the bread given to half the subjects in this experiment will have contained about 0·2 mg less than that given in the other experiments. The subjects for this experiment represented a larger range of haemoglobin levels than in the previous experiments and included four with gastric achlorhydria.

Experiment IV. This experiment was conducted to supplement the data on powdered iron and to examine further the effects of some of the factors which might have explained the low absorptions in the previous experiments. Eight haematologically 'normal' and four iron-deficient subjects were given 0·7–1·0 mg iron powder, labelled with Fe^{59}, incorporated into bread. Ferrous sulphate, with 0·7–1·0 mg iron labelled with Fe^{55}, was baked into bread and given to six subjects. The other six subjects were given the same amount of labelled ferrous sulphate in solution together with an extra 1 mg of inert iron as ferrous sulphate as a 'carrier' to minimize absorption on glass surfaces etc. The 'standard breakfast' was changed slightly in this experiment and for half the subjects contained no egg, and half received bread made from flour to which no calcium carbonate etc. had been added, as in Experiment III.

Experiment V. In this experiment the absorption of brown ferric ammonium citrate by normal subjects was examined, together with the absorption of reduced iron by normal and achlorhydric subjects. To simulate conditions within the flour milling industry in this country the flour to which the reduced iron had been added, was stored for six weeks (40 days) prior to baking. This was done in case, as had been suggested by Elwood (1965), surface oxidation of the particles of reduced iron might continue for a long time after its preparation, and might interfere with its absorption from the gastro-intestinal tract.

Subjects who co-operated in each of these five experiments are distinguished in Tables V–XIII in Appendix 1 by the appropriate number of the experiment.

The subjects

All the subjects included in these experiments were volunteers in the full meaning of the word. Approval for each experiment was sought, and granted, by the Radio-isotopes Advisory Panel of the Medical Research Council.

In the first two experiments and the pilot study, only males who were 'normal' haematologically were included. In subsequent experiments females were included; some of these subjects had low haemoglobin levels, low mean corpuscular haemoglobin concentrations (M.C.H.C.), low serum irons and high total iron binding capacity (T.I.B.C.), and so can be presumed to have been iron-deficient. A few other subjects, included only in Experiments III and V had proven Addisonian anaemia, and so can be presumed to have had gastric achlorhydria. Haematological data for each subject, obtained from a sample of venous blood taken at the time of the breakfast, are given in Table V, pp. 21, 22.

The number of iron-deficient subjects included in these experiments was small and few were grossly iron-deficient. This was partly because it was difficult to find iron-deficient subjects who were not undergoing treatment for other conditions at the time of each experiment. More particularly, however, we designed our studies to be as 'realistic' as possible with regard to the subjects included and the conditions under which they were studied, and haematologically 'normal' and mildly iron-deficient persons together make up the bulk of the community in this country.

For the same reasons, it was not intended to study in any detail the absorption of iron by subjects with achlorhydria. Interest in achlorhydria only concerned one point. There are reasonable *a priori* grounds to suppose that, however readily available powdered iron and reduced iron are to normal and to iron-deficient subjects, their availabilities to subjects with gastric achlorhydria will be extremely low. In one experiment in which powdered iron was examined (Experiment III) and in one in which reduced iron was examined (Experiment V) a few subjects who were known to have histamine fast achlorhydria were therefore included.

The standard breakfast

The standard breakfast consisted of:—

Orange juice (bottled) . . .	3¾ oz
Bread (total)	3 oz (84 g approx.)
Butter	1 oz (28 g approx.)
Marmalade	1 oz (28 g approx.)
Egg	one (see Experiments IV and V)
Coffee or tea	two cups

A few subjects declined to take a slice of ordinary bread which had been included simply to make up the weight of the radio-active portions.

The total iron content of the meal (including added iron) was estimated to be 5·5 mg and the total ascorbic acid content about 25–55 mg. Almost all of the ascorbic acid in the meal was present in the bottled orange juice and this is claimed to contain 20–50 mg ascorbic acid per 100 ml.

Subjects were asked not to have anything by mouth prior to the breakfast, other than a cup of tea if this were usually taken, and not to have anything following the breakfast for about four hours, other than perhaps a cup of coffee and a biscuit.

In Experiments III and IV half the subjects were given bread baked from flour with none of the additives (calcium carbonate etc.) specified in the Flour Order (1963). In Experiment IV an egg was given to only half the volunteers, and in Experiment V none of the subjects received an egg. In Experiment IV the 'control' iron preparation (ferrous sulphate) was given in liquid form to half the subjects.

Investigations other than the radio-active isotope studies

The studies described here had considerable disadvantages. They were costly, difficult to organize and volunteers were enlisted with a little difficulty. Furthermore, interpretation of results obtained in such experiments demands the assumptions that the absorption and utilization of radio-active iron is similar to that of inert iron, and that estimations of the relative availabilities of single doses of iron are a good guide to their absorptions and utilization over long periods of time. Other studies have therefore been conducted, or are planned, and some of these will be reported elsewhere, but are briefly described below.

In a small number of subjects, changes in serum iron level following a single large oral dose of each of the iron preparations of interest were studied. These experiments were basically unsatisfactory, as the large doses of iron (about 150 mg) which were necessary to give a detectable rise in serum iron level, made them 'unrealistic'. For this reason this approach was abandoned.

While very precise estimations of the absorption and utilization of a single small dose of iron can be made by a radio-active technique, more direct evidence of the value, if any, of small amounts of iron can be sought in changes in the circulating haemoglobin level of subjects in long-term feeding studies. Unfortunately it is difficult to make such studies entirely 'realistic', as very small amounts of iron are unlikely to give a detectable change in haemoglobin level within a reasonable time. We are at present running clinical trials in which small daily supplements of iron in tablet form are being given to groups of adolescent girls, and to adult women who have been anaemic but whose haemoglobin levels have first been raised by therapeutic doses of iron. It is expected that these studies will give some indication of the size of the iron supplement which will protect a community from iron deficiency.

We have recently begun a long-term community-based study in which the haematinic effect of bread made from white flour, the iron content of which has been restored in accordance with the present practice (i.e. with iron powder) will be compared with that of flour to which a similar amount of iron as ferric ammonium citrate B.P. has been added.

4. Results

Presentation of results

Detailed results for every subject are given in Tables VI to XIII in Appendix 1. The results given in Tables II and III and the results quoted in the text are estimations of mean absorption based on the original data in Tables VI to XIII.

Experiments were designed primarily to enable comparisons to be made between the proportionate absorptions of pairs of iron preparations, one of which was always ferrous sulphate. These comparisons are made 'within subjects', and in all but certain intentional exceptions, variation within subjects was controlled by giving the two iron preparations in the same meal.

It was obviously impossible to design the experiments so that all factors of interest could be examined factorially. Therefore, the effect of factors such as the presence of calcium in the flour etc. are examined by comparisons of mean absorptions in small subgroups of subjects.

Most of the results presented and the means quoted in this section are based on estimations of utilization for haemoglobin regeneration made on a sample of blood. In the original data in Tables VI to XIII estimations based on total body measurements are also given.

Reliable estimations of proportionate absorption based on blood sample measurements could only be made down to about 0·1 per cent, and although the errors in estimations by this method were large below 1 per cent, absorptions down to 0·1 per cent are quoted. Below this they are shown as <0·1 per cent, but where available in such cases estimations based on measurements of the total body content of Fe^{59} are used in the derivation of means.

Because of the marked inhibition of iron absorption caused by the inclusion of an egg in the standard breakfast (this is discussed on p. 14) mean absorptions are usually quoted separately for subjects who received an egg and for those who did not.

The composition of the groups of subjects who received the various iron preparations was not the same with respect to factors of relevance to iron absorption. In particular, the proportion of subjects likely to be iron-deficient differed considerably in the different experiments. Hence straight comparisons between the mean absorptions of the various iron preparations are invalid and are likely to be misleading. However, every subject received ferrous sulphate and so comparisons between the mean absorptions of any of the other iron preparations (ferric ammonium citrate, reduced iron and powdered iron) and the mean absorption of ferrous sulphate 'within the same subjects' are valid. If iron preparations other than these are compared allowance must be made in the interpretation for differences between the groups in their avidity for iron as shown by the mean absorptions of ferrous sulphate in the groups.

Results are quoted for only two types of subjects, those with gastric achlorhydria, and the other subjects. No attempt is made to distinguish between 'normal' and 'iron-deficient' or 'anaemic' subjects as this represents only a quantitative difference and not a qualitative difference in the context of iron absorption. However, detailed data relevant to the haematological state of each subject are given in Table V, pp. 21, 22.

The numbers of subjects in the various subgroups discussed in what follows are in Table I.

Table I. Numbers of subjects in the various subgroups receiving different iron preparations (other than ferrous sulphate which was given to every subject). Tables referred to give the original results for that subgroup in Appendix 1.

Subjects	Reduced iron	Ferric ammonium citrate		Powdered iron
		Green	Brown	
Normal or iron-deficient:				
Given an egg	13 (Table VI)	13 (Table VII)	3*	14 (Table VIII)
Not given an egg	9 (Table IX)	—	9 (Table X)	6 (Table XI)
Achlorhydric:				
Given an egg	—	—	—	4 (Table XIII)
Not given an egg	6 (Table XII)	—	—	—

* Pilot experiment—results given in footnote to Table II.

The absorption of the four iron preparations

The results for reduced iron, ferric ammonium citrate and powdered iron, are summarized in Tables II and III, and given in detail in Appendix 1, Tables VI to XIII. There is some evidence in the original results that the more avid subjects were for iron, the greater the difference in the percentage absorptions of powdered iron and ferrous sulphate (Tables VIII and XI).

Table II. Mean percentage absorption (calculated after arcsin transformations) of various iron preparations by subjects with and without achlorhydria, some of whom were given an egg as part of the standard breakfast. Mean absorptions in brackets refer to ferrous sulphate given to the same subjects as those on which the mean they follow were based. For methods of calculation, see pp, 6, 10; for numbers of subjects, see Table I.

Subjects	Reduced iron	Ferric ammonium citrate		Powdered iron
		Green	Brown	
Normal or iron-deficient:				
Given an egg	0·72 (0·87)	0·94 (0·80)	Note †	0·20 (2·05)*
Not given an egg	3·55 (7·95)	—	10·35 (12·60)	0·86 (18·30)*
Achlorhydric:				
Given an egg	—	—	—	0·13 (0·58)
Not given an egg	0·73 (3·40)	—	—	—

* Significant difference at $P < 0{\cdot}05$.
† Three results only from pilot experiment, 0·5 per cent, 0·9 per cent, 8·0 per cent.

Table III. Mean percentage absorptions (without arcsin transformation) of various iron preparations by subjects with and without achlorhydria, some of whom were given an egg as part of the standard breakfast. Mean absorptions in brackets refer to ferrous sulphate given to the same subjects as those on which the mean they follow were based.

Subjects	Reduced iron	Ferric ammonium citrate		Powdered iron
		Green	Brown	
Normal or iron-deficient:				
Given an egg	0·85 (0·95)	1·06 (0·88)	Note *	0·28 (3·45)
Not given an egg	5·10 (11·68)	—	12·24 (14·00)	1·13 (23·47)
Achlorhydric:				
Given an egg	—	—	—	0·14 (0·70)
Not given an egg	1·12 (3·50)	—	—	—

* Three results only from pilot experiment, 0·5 per cent, 0·9 per cent, 8·0 per cent.

The absorption of ferrous sulphate by the different groups of subjects is summarized in Table IV. However, for the reasons given on p. 10, these pairs of absorptions cannot be compared directly.

Table IV. Mean percentage absorption of ferrous sulphate by subjects with and without achlorhydria, some of whom were given an egg as part of the standard breakfast.

Subjects	Number	Absorption of ferrous sulphate (%)
Normal or iron-deficient:		
Given an egg	40	1·1
Not given an egg	24	12·0
Achlorhydric:		
Given an egg	4	0·6
Not given an egg	6	3·4

The differences in the mean absorption of reduced iron, brown and green ferric ammonium citrate and ferrous sulphate appear to be small, provided comparisons allow for differences due to the composition of the groups and the effect of egg on availability of iron, and none is statistically significant. However, the consistency in the signs of the differences does suggest that reduced iron may be a little less available than ferrous sulphate or ferric ammonium citrate to subjects without gastric achlorhydria and much less available than these salts to subjects with gastric achlorhydria. On the other hand, powdered iron appears to be very much less available than any of the other iron preparations and this difference appears to be greatest in conditions which favour iron absorption e.g. in anaemic subjects given no egg.

The effects of factors relating to the subjects

Iron deficiency. Examination of the original data in Tables VI to XIII, together with haematological data relating to the subjects in Table V, shows that subjects who were likely to be iron-deficient absorbed, on average, more of each iron preparation. This increase in absorption is barely apparent, however, for those who received powdered iron. It must be stressed, however, that the haematological data given in Table V give only a very indirect estimation of the degree of iron-deficiency in a subject, just as the proportionate absorption of a single dose of iron gives only a very approximate indication of a subject's 'avidity' for iron. A close correlation would not therefore be expected.

Gastrica chlorhydria. The reasons for including these subjects are given on p. 8. The results indicate that both powdered iron and reduced iron are very poorly available to these subjects.

The effects of factors relating to the bread

The presence of added calcium carbonate. Very few of our results relate to this factor. In experiments III and IV 12 subjects, at random, received bread made from ordinary flour and the other 12 bread made from flour with no added calcium carbonate (see p. 33). The 12 subjects in each group were homogeneous with regard to other factors of relevance (such as the number who received an egg, etc.). The mean absorption of ferrous sulphate in those whose bread contained added calcium carbonate was 2·6 per cent and in those whose bread was made from flour with no added calcium carbonate was 6·2 per cent. These means do not differ significantly. In fact, although both these means are based on very skewed distributions (see Tables VIII, XI and XIII), the distribution which gives the lower mean of 2·6 per cent has only one very high value (24·9 per cent) while that which gives the higher mean of 6·2 per cent has three very high values (31·1, 46·1 and 56·1 per cent). In spite of the transformations used, these means cannot be regarded as stable estimates nor can the results of the tests of significance used on the difference be accepted without considerable reservations. In any case, flour to which calcium carbonate had been added was used in all the other experiments, and as individual absorptions like those quoted above often occurred in the experiments in which an egg was not given as part of the breakfast, it seems unlikely that the added calcium carbonate in flour has any important effect on the availability of added iron.

The baking process. There are so few results relating to this factor that it barely deserves discussion. Six subjects in Experiment IV received the labelled ferrous sulphate with bread and six received it baked into bread. The mean absorptions in these groups were 4·0 per cent and 13·2 per cent respectively and these do not differ significantly. However, the variation in the results is so great that little reliance can be put upon these means or the difference between them.

Prolonged storage. In Experiment V flour, to which reduced iron had been added was stored for six weeks. No similar group of subjects was given the same iron preparation which had not been stored in flour, in the same breakfast, so that the results are inconclusive, but suggest that the effect of storage, if any, is very small.

The effects of factors relating to the meal

Interactions between iron preparations. Or results give relatively little information relating to this factor. In Experiment II 12 subjects received their two iron preparations separately, each on three alternate days. Ferrous sulphate was therefore given without another iron preparation at the same time to these subjects, and their mean absorption was 0·96 per cent. All the other subjects in Experiment II received two iron preparations together. The best comparison is restricted to a group of subjects homogeneous with respect to all related factors, such as sex, age and the presence of an egg in the meal, etc. The 12 subjects in Experiment I fulfil these conditions, and they all received ferrous sulphate together with either ferric ammonium citrate or reduced iron in the same meal (no subject in any experiment received two preparations in the same bread). The mean absorption of ferrous sulphate in this group was 0·74 per cent and this does not differ significantly from 0·96 per cent.

The presence of egg. The effect of the inclusion of an egg in the breakfast was examined in Experiment IV. Six of these subjects received an egg and their mean absorption was 1·75 per cent. The other group of six subjects, which was homogeneous in other relevant respects, absorbed 18·3 per cent. Despite the marked difference in these proportions they do not differ significantly. Tables II and IV show that in other subgroups of subjects egg appears to have had a very marked inhibitory effect on the absorption of iron, though for the reasons given on p. 10 direct comparisons between subgroups should not be made. However, the size of the differences between all subgroups who received egg and all subgroups who did not, together with the consistency in the sign of these differences, seems to indicate beyond reasonable doubt that egg does have a marked inhibitory effect on the absorption of reduced iron, ferric ammonium citrate and ferrous sulphate from bread. It should also be noted that even in the absence of an egg powdered iron was very poorly absorbed, the mean in six subjects being 0·86 per cent compared with 18·30 per cent for ferrous sulphate in the same subjects (Table II).

