ALSO PUBLISHED AS
SUPPLEMENT NUMBER 376 TO VOLUME 171
OF
ACTA MEDICA SCANDINAVICA

IRON ABSORPTION STUDIES
II.

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GÖTEBORG 1962

EXPERIMENTAL STUDIES ON ORAL IRON THERAPY

by

HANS BRISE

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AKADEMISK AVHANDLING

SOM MED TILLSTÅND AV
MEDICINSKA FAKULTETEN VID GÖTEBORGS UNIVERSITET
FÖR VINNANDE AV MEDICINE DOKTORSGRAD OFFENTLIGEN FÖRSVARAS Å
STORA AULAN, SAHLGRENSKA SJUKHUSET,
LÖRDAGEN DEN 24 MARS 1962 KL. 9 F. M.

av

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MED. LIC.

GÖTEBORG 1962
ELANDERS BOKTRYCKERI AKTIEBOLAG

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SAHLGRENSKA SJUKHUSET, GÖTEBORG, SWEDEN

EXPERIMENTAL STUDIES ON ORAL IRON THERAPY

BY

HANS BRISE

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The present thesis is based on the following papers published in Acta Medica Scandinavica, 1962, vol. 171, supplementum 376.

I. H. Brise and L. Hallberg, A method for comparative studies on iron absorption in man using two radioiron isotopes.

II. H. Brise and L. Hallberg, Absorbability of different iron compounds.

III. H. Brise, Influence of meals on iron absorption.

IV. H. Brise, Effect of surface-active agents on iron absorption.

V. H. Brise and L. Hallberg, Effect of ascorbic acid on iron absorption.

VI. H. Brise and L. Hallberg, Effect of succinic acid on iron absorption.

These papers are refered to in the text as I, II, III, IV, V and VI.

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INTRODUCTION

Iron deficiency is a very common deficiency disease in all parts of the world.[1] In most Western countries it is probably the only deficiency disorder of practical importance. In U. S. A. and Great Britain it is found that between 5—25 per cent of the population show evidence of iron deficiency.[2] This estimation is in accordance with the results obtained in Sweden, where one of the few thorough studies on iron deficiency was made in 1946.[3] It was found that among women of a rural population one third showed evidence of iron deficiency, amongst these one third had anaemia.

Iron deficiency may be the result either of a decreased absorption of iron or an increased need of it. A decreased absorption may be caused either by a low intake of iron or a reduced absorption as for instance after gastric resection, idiopathic sprue etc. An increased iron need may be due on one hand to blood loss (menorrhagia, bleeding peptic ulcer, tumor in the gastrointestinal tract, hookworm infestation etc.) and on the other hand pregnancy and rapid growth in children and adolescents. The treatment of iron deficiency thus must aim to treat both the underlying cause (if possible) and the iron deficiency as such.

Iron is administered either orally or parenterally. It is generally recommended that parenteral iron therapy be reserved for those subjects not responding to oral ron treatment (malabsorption syndromes) or those subjects not tolerating oral iron.[4 5 6] In most subjects a favourable therapeutic response is obtained with oral iron therapy which is considered as the standard method of treatment of iron deficiency.

However, there are a number of practical problems connected with oral iron therapy. The aim of the therapy is not only to normalize the hemoglobin level but also to restitute tissue iron and to some extent iron stores. To achieve this, the treatment should be continued for a considerable time after the hemoglobin level has been normalized. The only way to shorten this time is of course to increase the absorption of iron.

More iron will be absorbed when more iron is administered. However, by increasing the iron doses, not only the absorption but also the frequency and severity of side-effects are increased. As will be discussed later the side-effects are rather depending on the amount of iron given than on the kind of iron compound used.

The absorbability of the iron compound used, will thus be one of the main factors determining the therapeutic effectiveness of oral iron therapy. However, in spite of numerous studies the knowledge of the absorbability of different iron compounds is insufficient, mainly due to the methodological difficulties of making valid comparisons between the great number of iron compounds used today.

Moreover, a study of other factors which may influence the absorption of iron may also lead to an increased effectiveness of oral iron therapy including a shortening of the time of treatment. However, a comparison of the absorption of a great number of iron compounds and a more thorough study of factors influencing the absorption, necessitates a new methodological approach, in order to draw valid conclusions from studies of minor groups of subjects within reasonable time. This is impossible using current methods. The initial aim of the present investigation was thus to devise a method more suitable for comparative studies of the problems outlined.

METHOD

In studies of the absorbability of iron compounds the primary interest is the absorbability of one compound in relation to another.

Also in studies on factors influencing the absorption of iron the main interest is the relationship between the amounts of iron absorbed under the different conditions. In comparative studies on the absorption of iron there are a number of problems mainly due to the varying absorption of iron in different individuals and also due to the variation in absorption from day to day in the same individual. If two groups of subjects are each given one of two different compounds the groups must of necessity be large in order to detect even a fairly large difference in absorbability between the compounds.

In addition to the greatly variable absorption of iron within and between individuals there are also other difficulties related to the determination of the absorption of iron.

Earlier comparative studies of iron compounds have been based on determinations of the rate of hemoglobin response or reticulocyte increase in iron deficient subjects during treatment.[7 8 9] Another method used to compare the absorbability of iron is based on the plasma iron increase during the first few hours after the administration of an oral iron dose.[10] All these methods have considerable sources of error which may greatly invaliditate the results obtained as discussed in detail in paper II. It was thus necessary to devise a method which reduced the effect of the varying absorption within and between individuals as much as possible and which accurately determined the absorbability of one compound in relation to another.

In the method described in paper I, two different iron compounds were given

on alternate days for 10 days labelled with different radioiron isotopes (Fe^{55} and Fe^{59}). The relative amounts of iron absorbed can then be determined from the Fe^{55} and Fe^{59} activity in the blood.[11] By using this method comparative studies on iron absorption are greatly facilitated, inasmuch as

1) the effect of the variation in absorption of iron between individuals is eliminated since each subject serves as his own control;

2) the effect of the variation in absorption from one day to another is reduced by repeated administration of the iron doses;

3) the accuracy of the determination of absorbed iron is increased, as the determinations are made from one single blood sample.

To be able to compare the results obtained in different individuals, ferrous sulphate was used as a reference in all subjects.

In order to study the absorbability of iron under similar conditions as those present during oral iron therapy, a more laborious modification of the method described in paper I has also been used in papers II and III. Iron was given in tablet form three times a day for 24 days instead of the administration of iron solutions once a day for 10 days in the original method.

The absorption of one iron compound in relation to another compound or the absorbability of iron under two different sets of conditions was expressed as the absorption ratio. The relationship between the absorption level and the absorption ratio may need a special commentary.

When for instance the absorbability of two compounds is compared and the result is expressed as a ratio which is greater than 1 at a certain absorption level, the ratio will theoretically approach the value 1 when the basic absorption of the substance in the denominator approaches 100 per cent, and the ratio will approach infinity when the basic absorption approaches zero. However, it was found that there was no relationship between absorption ratio and absorption level within the range studied. This is evident from fig. 2 in paper I and from the comparison between ferrous and ferric iron in the same paper, fig. 5. In paper V, when the relationship between the amount of ascorbic acid and the absorption increase obtained was studied statistically, it was found that the rest variance in the correlation between these two parameters was not reduced when a multiple correlation study was made also including the basic absorption of iron. The same results were obtained in paper VI in the study of the effect of succinic acid on iron absorption.

The results thus mean that no systematic errors are included when forming mean values of absorption ratios obtained in different individuals with different basic absorption levels.

It is thus possible to draw valid conclusions regarding the absorbability of different iron compounds and/or the effect of different conditions on the absorption of iron, from studies made in relatively few subjects. An additional main advantage of the method is, that the composition of the material has little influence on the results.

THE CHOICE OF IRON PREPARATION IN ORAL IRON THERAPY

A great number of iron compounds are used today in oral iron therapy and new preparations are continuously put on the market with claims of superiority with respect to side-effects and absorbability. In the comparative studies published on side-effects from different iron compounds, where adequate control groups are included, no significant differences have been observed between different iron compounds.[12 13 14] The absorbability of an iron compound will thus be the main factor determining its therapeutic value.

There is a lack of knowledge concerning the absorbability of different iron compounds, mainly because of the great number of methodological difficulties discussed in paper II. Highly diverging results and views concerning the absorption of various compounds have been published.[15] An increased absorption of ferrous versus ferric iron seems to be the only point, where general agreement exists today.

The double radioiron method described in paper I greatly facilitates studies of the absorbability of different iron compounds. As ferrous sulphate was used as a reference in all subjects it was possible to compare all compounds with each other.

The results in the present study (paper II) in which 14 iron compounds were compared, showed that there were marked differences in absorbability between different iron compounds.

The four ferric compounds studied all showed significantly less absorbtion than the ferrous compounds. The absorbability of ferrous versus ferric iron was found to be dependent on the amount of iron administered (paper I). This was interpreted as being due to the fact that a much lower iron ion concentration occurs at the pH of the gastrointestinal tract when giving ferric iron than when giving ferrous iron. The interpretation that the iron ion concentration obtained is a determining factor for the absorbability of an iron compound is consistent with the observations that such ferrous compounds (citrate, tartrate and pyrophosphate) of which considerable amounts of the iron can be expected to be present as complex ions, were less absorbed than the other ferrous compounds. The greater absorbability of ferrous succinate when given in a solution compared with the totally dissociated ferrous sulphate could not be explained from this hypothesis. This was the starting point for further studies, published in paper VI. When iron was given in tablet form no difference in absorbability was found between ferrous sulphate and ferrous succinate, probably due to the lower rate of dissolution of the latter.

The hypothesis that the iron ion concentration in the gastrointestinal tract obtained with an iron compound is the main factor determining the absorbability of an iron compound, makes it improbable that iron compounds will be found which are better absorbed than an easily soluble totally dissociated iron compound as e. g. ferrous sulphate.

THE CHOICE OF METHOD OF ADMINISTERING THE IRON DOSES

There are a number of data suggesting that less iron is absorbed when given together with meals than when given between meals.[16 17] In spite of this it is generally recommended to take the iron with meals to reduce gastric irritation. However, the relationship between absorbability, side-effects, dosage and way of administration of iron are not yet known. Comparative studies of the frequency of side-effects and absorbability of iron when given with and between meals should be made, in order to design the most effective way of iron administration.

However, it was considered to be of practical value to get quantitative information on the effect of meals on iron absorption. The method described in paper I facilitates a comparative study of the absorbability of iron when given between and with meals, and the study presented in paper III is limited to this problem.

It was found that on an average iron absorption was reduced by half when given with meals. There seemed to be a much lower reduction, in the absorption of iron at high absorption levels however, which may be of great practical interest, inasmuch as the results indicate that when iron doses are given together with meals to iron deficient subjects it is probable that these doses are both well absorbed and well tolerated.

THE SEARCH FOR ABSORPTION-PROMOTING SUBSTANCES

The problem in oral iron therapy is to get as much iron as possible absorbed in as short a time as possible and with the fewest side-effects. When the absorbability of different iron compounds were compared in paper II it was found that there was no compound from which iron was better absorbed than from easily soluble quite dissociated ferrous compounds, as e. g. ferrous sulphate which is the iron compound most commonly used in oral iron therapy today.

It was also pointed out that it is improbable that any iron compounds will be found which are better absorbed than ferrous sulphate. From the preceding discussion on absorbability in relation to side-effects, it may be concluded that a more effective oral iron therapy might be obtained if a greater part of the dose administered is absorbed. The only way to achieve this is to try to find substances which in some way increase the absorption of iron.