5. Discussion

The average daily intake of iron in this country is estimated to be about 14·3 mg per person, and of this about 1·8 to 2·6 mg (12 to 18 per cent) is estimated to come from white bread, and a further 1·3 mg (10 per cent) from flour, or products of flour (Ministry of Agriculture, Fisheries and Food, 1965). The present level of extraction of white flour is about 72 per cent and this is fortified with iron to give a final iron content of 1·65 mg per 100 g flour; only between one-third and one-half of the iron content of white flour is artificially added. That is, each person receives on average about 1·3 mg of added iron each day or 9 per cent of their total daily intake of iron, because of the provisions of the Bread and Flour Regulations (Statutory Instruments, 1963).

The figures quoted above are derived from average household consumption of food (Ministry of Agriculture, Fisheries and Food, 1965). The more detailed analyses presented in the same report suggest that, while the annual consumption of white bread is showing a gradual downward trend over the years, households in certain sections of the community such as families with four or more children, families with low incomes, and families with several children and a pregnant mother, devote a greater than average proportion of their total food expenditure on white bread. Although there does not seem to be a 'social class' gradient in the distribution of haemoglobin level in the community in this country

(Elwood, 1965), nor, in a recent study in South Wales, did there appear to be a significant correlation in women between the amount of bread consumed and haemoglobin level (Elwood, unpublished), yet it is not unreasonable to assume that some, if not all, of the above sections of the community represent groups with a greater risk than average of becoming iron-deficient. The importance of bread therefore as a source of iron may in fact be greater than the average figures quoted above suggest. This would also be true if the availability of the added iron is higher than that of the iron obtained from other dietary sources.

The studies presented here establish with a reasonable degree of certainty that the availability to man of powdered, electrolytically precipitated iron, one of the main forms of iron added to flour in this and other countries, is considerably lower than that of the three other iron preparations examined. Furthermore, although our data are few, they clearly suggest that both in iron-deficient subjects and in subjects with achlorhydria, the availability from bread of powdered iron is even lower, compared with that of ferrous sulphate, than in 'normal' subjects. Our results also indicate that, while the absorption of ferrous sulphate was greater than that of powdered iron in the presence of egg, when the egg was omitted from the meal the difference between these preparations was very much greater. Consequently, it appears likely that most of our figures underestimate to a considerable degree the differences between powdered iron and the other iron preparations we examined.

It is clear therefore that if iron is to be added to flour, a preparation which is more readily available than powdered iron should be used, provided this is shown to cause no serious deleterious effect on the storing and baking qualities of the flour (Martin and Halton, 1964). The present results suggest that, while reduced iron may be readily available to normal and iron-deficient subjects, it is possibly less so than either ferric ammonium citrate or ferrous sulphate, and is certainly very poorly available to subjects with achlorhydria. The evidence is insufficient to warrant a comparison between the green and the brown forms of ferric ammonium citrate, but it seems that either is satisfactory.

It must be realised, however, that neither reduced iron nor ferric ammonium citrate are iron preparations which can be clearly defined in all respects. It is likely that the most important aspect of reduced iron which is relevant to its availability is its surface area, which is partly, but not necessarily wholly, dependent on particle size. The particle size certainly depends on the temperature at which the iron preparation is produced, and probably the surface area does also, and so we have given details relating to the preparation and the particle size distribution of the sample we used in the studies reported here (p. 31). On the other hand the physico-chemical nature of ferric ammonium citrate is not fully understood. It is believed to be a colloidal suspension of ferric hydroxide in ammonium citrate, but it is stoichiometric with a variable iron content.

Unfortunately, we have no results relating to sodium iron pyrophosphate. Although this is a more expensive preparation than reduced iron, it is said to be particularly suitable for use in the food industry as it has the merit of being light-coloured (Wilder and Williams, 1944). However, the availability to anaemic rats of sodium iron pyrophosphate or sodium ferric pyrophosphate appears to be low compared to that of a wide variety of iron preparations (Freeman and Burrill, 1945; Street, 1943; Blumberg and Arnold, 1947). On the other hand, Steinkamp, Dubach and Moore, (1955), on the basis of the absorption of a single radio-active dose, state that sodium iron pyrophosphate is as readily available to man from bread as a variety of other iron preparations, including ferrous sulphate and reduced iron, though only eight subjects were given sodium iron pyrophosphate in their studies. However, it seems that sodium iron pyrophosphate or sodium ferric pyrophosphate is not a single salt and the substance so called which was prepared and tested by Steinkamp, Dubach and Moore (1955) does not appear to be the same as that used for the enrichment of flour in the U.S.A. (p. 32). Because of the difficulty in defining a single iron salt with this name we decided not to include sodium iron pyrophosphate in our studies.

The findings, which we present here on the effect of a freshly boiled egg on the absorption of iron by our subjects, show how relatively little is known about iron absorption in man. The *in vitro* studies of Kirch *et al.* (1947) showed that while a wide variety of foods cause reduction of ferric to ferrous iron in pepsin-hydrochloric acid solutions, egg yolk has no such effect but causes almost complete 'complex' formation of an iron salt. The possible importance of this *in vivo* is supported by Bergeim and Kirch (1949) and also by Chodos *et al.* (1957). The latter authors found that between 8 and 75 per cent (mean 31 per cent, median 23 per cent) of an oral dose of 4·0–6·4 mg of iron as ferric chloride was absorbed by eight 'normal' subjects when given alone, but, if this was given with an egg to five 'normal' subjects, only 1–43 per cent (mean 13 per cent, median 4 per cent) of a total dose of about 8 mg of iron was absorbed. It is of further interest in this context that Choudhury and Williams (1959) mention that in preliminary studies they incorporated Fe^{59} into biscuits, but later abandoned biscuits in favour of bread as the absorption from biscuits, even by anaemic patients, was 'very poor'. The recipe they used for the biscuits is thought to have included egg, while that used for the bread did not (M. Freeman—personal communication). In spite of these reports (though we were not aware of the last until more recently), we decided to include an egg in our 'standard breakfast' to make it a 'realistic' meal. The consequent effect on the iron absorption of our subjects appears to have been far greater than we could possibly have anticipated on the basis of published work. The inhibitory effect of egg on iron incorporated into food would appear therefore to be much greater than on iron given with food, possibly because of greater dispersion throughout the meal. While it could be that our subjects absorbed the iron in egg in preference to the iron in the bread, this seems an unlikely explanation in view of findings of Moore and Dubach (1951), which suggest that the iron in eggs laid by radio-active hens is no more readily available than iron in any other foodstuff. Chodos *et al.* (1957) also found that egg iron, given in a similar experiment to that of Moore and Dubach (1951), was very poorly absorbed by their fourteen subjects.

On the other hand our results give no clear indication that the availability of iron is adversely affected to any important extent by the addition of calcium carbonate to the flour at the levels specified in the Bread and Flour Regulations (Statutory Instruments, 1963), nor by the baking process, nor by the effect of storage of flour to which reduced iron had been added six weeks prior to baking, but our data are limited. Further work should be done to elucidate the effect of egg, and possibly also of calcium carbonate, on the availability of iron. The importance of egg in this context seems to be considerable, both because of the apparent magnitude of its effect, and because of the very frequent use of egg and cereals together in certain breads, cakes and, possibly of even greater importance, infant foods. Ideally, studies of the interactions between foodstuffs, such as egg and iron, should be long-term community-based feeding trials. The results of animal studies or human 'laboratory' type experiments are often impossible to interpret in terms of a possible effect in the community.

The present policy in this country regarding the addition of iron to flour is one of 'restoration'. That is, the naturally-occurring iron in white flour of extraction rates below 80 per cent is 'restored' to that of 80 per cent extraction flour. The choice of the level of restoration (1·65 mg iron per 100 g flour) is only understandable against the historical background of the relevant legislation, as it is largely an empirical standard. In certain states of the U.S.A. the level chosen appears to be that of wholemeal flour, about 3·5 mg per 100 g flour (Wilder, 1956), and even the addition of iron to give a total level in flour comparable to that of 'entire' wheat grain, 5·7 mg per 100 g (Sherman, 1952), would still be, strictly, a 'restorative' measure. However, reference to the present policy as one of 'restoration' and distinction between this and 'fortification' seem to be meaningless unless account is taken of the availability of the iron. Radio-active studies suggest that naturally-occurring cereal iron is relatively poorly available to man (Hussain *et al.* 1965) and its absorption appears to be little enhanced in iron deficiency. It may be therefore that, if a readily available iron salt such as ferric ammonium citrate is used to replace some of the wheat iron, the resultant flour is, in effect, fortified. On the other hand, environmental factors, such as the presence of other foodstuffs, have been shown to affect the absorption of iron salts to a greater extent than food iron, and it has been suggested that very small doses of iron salts may be particularly sensitive to these effects (Choudhury and Williams, 1959). It could be therefore that in 'real life' situations the addition of small amounts of an iron salt to a foodstuff will have little effect on iron balance. Again the desirability of long-term community-based feeding trials is obvious.

However, the adoption of a measure at a national level aimed at the prevention of iron deficiency in the community, such as the addition of iron to flour, is only reasonable if it can be shown that iron deficiency is common and important in terms of morbidity or mortality, and if the measure adopted can be shown to have a beneficial effect.

Iron deficiency anaemia, as conventionally defined in clinical practice (World Health Organization, 1959), has been shown to be common in this country in women of all ages (Kilpatrick, 1961), and the elderly of both sexes (Kilpatrick and Hardisty, 1961; Parsons, Withey and Kilpatrick, 1965). However, the evidence presented in these studies is based simply on the proportion of subjects with levels of circulating haemoglobin below a 'lower limit of normal' which has been chosen in an arbitrary manner. The use of such a concept is misleading (Murphy and Abbey, 1967) and any single diagnostic criterion of anaemia in terms of a level of circulating haemoglobin concentration is unreasonable unless it is shown that levels below this are significantly associated with ill health; furthermore, the level within each sex varies with age, weight and phase of the menstrual cycle. Again the evidence presented by epidemiologists of a negatively skewed normal distribution of haemoglobin levels in women (Elwood, 1964), though not in adolescents (Elwood, Withey and Kilpatrick, 1964) and probably not in men, is of relevance to national food policy only if it is shown that the low levels indicate disease or ill health; evidence presented from a large-scale community study by Elwood *et al.* (1967) suggests that this is not true to any appreciable extent. Indeed recent reports of apparent associations, even if these are only indirect and not causal, between high haemoglobin levels or haematocrits and coronary artery occlusion (Dawber and Kannel, 1961; Burch and De Pasquale, 1962), cerebral vascular accidents (Eisenberg, 1966) and pre-eclamptic toxaemia (Thomson and Hytten, 1967) emphasize the necessity of an unprejudiced epidemiological approach to the interpretation of haemoglobin level as an index of 'health' at all levels of its distribution and not simply at lower levels. We have recently commenced a large community-based study along these lines.

We have attempted to 'validate' low haemoglobin levels as an index of ill health. So far we have failed to detect a significant association between the severity of a variety of symptoms and haemoglobin level, nor a beneficial effect on these symptoms of oral iron therapy (Wood and Elwood, 1966; Elwood and Wood, 1966, and more recent unpublished work). In one very small study we failed to detect a significant association between time off work and haemoglobin level. We feel that work along these lines should be extended to cover other 'real life' situations.

The other aspect of relevance to this report is the effect, if any, that the addition of iron to flour has on iron balance over long periods of time. While the results presented here are, on the whole, in keeping with those of Steinkamp, Dubach and Moore (1955) and Choudhury and Williams (1959), all these studies are unrealistic, as they attempt to measure the retention or utilization of only a single dose of labelled iron given in a 'standard' meal to a highly selected group of subjects. Even if we accept that a rise in haemoglobin level is indicative of an improvement in health, the conclusion based on the present studies, that the substitution of ferric ammonium citrate B.P. or ferrous sulphate B.P. for powdered iron used to fortify flour in this country will lead to a mean rise in haemoglobin level in the community, can be drawn only with considerable reservations. This is both because of the limitations of the present studies, but also because of the absence of satisfactory evidence in the more realistic long-term feeding trials of Mackay, Dobbs and Bingham (1945), Widdowson and McCance (1954) and Elwood (1963). While the absence of a favourable result in the trial of Elwood (1963), and possibly in the studies of Widdowson and McCance (1954), may have been due to the unwitting use of powdered iron instead of true reduced iron B.P., this cannot be the explanation of the negative results of Mackay, Dobbs and Bingham (1945) as they used an iron salt, albeit a rather unstable one (ferrous carbonate), for fortification of their bread. On the other hand it could be that the children in the studies of Mackay *et al.* (1945) and Widdowson and McCance (1954) received sufficient iron in the control diets for the maintenance of iron balance.

In fact, however, even the effect of the administration of small amounts of iron in tablet form over a long time seem to be uncertain. Garry *et al.* (1954), Antila (1962) and Natvig, Bjerkedal and Jonassen (1966) reported a significant effect on haemoglobin level of small doses of iron, although in earlier work Natvig, Bjerkedal and Jonassen (1963) had not detected any effect by supplements of similar size, nor had Scott and Heller (1964), Garby, Irnell and Werner (1967), De Leeuw, Lowenstein and Hsieh (1966), or Paintin, Thomson and Hytten (1966). We are at present conducting similar studies in which small iron supplements is being given to groups of female subjects, and we have chosen for our studies, groups, which, we think, should be sensitive to small changes in iron balance; viz: groups of adolescent girls, many of whom are likely to be in negative iron balance due to rapid growth, and groups of adult women who have been iron-deficient recently and are still likely to be in negative iron balance. However, the results of all such studies are difficult to interpret, as iron supplements, particularly if large, may have a haematinic effect in subjects who are not iron-deficient, (the evidence is controversial but is reviewed in Paintin, Thomson and Hytten (1966), and in De Leeuw, Lowenstein and Hsieh (1966). A rise in haemoglobin level following the administration of iron cannot therefore be accepted as unequivocal proof of iron-deficiency. While a dilemma of this kind is unlikely to arise in clinical work and is unimportant if it does, in the studies we refer to above, and in those we propose below, in which relatively small doses of added iron are given to subjects who are not grossly iron-deficient, the interpretation of small changes in mean haemoglobin levels may be exceedingly difficult.

We feel that long-term large-scale community-based trials must be conducted in which the effect of supplementation on the dietary intake of iron is examined, and we have recently commenced one such study. Ideally such trials should assess the beneficial effect, if any, on fully representative samples of the community in terms of a change in morbidity. However, indices of morbidity are relatively insensitive and a change in mean haemoglobin level may have to be used to assess the immediate effect. If so, the indirect nature of haemoglobin level as a measure of health must be kept in mind and parallel studies should be conducted to establish the relevance of haematological indices in this context.

6. Summary

1. The mean absorptions of powdered iron, reduced iron and ferric ammonium citrate from bread given together with an egg were 0·20, 0·72 and 0·94 per cent respectively. The mean absorptions by the same subjects of ferrous sulphate from bread were 2·05, 0·87 and 0·80 per cent respectively.

2. When given in bread as part of an egg-free breakfast the absorptions of the same iron preparations were 0·86, 3·55 and 10·35 per cent respectively compared with 18·30, 7·95 and 12·60 per cent for ferrous sulphate given to the same subjects.

3. The results suggest that all the iron preparations are more readily available to subjects with iron-deficiency though this effect with powdered iron is negligible. The results also indicate that both powdered iron and reduced iron are very poorly available to subjects with gastric achlorhydria.

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APPENDIX 1

Tables of data for individual subjects

Table V. Details of the haematological state of each subject at the beginning of each experiment.

Subject	Hb level (g/100 ml)	P.C.V. (%)	M.C.H.C. (%)	Serum iron (μg/100 ml)	T.I.B.C. (μg/100 ml)
A.1	15·8	48	33	135	380
D.1	16·4	48	35	145	350
F.1	13·3	40	33	135	420
G.1	15·4	45	34	74	270
J.1	14·1	44	32	135	370
L.1	14·5	44	33	74	370
N.1	14·7	44	33	80	340
E.1	16·1	46	35	200	400
M.1	14·8	47	32	145	400
H.1	14·7	45	33	66	370
B.1	15·5	47	33	100	320
C.1	15·1	45	33	54	320
K.1	15·1	45	34	100	350
O.1	16·1	48	34	88	260
A.2	15·5	46	34	171	—
B.2	15·5	47	33	121	—
D.2	16·1	46	35	138	—
F.2	14·1	44	32	104	—
G.2	14·5	43	34	82	—
K.2	15·8	47	34	93	—
C.2	15·4	47	33	93	—
E.2	15·7	48	33	69	—
H.2	14·1	44	32	85	300
J.2	15·7	50	31	70	340
L.2	13·9	43	32	140	360
M.2	14·5	46	32	43	—
A.3*	13·5	42	32	85	290
B.3	14·9	45	33	85	320
C.3*	13·5	42	32	125	240
D.3	16·0	48	33	125	310
E.3*	7·1	27	26	20	480
F.3*	8·4	31	27	14	420
G.3*	9·0	32	28	7	460
H.3*	12·1	39	31	34	320
A.4*	13·0	40	33	125	240
C.4	16·0	44	36	115	—
D.4*	14·7	42	35	85	250
F.4	15·4	44	35	100	—
G.4*	13·5	40	34	95	270
H.4	12·7	39	33	55	230
K.4*	15·7	44	36	75	270
M.4*	13·0	36	36	50	320
B.4*	11·8	38	31	40	290
E.4*	11·4	36	32	40	510
J.4*	11·7	38	31	105	360
L.4*	10·8	35	31	140	240
G.5	11·1	35	31	—	—
H.5*	14·1	44	32	95	420

Table V (continued)

Subject	Hb level (g/100 ml)	P.C.V. (%)	M.C.H.C. (%)	Serum iron (μg/100 ml)	T.I.B.C. (μg/100 ml)
J.5*	13·0	40	33	140	480
K.5*	13·9	43	32	78	549
L.5*	7·8	30	26	31	576
M.5*	9·3	31	30	39	432
N.5*	8·0	28	29	35	654
O.5*	9·5	34	28	—	—
P.5*	9·3	31	30	35	498
Q.5*	11·7	35	33	—	—
R.5*	11·4	37	31	39	498
S.5*	13·0	42	31	57	522
T.5*	12·1	37	33	45	510
U.5*	12·0	40	30	74	444
V.5*	11·6	36	32	35	459
W.5*	12·3	39	32	105	444
X.5*	12·9	40	32	61	444
Y.5*	13·3	41	32	52	444
Subjects with Gastric Achlorhydria					
J.3	16·0	50	32	95	370
K.3	14·5	46	32	100	410
L.3	15·1	47	32	85	100
M.3*	12·6	40	32	40	330
A.5*	8·4	32	26	39	444
B.5*	14·8	45	33	109	498
C.5*	15·7	47	33	122	483
D.5*	14·5	46	32	61	498
E.5*	15·7	47	33	175	540
F.5*	10·8	35	31	52	420

Note.—P.C.V. = Packed cell volume.
M.C.H.C. = Mean corpuscular haemoglobin concentration.
T.I.B.C. = Total iron binding capacity.

* Female subject.

Table VI. Estimated absorptions and percentage absorptions by subjects given ferrous sulphate B.P., labelled with Fe^{59}, and reduced iron, labelled with Fe^{55}, baked into bread as part of a breakfast which contained an egg. Absorption of reduced iron estimated from a sample of blood, and of ferrous sulphate from a sample of blood and by whole body measurements.

Subjects	Hb level (g/100 ml)	Absorption (nCi)			Percentage of oral dose absorbed
		Fe^{59} from ferrous sulphate		Fe^{55} from reduced iron	
		Blood	Whole body	Blood	
E.1	16·1	5·8	6·5 ± 0·3		0·6
				181·6	0·7
M.1	14·8	10·2	10·8 ± 0·3		1·1
				128·3	0·6
H.1	14·7	4·2	4·5 ± 0·6		0·4
				53·2	0·2
B.1	15·5	11·1	11·5 ± 0·4		1·2
				161·5	0·7
C.1	15·1	12·1	10·0 ± 0·4		1·2
				353·0	1·6
K.1	15·1	3·9	4·8 ± 0·5		0·4
				82·3	0·4
O.1	16·1	2·3	2·4 ± 0·3		0·2
				36·8	0·2
C.2	15·4	58·7	51·7 ± 0·5		1·0
				308·6	1·4
E.2	15·7	134·9	85·6 ± 0·9		2·4
				454·2	2·0
H.2	14·1	26·2	25·0 ± 0·3		0·5
				150·5	0·7
J.2	15·7	59·3	60·1 ± 0·6		1·1
				258·3	1·2
L.2	13·9	78·1	74·9 ± 0·7		1·4
				144·3	0·6
M.2	14·5	44·3	50·9 ± 0·5		0·8
				190·9	0·8

Table VII. Estimated absorptions and percentage absorptions by subjects given ferrous sulphate B.P., labelled with Fe^{59}, and green ferric ammonium citrate B.P., labelled with Fe^{55}, baked into bread as part of a breakfast which contained an egg. Absorption of ferric ammonium citrate was estimated from a sample of blood, and of ferrous sulphate from a sample of blood and by whole body measurements.

Subjects	Hb level (g/100 ml)	Absorption (nCi)			Percentage of oral dose absorbed
		Fe^{59} from ferrous sulphate		Fe^{55} from ferr. am. cit.	
		Blood	Whole body	Blood	
A.1	15·8	6·8	5·6 ± 0·3	113·1	0·7 0·5
D.1	16·4	26·1	19·1 ± 0·3	528·2	2·9 2·2
F.1	13·3	3·6	3·3 ± 0·3	140·3	0·4 0·6
G.1	15·4	7·2	6·7 ± 0·3	162·2	0·7 0·7
J.1	14·1	4·0	5·2 ± 0·3	38·9	0·5 0·2
L.1	14·5	6·2	7·0 ± 0·4	143·9	0·6 0·6
N.1	14·7	5·8	4·4 ± 0·4	84·9	0·6 0·3
A.2	15·5	37·5	45·9 ± 0·5	374·6	0·7 1·5
B.2	15·5	59·3	47·0 ± 0·5	600·6	1·1 2·3
D.2	16·1	21·9	22·7 ± 0·2	132·3	0·4 0·5
F.2	14·1	76·5	70·9 ± 0·7	603·4	1·4 2·4
G.2	14·5	52·7	44·6 ± 0·4	261·6	0·9 1·0
K.2	15·8	30·5	46·4 ± 0·5	245·2	0·5 1·0

Table VIII. Estimated absorptions and percentage absorptions by subjects given ferrous sulphate B.P., labelled with Fe^{55}, and powdered iron, labelled with Fe^{59}, baked into bread as part of a breakfast which contained an egg. Absorption of ferrous sulphate estimated from a sample of blood, and of powdered iron from a sample of blood and by whole body measurements.

Subjects	Hb level (g/100 ml)	Type of flour†	Absorption (nCi)			Percentage of oral dose absorbed
			Fe^{55} from ferrous sulphate	Fe^{59} from powdered iron		
			Blood	Blood	Whole body	
A.3*	13·5	Ordinary	272·0			1·6
		Ordinary		11·0	11·1 ± 0·3	0·4
B.3	14·9	Ordinary	114·3			0·7
		Ordinary		8·7	5·6 ± 0·3	0·3
C.3*	13·5	Pure	124·0			0·7
		Pure		2·7	1·7 ± 0·3	0·1
D.3	16·0	Pure	69·2			0·4
		Pure		—	1·8 ± 0·3	0·06
E.3*	7·1	Ordinary	3,878·8			24·9
		Ordinary		63·5	33·1 ± 0·5	1·7
F.3*	8·4	Ordinary	10·7			0·1
		Ordinary		1·9		0·1
G.3*	9·0	Pure	77·2			0·5
		Pure		—		<0·1
H.3*	12·1	Pure	989·4			5·5
		Pure		20·9	15·3 ± 0·4	0·7
A.4*	13·0	Ordinary	134·5			0·7
		Ordinary			2·3 ± 0·2	0·08
C.4	16·0	Pure	226·0			1·5
		Pure			4·9 ± 0·3	0·07
G.4*	13·5	Ordinary	687·7			5·4
		Ordinary		3·1	39·9 ±0·6	0·1
H.4	12·7	Pure	15·2			0·1
		Pure				<0·1
B.4*	11·8	Ordinary	992·9			5·6
		Ordinary			1·9 ± 0·2	0·07
J.4*	11·7	Ordinary	70·6			0·6
		Ordinary				<0·1

Notes.—Where activity was too low to give a realistic estimate, absorption is shown by '—', and, in the absence of an estimate of whole body content of Fe^{59}, is shown as 0·1 per cent. Where no result is shown, an estimate was not made.

* Female subjects.

† Ordinary = normally available flour.
Pure = flour with no calcium carbonate etc. added (see p. 33).

Table IX. Estimated absorptions and percentage absorptions by subjects given ferrous sulphate B.P., labelled with Fe^{55}, and reduced iron, labelled with Fe^{59}, baked into bread as part of a breakfast which did not contain an egg. Absorptions of ferrous sulphate estimated from samples of blood, and of reduced iron from samples of blood and by whole body measurements.

Subjects	Hb level (g/100 ml)	Absorption (nCi)			Percentage of oral dose absorbed
		Fe^{55} from ferrous sulphate	Fe^{59} from reduced iron		
		Blood	Blood	Whole body	
H.5*	14·1	33			0·3
			30	27·0 ± 4·0	0·6
J.5*	13·0	36			0·4
			28	24·0 ± 1·0	0·6
L.5*	7·8	745			12·1
			170	180 ± 20·0	3·7
O.5*	9·5	814			12·4
			260	260 ± 2·0	6·0
Q.5*	11·7	70			1·0
			11		0·3
S.5*	13·0	883			12·6
			312	420 ± 20·0	7·9
U.5*	12·0	224			3·1
			44	95 ± 5·0	1·1
V.5*	11·6	403			5·6
			111	142 ± 1·0	2·5
X.5*	12·9	4,096			57·6
			966		23·2

* Female subjects.
See footnotes to Table VIII.

Table X. Estimated absorptions and percentage absorptions by subjects given ferrous sulphate B.P., labelled with Fe^{55}, and brown ferric ammonium citrate B.P., labelled with Fe^{59}, baked into bread as part of a breakfast which did not contain an egg. Absorptions of ferrous sulphate estimated from samples of blood, and of ferric ammonium citrate from samples of blood and by whole body measurements.

Subjects	Hb level (g/100 ml)	Absorption (nCi)			Percentage of oral dose absorbed
		Fe^{55} from ferrous sulphate	Fe^{59} from ferric ammonium citrate		
		Blood	Blood	Whole body	
G.5*	11·1	704			6·5
			151		12·6
K.5*	13·9	688			8·3
			23	23 ± 4·0	1·5
M.5*	9·3	1,591			25·7
			319	400 ± 2·0	26·6
N.5*	8·0	355			5·6
			69	70 ± 7·0	5·3
P.5*	9·3	1,220			17·3
			233	340 ± 20·0	23·6
R.5*	11·4	411			6·1
			122	130 ± 7·0	13·5
T.5*	12·1	2,276			32·6
			291		21·7
W.5*	12·3	238			3·3
			53	70 ± 6·0	3·8
Y.5*	13·3	1,454			20·6
			26	16 ± 3·0	1·6

* Female subjects.
See footnotes to Table VIII.

Table XI. Estimated absorptions and percentage absorptions by subjects given ferrous sulphate B.P., labelled with Fe^{55}*, and powdered iron, labelled with* Fe^{59}*, baked into bread as part of a breakfast which did not contain an egg. Absorptions of ferrous sulphate estimated from samples of blood, and of powdered iron from samples of blood and by whole body measurements.*

Subjects	Hb level (g/100 ml)	Type of flour†	Absorption (nCi)			Percentage of oral dose absorbed
			Fe^{55} from ferrous sulphate	Fe^{59} from powdered iron		
			Blood	Blood	Whole body	
D.4*	14·7	Ordinary	671·8			3·8
		Ordinary		4·5	3·0 ± 0·3	0·2
F.4	15·4	Pure	6,612·1			46·1
		Pure		47·6	36·4 ± 0·5	1·7
K.4*	15·7	Pure	3,911·4			31·1
		Pure		61·3	57·8 ± 0·7	2·2
M.4*	13·0	Pure	284·1			2·3
		Pure		4·6		0·2
E.4*	11·4	Pure	8,039·9			56·1
		Pure		70·1		2·4
L.4*	10·8	Ordinary	181·2	—		1·4
		Ordinary				<0·1

* Female subjects.

† Ordinary = normally available flour.
Pure = flour with no calcium carbonate etc.
See footnotes to Table VIII.

Table XII. Estimated absorptions and percentage absorptions by subjects with gastric achlorhydria given ferrous sulphate B.P., labelled with Fe^{55}, and reduced iron, labelled with Fe^{59}, baked into bread as part of a breakfast which did not contain an egg. Absorptions of ferrous sulphate estimated from samples of blood, and of reduced iron from samples of blood and by whole body measurements.

Subjects	Hb level (g/100 ml)	Absorption (nCi)			Percentage of oral dose absorbed
		Fe^{55} from ferrous sulphate	Fe^{59} from reduced iron		
		Blood	Blood	Whole body	
A.5*	8·4	422			3·5
			26	35 ± 7·0	0·4
B.5*	14·8	334			3·9
			85	90 ± 8·0	3·6
C.5*	15·7	228			3·2
			36	72 ± 5·0	0·8
D.5*	14·5	509			5·8
			26	21 ± 3·0	0·5
E.5*	15·7	97			1·1
			15		0·3
F.5*	10·8	337			4·0
			10	12 ± 3·0	0·2

* Female subjects.
See footnotes to Table VIII.

Table XIII. Estimated absorptions and percentage absorptions by subjects with gastric achlorhydria given ferrous sulphate B.P., labelled with Fe^{55}, and powdered iron, labelled with Fe^{59}, baked into bread as part of a breakfast which contained an egg. Absorptions of ferrous sulphate estimated from samples of blood, and of powdered iron from samples of blood and by whole body measurements.

Subjects	Hb level (g/100 ml)	Type of flour†	Absorption (nCi)			Percentage of oral dose absorbed
			Fe^{55} from ferrous sulphate	Fe^{59} from powdered iron		
			Blood	Blood	Whole body	
J.3	16·0	Ordinary	204·8			1·2
		Ordinary		—	8·0 ± 0·3	0·3
K.3	14·5	Ordinary	207·6			1·2
		Ordinary		—	0·7 ± 0·3	0·02
L.3	15·1	Pure	43·0			0·3
		Pure		4·9		0·2
M.3*	12·6	Pure	23·0			0·1
		Pure		—		<0·1

* Female subjects

† Ordinary = normally available flour.
Pure = flour with no calcium carbonate etc.
See footnotes to Table VIII.

APPENDIX 2

Methods of preparation

A. The pharmaceutical methods

by K. C. James, Senior Lecturer in Physical Pharmacy, Welsh College of Advanced Technology, Cardiff.

Reduced iron. Reduced iron was prepared by D. J. Jenkins, Ph.D. of the Radiochemical Centre of the U.K.A.E.A. according to the specifications of the British Pharmacopoeia Codex (1949). Ferric oxide powder, labelled with Fe^{55}, was heated in a stream of hydrogen to about 500°C (the temperature was not measured exactly). This was later mixed with an inert sample of reduced iron prepared in the same way in the same laboratory to give the required activity for baking into bread. The particle size distribution of the inert sample (determined by V. Timbrell, Ph.D., of the Pneumoconiosis Research Unit of the Medical Research Council) is shown in Table XIV.

Table XIV. Particle size distributions of a sample of the reduced iron B.P.C. and powdered iron used in the absorption studies.

Reduced iron B.P.C.		Powdered iron	
μ	Number of particles (as percentage)	μ	Number of particles (as percentage)
		< 5	25·1
		5–	22·1
<2	7·4	10–	15·7
2–	50·4	15–	11·5
4–	29·3	20–	5·8
6–	11·2	25–	9·0
8–10	1·7	30–	3·2
		35–	2·2
		40–	1·2
		45–	1·2
		50+	3·0

Ferric ammonium citrate. Green ferric ammonium citrate B.P.C. (1954) was prepared by dissolving freshly made ferric hydroxide in citric acid solution and partially neutralising with ammonia. The compound was labelled with Fe^{55} by adding radio-active ferric citrate solution (obtained from the Radiochemical Centre of the U.K.A.E.A.), to the soluble ferric salt solution before precipitation as hydroxide. The iron content of the solution was estimated from the optical density at 535 mμ. after addition of ammonium thiocyanate and ammonium hydroxide. Ferric ammonium citrate described in this report as brown ferric ammonium citrate (B.P. 1963), was prepared by the same process, using insufficient citric acid solution to dissolve all the ferric hydroxide. Excess precipitate was removed after addition of ammonia. Specific radio-activity of these solutions was estimated from an aliquot, and the iron content was then adjusted by adding sufficient inert ferric ammonium citrate solution to give the required activity.

Powdered iron. A sample of powdered electrolytically precipitated iron (400 mesh) was obtained from a commercial source which supplies iron to the flour milling industry. Examination of a sample of this by L. Salmon, M.Sc., of the A.E.R.E. by activation analysis showed that the only important contaminant was cobalt of which about 0·006 per cent was present. A 50 mg sample of this powder was activated in a thermal neutron flux of 10^{14}n/cm^2/sec, by M. J. Whiting of the Radiochemical Centre, U.K.A.E.A., and then diluted with sufficient of the non-irradiated sample to give the required activity for baking into bread. During irradiation there was simultaneous production of Fe^{55}, and allowance for this had to be made during the calculation of the proportionate utilizations of the two iron preparations by each subject. Because of contamination there was also (at most) 0·05 μC of Co^{60} per 1 mg of iron also present. The particle size distribution of this preparation of iron is shown in Table XIV.