There are theoretically four principle ways of increasing the absorption of iron:

1) by increasing the area for absorption, e. g. by giving surface active agents,

2) by keeping the iron ion concentration as high as possible, for as long as possible, e. g. by keeping the iron in a ferrous state using reducing agents,

3) by increasing the transfer process through the mucosal cells,

4) by increasing the outflow from plasma of absorbed iron, e. g. by increasing the erythropoiesis.

The double radioiron method described in paper I greatly facilitates a study of factors influencing the absorption of iron, inasmuch as a single factor can be studied separately and as each individual serves as his own control.

In paper IV this method was used to study the effect of surface-active agents on iron absorption. The possibility of increasing the absorption of iron was studied in paper V in which the effect of ascorbic acid was studied. The results in paper VI showed that a stimulation of the transfer of iron across the mucosal cells is also possible, as it was observed that succinic acid had such a marked effect. The last mentioned possibility of increasing the absorption, namely a stimulation of the erythropoiesis has not been studied in the present investigation. Attempts have been made to increase the absorption by giving cobalt in order to increase the erythropoietin activity. However, a number of serious objections have been raised against the use of cobalt for increasing the absorption of iron.[6] The results of the present studies on potential absorption-promoting substances will be briefly summarized.

1. STUDIES ON THE EFFECT OF SURFACE-ACTIVE AGENTS ON IRON ABSORPTION (Paper IV)

In studies on hamsters it was observed that increased amounts of iron were present in the liver when Tween 20 (polyoxyethylene sorbitan monolaurate) was given together with the rations for long time.[18] It was concluded that this effect was due to an extension of the intestinal area of absorption. Based on these observations Tween 20 has been added to pharmaceutical iron preparations.

The present study showed that no absorption increase was obtained in humans by the addition of surface-active agents of various kinds. In the discussion in paper IV it was concluded that the effect in animals was probably due to a toxic action of Tween 20 when given in huge amounts, inasmuch as cirrhotic changes were noted, a fact which is in accordance with previous observations by other workers.[19] It was concluded therefore that there is no rational basis for using Tween 20 in pharmaceutical iron preparations.

2. STUDIES ON THE EFFECT OF ASCORBIC ACID ON IRON ABSORPTION (Paper V)

The determining influence of the iron ion concentration in the intestinal tract on the absorption of iron makes it probable

that more iron will be absorbed if it is kept in the ferrous state. Reducing substances such as ascorbic acid and cystein have been shown to increase the absorption of food iron and ferric iron.[10 20] However, diverging results have been obtained when ascorbic acid was added to ferrous iron. The general view held today is that in oral iron therapy the addition of ascorbic acid offers no significant advantage.[21]

In the present study it was found that the addition of ascorbic acid increased the absorption of iron and that the increase was related to the amount of ascorbic acid given. A significant effect was observed when a dose of 200 mg or more was given.

It is known that ascorbic acid plays an important role in several intracellular redox systems[22] but little is known about its action on the absorption of iron. However, from the studies in paper V it can be concluded that its absorption promoting effect is exerted in the gastrointestinal lumen, and is thus probably mainly due to its reducing action. This fact further supports the conclusion in papers I and II that the concentration of iron ions in the gastrointestinal lumen is the most important factor determining the magnitude of the iron absorption.

3. STUDIES ON THE EFFECT OF SUCCINIC ACID ON IRON ABSORPTION. (Paper VI)

The hypothesis that the iron ion concentration obtained in the gastrointestinal lumen was the main factor determining the absorbability of an iron compound, could not explain the observation in paper II that more iron was absorbed from a solution of ferrous succinate than from a solution of ferrous sulphate. Because of that, this finding was the starting point for further studies.

It was found that succinic acid increased the absorption of iron and that this increase was related to the amount of succinic acid given. A number of studies were made to analyze the way of action of succinic acid. It was found that a markedly increased absorption was also obtained from an oral iron dose when succinic acid was given intravenously. Moreover it was found that succinic acid did not influence the plasma iron turnover. By a process of elimination it could be concluded, that the probable action of succinic acid was a direct stimulation of the transfer of iron through the mucosal cells. It has recently been suggested that the absorption of iron is an active process dependent upon oxidative metabolism and the generation of phosphate-bound energy.[23] In view of this attempts were also made to locate further the action of succinic acid on energy systems within the cells.

Succinic acid is an integrating part of a number of electron transfer systems within the cells. When other acids comprised in the Krebs' cycle were given orally together with iron no increased absorption was obtained. It is thus probable that succinic acid is comprised in another system which limits the rate of transfer of iron through the mucosal cells.

CONCLUDING COMMENTS

The data obtained in the present series of papers may be of both theoretical and practical importance and may initiate further work in different directions.

Of special theoretical interest is the fact that the transfer process of a substance through the mucosal cells can be affected by succinic acid. This may suggest that it is possible to affect also the absorption of other substances. Another theoretical possibility is that internal transfer systems in other cells also may be influenced inasmuch as the energy-metabolism follows the same general pathways in all cells in the body.

Of special practical interest in oral iron therapy is the possibility of increasing the absorption of iron using absorption promoting substances. In a preliminary communication it was reported that in long term experiments, the absorption of iron was increased by 50 to 100 per cent by including ascorbic and succinic acids in ferrous sulphate tablets and that such an increased absorption was obtained without increasing the side-effects.[24] These results thus illustrate the practical importance of using absorption promoting substances in oral iron therapy and suggest that this is a possible way of attaining a more effective oral iron therapy, where more iron is absorbed within a shorter time. Therefore it also seems possible to solve the main practical problem in oral iron therapy today, the restitution of iron stores, because the absorption promoting effect is not reduced when the hemoglobin level is normalized. In oral iron therapy future work with absorption promoting substances thus seems to be promising.

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GÖTEBORG
ELANDERS BOKTRYCKERI AKTIEBOLAG
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IRON ABSORPTION STUDIES

II*

H. BRISE AND L. HALLBERG: A method for comparative studies on iron absorption in man using two radioiron isotopes.

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H. BRISE AND L. HALLBERG: Effect of succinic acid on iron absorption.

* A series of papers have earlier been published under the heading "Iron Absorption Studies" as Supplement 358 to volume 168, 1960, of ACTA MEDICA SCANDINAVICA

These investigations have been supported by grants from the Swedish Medical Research Council, the Göteborg Medical Society, and AB Hässle, Göteborg.

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FROM THE DEPARTMENT OF MEDICINE II (HEAD: PROFESSOR ERIK WASSÉN), UNIVERSITY OF GÖTEBORG, SAHLGRENSKA SJUKHUSET, GÖTEBORG, SWEDEN.

A METHOD FOR COMPARATIVE STUDIES ON IRON ABSORPTION IN MAN USING TWO RADIOIRON ISOTOPES

By

HANS BRISE AND LEIF HALLBERG

INTRODUCTION

The amount of iron absorbed at one occasion can be accurately determined using available radioiron methods[1, 2, 3]. In comparative studies (e.g. the absorbability of different iron compounds) other methodological problems will arise. When comparing the absorption of iron in two groups of individuals treated in different ways, the great variation in absorption between individuals will make the results from such studies very difficult to interprete even when using accurate methods to determine the absorption. Because of this, comparisons have often been made in the same subject. However, great variation in the absorption of iron occurs not only between individuals but also within a single individual on different days. Both these sources of variation were considered when the present method for comparative studies on iron absorption was designed.

The method was based on a repeated administration of iron to each individual (one dose on each of 10 days), giving on alternate days two iron compounds, each compound labelled with a different radioiron isotope (Fe^{55} or Fe^{59}). Determinations of Fe^{55} and Fe^{59} activities were made in a blood sample drawn 2 weeks after the last oral iron dose, when an optimal utilization of absorbed iron for hemoglobin synthesis could be expected to have taken place[4—7]. From these determinations the relative absorbability of the two compounds could be calculated. By giving repeated iron doses (5 doses of each compound on alternate days) the error due to the variation in absorption on different days could be reduced by more than half, and by making the comparisons within the same subject the variation in absorption between individuals was eliminated.

In 1958 a preliminary report was given on the method[8]. In the present paper the details of the experimental procedure are given and the validity of the method is more thoroughly tested. As an example of the application of the method, a study of the relative absorbability of ferrous- and ferric iron is included.

MATERIAL AND METHODS

MATERIAL

Sixtytwo subjects were included in this study. One subject (1-M-T) had a hypernephroma without demonstrable metastasis. One subject (26-M-BII) had a Biliroth II gastric resection several years ago. Three subjects had an iron deficiency anemia after acute blood loss (ID). The other subjects were healthy volunteers (N), some of whom had served as blood donors (BD). In the tables (M) denotes male and (F) female subjects.

PRINCIPLE OF METHOD AND EXPERIMENTAL PROCEDURE

The experimental design is outlined in figure 1. Unless otherwise stated, the same amount of elemental iron was given every morning for 10 days after an overnight fast. The iron was labelled with Fe^{55} and Fe^{59} on alternate days. When comparing ferric and ferrous iron, for instance, the compounds were labelled with different radioiron isotopes and were given on alternate days. To reduce systematic errors the first iron dose was alternately labelled with Fe^{55} and Fe^{59}, and was also alternately ferrous and ferric iron. From analysis of Fe^{55} and Fe^{59} activity in a blood sample drawn 2 weeks after the last oral dose the mean absorption ratio was calculated.

Each subject received a box containing 10 consecutively numbered 25 ml flasks which were taken in order. Detailed written and oral instructions were given for the experiment.

The iron solution was taken directly from the flask. This was then filled with tap water and the rinse water was also taken. This procedure was repeated so that the total volume consumed was 75 ml. No food or drink was taken for an additional two hours.

The residual radioactivity in the flasks was less than 0.5 per cent of the original content. This determination was made using a scintillation detector with a 5 inch. $\times$ 6 inch. plastic crystal with a well to contain the whole flask.

ORAL IRON DOSES

The ferrous iron in this study was $FeSO_4 \cdot 7\ H_2O$ (Merck, pro analysi). The ferric iron content was less than 1 per cent as found by analysis using the thiocyanate method[10].

The ferric iron salt administered was $Fe_2\ (SO_4)_3\ 6\ H_2O$ (Union Chimique Belge: pour analyse).

The volume of the solution in each flask was 25 ml and contained 30 mg of elemental iron, 10 mg of ascorbic acid (to prevent oxidation of ferrous iron — no ascorbic acid was added to ferric iron solutions), 4 gram of sucrose and 4—5μC of radioiron. In the solutions containing 5 mg of iron the ascorbic acid content was reduced proportionally to 1 2/3 mg.

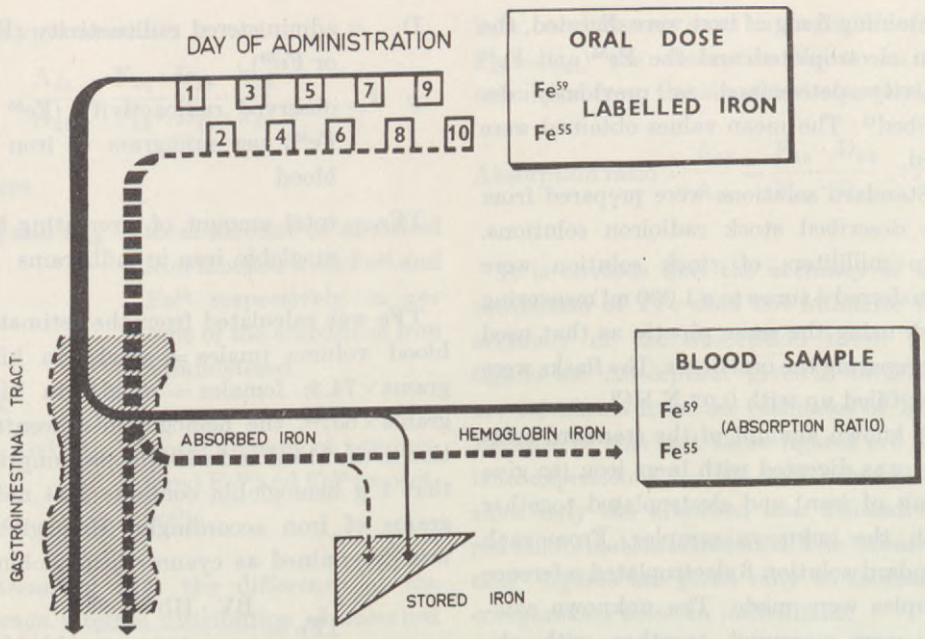

Fig. 1. Experimental design.

Freshly boiled distilled water was used in the preparation of the solutions and nitrogen was bubbled through the solutions in the flasks before closing. The ferric iron content in the ferrous sulphate solutions in these 25 ml flasks was less than 1 per cent after 4—6 weeks storage in room temperature.

The Fe^{55} and Fe^{59} was obtained from Abbott Laboratories, Oak Ridge, Tennessee, U.S.A. as a solution of $FeCl_3$ (pH less than 1.5). The specific activity of Fe^{59} was 5—13 microcuries per microgram and the specific activity of Fe^{55} was 2—3 microcuries per microgram respectively. Stock solutions of Fe^{55} and Fe^{59} in 0.02 N HCl containing 2—3 μC of radioiron per ml were prepared from the original solutions.

The final pH in the administered solutions was 2.5. The total amount of radioactivity administered to each subject was less than 25 μC Fe^{59} and 25 μC Fe^{55}.

As ferric chloride was used to label the ferrous sulphate the isotope exchange was tested. An acid solution containing the two compounds was transferred to a separatory funnel and extracted with isopropylether, which will extract only ferric ions under the conditions used[9]. A complete exchange was found to have taken place as the radioactivity in the isopropylether layer was less than 2 per cent of the original amount.

RADIOACTIVE ANALYSIS

From the blood sample (150 ml), drawn in 50 ml ACD-solution[1]) 2 weeks after the last oral iron dose, four samples each

[1]) 1.32 g of sodium citrate, 0.48 g of citric acid, 1.47 g of glucose to 100 ml with water.

containing 5 mg of iron were digested, the iron electroplated and the Fe^{55} and Fe^{59} activity determined as previously described[11]. The mean values obtained were used.

Standard solutions were prepared from the described stock radioiron solutions. Two milliliters of stock solution were transferred 4 times to a 1 000 ml measuring flask using the same pipette as that used in preparing the oral doses. The flasks were then filled up with 0.02 N HCl.

A known amount of the standard solution was digested with inert iron (to give 5 mg of iron) and electroplated together with the unknown samples. From each standard solution 6 electroplated reference samples were made. The unknown samples were measured together with the reference samples in an automatic sample changer.

CALCULATIONS

The Fe^{55} and Fe^{59} activities per 5 mg of iron in circulating red cells were determined according to formulas given in an earlier paper[11].

The amount of absorbed iron labelled with Fe^{55} or with Fe^{59} in circulating red cells was calculated from the activities of Fe^{55} and Fe^{59} in the administered doses, and from the activities in the blood according to the following equation:

$$\frac{a \cdot D}{100} = F \cdot TFe \quad \ldots\ldots\ldots\ 1$$

where

a = per cent of the administered iron in the circulating hemoglobin mass

D = administered radioactivity (Fe^{55} or Fe^{59}).

F = observed radioactivity (Fe^{55} or Fe^{59}) per milligram of iron in blood

TFe = total amount of circulating hemoglobin iron in milligrams

TFe was calculated from the estimated blood volume (males = weight in kilograms $\times$ 74.2; females = weight in kilograms $\times$ 65)[26], the hemoglobin concentration in the blood and with the presumption that 1 g hemoglobin contains 3.34 milligrams of iron accordingly. Hemoglobin was determined as cyanmethemoglobin[12].

$$TFe = \frac{BV \cdot Hb \cdot 3.34}{100} \quad \ldots\ldots\ 2$$

where

BV = estimated blood volume in milliliters

Hb = hemoglobin concentration in grams per 100 ml blood.

When two compounds were compared in this experimental design a figure of the relative absorbability of the two compounds was obtained according to the following equations:

$$A = \frac{a}{k} \quad \ldots\ldots\ldots\ 3$$

where

A = total amount of absorbed iron in per cent of the amount administered

k = fraction of absorbed iron in the circulating hemoglobin mass

a was calculated from Equation 1.

and,

Absorption ratio:

$$\frac{A_{55}}{A_{59}} = \frac{F_{55} \cdot D_{59} \cdot k_{59}}{F_{59} \cdot D_{55} \cdot k_{55}} \quad \ldots\ldots \quad 4$$

where

A_{55} and A_{59} = total amount of absorbed iron labelled with Fe^{55} and Fe^{59} respectively in per cent of the amount of iron administered,

and,

D_{55} and D_{59} = total amount of administered Fe^{55} and Fe^{59} respectively.

Assuming that the difference of the average internal distribution of absorbed iron on different days is negligible (i.e. $k_{59} = k_{55}$) the absorption ratio can be calculated from the simplified equation.

$$\text{Absorption ratio} = \frac{A_{55}}{A_{59}} = \frac{F_{55} \cdot D_{59}}{F_{59} \cdot D_{55}} \quad \ldots \quad 5$$

It is obvious that the accuracy of the estimation of TFe does not influence the accuracy of the absorption ratio. The figures for "Absorption" given in the tables are calculated from the estimates of TFe. Because of this fact these figures are not true expressions for the total absorption since only the absorbed iron utilized for red cell formation is included. The "Absorption" figures are given only to facilitate comparisons between individuals.

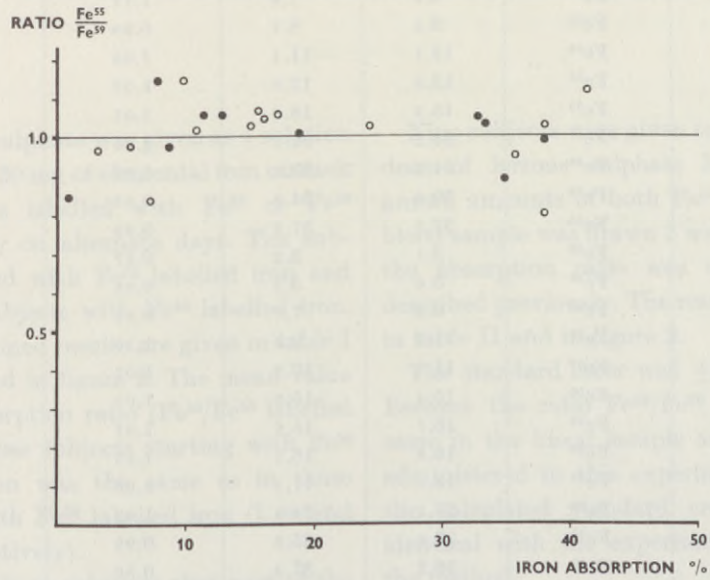

Fig. 2. Precision and accuracy of method. Absorption ratio of Fe^{55} labelled and Fe^{59} labelled ferrous sulphate administered on alternate days for 10 days. (Each dose was equivalent to 30 mg of elemental iron.) Observed absorption ratio values were plotted against estimated absorption. Results in subjects starting with Fe^{55} were indicated as dots •, in those starting with Fe^{59} as rings ○.

RESULTS

CONTROL STUDIES

Even when the foregoing experimental design is used, the accuracy of comparisons of the absorbability of different iron compounds is limited by (a) the day to day variation in the absorption of iron and (b) the variation of the internal distribution of the absorbed iron to erythropoiesis and storage. In order to be able to calculate the magnitude of the total variation the following studies were made.

TABLE I

Precision and accuracy of method. Administration of Fe^{55} and Fe^{59} labelled ferrous sulphate administered on alternate days for 10 days. Each dose equivalent to 30 mg of elemental iron.

SUBJECT	First dose	"ABSORPTION" (per cent)		ABSORPTION RATIO Fe^{55}/Fe^{59}	
		Fe^{55}	Fe^{59}	Individual value	Mean value
1-M-T	Fe^{55}	0.7	0.9	0.86	
2-M-N	Fe^{55}	8.7	7.8	1.11	
3-M-BD	Fe^{55}	8.5	8.7	0.98	
4-M-N	Fe^{55}	12.1	11.4	1.06	
5-M-BD	Fe^{55}	13.6	12.8	1.06	
6-M-BD	Fe^{55}	18.9	18.8	1.01	
7-M-BD	Fe^{55}	34.2	32.7	1.05	
8-M-BD	Fe^{55}	34.2	33.3	1.03	
9-F-BD	Fe^{55}	30.8	34.8	0.89	
10-M-BD	Fe^{55}	37.5	37.9	0.99	1.00
11-M-N	Fe^{59}	3.1	3.2	0.97	
12-M-N	Fe^{59}	5.6	5.7	0.98	
13-M-N	Fe^{59}	6.5	7.7	0.84	
14-F-N	Fe^{59}	11.3	9.8	1.15	
15-M-BD	Fe^{59}	11.1	10.8	1.02	
16-M-BD	Fe^{59}	15.4	15.0	1.03	
17-F-BD	Fe^{59}	16.7	15.6	1.07	
18-F-BD	Fe^{59}	16.8	16.1	1.05	
19-M-BD	Fe^{59}	18.1	17.1	1.06	
20-M-BD	Fe^{59}	25.1	24.4	1.03	
21-F-N	Fe^{59}	33.9	35.6	0.95	
22-M-BD	Fe^{59}	30.2	37.9	0.80	
23-M-BD	Fe^{59}	39.0	37.9	1.03	
24-M-BD	Fe^{59}	46.1	41.2	1.12	1.01

Absorption ratio: Mean value: 1.01
Standard error of mean: ±0.02
Standard error: ±0.09

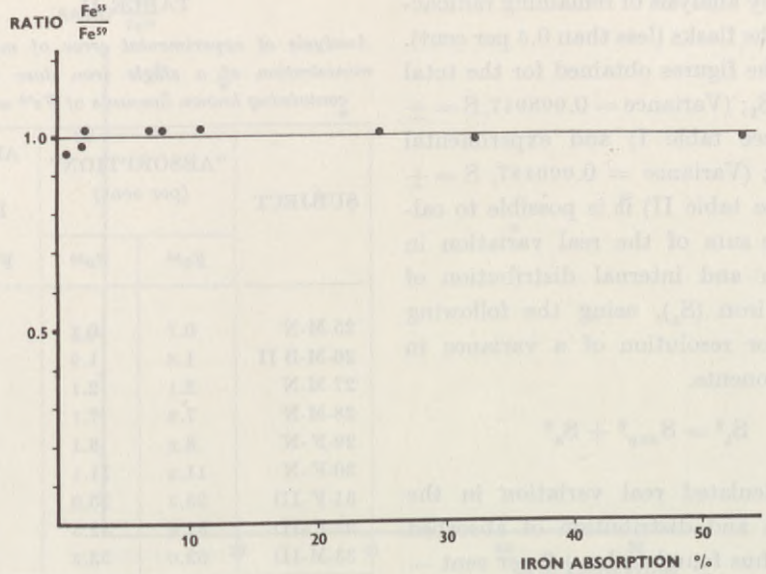

Fig. 3. Analysis of experimental error of method. Absorption ratio of Fe^{55} and Fe^{59} labelled iron from a single dose (30 mg Fe) containing known amounts of both isotopes. Results plotted against estimated absorption.