Ferrous sulphate. Ferrous sulphate B.P. labelled with a radio-active isotope of iron (Fe^{59} or Fe^{55}) was obtained from the Radiochemical Centre of the U.K.A.E.A. The activity of this preparation relative to its iron content was always higher than required and was adjusted to the desired proportions by dilution with a 2·5 per cent (w/v) aqueous solution of ferrous sulphate containing 2·5 per cent (v/v) of dilute hypophosphorous acid B.P. as stabilizing agent.

Sodium ferric pyrophosphate. This preparation appears to be used for the enrichment of flour in some countries (Wilder and Williams, 1944). It is described as a 'light tan powdered material . . . insoluble in water'. Steinkamp, Dubach and Moore (1955) give a method of preparation of sodium iron pyrophosphate from ferric hydroxide, but in our laboratory this gave a green crystalline material, soluble in water, resembling ferric pyrophosphate B.P.C. (1949). A sample of an iron preparation known as sodium iron pyrophosphate was obtained from a commercial source which supplies the flour milling industry in the U.S.A. and examination of this confirmed that in physical characteristics, at any rate, the two substances, both known as sodium iron pyrophosphate, or sodium ferric pyrophosphate, are very different. It was decided not to investigate either of these substances further.

B. The baking methods

by B. M. Bullock, Head of Bakery Department, College of Food Technology, and College of Commerce, Cardiff.

The recipe and methods adopted for the production of bread simulated the industrial method of bread-making in this country except that large-scale machines and a long bulk fermentation system were not used. The bread produced was an acceptable commercial sample with characteristics typical of its variety.

The basic recipe was as follows:—

Flour	3 lb 8 oz
Fat	½ oz
Salt	1 oz
Yeast	2 oz
Water	1 lb 15½ oz

A dough temperature of 80°F (27°C) was used and the dough was fermented in bulk before being moulded, tinned, proved and baked. The variety of bread produced was the 'box sandwich' or 'under tin' as this was convenient for handling during subsequent stages. The proving was carried out at an approximate temperature of 105°F (41°C) and a relative humidity of 85 per cent. Baking was achieved in 40 minutes at a temperature of 450°F (232°C).

Apart from half the bread used in experiments III and IV the flour used throughout was ordinary 'Bakers Grade' with an extraction rate of approximately 65–70 per cent. In experiments III and IV half the bread was made with flour which had been specially obtained and which was free from added calcium. In all other respects this was identical to the normal 'Bakers Grade' flour. The calcium content of the two types was determined by D. S. Tucker, Ph.D., of the College of Food Technology and College of Commerce, Cardiff, and found to be 0·32 per cent (w/w) in the normal flour and 0·075 per cent (w/w) in the calcium-free flour.

The fat used throughout was a normal white shortening composed chiefly of hydrogenated vegetable oils and fats. The soluble radio-active iron preparations were added to the water used in the preparation of the dough, and the powdered preparations were well mixed with the dry flour immediately prior to baking except in experiment V (see below). Distribution of the iron throughout the resultant loaves was examined by determining the content of radio-active isotope in numerous small samples taken at random from several loaves. There was evidence of marked concentrations of radio-active iron in the crust, so this was always discarded. Otherwise distribution throughout the loaf appeared to be reasonably good (coefficients of variation of a series of samples were 2 per cent and 4 per cent in loaves in which a soluble iron preparation had been incorporated, and 12 per cent in a loaf in which a powdered preparation had been incorporated.

In order to simulate more exactly conditions in the flour milling industry in this country, flour for experiment V, to which the radio-active reduced iron had been added, was stored for six weeks (40 days) before being baked into bread, (see p. 8). Storage conditions during this time were similar to those in which flour is stored commercially in this country.

PART II

Storage of flour containing different iron preparations

by E. A. Farrand (Rank, Hovis, McDougall Ltd) and
J. Pace and B. A Stewart (Research Association of British Flour Millers)

The National Association of British and Irish Flour Millers have investigated under experimental conditions the addition to flour of ferrous redactum, ferric ammonium citrate (green), ferric ammonium citrate (brown) and ferrous sulphate. Rank, Hovis, McDougall Limited kindly agreed to carry out these tests ,on behalf of the National Association, and they were organized by Mr. E. A. Farrand. The Cereals Research Station, of the Research Association of British Flour Millers, cooperated in the investigation.

1. General Plan of the Investigation

Two types of flour were investigated—a normal commercial baker's grade of flour and a commercial cake flour. To samples of each of these flours were added in an appropriate mastermix: ferrum redactum, ferric ammonium citrate (green), ferric ammonium citrate (brown) and ferrous sulphate. Samples of these fortified flours, together with the control flours without added iron, were stored at normal temperature, at the mill where they were prepared, and at 80°F at (a) the Deptford Laboratory of Rank, Hovis, McDougall Ltd. (*b*) the Cereals Research Station, St. Albans. All the flours were examined and tested at approximately monthly intervals independently at both Deptford and St. Albans over a period of six months. Dr. Berry of the Ministry of Health saw the tasting and baking tests at Deptford and Mr. George (Ministry of Agriculture, Fisheries and Foods) those carried out at St. Albans.

2. Materials

Flours

For bread-making. This was a typical baker's grade flour, bleached and treated with the usual oxidizing agents at the normal commercial level.

For cake-making. This was a conventional type of cake flour, treated with chlorine at the usual commercial level.

Flour storage

For storage at normal temperatures the flour samples were packed in multiwall paper bags of 70-lb capacity and held in the mill warehouse.

The samples stored at 80°F were packed in polythene bags of 6-lb capacity.

Master mixes

Four master mixes were prepared at the Deptford Laboratory containing:—

(1) Reduced iron, vitamin B_1, nicotinic acid.

(2) Green ferric ammonium citrate, vitamin B_1, nicotinic acid.

(3) Brown ferric ammonium citrate, vitamin B_1, nicotinic acid.

(4) Ferrous sulphate, vitamin B_1, nicotinic acid.

The iron salts were ground in a pestle and mortar to pass through a standard No. 10 silk. The hygroscopic nature of the ferrous sulphate ($FeSO_4.7H_2O$) made this a difficult operation. The master mixes were made up to contain 3·15 per cent iron, 3·55 per cent nicotinic acid, 0·74 per cent vitamin B_1, in a base of patent flour dried to a moisture content of 10 per cent. Master mix of this composition when added to flour at the conventional commercial level of 1 oz master mix per sack (280 lb) of flour provided in mg per 100 g flour:—iron 0·70, nicotinic acid 0·79, vitamin B_1 0·165. The master mixes were prepared by blending the components in a Simon Rota-Blender until homogeneous. After preparation they were analysed and found to contain:—

Master mix containing:	Percentage of: iron	nicotinic acid	B_1
Reduced iron	3·00	3·65	0·74
Green ferric ammonium citrate. . . .	3·04	3·60	0·74
Brown ferric ammonium citrate . . .	3·04	3·65	0·76
Ferrous sulphate	3·10	3·65	0·74

Each iron component was tested for copper content, which in all cases was < 1 p.p.m.

The master mixes were blended into the flour in a batch mixer, at a level of 1 oz master mix per 280 lb flour.

The master mixes were stored at room temperature at the Deptford Laboratory.

3. Tests at St. Albans

One 70 lb bag of each of the flours containing the different iron preparations was sent to St. Albans from the mill warehouse on the dates shown in the following Table which also shows the corresponding dates on which the flours were examined and baked.

Batch No.	*Date of arrival at St. Albans*	*Date of examination*
1	11.11.65	15.11.65
2	10.12.65	15.12.65
3	21. 1.66	26. 1.66
4	2. 3.66	7. 3.66
5	4. 4.66	5. 4.66
6	31. 5.66	6. 6.66

The samples stored at St. Albans at 80°F were taken from Batch 1.

Odour tests

Storage at normal temperature. All the samples of bread flour smelled slightly stale towards the end of the experiment. All the cake flours had developed a 'tallowy' smell by the end of the experiment.

No differences between the controls and any of the flours containing the different iron preparations were detected.

Storage at 80°F. All the samples of bread flour developed a slightly stale smell after 2 months. After 5 months' storage some, but not all, of the subjects smelling the flours considered that the sample containing ferrous sulphate had a very slightly rancid odour. All the other samples were considered to have a 'musty' smell.

All the cake flours developed a tallowy smell but no differences between samples containing different iron preparations were detected.

Baking tests

Bread from flours stored at normal temperatures. All the breads were satisfactory in appearance, smell and taste.

Bread from the flours stored at 80°F. Deterioration in baking quality was observed after 2 months' storage in bread from all the flours. This deterioration increased to the end of the experiment when all the breads were also considered to have a musty smell and a 'stale' taste.

No differences were noted between flours containing the different preparations of iron.

Cakes from flours stored at normal temperature. Sponge cakes, made from the following formula, were used in the tests:—4 oz sugar, 4 oz flour, 5 oz egg.

All the sponges were satisfactory in appearance, taste and smell.

Cakes from flours stored at 80°F. Sponges from all the flours deteriorated in baking quality towards the end of the experiment, and developed a slightly 'off' smell. No differences were detected between flours containing the different preparations of iron.

Grade colour value

There were no differences detectable by the eye in the colour of any of the flours or in the breads made from them.

In commercial practice it is usual to measure a grade colour value using an instrument called a Kent-Jones & Martin Flour Colour Grader. For testing colour values with this instrument flour is made into a batter with water, and the colour measurement is made on the batter. Values for the different flours obtained with this instrument are shown below.

	Grade Colour Value (18.11.65)	
	Baker's flour	*Cake flour*
Control	2·75	1·0
Reduced iron	2·80	1·0
Brown citrate	2·95	1·05
Green citrate	2·90	1·05
Ferrous sulphate . . .	3·20	1·30

In terms of measurement on this instrument, ferrous sulphate produces a significant increase, of about 0·5 unit, in grade colour value.

The observation was checked by examining the effect of adding the iron preparation to an untreated Manitoba flour, milled in the laboratory, with the following results:—

	Grade Colour Value
Control	3·3
Brown citrate	3·3
Green citrate	3·3
Ferrous sulphate	3·8

The effect occurs with other ferrous salts. This was shown by adding several ferrous salts to another sample of untreated flour, milled in the laboratory, with the following results:—

	Grade Colour Value
Untreated flour	1·7
+ ferrous sulphate	2·2
+ ferrous gluconate	2·2
+ ferrous fumarate	2·1
+ ferrous chloride	2·2

No effect was observed when ferrous sulphate was added to a starch paste instead of a flour paste.

Peroxide values

After three months' storage none of the flours had a measurable peroxide value when tested by a modified Lea Procedure. No positive values were found with the samples of baker's flour stored at 80°F after six and seven months. All the cake flours stored at 80°F had a small positive value of about 8 m.eq oxygen per kg fat and, there was no difference between any of the iron preparations.

The baker's flours and the cake flours stored at normal temperatures had the following peroxide values after seven months.

	Peroxide Values (m.eq oxygen/kg fat)	
	Bakers' flour	*Cake flour*
Reduced iron	46	28
Ferrous sulphate	52	42
Green citrate	66	43
Brown citrate	41	41

The fact that these samples showed higher peroxide values than the samples stored at 80°F is considered to be due probably to the differences in packaging—in paper bags at normal temperature compared with polythene bags at 80°F.

There were, however, no significant differences between the different iron preparations.

4. Tests at Deptford

Odour tests on master mixes

The master mix containing ferrous sulphate developed a strong rancid odour within a few hours of preparation. All master mixes were examined for odour during the storage period with the following results:—

	Odour after period of storage (months)			
	1	4	5	6
Reduced iron	not rancid	slightly stale	stale	stale
Green citrate	rancid	rancid	rancid	rancid
Brown citrate	slightly rancid	slightly rancid	fairly rancid	fairly rancid
Ferrous sulphate	very rancid	very rancid	very rancid	very rancid

Baking tests

The flours were examined and baking tests carried out at approximately the same time intervals during the storage period as at St. Albans.

The formula used for cake-making was:—

Sponge recipe	
Frozen egg	312 g
Sugar	252 g
*Reconstituted milk powder	132 g
Flour	288 g
Baking powder	4 g

Yellow slab recipe	
Frozen egg	204 g
Sugar	336 g
Flour	300 g
Fat	144 g
Milk powder	30 g
Salt	4 g
Baking powder	7 g
Water	240 g

The results obtained were broadly similar to those found at St. Albans. In particular no significant differences in storage behaviour were observed between the flours containing the different preparations of iron.

Other tests

Vitamin B_1 tests on the bakers' flours carried out at the beginning and end of the storage period showed no significant changes that could be associated with the type of iron addition.

5. Summary

1. A typical commercial bakers' flour and a typical commercial cake flour were used to examine the effect of addition of various preparations of iron upon their behaviour during storage.

2. The iron preparations used were: reduced iron, green ferric ammonium citrate, brown ferric ammonium citrate and ferrous sulphate.

3. Samples of the flours were stored at normal temperature and at 80°F for six months. The flours were examined and baking tests carried out at approximately monthly intervals. Peroxide values on the flours were determined after three months and at the end of the storage period. None of these tests revealed any difference in the storage behaviour of the flours which could be attributed to the different preparations of iron used.

4. Grade colour values determined on batters of the flours showed that ferrous sulphate increased the colour value slightly.

5. The master mix containing ferrous sulphate became rancid shortly after preparation and the master mixes containing the two forms of ferric ammonium citrate became rancid during the storage period.

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* 1 part spray-dried milk powder + 10 parts water.

PART III

Radioisotope techniques in a study of the absorption of iron from bread by D. Newton, J. D. Eakins and D. A. Brown (Atomic Energy Research Establishment, Harwell) and G. M. Owen (Velindre Hospital, Cardiff)

1. Introduction

The use of radioactive isotopes of iron in the study of iron absorption and blood disorders is well established. Because of their convenient physical half-lives, the two nuclides employed in such investigations are Fe^{55} (2·6 years) and Fe^{59} (45 days). Fe^{59} decays by beta-emission and it can be detected in blood through its beta-particles or alternatively through its energetic gamma-rays, which also enable its behaviour *in vivo* to be studied using detectors external to the body. The decay of Fe^{55} occurs by electron capture, without emission of gamma-radiation; its concentration in blood can be estimated, with some difficulty, from the low energy X-rays which follow this process, but it cannot be assayed *in vivo* by external counting.

Estimates of iron absorption are frequently based either on measurements of activity in blood samples following administration of Fe^{55} and/or Fe^{59}, or on the retention of Fe^{59} assayed in a 'whole-body counter'. This second method, while subject to uncertainties imposed by calibration difficulties, will generally give the more accurate result, since it avoids assumptions about the total blood volume and the fraction of the whole-body iron content which is present in blood. The determination of whole-body Fe^{59} by external counting, together with estimation of the relative uptakes of Fe^{55} and Fe^{59} from blood samples, is a logical combination of the two methods when accurate values for the simultaneous uptake of two labelled preparations are required.

The measurements we shall describe were made in a study of the availabilities of various iron preparations baked into bread. A full account of the objects of these experiments, the detailed results and their interpretation is given by Elwood in Part I of this report. Details of the pharmaceutical methods employed are described on p. 31, and the methods used to incorporate the radioactive iron into bread on p. 32. We shall be concerned here only with the methods used to estimate the absorbed Fe^{55} and Fe^{59}.

2. Methods

The amounts of both nuclides administered to each subject were known from measurements of the specific activity of the bread, by methods similar to those to be described for blood; the Fe^{59} content of each subject's bread was also measured directly using a ring of Geiger counters similar to that described by Veall and Vetter (1952). After an interval of two weeks to allow for the excretion of unabsorbed iron, subjects visited the Atomic Energy Research Establishment at Harwell for body radioactivity measurements, which enabled their retained Fe^{59} to be estimated. At about the same time, a 10 ml sample of each subject's blood was analysed for both nuclides.

Body radioactivity measurements

The equipment in use at A.E.R.E., Harwell, consists of an array of four sodium iodide scintillation crystal detectors, each 15 cm in diameter and 9 cm thick, housed inside a massive lead shield to reduce their background response as far as possible and so increase their sensitivity to activity *in vivo* to a maximum. Two of these detectors are positioned above and two below the supine subject, as illustrated in Plate 1 and described by Rundo (1958). The combined response of these counters, suitably amplified, is examined by a multi-channel pulse height analyser, which accumulates a spectrum arising from the gamma-radiation emitted by the subject.