Ferrous sulphate was given as a solution containing 30 mg of elemental iron on each of 10 days labelled with Fe^{55} or Fe^{59} respectively on alternate days. Ten subjects started with Fe^{55} labelled iron and fourteen subjects with Fe^{59} labelled iron.

The obtained results are given in table I and graphed in figure 2. The mean value of the absorption ratio (Fe^{55}/Fe^{59} labelled iron) in those subjects starting with Fe^{55} labelled iron was the same as in those starting with Fe^{59} labelled iron (1.00 and 1.01 respectively).

To be able to calculate that part of the variation which is due to a varying absorption and internal distribution of iron on different days, the experimental error was calculated in the following way:

Nine subjects were given one 30 mg iron dose of ferrous sulphate labelled with known amounts of both Fe^{55} and Fe^{59}. A blood sample was drawn 2 weeks later and the absorption ratio was calculated as described previously. The results are given in table II and in figure 3.

The standard error was ± 2.2 per cent. Because the ratio Fe^{55}/Fe^{59} must be the same in the blood sample as in the dose administered in this experimental design the calculated standard error must be identical with the experimental error of the method.

This experimental error does not include the variation of emptying and rinsing of the flasks containing the iron doses. However, this latter error is quite negligible

as found by analysis of remaining radioactivity in the flasks (less than 0.5 per cent).

From the figures obtained for the total variation S_t; (Variance = 0.008017, S = ± 0.09 — see table I) and experimental error S_{exp}; (Variance = 0.000487, S = ± 0.02 — see table II) it is possible to calculate the sum of the real variation in absorption and internal distribution of absorbed iron (S_a), using the following formula for resolution of a variance in two components.

$$S_t^2 = S_{exp}^2 + S_a^2$$

The calculated real variation in the absorption and distribution of absorbed iron was thus found to be ±9 per cent — variance 0.007530. This means that the experimental error only forms a negligible part of the total variation.

TABLE II

Analysis of experimental error of method. Administration of a ***single*** *iron dose (30 mg Fe) containing known amounts of Fe^{55} and Fe^{59}.*

SUBJECT	"ABSORPTION" (per cent)		ABSORPTION RATIO
	Fe^{55}	Fe^{59}	Fe^{55}/Fe^{59}
25-M-N	0.7	0.7	0.96
26-M-B II	1.8	1.9	0.98
27-M-N	2.1	2.1	1.02
28-M-N	7.2	7.1	1.02
29-F-N	8.2	8.1	1.02
30-F-N	11.3	11.1	1.02
31-F-ID	25.3	25.0	1.01
32-F-ID	32.3	32.5	0.99
33-M-ID	53.0	53.5	0.99

Absorption ratio: Mean value: 1.00
Standard error of mean: ±0.01
Standard error: ±0.02

TABLE III

Precision and accuracy of method. Administration of Fe^{55} and Fe^{59} labelled ferrous sulphate administered on alternate days for 10 days. Each dose equivalent to 5 mg of elemental iron.

SUBJECT	First dose	"ABSORPTION" (per cent)		ABSORPTION RATIO Fe^{55}/Fe^{59}	
		Fe^{55}	Fe^{59}	Individual value	Mean value
34-M-N	Fe^{55}	7.6	7.6	1.01	
35-M-N	Fe^{55}	9.1	7.8	1.17	
36-M-N	Fe^{55}	9.9	9.0	1.10	
37-M-BD	Fe^{55}	19.9	17.8	1.12	
38-M-BD	Fe^{55}	37.5	35.7	1.05	1.09
39-M-N	Fe^{59}	13.0	10.5	1.23	
40-F-N	Fe^{59}	19.8	17.3	1.14	
41-M-BD	Fe^{59}	27.3	32.1	0.85	
42-F-BD	Fe^{59}	47.0	61.6	0.76	
43-M-BD	Fe^{59}	66.0	78.7	0.84	0.96

Absorption ratio: Mean value: 1.03
Standard error of mean: ±0.05
Standard error: ±0.16

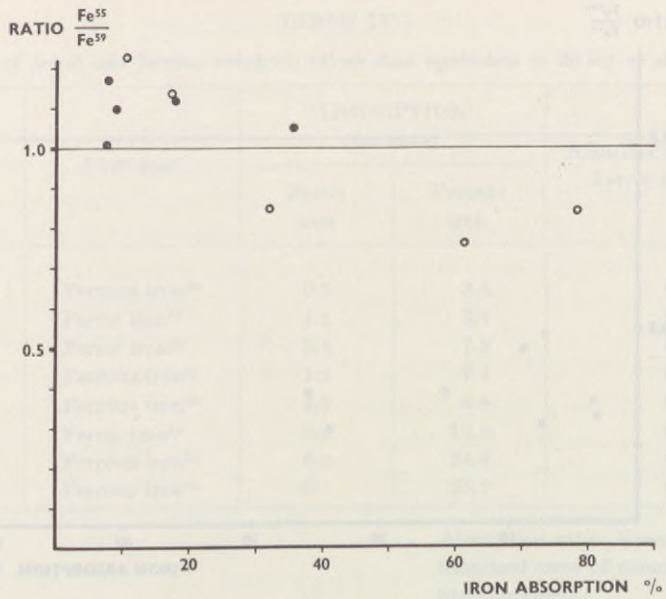

Fig. 4. Precision and accuracy of method. Absorption ratio of Fe^{55} labelled and Fe^{59} labelled ferrous sulphate administered on alternate days for 10 days. (Each dose was equivalent to 5 mg of elemental iron.) Observed absorption ratio values were plotted against estimated absorption. Results in subjects starting with Fe^{55} were indicated as dots •, in those starting with Fe^{59} as rings ○.

As S_a is obtained from a mean value of 5 pairs of comparisons the variation in absorption and utilization of absorbed iron from one day to another within the single individual can be calculated as $\sqrt{5 \times 0.007530} = \pm 20$ per cent (coefficient of variation).

The variation in absorption on different days was also studied when using 5 mg doses, because such a dose is more closely related to physiological conditions and has been recommended as the most satisfactory dose for testing iron absorption[13].

In this series comprising 10 subjects the 5 mg iron dose was given for 10 days in the same way as in the first series. The results obtained are given in table III and figure 4.

The observed standard deviation of the absorption ratio was 16 per cent. By resolution of the variance in the two components as above the variation in absorption on different days within the single individual using 5 mg doses was $\sqrt{5 \times 0.024794} = \pm 35$ per cent (coefficient of variation). As found by an F — test the standard deviation was greater when 5 mg doses were used than when 30 mg doses were used ($p < 0.05$).

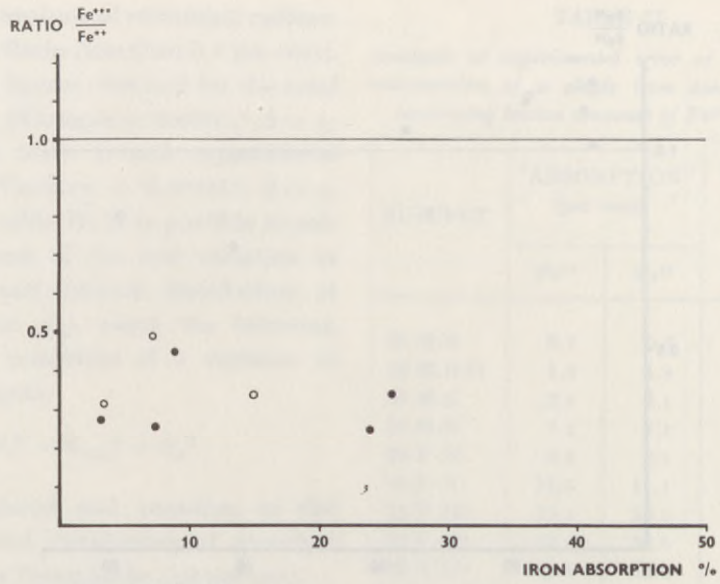

Fig. 5. Absorption of ferric versus ferrous iron at different estimated absorption levels. Each iron dose was equivalent to 40 mg of elemental iron. (• indicate subjects starting with ferrous sulphate, ○ indicate subjects starting with ferric sulphate.)

COMPARISON OF THE ABSORPTION OF IRON FROM FERROUS AND FERRIC SULPHATE

As an example of the application of this double isotope method a comparison of the absorption of iron from ferrous and ferric sulphate is included in the present paper.

It has repeatedly been shown, using different methods that ferrous iron is more readily absorbed than ferric iron[14—17]. Because of this a comparison of ferrous and ferric iron may also serve as an indirect check of the method. The data on the quantitative importance of the valency of iron are greatly diverging. The present method can be expected to give more exact information on the long debated problem.

a. Oral iron dose 30 mg.

Eight subjects were included in this study. The solutions were prepared as previously described (ascorbic acid was not added to the ferric sulphate solutions) and each dose contained 30 mg of elemental iron. In five subjects the ferrous iron was labelled with Fe^{59}, in three subjects with Fe^{55}. In order to further reduce the possibility of systematic errors in this comparison 5 subjects started with the ferrous dose and 3 subjects with the ferric dose.

The results are given in table IV and are illustrated in figure 5. In the figure the absorption ratio ferric/ferrous iron is graphed against the absorption of iron from the ferrous sulphate solution. The term "absorption" is used to mean the

TABLE IV

Absorbability of ferric and ferrous sulphate. (Each dose equivalent to 30 mg of elemental iron.)

SUBJECT	First dose	"ABSORPTION" (per cent)		ABSORPTION RATIO Ferric/Ferrous iron
		Ferric iron	Ferrous iron	
44-M-N	Ferrous iron^{59}	0.9	3.2	0.28
45-M-N	Ferric iron^{55}	1.1	3.4	0.32
46-M-BD	Ferric iron^{55}	3.5	7.2	0.49
47-M-BD	Ferrous iron^{59}	1.9	7.4	0.26
48-F -BD	Ferrous iron^{59}	4.0	8.9	0.45
49-M-BD	Ferric iron^{59}	5.2	15.0	0.34
50-M-BD	Ferrous iron^{55}	6.0	24.0	0.25
51-M-BD	Ferrous iron^{55}	8.7	25.7	0.34

Absorption ratio: Mean value: 0.34
Standard error of mean: ±0.03
Standard error: ±0.09

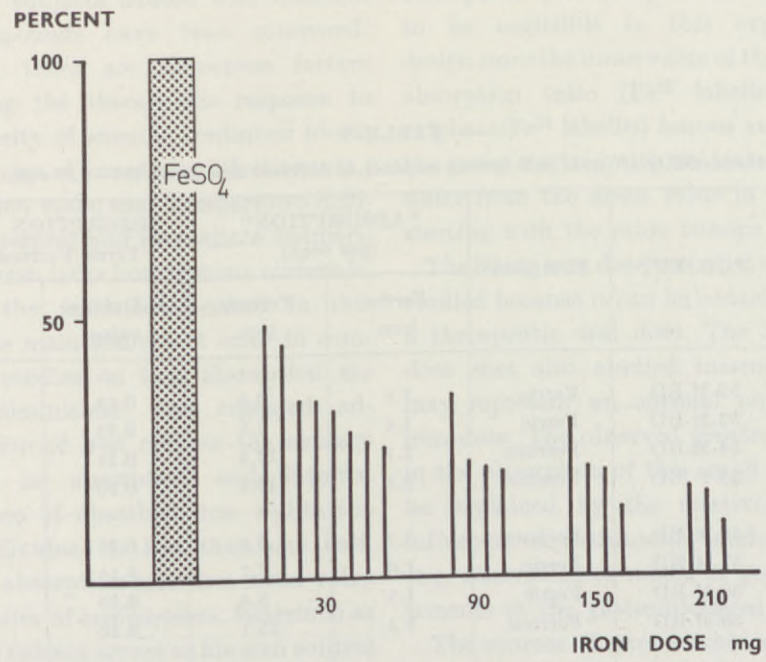

Fig. 6. Absorption of ferrous and ferric iron at different dosage levels. The same amount of iron was given each day. Each line represents absorption of ferric iron as a percentage of the absorption of ferrous iron in the same subject.

per cent of absorbed iron in circulating red cells 2 weeks after the administration of the last oral iron dose.