Part of the nett* gamma-ray spectrum obtained from Subject AP, who was involved in a pilot experiment, appears in Fig. 1. This was observed in a measurement of 35 minutes duration 14 days after he had ingested bread containing 867 nCi Fe^{59}; at this time only 3·4 nCi (0·4 per cent) of the administered activity remained. The most prominent features of Fig. 1 are the peaks due to 29 nCi caesium^{137} (0·662 MeV gamma-rays) arising from nuclear weapons fall-out, and to 150 g of potassium (1·46 MeV gamma-rays from natural K^{40}); these nuclides provide an additional 'background' response on which contributions from administered activities are superimposed. The peak at 1·11 MeV

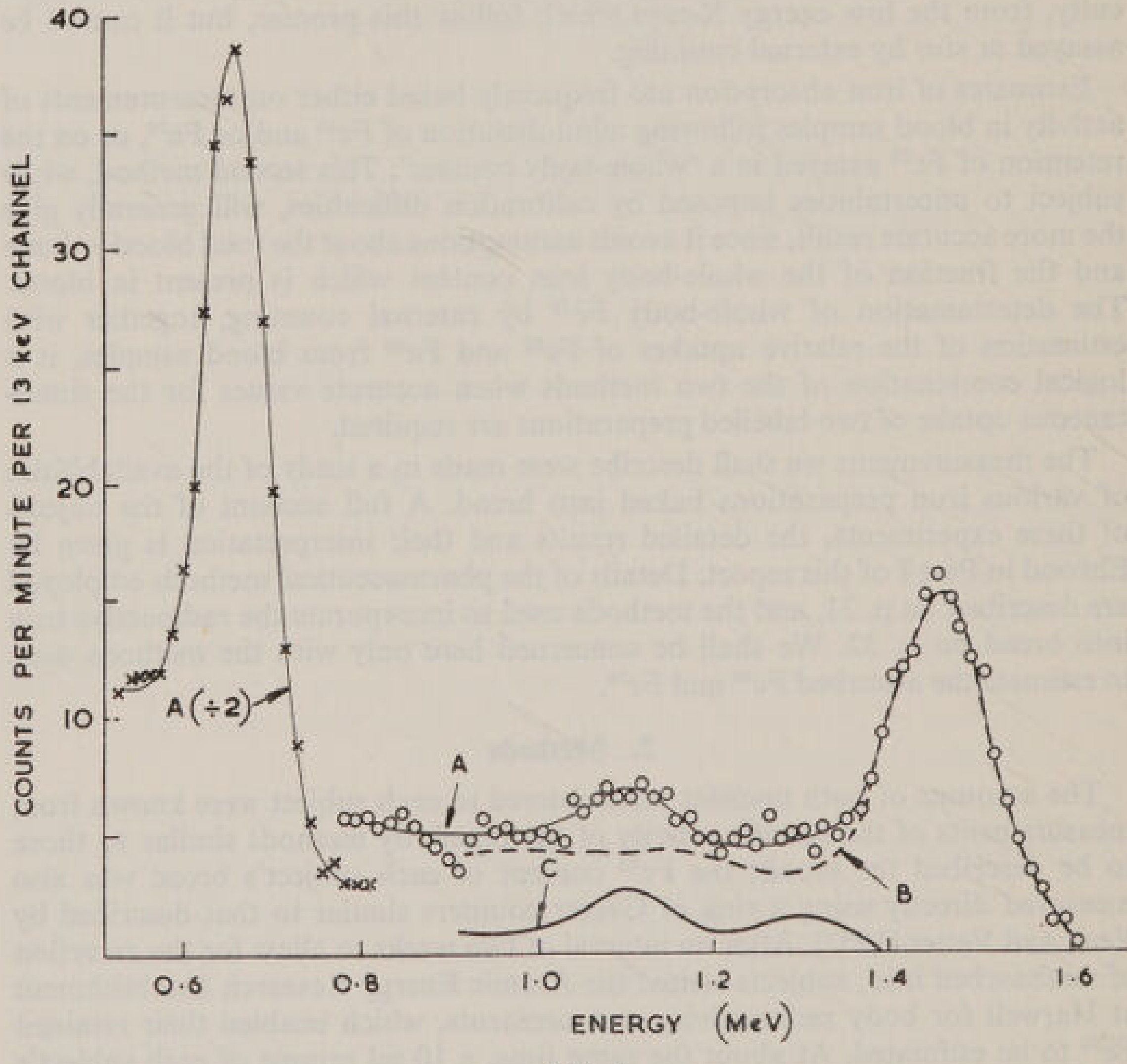

Fig. 1

Gamma-ray spectrum observed from Subject AP two weeks after ingestion of Fe^{59} (experimental points); the smooth curve A through these points was fitted by least squares analysis (see text). The computed contributions from natural K^{40} (curve B) and from the retained Fe^{59} (curve C) are also shown.

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* i.e., with the counter background response subtracted.

is due to the retained 3·4 nCi Fe^{59}; at this level the weaker 1·29 MeV gamma-rays of Fe^{59} are not readily distinguished in the presence of the dominant contribution from K^{40}.

Calibration of the equipment and analysis of the subjects' spectra were achieved as follows. Standard spectra for Fe^{59}, K^{40} and caesium^{137} were obtained using 'phantoms' similar to that shown in Plate 1, filled with solutions containing known amounts of each nuclide. Each phantom consisted of an assembly of polythene vessels which in shape resembled the human body; the dimensions of the component parts were based on those given by Bush (1949). These standard spectra enabled each subject's spectrum to be analysed for its constituent activities, using the computer programme GASP (Salmon, 1965). This programme calculated the amounts of each activity whose combined spectrum would provide the best fit, in the least squares sense, to the subject's spectrum; it also performed other useful operations, such as adjustment of spectra when small instrumental drifts had occurred during a measurement, and correction for radioactive decay of the absorbed Fe^{59} between its ingestion and subsequent measurement *in vivo*. In Fig. 1 the calculated contributions from Fe^{59} and K^{40} are shown; the continuous curve through the experimental points is the fitted spectrum, i.e., the sum of the calculated contributions from Fe^{59}, K^{40} and caesium^{137}.

The sensitivity of the equipment is such that the statistical uncertainties (i.e., those attributable to the random nature of radioactive decay) associated with the estimates of Fe^{59} retained were generally small. A retention of only 2 nCi could be measured with a statistical standard error of no more than 15 per cent, despite the predominant response from K^{40}, and the minimum detectable activity is evidently below 1 nCi. When larger amounts of Fe^{59} were found the K^{40} contributions were less important and, for amounts of 10 nCi or more, the statistical uncertainties became negligible; this may be seen from Table XV which summarizes the results for experiment I (pp. 7, 23, 24).

Table XV. Estimates of whole-body Fe^{59} and Fe^{55}, made two weeks after ingestion of approximately 1 μCi Fe^{59} and 25 μCi Fe^{55} by normal subjects.

Subject	Body content (nCi) from external measurements $\pm\ \sigma$ *	Body content (nCi) based on activity in blood $\pm\ \sigma$ *	
	Fe^{59}†	Fe^{59}†	Fe^{55}†
A.1	5·6 ± 0·3	6·8 ± 0·8	113 ± 1
B.1	11·5 ± 0·4	11·1 ± 0·8	161 ± 1
C.1	10·0 ± 0·4	12·1 ± 0·8	353 ± 2
D.1	19·1 ± 0·3	26·1 ± 1·0	528 ± 3
E.1	6·5 ± 0·3	5·8 ± 0·8	182 ± 1
F.1	3·3 ± 0·3	3·6 ± 0·7	140 ± 2
G.1	6·7 ± 0·3	7·2 ± 0·8	162 ± 1
H.1	4·5 ± 0·6	4·2 ± 0·7	53 ± 1
J.1	5·2 ± 0·3	4·0 ± 0·8	39 ± 2
K.1	4·8 ± 0·5	3·9 ± 0·6	82 ± 1
L.1	7·0 ± 0·4	6·2 ± 0·7	144 ± 1
M.1	10·8 ± 0·3	10·2 ± 0·8	128 ± 2
N.1	4·4 ± 0·4	5·8 ± 0·7	85 ± 2
O.1	2·4 ± 0·3	2·3 ± 0·8	37 ± 1

* σ = statistical standard uncertainty.

† Corrected for radioactive decay between administration and measurement.

The major uncertainties in estimating the whole-body content of Fe^{59} were usually systematic errors arising from the calibration procedure. The response of a single counter to a point source of radioactivity is governed approximately by the inverse square law. However, the arrangement of multiple counters which we have adopted gives a response largely independent of the location of activity in the body, except for regions close to an individual detector (Rundo, 1958; Rundo, 1962); therefore, unless there is a marked concentration of a radionuclide in a particular organ, such as the liver, which may be in the immediate vicinity of a detector, the distribution of activity in the phantom need not match closely that *in vivo*. Two weeks after intake, the distribution of Fe^{59} will normally be widespread; the assumption of a uniform dispersal throughout the body for calibration purposes should not result in an error of more than ±5 per cent. Larger errors may however arise if a subject is much heavier or lighter in build than the phantom, because gamma-rays will be more severely attenuated in the heavier source. Errors in calibration could be minimized by the use of a phantom selected to match an individual subject, but this was considered unnecessary for these experiments. Rundo and Taylor (1964) showed that estimates of caesium isotopes could be obtained with an overall error of less than ±12 per cent with this type of equipment, using a single phantom calibration, except for subjects near the extremes of body build. For Fe^{59} this overall error should not normally exceed 10 per cent, because of its more penetrating gamma-radiation, and this accuracy was quite acceptable for our investigations.

Determination of Fe^{55} and Fe^{59} in blood

Several procedures have been described for the simultaneous determination of Fe^{55} and Fe^{59} in blood. All involve an initial oxidation stage to obtain the iron in an inorganic form and differ only in the subsequent sample preparation and the technique used to detect the weak Fe^{55} X-rays. In some of them the iron is electrodeposited and the X-rays are detected with Geiger-Muller counters (Peacock *et al.*, 1946; Hallberg and Brise, 1960); in others the electrodeposited iron is dissolved and its solution is mixed with a liquid organic scintillator (Dern and Hart, 1961). The electrodeposition stage is eliminated in the liquid scintillation method of Perry and Warner (1963), the Fe^{55} being assayed as a colourless complex.

A common factor in these methods is the poor detection efficiency for Fe^{55}; the technique of Dern and Hart (1961) is the best in this respect, giving 7 per cent efficiency for sources containing 5 mg of inactive iron. This insensitivity arises largely from the 'quenching' of the light output from low energy electrons when aqueous solutions are added to liquid scintillation systems. We prefer to measure the activity as a precipitate held in suspension in the scintillator by a thixotropic silica gel; this enables more of the sample to be mixed with the scintillator, and if it can be obtained as a pure white precipitate, quenching is often less severe than in more conventional methods. With the particular requirements of the iron absorption studies in mind, we have isolated such a compound, believed to be an ammonium salt of a complex ferriphosphoric acid; this has enabled us to develop an improved method for the simultaneous determination of Fe^{55} and Fe^{59} in blood, giving counting efficiencies approaching 20 per cent and 33 per cent respectively. The preparation of this compound from whole blood and the procedure for mixing it with the liquid scintillator have been published with other details of the method by Eakins and Brown (1965, 1966).

The activity was measured using a Packard Tricarb type 3214 liquid scintillation spectrometer (Plate 2). With this fully automatic instrument up to 200 samples, suitably prepared and mixed with the liquid scintillator, could be loaded for sequential counting, either for a preset time or until a specified number of counts had been accumulated. At the output of the detector the beta-particles from Fe^{59} gave rise to pulses whose average size was much greater than those due to the less energetic Fe^{55} X-rays. The amplified pulses whose size fell within a selected range, or 'window', could be recorded, and with this range suitably defined, the counting-rate from each nuclide was found to vary with the amplifier gain in the manner shown in Fig. 2. The response from Fe^{59} was highest with the amplifier adjusted to give 12·5 per cent of its maximum gain; under these conditions the contribution from Fe^{55} X-rays was negligible and Fe^{59} could be determined uniquely by comparison with a suitable standard. The maximum response from Fe^{55} occurred for 100 per cent amplifier gain; at this setting there was a contribution from Fe^{59}, but this could be assessed from the observed counting rate at 12·5 per cent gain. Two independent amplifying and recording systems, or 'channels', one operating at 12·5 per cent gain and the other at 100 per cent gain, enabled the two measurements to proceed simultaneously.

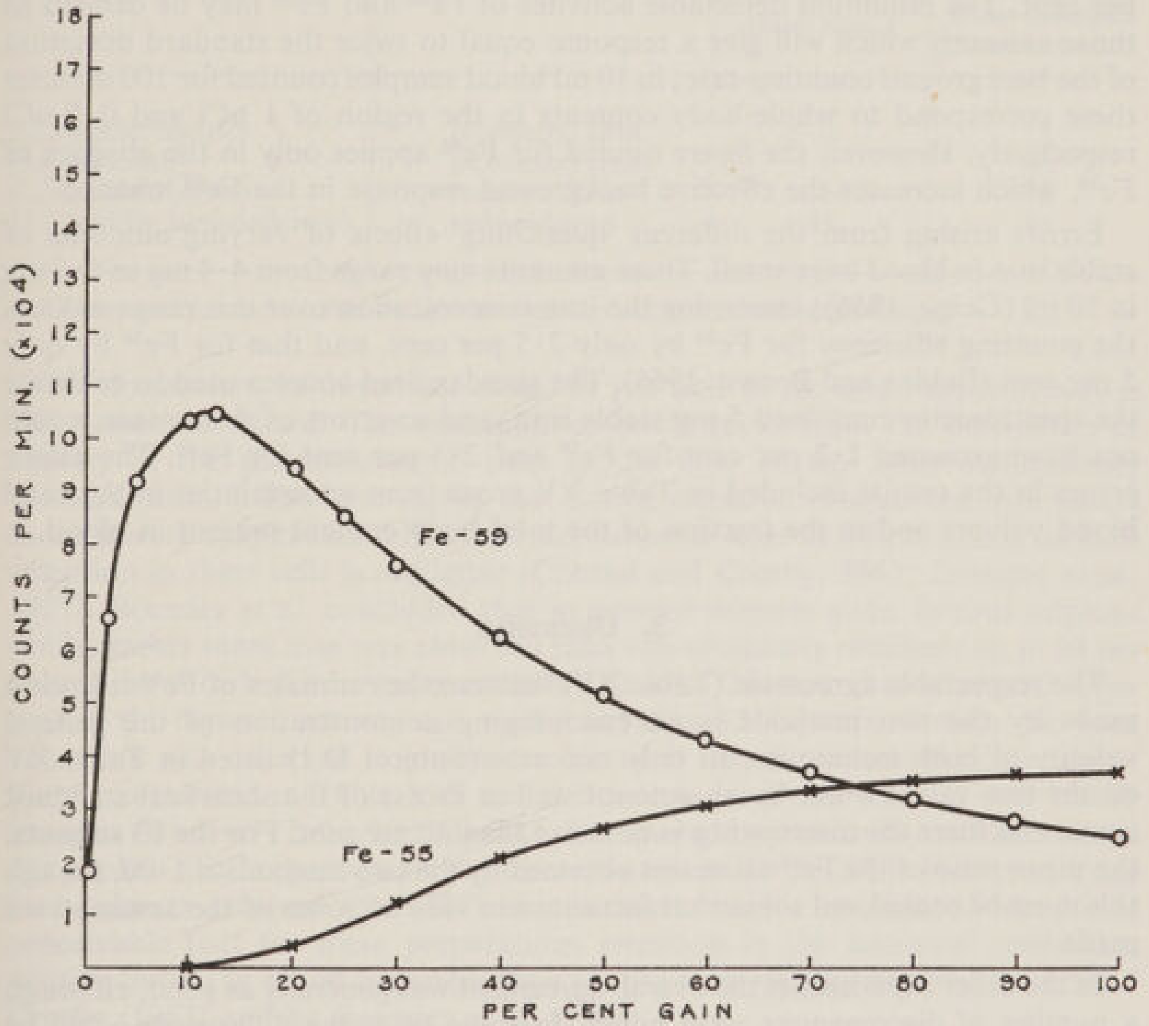

FIG. 2

Response of the liquid scintillation spectrometer to sources of Fe^{55} and Fe^{59}, as a function of amplifier gain

The results for the subjects of the first experiment are included in Table XV (p. 43); the estimates of whole-body content have been calculated with the assumption that the blood volume in litres is 7·42 per cent of the body weight expressed in kilograms, and that the blood contains 80 per cent of the body content of iron. In Table XV only the 'statistical' uncertainties, arising from the random occurrence of radioactive disintegrations, have been included. For Fe^{55} these were in every case quite negligible, even though the retention ranged from only 0·2 per cent to 2 per cent; for Fe^{59} these errors were considerable only when values below 5 nCi were encountered, but even amounts of 2 nCi (0·2 per cent retention) were estimated with a statistical standard uncertainty of less than 50 per cent. The minimum detectable activites of Fe^{55} and Fe^{59} may be defined as those amounts which will give a response equal to twice the standard deviation of the background counting-rate; in 10 ml blood samples counted for 100 minutes these correspond to whole-body contents in the region of 1 nCi and 0·5 nCi respectively. However, the figure quoted for Fe^{55} applies only in the absence of Fe^{59}, which increases the effective background response in the Fe^{55} 'channel'.