The mean value of the absorption ratios in these 8 subjects was 0.34 ± 0.03 and it is thus quite clear that ferrous iron is much more readily absorbed than ferric iron.

b. Oral iron dose 90—210 mg.

It is possible that the magnitude of the iron dose may influence the relative absorbability of ferrous and ferric iron. An additional study was thus made in which ferrous and ferric iron were compared at higher dose levels (90, 150 and 210 mg of elemental iron). The same amounts of ferrous and ferric iron were thus given to each subject.

The results are given in table V and are also graphed in figure 6 where each bar represents one subject. It is evident that the greater absorbability of ferrous iron was more pronounced the higher the iron dose. A correlation analysis between absorption ratio and iron dose gave the following results: $r = -0.50$ and $p < 0.05$.

When 30 mg iron doses were compared, 3 times more ferrous iron was absorbed. When 90, 150 and 210 mg doses of iron were studied, respectively 4, 5 and 7 times more ferrous than ferric iron was absorbed.

TABLE V

Absorbability of ferric and ferrous sulphate at different dose levels (90—210 mg).

Daily oral dose (mg Fe)	SUBJECT	First dose	"ABSORPTION" (per cent)		ABSORPTION RATIO Ferric/Ferrous iron	
			Ferric iron	Ferrous iron	Individual value	Mean value
90	52-M-BD	Ferric	1.0	3.0	0.35	
90	53-M-BD	Ferric	1.6	7.5	0.22	
90	54-M-BD	Ferrous	2.7	12.8	0.21	
90	55-F-BD	Ferrous	3.3	16.6	0.20	0.25
150	56-M-BD	Ferrous	1.3	6.1	0.21	
150	57-M-BD	Ferric	1.0	7.7	0.13	
150	58-M-BD	Ferric	1.7	8.5	0.20	
150	59-M-BD	Ferrous	7.5	25.1	0.30	0.21
210	60-M-BD	Ferrous	0.5	4.4	0.10	
210	61-M-BD	Ferrous	1.0	6.1	0.17	
210	62-M-BD	Ferric	1.2	7.5	0.16	0.14

COMMENT

This method was devised in an attempt (a) to make more valid comparisons of the absorption of iron from different iron compounds and (b) to facilitate the quantitation of factors influencing the absorption of iron. An example of the latter application of the method is a study of the effect of meals on iron absorption presented in a preliminary report[8].

Earlier comparative studies have almost exclusively been devoted to the relative absorbability of different iron compounds [18—24]. The comparisons have usually been based on determinations of the regeneration rate of hemoglobin during iron therapy in iron deficient subjects. Two or more groups of subjects treated with different iron compounds have been compared. However, there are numerous factors influencing the therapeutic response to iron (severity of anemia, continued bleeding, condition of iron stores, infections etc.) which often make such comparisons difficult to interpret and necessitate comparisons between large homogenous materials.

Using the method described in this paper, the main sources of error in comparative studies on iron absorption are greatly diminished. The repeated administration of iron reduces the average variation in absorption and internal distribution of absorbed iron within the single individual to less than one half, since the absorption ratio is a mean value of five pairs of comparisons. Inasmuch as the single subject serves as his own control valid conclusions can be drawn from materials containing relatively few individuals. For the same reason the requirements of a selection and classification of subjects for comparative iron absorption studies are also markedly reduced.

The method is convenient since it is not necessary to quantitate the total absorption (e.g. by faeces collection) to be able to study the effect of a substance on iron absorption or the relative absorbability of iron from two compounds.

Iron doses labelled with different isotopes were not given on the same day in order to diminish the possibility of an exchange of radioiron between different doses in that part of the intestine where a measurable absorption could take place.

The effect of a preceding dose on the absorption of a subsequent dose was found to be negligible in this experimental design since the mean value of the obtained absorption ratio (Fe^{55} labelled ferrous sulphate/Fe^{59} labelled ferrous sulphate) in the group starting with one isotope did not differ from the mean value in the group starting with the other isotope.

The 30 mg iron dose was most thoroughly studied because it can be considered to be a therapeutic oral dose. The 5 mg iron dose was also studied inasmuch as it may represent an optimal physiological iron dose. The observed greater variation in the absorption of this small dose, may be explained by the relatively greater influence of extraneous random factors (e.g. adsorption to mucin or protein components in the gastrointestinal tract).

The sources of error in this method are of two kinds. One kind consist in analytical errors and errors in the administration of the iron doses. The magnitude of these

errors was found to be only about 2.5 per cent. The other and main source of error is the variation in absorption and distribution of absorbed iron. This error can be further reduced only by giving more iron doses for longer time.

In 10 subjects a blood sample was drawn not only 2 weeks after the last oral dose but also after 3 weeks and in 5 of the subjects at times up to 2 months after the last dose. The difference between the absorption ratios within the single subject was of the same magnitude as the experimental error. The effect of a variation in internal distribution of absorbed iron on the real absorption ratio can be expected to decrease in time. The fact that no significant difference between absorption ratios was found, when followed for longer time indicates that the main part of the observed total variation is related to a variation in absorption from day to day. This variation was about ± 20 per cent when 30 mg iron doses were given and about ± 35 per cent when 5 mg doses were given. This great variation means that it is very difficult or impossible to demonstrate minor differences in absorbability of two compounds, if such a comparison is based on determinations of iron absorption on two occasions in the same subject, even if these determinations are made with a very accurate method. The great variation in absorption of iron on different days in the same subject stresses the importance of giving iron in repeated doses in comparative studies in the same individual.

The degree of underestimation of the real absorption, as calculated from the radioactivity in the red cell mass 2 weeks after the last oral dose, does not influence the absorption ratio. These "absorption figures" have only been given as a rough classification of the subjects' avidity to absorb iron.

The observed lower absorption of ferric iron (compared with ferrous) is consistent with earlier observations[14–17]. From the observed difference in absorbability it is not necessary to postulate that iron is absorbed only in the ferrous state. The difference can most easily be explained from the well known physico-chemical difference between ferric and ferrous ions. At the pH existing in the gastrointestinal tract, a considerably greater amount of the ferric than of the ferrous iron will be present as undissociated hydroxide. Moreover ferric iron has a greater avidity to form insoluble compounds or complex compounds than ferrous iron. The average ionic concentration of iron in the upper part of the intestinal tract, where the absorption of iron mainly takes place, can thus be expected to be much higher when ferrous iron is given than when ferric iron is given.

The difference in relative absorbability between ferrous and ferric iron can be expected to be more pronounced the higher the iron dose because at higher dose levels the ferric ion concentration will remain constant while more and more undissociated ferric hydroxide will be formed.

This reasoning is consistent with the observed decrease of the ferric/ferrous iron absorption ratio with increased iron doses (0.34—0.14 at the dose levels 30 and 210 mg of iron respectively).

It is also consistent with the observation by Bonnet, Hagedorn and Owen who found no difference in absorbability of ferrous and ferric iron when very small amounts (50 μg) of elemental iron were

used[25]. At the much lower concentration of iron achieved in the gastrointestinal tract with this extremely small iron dose, it can be expected that ferrous and ferric iron will both be present in ionic form to the same degree (the solubility product of ferric hydroxide will not be exceeded).

From the present studies it can be concluded that considerably more iron is absorbed from ferrous than from ferric sulphate. — At therapeutic dose levels (30 mg of iron or more) at least 3 times more iron will be absorbed if given in the ferrous state. This difference in absorbability between ferrous and ferric iron is of such a magnitude that it can be concluded that ferric iron has no place in oral iron therapy.

SUMMARY

A method is described which is especially devised for comparative studies of the absorbability of different iron compounds and for a quantitation of the influence of various factors on iron absorption.

Two radioiron isotopes are used — Fe^{55} and Fe^{59}. One iron compound is labelled with one isotope and one compound with the other. The compounds (and isotopes) are administered on alternate days for ten consecutive days.

From analysis of Fe^{55} and Fe^{59} in one blood sample drawn two weeks after the last oral dose the relative absorbability of different iron compounds can be determined.

By giving ferrous sulphate labelled with the two isotopes on alternate days the accuracy and precision of the method has been determined. The average day to day variation in absorption of iron in the single individual was found to be about ± 20 per cent using 30 mg doses and ± 35 per cent using 5 mg doses.

As an example of the application of the method the absorbability of iron from ferrous and ferric sulphate has been studied at different dosage levels. It was found that about 3—7 times more iron was absorbed from ferrous sulphate than from ferric sulphate.

The results show that the method will greatly facilitate comparative iron absorption studies since each subject serves as his own control.

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ABSORBABILITY OF DIFFERENT IRON COMPOUNDS

By

HANS BRISE AND LEIF HALLBERG

INTRODUCTION

A great number of iron compounds are used in the treatment of iron deficiency. With the dosage commonly employed to-day side-effects are fairly uncommon. In a well controlled study of side-effects in oral iron therapy no significant difference was observed between some commonly used iron compounds when the same amount of elemental iron was given[1]. With increasing dose of iron more side-effects are encountered[2]. Because of this, the therapeutic value of an iron compound is determined mainly by its absorbability. However, inasmuch as it is very difficult to compare the absorbability of iron compounds, it is also difficult to get an objective measurement of the therapeutic value of different iron compounds.

The absorbability of an iron compound has usually been evaluated from the regeneration rate of hemoglobin in cases with iron deficiency anemia during iron therapy[3—8]. However, the regeneration rate is not only determined by the amount of iron absorbed but also by a number of other factors, such as the severity of anemia, the condition of the iron stores and the presence of concurrent other diseases (e.g. infections). A false value of the absorption can also be obtained if the amount of iron absorbed is greater than the amount utilized by the bone marrow. Such sources of error in estimating absorption of iron from the regeneration rate of hemoglobin will make it necessary to have fairly large homogenous materials when the absorbability of different iron compounds are to be compared.

Other methods, which have been used to compare the absorbability of iron compounds (e.g. the plasma iron increase after oral administration of different compounds), also have considerable sources of error. These will be discussed later.

The difficulties in evaluating the relative absorbability of different iron compounds can perhaps account for the diverging results obtained by different authors and the numerous iron preparations used to-day, most of which have been introduced with claims of superiority. A critical review of the present confused situation has been published by BEUTLER 1960[9].

The double radioiron method offers the possibility of making a more valid evaluation of the absorbability of different iron compounds since two compounds can be compared in the same individual within the same period thereby reducing or eliminating some of the main sources of error in earlier methods[10 11 12]. Actually, the difficulties in comparing the absorbability of iron compounds initiated the devising of the double radioiron method.

In this paper the absorbability of 14 iron compounds are reported. In each individual two compounds, labelled with different radioiron isotopes, have been compared. — One of these two compounds has always been ferrous sulphate. Thus, ferrous sulphate served as a reference in all subjects. A preliminary report including only 4 compounds was published in 1958 as an example of possible applications of this double radio-isotope method[10 11].

METHODS AND MATERIAL

METHODS

The experimental design of this study and the details of the method were the same as previously described.[12] A solution of 25 ml containing 30 mg of elemental iron was given every morning after an overnight fast for 10 days. Ferrous sulphate was labelled with one radioiron isotope and the iron compound under study was labelled with the other isotope. The two compounds were given on alternate days. This design (giving ferrous sulphate to all subjects on alternate days) will thus make possible a comparison between individuals by making ferrous sulphate a common reference.

In order to reduce systematic errors the first dose was alternately ferrous sulphate and the compound under study, and the ferrous sulphate was alternately labelled with Fe^{55} and Fe^{59}. The solutions containing ferrous iron also contained 10 mg of ascorbic acid for every dose in order to prevent oxidation of the ferrous iron. Repeated analyses using the thiocyanate method[13] showed that in these flasks less than 3 per cent of the iron was present in ferric form even after one month's storage at room temperature.

Also included in this paper is a separate study concerning the absorbability of iron from different iron compounds when given as tablets for a longer time. The same general experimental design was employed using ferrous sulphate tablets as a reference in all subjects. One tablet containing ferrous sulphate corresponding to 30 mg of elemental iron was given 3 times a day on alternate days. These tablets were labelled with one of the radioiron isotopes. On the other days the compound under study, labelled with the other radioiron isotope, was also given as tablets 3 times a day. All tablets contained 30 mg of elemental iron and had a disintegration time of 15 minutes[14]. Tablets were given between meals for 24 days as outlined in figure 1. A blood sample was drawn 2 weeks after the last oral dose for deter-

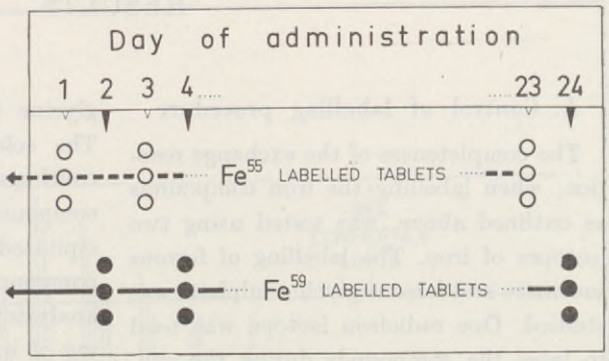

Fig. 1. Dosage schedule.

mination of Fe^{55} and Fe^{59} activity. Four compounds were compared in this way: ferrous sulphate, ferrous succinate, ferrous gluconate and ferrous glycine sulphate.

Preparation and labelling of iron compounds

All iron compounds used were reagent grade or prepared from reagent grade chemicals. The iron content was determined with the thiocyanate method using hydrogen peroxide as oxidant.[13].

The compounds were labelled by isotope exchange. Radioiron with high specific activity (not less than 2—3 μC/μg) was added to the solutions containing non-radioactive iron[12]. A complete exchange took place also with the complex iron compounds. A separate study of the completeness of the exchange was made (reported below).

MATERIAL

The subjects in this study were 15 normal healthy volunteers (N), 54 blood donors (BD), who had served as blood donors for varying time and never received any iron supplementation. Moreover, 11 patients (ID) with iron deficiency anemia or post haemorrhagic anemia without signs of continued bleeding were included in the material. The 8 subjects in whom ferric and ferrous sulphate were compared have been reported earlier[12].

RESULTS

A. Control of labelling procedure

The completeness of the exchange reaction, when labelling the iron compounds as outlined above, was tested using two isotopes of iron. The labelling of ferrous succinate and ferrous glycine sulphate was studied. One radioiron isotope was used to label the compounds during the synthesis. The compound was purified and dissolved together with a trace amount of an other radioiron isotope. The solution was given orally as a single dose and the ratio of the radioisotopes in a blood sample drawn 2 weeks later will thus be an expression for the precision of the analysis and the completeness of the exchange reaction.

Ferrous glycine sulphate was prepared in the following manner: Ferrous sulphate ($FeSO_4 \cdot 7H_2O$ pro analysi) was dissolved in boiling water under nitrogen, together with Fe^{59} as ferric chloride with the same specifications as previously. A warm solution containing equivalent amounts of glycine (Merck pro analysi) was added. The solution was slowly chilled under continued nitrogen bubbling and the iron compound, which was formed, was precipitated with 95 % ethyl alcohol. The compound was purified and found by analysis to be $FeSO_4$-glycine $\cdot 5H_2O$. Thirty mg of iron as Fe^{59} labelled ferrous glycine sulphate was dissolved together with a tracer dose of Fe^{55} as ferric chloride (see above). This mixture was given to three subjects. The results are shown in table I.

Ferrous succinate was prepared in the following way: to a solution of ferrous sulphate labelled with Fe^{59} a solution of equivalent amounts of sodium succinate was added. The precipitate was purified and dissolved together with a trace amount of Fe^{55} (as above). This solution was given to three subjects. The results are given in table II.

It is evident from table I and II that a complete exchange took place between the radioiron used in the synthesis of the

TABLE I.

Labelling of ferrous glycine sulphate with two radioiron isotopes. (For details see text.)

SUBJECT	"ABSORPTION" (per cent)		RATIO Fe^{55}/Fe^{59}
	Fe^{59}	Fe^{55}	
1-M-N	3.4	3.5	1.04
2-M-N	10.2	10.0	0.98
3-M-ID	27.1	26.3	0.97
Mean value: 1.00			

TABLE II.

Labelling of ferrous succinate with two radioiron isotopes. (For details see text.)

SUBJECT	"ABSORPTION" (per cent)		RATIO Fe^{55}/Fe^{59}
	Fe^{59}	Fe^{55}	
4-M-N	7.6	7.3	0.97
5-M-ID	14.5	14.3	0.98
6-M-ID	43.0	42.7	0.98
Mean value: 0.98			

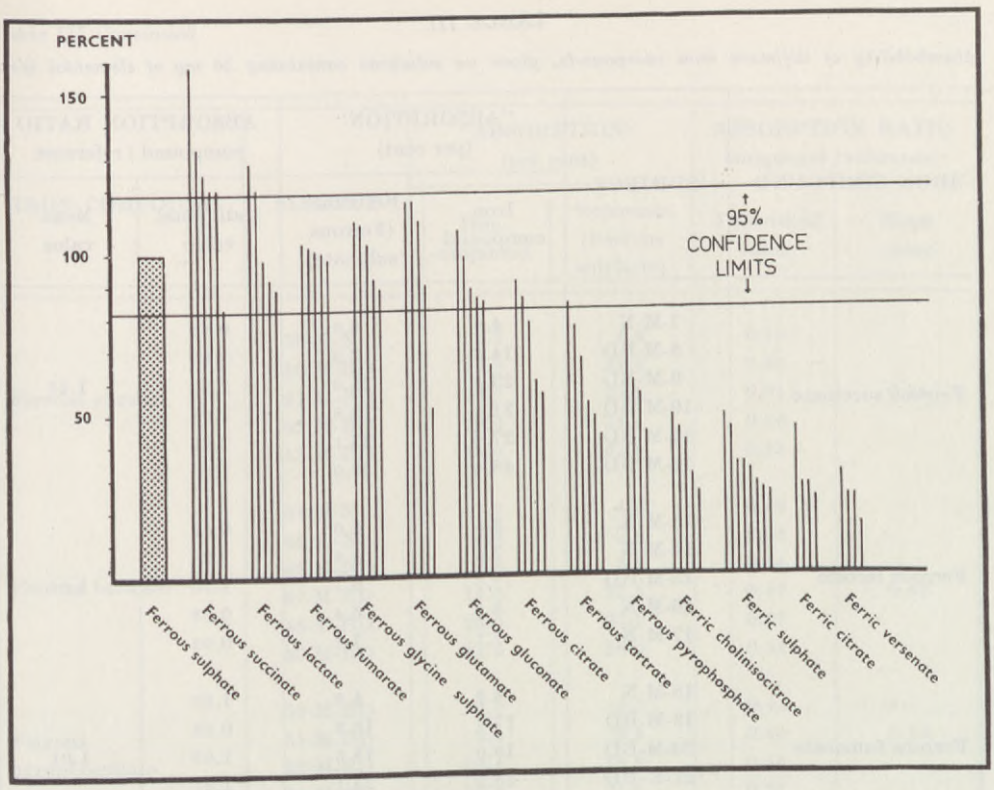

Fig. 2. Absorbability of different iron compounds (solutions). Individual values in relation to ferrous sulphate.

compounds and the trace amount of radioiron added to the labelled compound. The variation observed is not greater than that which can be explained by analytical errors (cf. labelling of ferrous sulphate with both isotopes in a previous study[12]).

B. Absorption of different iron compounds (solutions)

In this part of the study 30 mg of elemental iron was given as a solution for 10 days. Ferrous sulphate was labelled with one isotope and the compound under study was labelled with the other isotope. The solutions were given on alternate days for 10 days as described earlier[12]. The results are given in table III and graphed in figure 2. In this figure the individual absorption figures for the different compounds are graphed, expressed as a percentage of the absorption of iron from ferrous sulphate in the same individual. In figure 2 are also graphed the 95 per cent confidence limits of the individual day to day variation of the absorption of iron from ferrous sulphate, as found in a previous study in which this experimental design was used[12].

It is evident from figure 2 that the

TABLE III

Absorbability of different iron compounds, given as solutions containing 30 mg of elemental iron.

IRON COMPOUND	SUBJECT	"ABSORPTION" (per cent)		ABSORPTION RATIO compound / reference	
		Iron compound	Reference (Ferrous sulphate)	Individual value	Mean value
Ferrous succinate	7-M-N	4.9	6.0	0.83	1.23
	8-M-BD	14.8	12.3	1.20	
	9-M-BD	23.8	18.0	1.33	
	10-M-BD	31.4	19.8	1.58	
	11-M-BD	27.6	23.1	1.20	
	12-M-BD	48.8	38.9	1.25	
Ferrous lactate	13-M-N	3.5	4.0	0.89	1.06
	14-M-N	7.4	5.8	1.28	
	15-M-BD	7.3	6.1	1.20	
	16-M-N	6.3	6.4	0.98	
	17-M-N	7.1	7.8	0.92	
Ferrous fumarate	18-M-N	4.8	4.8	1.00	1.01
	19-M-BD	17.7	18.2	0.98	
	20-M-BD	19.0	18.6	1.03	
	21-F-BD	23.5	23.1	1.01	
	22-F-BD	34.6	34.2	1.02	
Ferrous glycine sulphate	23-F-N	4.2	3.8	1.09	1.01
	24-M-N	10.1	9.2	1.10	
	25-M-ID	48.2	52.7	0.92	
	26-M-ID	41.4	46.6	0.89	
	27-M-ID	69.9	67.6	1.03	
Ferrous glutamate	28-M-BD	22.1	19.0	1.16	0.97
	29-M-BD	10.0	19.3	0.52	
	30-M-BD	30.1	25.9	1.16	
	31-M-BD	33.4	30.2	1.10	
	32-M-BD	51.1	56.6	0.90	
Ferrous glyconate	33-M-BD	3.2	3.7	0.86	0.89
	34-M-BD	5.8	6.5	0.89	
	35-M-BD	15.2	15.3	0.99	
	36-M-BD	19.1	22.6	0.85	
	37-M-BD	16.5	25.3	0.65	
	38-F-BD	34.9	32.5	1.07	