Errors arising from the different 'quenching' effects of varying amounts of stable iron in blood were small. These amounts may range from 4·4 mg to 5·6 mg in 10 ml (Geigy, 1956); increasing the iron concentration over this range reduces the counting efficiency for Fe^{59} by only 2·5 per cent, and that for Fe^{55} by only 5 per cent (Eakins and Brown, 1966). The standardized sources used to calibrate the spectrometer contained 5 mg stable iron, and so errors of this nature would not have exceeded 1·2 per cent for Fe^{59} and 2·5 per cent for Fe^{55}. The major errors in the results included in Table XV arose from uncertainties in the total blood volume and in the fraction of the total body content present in blood.

3. Discussion

The respectable agreement (Table XV) between the estimates of Fe^{59} retention made by the two methods is an encouraging demonstration of the general validity of both techniques. In only one case (Subject D.1) listed in Table XV do the two values differ by an amount well in excess of the statistical standard error, and there the discrepancy is no more than 30 per cent. For the 13 subjects, the mean ratio of the Fe^{59} estimates obtained by the two methods is 1·00, though this must be considered somewhat fortuitous in view of some of the assumptions made.

In the other experiments the overall agreement was generally as good, although a number of discrepancies were noted. In some cases the differences could be related to peculiar physical characteristics in the subject. Thus, in obese subjects, the effects of self-absorption of the Fe^{59} gamma-rays would be larger than in the phantom used for calibration, and so body radioactivity measurements would underestimate the retention; on the other hand, their assumed blood volume, based on body weight, might well be overestimated, leading to an error in the opposite direction for Fe^{59} retention based on activity in blood.

However, in a few instances large discrepancies were encountered which could not be explained on these grounds. In Table XVI we have listed details of the four cases in which the disagreement was outstanding: in each of these body radioactivity measurements gave results at least twice as large as the values obtained from measurements of blood. While it is conceivable that in each case errors arose because of instrumental defects or faulty procedures, this seems unlikely since subjects in three different experiments, each conducted on separate occasions months apart, were involved, and the results for their fellow subjects were acceptable.

Table XVI. Absorption of Fe^{59}: inconsistent results obtained from body radioactivity measurements and measurements of activity in blood.

Subject	Fe^{59} administered as:	Body content (nCi)*	
		from blood	from external measurements
J.3 (achlorhydric)	powdered iron	< 2	8
G.4 (normal)	powdered iron	3	40
C.5 (achlorhydric)	reduced iron	36	72
U.5 (mildly iron-deficient)	reduced iron	45	95

* Corrected for radioactive decay between administration and measurement.

We doubt that, in these four cases, 80 per cent of the absorbed iron was in fact present in blood. The discrepancies could result from the persistence of activity in the gastro-intestinal tract at the time of the body radioactivity measurements. It has been shown that partial retention of absorbed iron occurs in the intestinal epithelial cells of normal subjects, while in iron-deficient subjects retention in these cells is negligible (Conrad and Crosby, 1963; Boender *et al.*, 1967). Boender *et al.* concluded that in normal subjects given ferrous sulphate orally, rather more iron was absorbed than was ultimately retained; up to 64 per cent of the absorbed iron was excreted within 10–15 days, and as much as 2·6 per cent of the ingested activity was voided in faeces 12 days after intake. No such behaviour was observed in anaemic subjects. It may be noted that only one of the subjects listed in Table XVI was deficient in iron, and she was only mildly anaemic (Hb level 12·0 g/100 ml). The data quoted by these other workers would not explain the magnitude of the discrepancies in Table XVI. However, these four subjects received their Fe^{59} as powdered iron or as reduced iron; it is conceivable that for these preparations retention in the intestinal epithelium relative to that in blood is greater than for the compounds used by Conrad and Crosby (1963) and by Boender *et al.* (1967).

Although in these experiments the uptakes of both nuclides were frequently below 1 per cent, the sensitivity of the methods enabled satisfactory results to be obtained with the administration of under 5 μCi Fe^{59} and 25 μCi Fe^{55}. The radiation doses resulting from these amounts were within acceptable limits. The largest dose (up to 0·33 rem) was in most cases due to irradiation of the lower large intestine by unabsorbed activity. According to the International Commission on Radiological Protection (1959), the principal dose from absorbed Fe^{55} and Fe^{59} would be received by the spleen; in one subject who showed unexpectedly high uptakes of both nuclides, this amounted to 0·54 rem, though in most cases it did not exceed 0·3 rem. For occupationally exposed workers the recommended maximum permissible dose to these organs is 15 rem in any period of one year (International Commission on Radiological Protection, 1966).

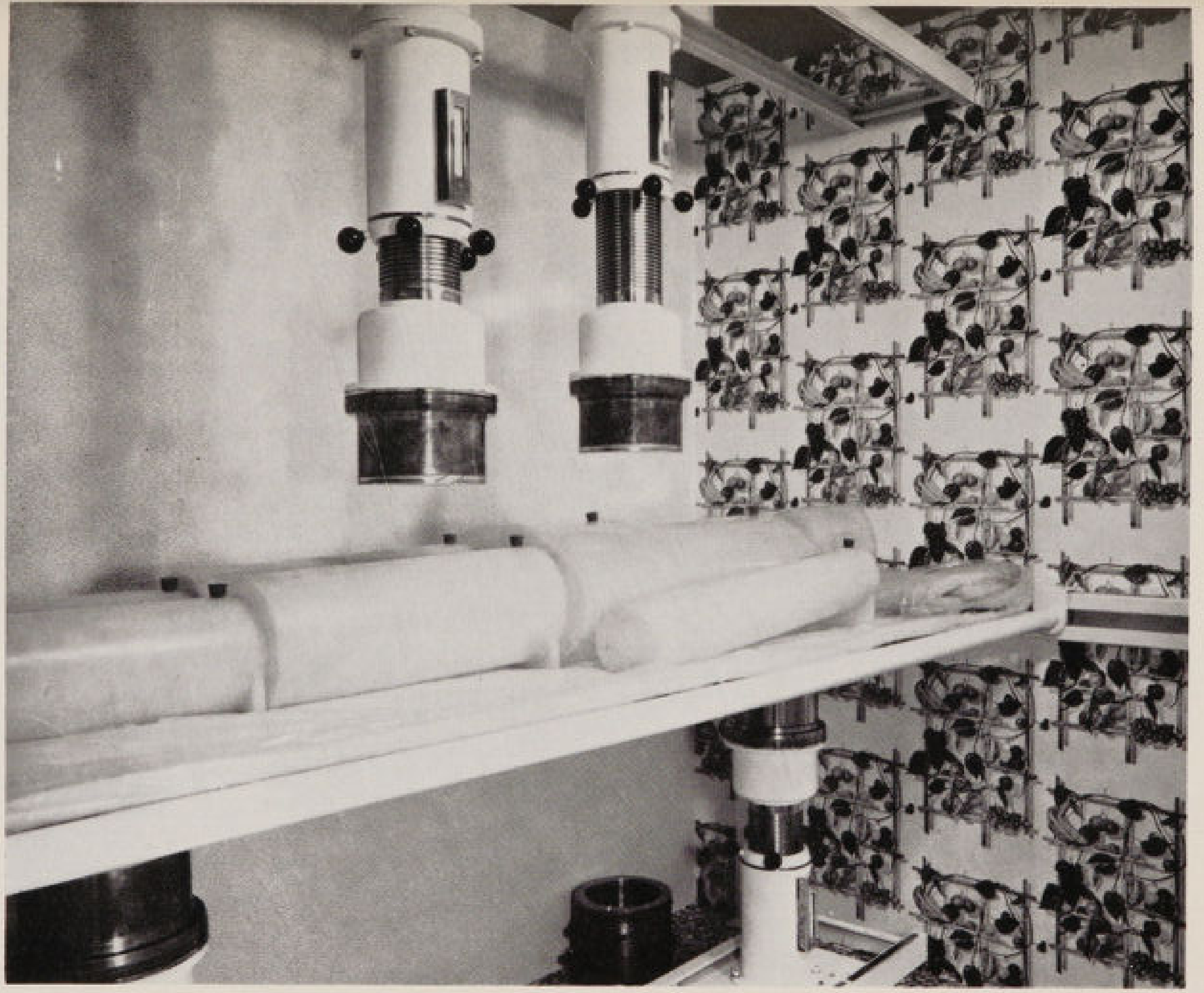

PLATE 1

The equipment used for body radioactivity measurements at A.E.R.E., Harwell. A 'phantom' similar to that shown was used for calibration measurements

PLATE 2

The liquid scintillation spectrometer used for the measurements of Fe^{55} and Fe^{59} in blood

BIBLIOGRAPHY

Antila, V., 1962. *Acta med. scand., Suppl.*, **393**, 1.

Bergeim, O. and Kirch, E. Z., 1949. *J. biol. Chem.*, **177**, 591.
Blumberg, H. and Arnold, A., 1947. *Cereal Chem.*, **24**, 303.
Boender, C. A., *et al.*, 1967. *Nature*, **213**, 1236.
Brise, H., 1962. *Acta med. scand. Suppl.*, **376**, 39.
Brise, H. and Hallberg, L., 1962. *Acta med. scand. Suppl.*, **376**, 7.
Burch, G. E. and De Pasquale, N. P., 1962. *Am. J. Med.*, **32**, 161.
Bush, F., 1949. *Br. J. Radiol.*, **22**, 96.

Chodos, R. B., *et al.*, 1957. *J. clin. Invest.*, **36**, 314.
Choudhury, M. R. and Williams, J., 1959. *Clin. Sci.*, **18**, 527.
Conrad, R. E. and Crosby, W. H., 1963. *Blood*, **22**, 406.
Cook, J. D., Brown, G. M. and Vallberg, L. S., 1964. *J. clin. Invest.*, **43**, 1185.

Dawber, T. R. and Kannel, W. B., 1961. *Mod. Concepts cardiovasc. Dis.*, **30**, 671.
De Leeuw, N. K. M., Lowenstein, L. and Hsieh, Y.-S., 1966. *Medicine, Baltimore*, **45**, 291.
Dern, J. R. and Hart, W. L., 1961. *J. Lab. clin. Med.*, **57**, 322.

Eakins, J. D. and Brown, D. A., 1965. *The simultaneous determination of iron-55 and iron-59 in blood by liquid scintillation counting.* (United Kingdom Atomic Energy Authority, Research Group Report, AERE–R.4945.)
Eakins, J. D. and Brown, D. A., 1966. *Int. J. appl. Radiat. Isotopes*, **17**, 391.
Eisenberg, S., 1966. *Circulation*, 33, *Suppl.* 2, 10.
Elwood, P. C., 1963. *Br. med. J.*, i, 224.
Elwood, P. C., 1964. *Br. J. prev. soc. Med.*, **18**, 81.
Elwood, P. C., 1965. *Proc. Nutn. Soc.*, **24**, 112.
Elwood, P. C., 1966. *Nutritio Dieta*, **8**, 210.
Elwood, P. C., *et al.*, 1967. *Br. med. J.*, iv, 714.
Elwood, P. C., Withey, J. L. and Kilpatrick, G. S., 1964. *Br. J. prev. soc. Med.*, **18**, 125.
Elwood, P. C. and Wood, M. M., 1966. *Br. J. prev. soc. Med.*, **20**, 172.

Freeman, S. and Burrill, M. W., 1945. *J. Nutn.*, **30**, 293.

Garby, L., Irnell, L. and Werner, I., 1967. *Acta Soc. Med. upsal.*, **72**, 91.
Garry, R. C., *et al.*, 1954. *Br. J. Nutn.*, **8**, 253.
Geigy, 1956. *Documenta Geigy: scientific tables. 5th edition.* Basle, Geigy, p. 330.
Goldberg, A., Lockhead, A. C. and Dagg, J. H., 1963. *Lancet*, **i**, 848.

Hallberg, L. and Brise, H., 1960. *Int. J. appl. Radiat. Isotopes*, **9**, 100.
Harril, I. K., Hoene, A. E. and Johnston, F. A., 1957. *J. Am. diet. Ass.*, **33**, 1010.
Hinton, J. J. C., Carter, J. E. and Moran, T., 1967. *J. Fd. Technol.*, **2**, 129.
Hinton, J. J. C. and Moran, T., 1967. *J. Fd. Technol.*, **2**, 135.
Hussain, R., *et al.*, 1965. *Am. J. clin. Nutn.*, **16**, 464.

International Commission on Radiological Protection, 1966. *Radiation protection: recommendations . . . adopted September 17, 1965.* (Publication 9.) Oxford, etc., Pergamon Press.
International Commission on Radiological Protection, 1959. *Report on permissible dose for internal radiation.* London, etc., Pergamon Press.

Kilpatrick, G. S., 1961. *Br. med. J.*, ii, 1736.
Kilpatrick, G. S. and Hardisty, R. M., 1961. *Br. med. J.*, i, 778.
Kirch, E. R., *et al.*, 1947. *J. biol. Chem.*, **171**, 687.

Mackay, H. M. M., Dobbs, R. N. and Bingham, K., 1945. *Archs Dis. Childh.*, **20**, 56.
Martin, H. F. and Halton, P., 1964. *J. Sci. Fd. Agric.*, **15**, 464.
Ministry of Agriculture, Fisheries and Food, 1960. *Report on bread and flour.* London, H.M.S.O.
Ministry of Agriculture, Fisheries and Food, 1965. *Domestic food consumption and expenditure: 1963. Annual report of the National Food Survey Committee.* London, H.M.S.O., p. 103.

Moore, C. V., 1955. *Am. J. clin. Nutn.* **3,** 3.
Moore, C. V., 1961. *Harvey Lect.*, **55,** 67.
Moore, C. V. and Dubach, R., 1951. *Trans. Ass. Am. Physns.*, **64,** 245.
Moore, C. V., Minnich, V. and Dubach, R., 1943. *J. Am. diet. Ass.*, **19,** 841.
Murphy, E. A. and Abbey, H., 1967. *J. chron. Dis.*, **20,** 79.

Nakamura, F. I. and Mitchell, H. H., 1943. *J. Nutn.*, **25,** 39.
Natvig, H., Bjerkedal, T. and Jonassen, O., 1963. *Acta med. scand.*, **174,** 341.
Natvig, H., Bjerkedal, T. and Jonassen, O., 1966. *Acta med. scand.*, **180,** 605.

Paintin, D. B., Thomson, A. M. and Hytten, F. E., 1966. *J. Obstet. Gynaec. Br. Commonw.*, **58,** 173.
Parsons, P. L., Withey, J. L. and Kilpatrick, G. S., 1965. *Practitioner*, **195,** 656.
Peacock, W. C., *et al.*, 1946. *J. clin. Invest.*, **25,** 605.
Perry, S. W. and Warner, G. T., 1963. *Int. J. appl. Radiat. Isotopes*, **14,** 397.
Pirzio-Biroli, G., Bothwell, T. H. and Finch, C. A., 1958. *J. Lab. clin. Med.*, **51,** 37.

Rundo, J., 1958. *Proc. 2nd Int. Conf. peaceful Uses atom. Energy*, **23,** 101.
Rundo, J., 1962. In: *Whole-body counting.* Vienna, International Atomic Energy Agency p. 121.
Rundo, J. and Taylor, B. T., 1964. In: *Assessment of radioactivity in man.* Vienna, International Atomic Energy Agency, Vol. 2, p. 3.

Salmon, L., 1965. In: *Radiochemical methods of analysis.* Vienna, International Atomic Energy Agency, Vol. 2, p. 125.
Scott, E. M. and Heller, C. A., 1964. *Am. J. clin. Nutn.*, **15,** 282.
Sharpe, L. M., *et al.*, 1950. *J. Nutn.*, **41,** 433.
Sherman, H. C., 1952. *Chemistry of food and nutrition.* 8th edition. New York, MacMillan & Co.
Snedecor, G. W., 1956. *Statistical methods applied to experiments in agriculture and biology. 5th edit.* Ames, Iowa State College Press.
Sorensen, E. W., 1964. *Acta med. scand.*, **175,** 763.
Statutory Instruments, 1963. *The Bread and Flour Regulations, 1963.* (S.I. 1963, No. 1435), H.M.S.O.
Steinkamp, R., Dubach, R. and Moore, C. V., 1955. *Archs intern. Med.*, **95,** 181.
Stott, G., 1960. *Bull. Wld. Hlth. Org.*, **23,** 781.
Street, H. R., 1943. *J. Nutn.*, **26,** 187.

Thomson, A. M. and Hytten, F., 1967. *Personal communication.*

Veall, M. and Vetter, H., 1952. *Br. J. Radiol.*, **25,** 85.