Table III Continued

Table III Continued

IRON COMPOUND	SUBJECT	"ABSORPTION" (per cent)		ABSORPTION RATIO compound / reference	
		Iron compound	Reference (Ferrous sulphate)	Individual value	Mean value
Ferrous citrate	39-M-N	2.3	4.0	0.56	0.74
	40-M-BD	12.8	21.4	0.60	
	41-M-BD	23.2	25.5	0.91	
	42-M-BD	29.6	34.4	0.86	
	43-M-BD	49.0	62.8	0.78	
Ferrous tartrate	44-M-N	1.9	4.0	0.49	0.62
	45-F-N	4.2	7.8	0.53	
	46-F-BD	3.8	8.8	0.43	
	47-M-BD	14.9	22.3	0.67	
	48-M-BD	25.0	32.5	0.77	
	49-M-BD	31.9	38.0	0.84	
Ferrous pyrophosphate	50-M-BD	2.0	4.7	0.44	0.59
	51-M-BD	3.4	5.8	0.60	
	52-M-BD	10.4	18.6	0.56	
	53-M-BD	16.3	21.0	0.77	
Ferric cholin-isocitrate	54-M-BD	8.1	26.4	0.30	0.38
	55-M-BD	12.6	27.8	0.45	
	56-F-BD	15.4	31.7	0.49	
	57-M-BD	13.5	32.7	0.41	
	58-M-BD	9.0	36.4	0.25	
Ferric citrate	59-M-BD	3.6	15.4	0.23	0.31
	60-M-BD	6.2	22.8	0.27	
	61-M-BD	14.8	32.6	0.45	
	62-M-BD	12.8	47.6	0.27	
Ferric versenate	63-M-BD	3.8	15.5	0.24	0.24
	64-M-BD	3.3	21.3	0.15	
	65-M-BD	8.6	27.9	0.31	
	66-M-BD	10.6	43.5	0.24	

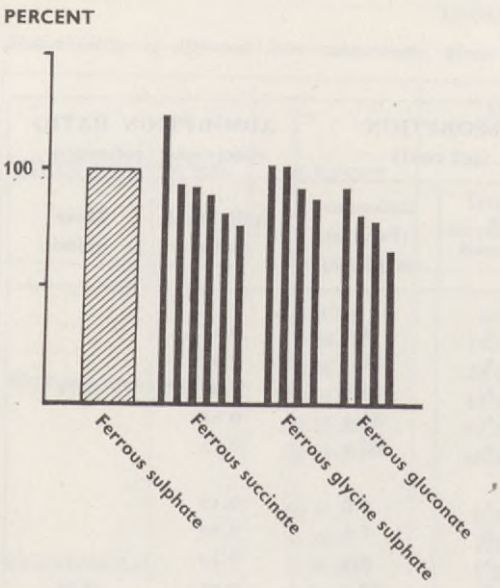

Fig. 3. Absorbability of different iron compounds (tablets). Individual values in relation to ferrous sulphate.

absorbability of iron from different iron compounds differs markedly. The ferric compounds are less absorbed than the ferrous. Of the ferrous compounds ferrous citrate, tartrate and pyrophosphate are significantly less absorbed than the other. Only one compound, ferrous succinate, was more absorbed than ferrous sulphate (when solutions were compared).

The amount of iron absorbed, expressed as per cent of the amount of iron administered, is given in the tables as "Absorption" (per cent). These figures are only estimates as discussed in a previous paper[12] but the errors in these estimates do not influence the absorption ratio.

C. Absorption of different iron compounds (tablets)

In this part of the study 30 mg of elemental iron was given in tablet form 3 times a day between meals. On every second day ferrous sulphate tablets were given and on the other days the tablets containing the iron compound under study were given. This dosage schedule is shown in figure 1.

The results are given in table IV and are graphed in figure 3. There is probably no real difference in absorbability of iron from these 4 compounds.

The mean value of the absorption ratios of ferrous succinate, glycine sulphate and gluconate are not significantly different statistically. However, since the variation of the absorption of iron from ferrous sulphate tablets has not been studied, it is impossible to decide if the absorption of iron from any one of the three compounds is significantly lower than from ferrous sulphate.

TABLE IV

Absorbability of different iron compounds, given as tablets containing 30 mg of elemental iron three times a day.

IRON COMPOUND	SUBJECT	"ABSORPTION" (per cent)		ABSORPTION RATIO compound / reference	
		Iron compound	Reference (Ferrous sulphate)	Individual value	Mean value
Ferrous succinate	67-F-N	3.7	3.9	0.94	0.93
	68-M-BD	7.8	8.4	0.93	
	69-M-BD	8.9	10.9	0.82	
	70-M-ID	21.8	17.7	1.23	
	71-M-ID	25.1	28.3	0.89	
	72-M-BD	25.4	33.4	0.76	
Ferrous glycine sulphate	73-F-BD	10.8	10.6	1.03	0.97
	74-F-BD	9.7	10.8	0.89	
	75-F-BD	17.2	18.5	0.93	
	76-F-ID	23.4	22.7	1.03	
Ferrous glyconate	77-M-BD	5.7	8.9	0.65	0.84
	78-M-BD	8.5	11.0	0.78	
	79-M-BD	11.9	14.9	0.80	
	80-M-ID	20.8	22.3	0.93	

DISCUSSION

Introduction

The great number of iron compounds used to-day in the oral treatment of iron deficiency and the disparity in views regarding their therapeutic value are probably mainly a result of the difficulties in making a valid evaluation of the relative merits of different compounds.

When the same amount of iron is given, no differences in side-effects have been observed in the few published controlled studies[1]. With increasing doses of iron, more side-effects were encountered in a double-blind study in which ferrous sulphate tablets were given in doses of 30 mg of elemental iron three times a day to 100 mg three times a day[2]. Because of these facts the therapeutic value of different iron compounds will be determined mainly by their relative absorbability. In almost all previous studies on the therapeutic value of different compounds such comparisons have been based on determinations of the relative absorbability using one method or another.

The therapeutic value of iron compounds

have also been assessed from toxicity determinations in animals. Acute oral iron toxicity studies in animals have occasionally served as a basis for postulating differences in tolerance of therapeutic doses of various iron compounds in man. The validity of such conclusions must be questioned since the factors determining the acute oral iron toxicity (e.g. the solubility and dissociation of an iron compound) are not identical with the factors determining the side-effects in oral iron therapy. Although the solubility of an iron compound in the gastric and/or intestinal juices may in some cases be a determining factor with respect to the acute oral iron toxicity, it does not necessarily have any connection with the side-effects seen in therapeutic dosage.

The preceding reasoning justifies the contention that the absorbability of iron from a compound is the main factor determining the therapeutic value.

It has repeatedly been shown that less iron is absorbed from ferric than from ferrous compounds. Despite numerous studies of the absorption of iron from various ferrous compounds it may be summarized that there is no evidence in the literature in favour of one compound or another. Iron compounds and preparations have often been introduced with claims of superiority with respect to absorbability. However, such claims have usually been based on studies lacking adequate controls.

There is thus a need of a critical evaluation of the absorbability of different iron compounds especially with regard to present disparities in view and the frequent occurrence of iron deficiency. A comprehensive discussion of various sources of errors in previous methods used to study the absorbability of iron compounds has not been published. Because of this such a review is included in this paper as a background for the discussion of the results obtained.

Earlier methods

In most previous studies the regeneration rate of hemoglobin during iron therapy in subjects with iron deficiency anemia has been the basis for comparisons of the absorbability of iron from different compounds[3—8].

The regeneration rate of the hemoglobin concentration will be an expression for the absorption of iron under the following conditions:

1. All absorbed iron must be utilized for synthesis of hemoglobin and must be the only limiting factor for the regeneration rate, i.e. the erythropoiesis must not be depressed by other diseases such as infections, renal disorders etc. This condition also demands that the magnitude of the oral dose be chosen in such a way that the optimal amount absorbed is not greater than that needed for an optimal erythropoiesis.

2. Extraneous loss of iron must be excluded during the period studied (e. g. bleeding, pregnancy). Moreover, there should be no increased random destruction of red cells.

3. Cases with a considerable amount of iron in the iron stores must be excluded since otherwise it would be impossible to know how much iron, utilized in the regeneration of hemoglobin, comes from

absorption and how much comes from stores.

4. The blood volume must be constant. To calculate the absolute amount of iron absorbed from the increase of the hemoglobin concentration the blood volume must be known.

When comparing the absorbability of iron from different iron compounds the same amount of elemental iron must be given to the different groups. The dosage schedule must be the same, the disintegration time of the tablets identical etc. Besides the conditions mentioned above (1—4) the following considerations have to be taken into account:

5. The initial hemoglobin level should be about the same in groups compared since the regeneration rate is faster when the hemoglobin level is lower. Preferably, the hemoglobin level should be of the same *relative* magnitude, because for example — a regeneration rate from 8 to 12 g hemoglobin per 100 ml blood will probably not be the same in two subjects in whom the final normal hemoglobin concentration is 12 and 16 g per 100 ml respectively. A method for comparisons between individuals of the regeneration rate has previously been described[15].

6. The observation period has to be limited to the regeneration period and must be finished before some individual reaches his normal hemoglobin level.

7. The age and sex distribution should be about the same in compared groups. The degree of erythropoietic stimulation cannot be considered to be independent of sex and age as these factors are known to influence the individual normal hemoglobin concentration.

8. Besides the above mentioned known factors, which influence the regeneration rate of hemoglobin, it is important to randomize the individuals between groups to decrease the influence of other more or less unknown factors.

The great number of factors influencing a measurement of the absorption of iron from the regeneration rate of hemoglobin makes it almost impossible to draw valid conclusions of the absorbability of different iron compounds. This is especially true when materials studied by different authors are to be compared, inasmuch as such comparisons are further obscured by the fact that the method of chosing and selecting the material is very seldom described.

The selection of a suitable material and its division into comparable groups may be very difficult. The great individual variation in absorption of iron and regeneration of hemoglobin thus necessitates comparisons between fairly large groups in order to be able to draw valid conclusions of the absorbability of different iron compounds. Some of the above mentioned ideal conditions for comparative studies may be of more theoretical than practical importance. However, the great number of factors which have to be taken into consideration is the probable reason why so few quite adequate comparative studies of the absorbability of iron from different compounds have been published. An example of a study in which most of these factors have been considered is the on by O'Sullivan, Higgins and Wilkinson[8].

The utilization coefficient is a concept

which has led to much confusion in comparative evaluations of iron compounds. This coefficient was originally defined as the percentage of iron absorbed from a dose which was just sufficiently large to give a regeneration rate of hemoglobin corresponding to 1 per cent per day of the final hemoglobin mass when the severity of anemia was about 50 %[4 5 6].

However, it is almost impossible to find such a dosage level for a certain individual and in almost all studies no attempts have been made to find out this minimum dosage. Usually the amount of iron given was more than that which could be expected to be necessary for an optimal regeneration rate. Under such conditions the utilization coefficient will be proportional to the ratio of the regeneration rate of hemoglobin to the amount of iron administered. This coefficient will thus be a meaningless expression without any relation to the real absorption of the iron compounds and mainly a figure inversely related to the dose.

Quite paradoxical utilization coefficient (> 100 per cent) have been reported in materials treated with small iron doses and including subjects with acute posthaemorrhagic anemia with probably normal iron stores. Most of the iron utilized in the regeneration of hemoglobin must have been derived from these iron stores.

The *reticulocyte response* to oral iron therapy has also been used to quantitate iron absorption.[4] The same general sources of error are inherent in this method. Moreover, since additional factors besides iron absorption may influence the reticulocyte response, this method does not offer any advantage in quantitative studies.