Widdowson, E. M. and McCance, R. A., 1942. *Lancet*, i, 588.
Widdowson, E. M. and McCance, R. A., 1954. *Spec. Rep. Ser. med. Res. Coun.* (Lond.), No. 287.
Wilder, R. M., 1956. *J. Am. med. Ass.*, 162, 1539.
Wilder, R. M. and Williams, R. R., 1944. *Bull. natn. Res. Coun.*, Wash., **110,** 38.
Wood, M. M. and Elwood, P. C., 1966. *Br. J. prev. soc. Med.*, **20,** 117.
World Health Organization, 1959. *Tech. Rep. Ser. Wld. Hlth. Org.*, No. 182.

The addition of iron to flour

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The addition of iron to flour

I. The solubility and some related properties of iron powders including reduced iron

by J. J. C. Hinton*, J. E. Carter† and T. Moran‡

Introduction

Since 1953 there has been a statutory minimum iron content for flour of 1·65 mg/100 g. White flour of about 70–72 per cent extraction, which has a natural iron content of 1–1·2 mg/100 g must, therefore, be enriched in order to reach the statutory minimum. The most recent Order—*The Bread and Flour Regulations* (Statutory Instruments, 1963)—lays down that the iron addition shall be in the form of ferric ammonium citrate (current B.P. or B.P. Codex) or reduced iron made by the action of hydrogen on ferric oxide. Another form of iron powder is that produced by the electrolysis of an iron salt. Both forms have been included in the present experiments since when they dissolve in dilute hydrochloric acid or gastric juice they both produce the same primary material in the absorption process, *viz*. ferrous chloride.

At present much of the flour in this country is enriched with reduced iron. Recently, however, Elwood (1963, 1965) from studies on a group of adult patients in a mental hospital, has raised doubts about the value of the present level of enrichment with this form of iron. Following on this publication we have studied some of the physical properties of different commercial samples of reduced iron, particularly their relative solubilities in gastric or simulated gastric juice. Our results are described in this paper.

Reduced iron

The literature on the chemistry and technology of the production of reduced iron is scanty. It can be obtained as a black powder by reducing one of the oxides (magnetite Fe_3O_4 or haematite Fe_2O_3) in a current of dry hydrogen. The temperature of reduction, according to Mellor (1932), may be from about 350° to 1150°C the lower the temperature the longer the time for the reduction. Below about 600°C the resulting finely divided powder is pyrophoric and when exposed to the air will oxidize extremely rapidly, and may take fire. At high temperatures the particles of iron are sintered, i.e. they agglomerate to form larger particles. It would be expected, therefore, that the particle size, the porosity of the particles, the surface area per gram and in turn the solubility of a sample of iron powder all depend on the conditions and particularly in the case of reduced iron the temperature of manufacture. *The Bread and Flour Regulations* (1963) do not suggest any limiting temperature but the B.P. Codex 1934 mentions that overheating produces a coarse preparation and suggests heating to 'dull' redness.

Leadbeater, Northcott and Hargreaves (1947) carried out an extensive examination of 12 reduced iron powders and 13 powders prepared electrolytically. Certain of their results are shown in Table 1.

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Table 1. Type of powder

Property	Reduced iron		Electrolytic	
	Range	Average	Range	Average
Iron content (%) . .	96·7 –98·7	98·0	98·5 – 99·6	99·2
Median size (μ) . . .	6 –95	64	47 –114	66
Particle porosity (%) .	0·19 – 3·31	1·97	0·78 – 3·81	2·13
Specific surface (m^2/g) .	0·448– 5·16	1·036	0·265– 1·149	0·534

These authors did not measure solubility, but their results demonstrate the variable nature of commercial samples of iron powder in either category.

Experimental

We have examined two samples of reduced iron manufactured in the U.S.A. (A.1 and A.2)*, five samples of reduced iron made in this country (B.1–B.5), four samples of electrolytic iron also made in this country (C.1–C.4) and one of reduced iron (L) specially prepared in the laboratory at a low temperature and supplied to us by Dr. Elwood.

Determination and recovery of iron

Throughout, iron was determined as the *o*-phenanthroline complex after solution in 1:1 concentrated HCl or evaporation of solutions, ashing and solution of the ash in 1:1 HCl (Pringle, 1946).

The recovery of metallic iron. In some of the experiments iron metal was extracted magnetically from suspensions or digestions. A U-shaped magnet 5 × 3·5 × 3·5 cm was wrapped in polythene film and passed, systematically and repeatedly, through the liquid in a shallow dish. After rinsing, the magnet was transferred to a dish of water or with flour suspensions, petrol ether, removed from the polythene wrapper and the metal brushed from the latter into the liquid. Preliminary experiments showed that the efficiency of recovery of weighed amounts of iron powder added to homogenates and suspensions was 90 per cent approximately. Iron A.1 because of its extreme fineness was exceptional in that the recovery was only 50 per cent. These factors were applied to the results.

Recovery of iron from bread and flour. The crumb was broken down either: (i) in water in a laboratory homogenizer for 15 min, or (ii) by digesting a 5-g crumb with 100 mg papain and 100 mg fungal amylase in 100 ml water (pH about 5) for 18 hr at 37°C followed by homogenizing for a short period. Flour was suspended in CCl_4.

Determination of solubility

(a) *In vitro.* The determination of solubility was very sensitive to conditions of temperature, agitation, type of vessel, etc.

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*A.1 was sent over by one of us (T.M.) from the U.S.A. in 1944. Some was used for the enrichment of the flour used in the Widdowson–McCance investigation (Widdowson and McCance, 1954) and the remainder kept in a stoppered bottle. Sample A.2 from the same firm, but now made for them by an outside company, was sent over in 1964.

All the samples contained at least 95 per cent iron and were almost completely soluble in 0·1 N HCl given sufficient time, e.g. in some cases 24 hr.

HCl alone. Quickfit stoppered tubes MF/24/3/8 of 85 ml capacity containing 4 mg iron powder and 50 ml 0·1 N HCl were rotated end over end at 17 rev/min in a constant temperature room at 23°C for times varying from 30 min to 2 hr.

HCl in the presence of pepsin. Two milligrams iron powder, 100 mg of pepsin and 25 ml 0·1 N HCl were maintained at 37°C for 2 hr. The conditions were chosen to resemble more closely normal gastric digestion. The reaction was carried out in conical flasks which were gently swirled at 30-min intervals. Pepsin B.P. containing lactose as diluent was used in these experiments. Both this product and pure crystalline pepsin depressed the solubility of iron powder in 0·1 N HCl.

(b) *In vivo.* Bread was baked from flour containing 21 ppm of the different samples of iron powder and fed to rats as described in the following paper. Food intake was measured daily and the metallic iron excreted was also determined daily by recovery from the faeces (homogenized with water) from all the rats on a particular bread. The experimental period was from 1 to 2 weeks, six rats being included for each experiment. At the end of the experimental period the rats were given non-enriched bread but the faeces were analysed for excreted metallic iron for a further two days.

Discussion

The results of the solubility tests are given in Table 2.

The relative solubilities by all three methods are in good agreement and the fact that the figures for simulated gastric juice are in accord with those from the digestion of the iron enriched breads by the rat suggests that the bread as such does not interfere with the solution and absorption of its added iron.

Table 2. Solubility of iron powders under different conditions

Sample	0·1 N HCl 30 min at 23°C (%)	0·1 N HCl, pepsin 0·4% 2 hr at 37°C (%)	*In vivo* in the rat (%)
A.1	93	82	90 81
A.2	37	28	39
B.1	30	20	19 15
B.2	—	23	—
B.3	39	27	36
B.4	34	27	27
B.5	30	—	—
C.1	53	69	58 42
C.2	44	53	—
C.3	42	38	—
C.4	52	58	60
L	100	99	100

The A, B and L samples of reduced iron vary widely in solubility. There is also a marked difference between the two samples A.1 and A.2 but we have been unable to obtain details of the manufacture of either sample. The C samples of electrolytic iron powder, generally have a higher solubility than the B samples. Sample L, completely soluble in gastric juice, was freshly prepared at a low temperature in the region of 500°C. As would be expected, however, it oxidized rapidly and after a few weeks (in an envelope) at ordinary temperature its solubility in pepsin–HCl had fallen from 99 to 53 per cent. Incidentally, the solubility of a sample of Fe_2O_3 was found to be only 5 per cent.

Solubility would be expected to go hand in hand with susceptibility to oxidation. Thus samples of A.1 and B.4 were stored at room temperature in a water saturated atmosphere and the figures in Table 3 for solubility obtained.

These of course were exaggerated conditions, never likely to be encountered in practice.

On the other hand a low solubility of a particular sample of iron powder is not necessarily due to surface oxidation. Thus sample B.1 was heated in a current of hydrogen for 30 min at 600°C. It was then cooled in hydrogen and its solubility determined immediately in pepsin–HCl at 37°C. It was found to be 18 per cent compared with the original figure of 20 per cent.

Table 3. Solubility in 0·1 N *HCl, pepsin 0·4 per cent, 2 hr at 37°C*

	A.1 (%)	B.4 (%)
Initially	82	27
After 45 hr storage	61	23
After 140 hr storage	53	25

Particle shape and size

A marked difference in particle shape was observed between the iron powders prepared electrolytically and by reduction. The former were mainly prismatic in shape with sharp angular outlines typical of a shattered crystalline mass. The latter were in general spherical with smooth rounded outlines, samples B.1–B.5 and A.2 being typical sintered aggregates, much larger in size than samples A.1 and L of greater solubility.

As would be expected, other things being equal, a rough relationship does exist between size and solubility. This was shown on a coarse and fine fraction from sample C.1:

	Solubility (percentage)	
	Coarse	Fine
0·1 N HCl, 0·4 per cent pepsin, 2 hr at 37°C	57	72

Nevertheless, an analysis of particle size distribution, using the Sartorius sedimentation balance, did not show any clear cut relationship between size distribution and solubility for powders prepared in different ways. Surface area per gram of powder was, however, more revealing. It ranged from 102 m^2/g for the very soluble powder L to 0·1 m^2/g for sample B.1. The figure for A.1 was 4·8. This work on particle size and surface area was carried out by Dr. J. Butcher and will be published separately.

Changes in solubility of added iron during the storage of flour

Direct experiment showed that more than 90 per cent of the iron powder added to flour could be recovered unchanged from the resulting bread. Table 2 also confirms this finding.

In the United Kingdom white flour is enriched by the addition of a 'master mix' to the flour stream at the rate of 1 oz per sack of 280 lb of flour. The master mix has the following composition:—

	Percentage by weight
Vitamin B_1	0·74
Nicotinic acid (or amide)	3·53
Reduced iron	3·14
Flour diluent	92·59

It was found that the solubility of the iron (B.4) extracted from a sample of master mix freshly made and after 15 months storage was the same.

On the other hand, in the case of flour enriched with the highly soluble iron powder (A.1) the solubility in pepsin–HCl of the powder, extracted after storage for 14 weeks had fallen by about 10 per cent.

All these results fit into a logical picture. From the point of view of the enrichment of flour with reduced iron it is clear that the desirable standard for this form of iron should be one combining the maximum solubility or ease of solution consistent with the minimum tendency to become oxidized during its storage either in air or in flour. The temperature to which the iron oxide is submitted either before or during the reduction process is probably the critical factor.

References

ELWOOD, P. C., 1963. *Br. med. J.* **i**, 224.

ELWOOD, P. C., 1965. *Proc. Nutr. Soc.*, **24**, 112.

LEADBEATER, C. J., NORTHCOTT, L. and HARGREAVES, F., 1947. *Some Properties of Engineering Iron Powders.* Iron and Steel Institute, London, Special Report No. 38.

MELLOR, J. W., 1932. *A Comprehensive Treatise on Inorganic and Theoretical Chemistry*, Vol. 3, p. 805. Longmans Green, London.

PRINGLE, W. J. S., 1946. *Analyst, Lond.*, **71**, 490.

STATUTORY INSTRUMENTS, 1963. No. 1435. *The Bread and Flour Regulations.* H.M.S.O., London.

WIDDOWSON, E. M. and MCCANCE, R. A., 1954. *M.R.C. Special Report No.* 287. H.M.S.O., London.

The addition of iron to flour

II. The absorption of reduced iron and some other forms of iron by the growing rat

by J. J. C. Hinton* and T. Moran†

Introduction

The previous paper showed that reduced iron is not a standard product in respect of the ease with which it dissolves in dilute hydrochloric acid, in simulated gastric juice or in the stomach of the rat. This work has been extended to measurements of the actual total absorption of iron by the rat, from different samples of reduced and electrolytic iron powders, and also from other forms of iron that are used or have been suggested for the enrichment of flour.

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Experimental

The different forms of iron were incorporated into a bakers grade flour containing no chalk, vitamin or other additions, and this flour was baked into bread. A small and variable amount of metallic iron (ranging in seven samples from 0·05 to 0·43 mg/100 g) was present as a contaminant from the steel rolls in the mill.

Normal iron enrichment of flour in this country is 7 ppm, but generally we have used 21 ppm in an attempt to reduce the experimental errors which beset this type of work. Furthermore, it is a reasonable level since to reach the iron content of whole wheat would require about 30 ppm. Also in the U.S.A. the level in practice ranges from 19 ppm (minimum) to 27 ppm (maximum).

Care of the animals

Female rats, which in each experiment were matched from the same litters, were housed, three to a cage, in specially made aluminium cages. Six rats (twelve in Experiment 3) were used for each diet and in every case a 'control' diet was included in which the rats were fed non-enriched bread.

The rats (Norwegian strain) were transferred at weaning (3–4 weeks old) to a bread diet made with flour containing no added iron (Experiments 1–9) until the start of the experiment.

In the final experiment (Experiment 10) the rats were made iron-deficient by transferring them at weaning to a milk diet for 3 weeks. They were then fed the experimental bread diets. The liquid milk diet of Elvehjem & Kemmerer (1931) was inadequate to maintain health, excessive diarrhoea and dropsical swellings of the feet occurring after 4 or 5 days. The milk diet of McCall *et al.* (1962), however, slightly simplified as shown below, and fed *ad libitum*, supported growth and health during the 3-week experimental period.

Diets

The bread was air dried to about 10 per cent moisture and coarsely ground.

	Bread diet	Milk diet
Dried crumb	87·5%	—
Arachis oil	5·0%	—
Salt mixture (Jones & Foster) (omitting iron)	2·0%	—
Liver powder	0·4%	—
Choline chloride	0·2%	—
L-Lysine hydrochloride	0·3%	—
Vitamin B_1	0·5 mg/100 g	1·5 mg
Vitamin B_6	0·5 mg/100 g	1·5 mg
Nicotinic acid	0·5 mg/100 g	1·5 mg
Riboflavin	1·0 mg/100 g	3·0 mg
Calcium pantothenate	2·5 mg/100 g	7·5 mg
p-Aminobenzoic acid	15·0 mg/100 g	45·0 mg
Folic acid	—	1·5 mg
Sucrose as diluent for the vitamins to 100%		
Dried skim milk	—	50·0 mg
Liquid milk	—	1000ml

The normal supplements of vitamins A, D and E were given orally at weekly intervals.

Intake of food, fed *ad libitum*, was recorded daily and the faeces were collected at the same time. Distilled water was provided; urine was not collected, the amount of iron excreted in this form being negligible compared with that in the faeces.

The experimental diet was fed for approximately one week followed by a period on non-enriched diet. The iron excreted, recorded in Tables 1 and 3, included a period of 2–4 days after the termination of the experimental diet by which time it had fallen to a relatively constant value.

Determination of iron

Iron was determined as the *o*-phenanthroline complex as described in the previous paper.

Diets and other dry samples were ashed directly.

Metallic iron was first extracted from the faeces* (the total for any one group) by the magnetic method described in the previous paper, the faeces being soaked in water for 2 or 3 hr and suspended in water using a laboratory homogenizer. In the next step the suspension was again agitated and any ingested aluminium allowed to settle for a few moments; an aliquot of the suspension was then withdrawn for the determination of non-metallic iron. The aluminium interfered because of its small iron content (approximately 0·5 per cent) and its tendency to precipitate phosphate during colour formation.

Determination of haemoglobin

In 0·02 ml blood taken from the tail as cyanmethaemoglobin using the International Standard procedure (C. Davis Keeler Ltd, 47 Wigmore Street, London, W.1).

Results

The forms of iron enrichment included six of the samples of iron powder dealt with in the previous paper, ferrous sulphate, sodium iron pyrophosphate and ferric ammonium citrate (green). As a matter of interest wholemeal flour containing no added iron was included, but in one experiment only.

The green citrate was used in preference to the brown because experiments have shown that it is not so conducive to the production of rancidity in flour stored for long periods (Martin and Halton, 1964).

Normal rats

In nine experiments the results of which are given in Tables 1 and 2, the animals commenced with iron status only a little below normal (blood haemoglobin averaged 12 g/100 ml) so that their requirements would, therefore, depend mainly upon growth. Table 1 gives the intake and excretion of total dietary iron and in Table 2 the iron retained is expressed as per cent of the total iron ingested. Fig. 1 shows the course of a typical experiment.

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* To provide some of the figures given in Table 2 in the preceding paper.

Anaemic rats

In a final experiment (Experiment 10) two samples of reduced iron, one of high (L) and the other of low solubility (B.2), ferrous sulphate and ferric ammonium citrate were compared in partially anaemic animals. At the start of the experiment the mean haemoglobin content was 8·7 g/100 ml blood with no significant differences between the means of the rats on the different diets. Table 3 gives the figures for iron intake and retention.

Table 1. Retention of iron by normal rats from enriched bread

Experiment. Enriched with	Period on experimental diet (days)	Average age of rats (weeks)	Per rat per day			
			Total food intake (g)	Total iron intake (mg)	Total iron excreted (mg)	Total iron retained (mg)
1 (a) NE	9	9	11·7	0·152	0·112	0·040
(b) 7 ppm sample B.4 . .	9	9	11·4	0·205	0·136	0·069
(c) 21 ppm sample B.4 . .	9	9	13·5	0·304	0·236	0·068
2 (a) NE	11	15	12·7	0·157	0·117	0·040
(b) 70 ppm sample B.4 . .	11	15	12·9	0·609	0·564	0·045
3 (a) NE	8	10	14·4	0·214	0·188	0·026
(b) 30 ppm sample A.1 . .	8	10	13·1	0·471	0·400	0·071
(c) Wholemeal . . .	8	10	12·6	0·432	0·355	0·077
4 (a) NE	9	8	11·7	0·155	0·092	0·063
(b) 21 ppm sample A.1 . .	9	8	12·6	0·353	0·242	0·111
(c) Ferric ammonium citrate	9	8	13·4	0·398	0·182	0·216
5 (a) NE	10	5	11·1	0·153	0·098	0·055
(b) 21 ppm sample B.1 . .	10	5	11·5	0·291	0·232	0·059
(c) 21 ppm sample C.1 . .	10	5	11·7	0·297	0·232	0·065
6 (a) 21 ppm sample B.1 . .	5	5	9·5	0·210	0·171	0·039
(b) 21 ppm sample C.1 . .	5	5	10·1	0·213	0·154	0·059
7 (a) NE	8	8	12·8	0·168	0·112	0·056
(b) Sodium iron pyrophosphate	8	8	13·0	0·372	0·262	0·110
8 (a) NE	7	4	8·2	0·116	0·088	0·028
(b) Ferric ammonium citrate	7	4	8·0	0·245	0·160	0·085
(c) Ferrous sulphate . .	7	4	8·2	0·287	0·214	0·073
9 (a) NE	9	5	9·5	0·134	0·091	0·043
(b) 21 ppm sample C.4 . .	9	5	9·7	0·282	0·209	0·073
(c) Ferric ammonium citrate	9	5	10·8	0·292	0·177	0·115
(d) $FeSO_4$	9	5	10·7	0·271	0·184	0·087

NE signifies 'no enrichment'. The sample numbers refer to iron powder samples numbered as in the previous paper. The three salts were added in a concentration to correspond to 21 ppm of iron.

Table 2. Iron retained as per cent total iron ingested

Experiment	NE	Sample B.1	Sample B.4	Sample C.1	Sample C.4	Sample A.1	Ferric ammonium citrate	$FeSO_4$	NaFe pyrophosphate	Wholemeal
1	26		34 (7) 12 (21)							
2	25		7 (70)							
3	12					15 (30)				18
4	40					32 (21)	54			
5	36	20		22						
6	—	19		28						
7	33								29	
8	24						35	25		
9	32				26		39	32		
Average .	28									

Figures in parentheses indicate degree of enrichment (ppm); in all the other cases, except for those marked NE, it is 21 ppm.

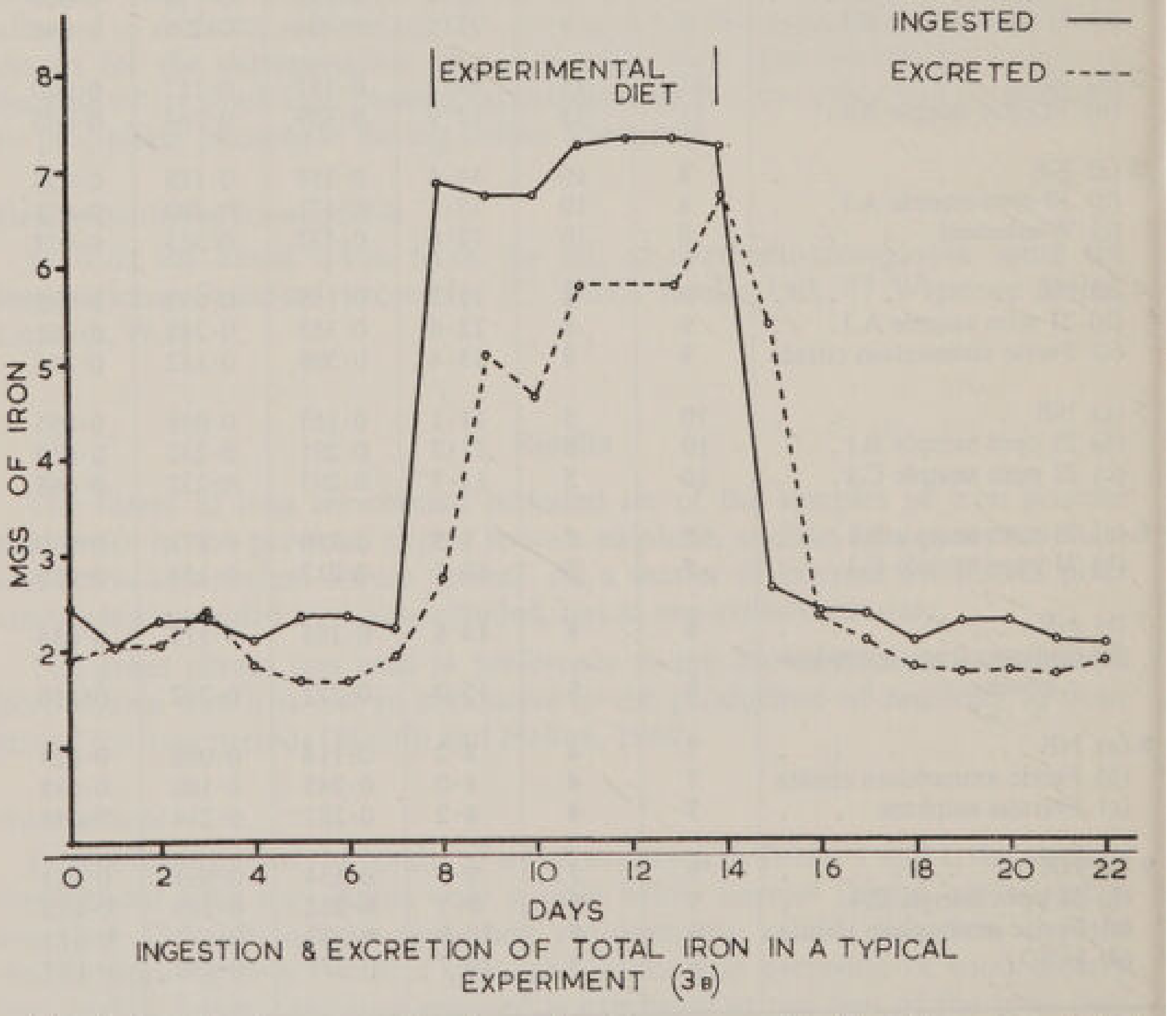

Fig. 1. Ingestion (————) and excretion (- - - - - - - - -) of total iron in a typical experiment (Experiment 3b).

Table 3. Retention of iron from enriched bread by anaemic rats

Enrichment (21 ppm)	Experiment No.	Period on experimental diet (days)	Average of rats (weeks)	Per rat per day					Iron retained as percentage of	
				Total food intake (g)	Total Fe intake (mg)	Total Fe excreted (mg)	Total Fe retained (mg)	Added Fe* retained (mg)	Total intake	Added iron
NE . .	10A	15	7	13·0	0·182	0·112	0·070	—	38	—
Sample B.2	10B	18	7	11·8	0·403	0·298	0·105	0·402	26	18
Sample L .	10C	13	7	11·9	0·375	0·244	0·131	0·067	35	32
Sulphate .	10D	18	7	12·1	0·385	0·235	0·150	0·085	39	39
Citrate .	10E	17	7	14·2	0·438	0·238	0·200	0·124	46	52

* Obtained from the difference between non-enriched and enriched diets after making allowance for the difference in food intake.

The haemoglobin content of the blood of each rat was also determined at intervals during the course of the experiment. Unfortunately our stock of iron powder L was small and was exhausted within 12 days. Table 4 gives the changes in haemoglobin content during the first 11 days whilst the individual changes in the four groups are shown in Fig. 2.

Table 4. Increase in blood haemoglobin values of anaemic rats

Diet	Haemglobin levels at 11 days (g/100 ml)
Non-enriched	9·6
Reduced iron B.2	9·4
Reduced iron L	12·5
Ferrous sulphate	13·35
Ferric ammonium citrate (green)	13·65

Discussion

In this country, on the average, the iron added to bread supplies about 10 per cent of out total intake. In the present experiments at the levels of enrichment corresponding to 70, 30, 21 and 7 ppm the percentages of the total intake due to added iron were approximately 80, 70 60 and 30 per cent, respectively. The results might, therefore, be expected to emphasize any marked differences in the availability of the different forms of added iron. Although it was not feasible to do the iron balance studies on each rat, the averages for each group, in comparable experiments, indicate that in general the different forms of iron when baked into bread were all well absorbed. The experiments, therefore, provide further evidence in favour of the enrichment of bread-making flour with iron.

Also another interesting result is the comparative constancy of the absorption of iron from non-enriched bread averaging 28 per cent. Moore *et al.* (1944) claim that there is a species difference in iron absorption. Even so it would be exceptional if the present experiments did not give pointers applicable to man.

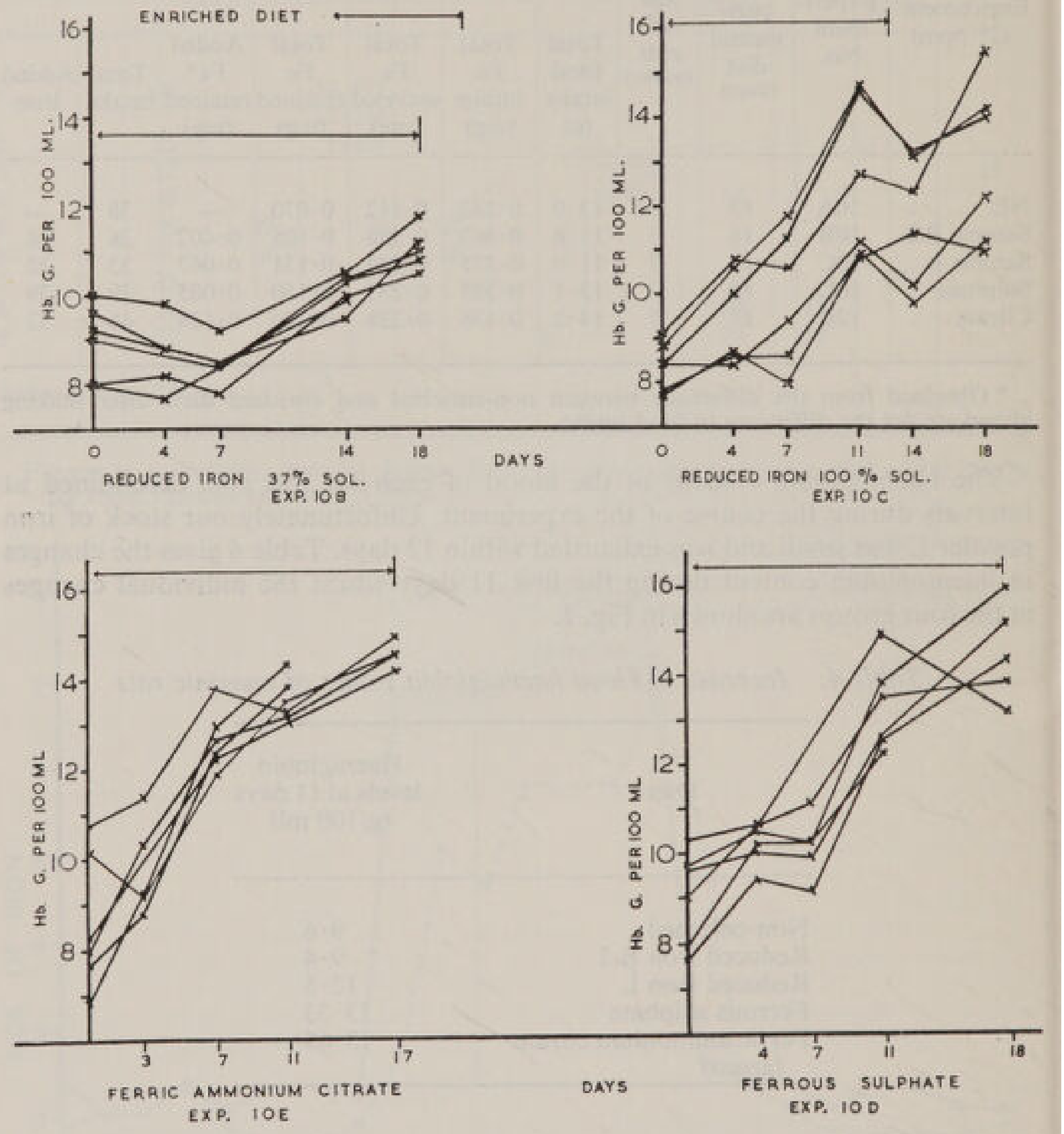

Fig. 2. Changes in haemoglobin content in four groups: (a) Reduced iron 37 per cent solubility, Experiment 10 (b); (b) reduced iron 100 per cent solubility, Experiment 10 (c); (c) ferric ammonium citrate, Experiment 10 (e); (d) ferrous sulphate, Experiment 10 (d). Arrow indicates period of enriched diet.

Statistical examination of the figures for the haemoglobin content in the individual animals at 11 days showed no significant difference between ferric ammonium citrate and ferrous sulphate. On the other hand, that between citrate and reduced iron L just reached the 5 per cent level of significance, whilst that between ferrous sulphate and L was less significant. The broad conclusion is that any differences between these three sources of iron are unimportant. By contrast the haemoglobin contents for citrate, sulphate and iron powder L are all significantly higher ($P < 5$ per cent) than those for iron powder B.2.

There are the usual apparent paradoxes found in most published papers on this problem. Thus: (i) In man it is generally accepted that ferrous salts are much more readily absorbed than ferric salts both by normal and by anaemic patients, cf Keele and Neil (1961). Yet for the rat, ferric ammonium citrate is as well absorbed as ferrous sulphate. Likewise, Steinkamp, Dubach and Moore (1955) found the same degree of absorption with single doses of radioactive reduced iron, ferrous sulphate, ferric orthophosphate and sodium iron pyrophosphate baked into bread and fed to 32 healthy young men and women. These results also seem to be incompatible with the practice of giving a supplement of ascorbic acid in iron therapy because of its reducing action. (ii) Despite its high content of phytates the absorption from wholemeal bread is sensibly the same as that from white bread enriched to wholemeal level with the relatively soluble reduced iron A.1. This however is in accord with the recent work of Cowan *et al.* (1966) showing that dietary phytate has no effect on iron absorption in the rat. (iii) The poor performance of iron powder B.2. Although the apparent drift downwards in the first seven days is within the experimental error the overall increase in haemoglobin content over the full experimental period of 18 days is considerably less than would be expected.

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References

Cowan, J. W., Esfahani, M., Salji, J. P. and Azzam, S. A., 1966. *J. Nutr.*, **90,** 423.
Elvehjem, C. A. and Kemmerer, A. R., 1931. *J. biol. Chem.*, **93,** 189.
Keele, C. A. and Neil, E., 1961. *Samson Wright's Applied Physiology, p.* 88. Oxford University Press, London.
Martin, H. F. and Halton, P., 1964. *J. Sci. Fd. Agric.*, **15,** 464.
McCall, M. G., Newman, G. E., O'Brien, J. R. P., Valberg, L. S. and Witts, L. J., 1962. *Br. J. Nutr.*, **16,** 297.
Moorf, C. V., Dubach, R., Minnich, V. and Roberts, H. K., 1964. *J. clin. Invest.*, **23,** 755.
Steinkamp, R., Dubach, R. and Moore, C. V., 1955. *Arch. intern. Med.*, **95,** 181.

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