The *plasma iron increase* after a single oral iron dose has also been used as a basis for a comparison of the absorbability of different iron compounds. However, the magnitude of the plasma iron increase is determined by a number of other factors besides absorption since the plasma iron level is a resultant not only of the absorption rate of iron but also of the rate of inflow of iron from storage compartments and the rate of the outflow of iron to bone marrow and stores[16 17]. The greatly varying absorption on different days within the same individual[12 18], reduces further the practical possibilities of comparing the absorbability of different compounds. Qualitative information of relative absorbability may be obtained if repeated studies are made in the same individual and if the material is sufficiently large. However, fairly small iron doses have to be given. The maximal plasma iron increase may otherwise be merely a measure of the unsaturated iron binding capacity (UIBC)[19].

Own results

From the preceding discussion of earlier methods and their sources of error it is evident that a method, in which each subject serves as his own control, offers many advantages. The homogenity of the material with respect to severity of anemia, age, sex, erythropoietic activity, blood volume changes etc. will not fundamentally affect the results employing the experimental design of the present study.

The ratio figures obtained will be an expression for the average absorption and the average utilization of the absorbed iron from the different compounds. When

the absorbability of different iron compounds given in tablet form was studied, blood samples were drawn not only 2 weeks after the last oral dose but also 1 and 3 months after the last oral dose. Since the same absorption ratio was obtained on the three occasions, it can be concluded that there was no difference in utilization of absorbed iron from different compounds. The ratio figures will then be a real expression for the absorbability of iron from different compounds.

The results obtained in this study showed that much less iron was absorbed from the ferric compounds than from the ferrous compounds. The differences between the ferric compounds were not great. However, it is interesting to observe that the lowest absorbability was shown by ferric versenate, a compound in which iron is strongly complex-bound. The fact that iron was absorbed at all from this compound suggests a splitting of the complex in the gastrointestinal tract as discussed by WILL and VILTER[20], and LARSEN, BIDWELL and HAWKINS[21].

As the absorbability of the ferric compounds was only 1/3—1/7 of ferrous sulphate the use of these compounds in oral iron therapy cannot be considered rational. This point has been discussed in a previous paper[12].

Three of the ferrous compounds were found to be less absorbed than the others (ferrous tartrate, citrate and pyrophosphate). In these compounds an appreciable part of the iron exists as complex ions. It is probable that the lower absorbability of these compounds is related to this common physico-chemical property.

Ferrous succinate was the only compound which was better absorbed than the totally dissociated ferrous sulphate. The average figure was lower than reported earlier[10 11], probably due to methodological improvements. Further studies are necessary in order to be able to interpret the observed increased absorption of iron from a solution of ferrous succinate since there is no immediate simple explanation.

Treatment with iron tablets is the usual form of oral iron therapy. For this reason a comparative study of the absorbability of iron from some compounds administered in tablet form was included in the present investigation. Four common compounds were studied and 30 mg of elemental iron (one tablet) was given 3 times a day.

Tablets were given for 24 days to reduce a possible increase in the variation of the basic absorption of iron from tablets due to variation in disintegration time of the tablets of the same compound, variation in transfer velocity of the tablets along the gastrointestinal tract, variation in interval of time between intake of tablets and meals etc. Moreover, a 24 day experimental period corresponds more closely to conditions during oral iron therapy than the 10 day period used in our earlier studies. However, the 24 day studies are much more difficult to perform and have thus been limited to these 4 compounds.

The absorption of iron from tablets containing ferrous succinate was not greater than from ferrous sulphate tablets, in contrast to the observed difference in absorbability of iron from solutions of these compounds. This may probably be due to the considerably lower rate of dissolution of ferrous succinate (in relation to ferrous sulphate) which may counteract

its slightly increased absorbability. There were no statistically significant differences in absorbability between these four compounds when administered as tablets.

Some of the results obtained in this study support earlier observations by other authors, other results are opposite to earlier findings and views. It is probable that the discrepancies are mainly due to the numerous sources of error in earlier methods which have been decreased or eliminated with the double radioiron method used in the present study as already discussed.

The results show that there may be great differences in absorbability of iron from different iron compounds. These differences are of such a magnitude that they have to be considered in the practical selection of an iron compound for oral iron therapy.

SUMMARY

The therapeutic value of an iron compound was concluded to be mainly related to its absorbability. A double radioiron method in which each subject served as its own control, facilitated a quantitative comparison of the absorbability of different iron compounds.

A total of 14 iron compounds were studied. Ferrous compounds were clearly better absorbed than ferric. Of the 4 ferric compounds studied the lowest absorption was obtained from ferric versenate, a compound in which iron is strongly complex-bound.

Of the 10 ferrous compounds studied, a lower absorption was obtained from ferrous citrate, ferrous tartrate and ferrous pyrophosphate, compounds in which a considerable part of the iron is complex-bound.

Slightly but significantly more iron was absorbed from ferrous succinate in relation to ferrous sulphate when the compounds were given as solutions. When these same compounds were compared in tablet form no difference was observed.

No iron compound was thus found to be better absorbed than slightly soluble, quite dissociated ferrous compounds (such as ferrous sulphate) under therapeutical conditions. The observed differences in absorbability of different iron compounds are of such a magnitude that they have practical importance in oral iron therapy.

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FROM THE DEPARTMENT OF MEDICINE II (HEAD: PROFESSOR ERIK WASSÉN), UNIVERSITY OF GÖTEBORG, SAHLGRENSKA SJUKHUSET, GÖTEBORG, SWEDEN.

INFLUENCE OF MEALS ON IRON ABSORPTION IN ORAL IRON THERAPY

By

HANS BRISE

INTRODUCTION

In order to achieve the most effective oral iron therapy, it is important to study factors influencing the absorption of iron. It is known that certain components in the food may interfere with the absorption of iron (e. g. phosphates[1 2] phytates[3 4] eggs[5] and bread[6]) and because of that it has been suggested that iron should be taken between meals[3 7 8]. However, to day it is generally recommended to take the iron doses with or immediately after meals to reduce gastric irritation[9—14].

In order to design the most rational oral iron therapy, which will give the greatest absorption of iron with the fewest side-effects, it is necessary to know:

(a) the relationship between the magnitude of the dose and the frequency and severity of side-effects, (b) the dependence of this relationship on the way iron is given in relation to meals, and (c) the effect of meals on the absorption of iron. There is a lack of quantitative information on all three points.

The purpose of the present paper is to quantitate the effect of meals on the absorption of iron using an iron dose within the therapeutic range. Iron absorption was compared when iron was administered in two different ways in relation to meals within the same subject according to the same experimental design as previously applied[15]. Iron was labelled with two different radioiron isotopes when administered in two different ways in relation to meals.

The investigation was devided into two parts. In the first part (A), an iron solution was given once a day for 10 days, in a fasting state or after a standardized light meal. In the second part (B), iron tablets were given three times a day for 24 days, between or with the ordinary meals.

It was found that less iron was regularly absorbed when given with meals than when given in a fasting state or between meals.

METHODS AND MATERIAL

The general experimental outline was the same as in previous studies using the double radioiron method[15]. The details of the experimental design and the material in the two parts of this study are reported together with the results.

The methods used in preparation of blood samples, analysis of Fe^{55} and Fe^{59} and calculations were the same as earlier published[15 16].

RESULTS

A. Effect of standardized light meal.

Nine healthy volunteers were included in this study. Six of these were blood donors (BD).

A solution containing 30 mg of elemental iron (ferrous sulphate) was given in the morning for 10 days. On alternate days when the iron solutions were labelled with one of the two radioiron isotopes (Fe^{55} or Fe^{59}) used, the solution was given after an overnight fast and no food or drink (except the usual rinse water[15]) was allowed for an additional 2 hours. On the other alternate days, when the iron solutions were labelled with the other radioiron isotope a light meal was given 1/2 hour before administration of the iron solutions. This meal was composed of 1 glass of milk, 1 sandwich (white bread) with cheese and 1 cup of coffee without sugar and cream. A blood sample was drawn 2 weeks after the last oral iron dose for analysis of Fe^{55} and Fe^{59}. The results are given in table I.

A reduced absorption of iron was found in all cases when the iron solutions were given half an hour after the meal compared with the absorption in the fasting state. The relative inhibiting effect of food on iron absorption seems to be greater (on a percentage basis) in individuals with lower absorption of iron. However, the decrease in amount of iron absorbed when given after the meal tended to be greater at high absorption levels. In the six blood donors on an average 2.6 mg less iron was absorbed of the 30 mg iron dose when given after the meal.

B. Absorption of iron from tablets given three times a day with or between meals.

Four healthy blood donors (BD) were included in this study.

One tablet containing 30 mg of elemental iron (as ferrous sulphate) was given 3 times a day for 24 days. On alternate days when the tablets were labelled with one of the radioiron isotopes the tablets were taken together with breakfast, lunch and dinner. On the other alternating days the tablets

TABLE I

Influence of a light meal on iron absorption. Iron administered 2 hours before and 1/2 hour after a standardized meal on alternate days for 10 days in each subject.

SUBJECT	"ABSORPTION" (per cent)		ABSORPTION RATIO after/before	Absorption decrease (estimated) mg Fe
	2 hs before meal	1/2 h after meal		
1-M-N	4.6	2.0	0.44	0.8
2-F-N	4.7	1.1	0.23	1.1
3-F-N	5.3	2.2	0.42	0.9
4-F-BD	16.6	6.9	0.42	2.9
5-M-BD	18.0	15.0	0.83	0.9
6-F-BD	21.5	13.7	0.64	2.3
7-M-BD	24.2	13.5	0.56	3.2
8-M-BD	33.9	27.0	0.80	2.1
9-M-BD	43.5	30.9	0.71	3.8
ABSORPTION RATIO, after/before: Mean value 0.56				

were labelled with the other radioiron isotope and taken "between meals" according to the following schedule: the first tablet was taken in the morning after an overnight fast and breakfast was eaten 1—2 hours later; the second tablet was taken in the afternoon 1—3 hours after lunch (2—4 hours before dinner); the third tablet was taken 2—3 hours after dinner. The dosage schedule is outlined in figure 1. Each subject noted in a record every day the time for the meals and the time he took the tablets. A blood sample was drawn 2 weeks after the end of the medication. Standard solutions were prepared from 6 tablets. The total activity given to each subject was 20 μC of each isotope. The standard variation of the activity of

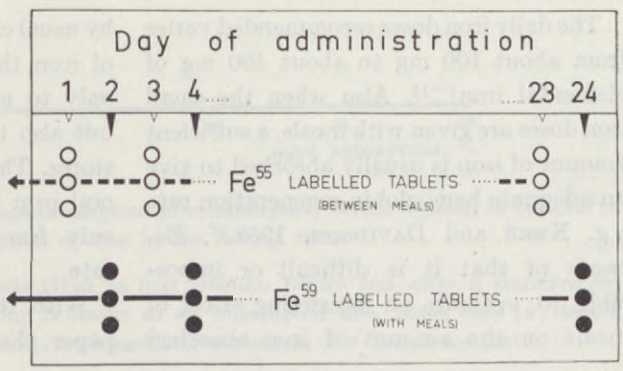

Fig. 1. *Dosage schedule.* One iron tablet 3 times a day given between and together with meals on alternate days for 24 days and labelled with two different radioiron isotopes.

TABLE II

Comparison of iron absorption at two different dosage schedules. One iron tablet 3 times a day given between and together with meals on alternate days for 24 days in each subject.

SUBJECT	"ABSORPTION" (per cent)		ABSORPTION RATIO	Absorption decrease (estimated) mg Fe
	between meals	with meals	with meals/between meals	
10-M-BD	6.0	3.6	0.60	2.2
11-M-BD	15.6	7.8	0.50	7.0
12-M-BD	15.9	7.9	0.49	7.2
13-M-BD	33.2	30.3	0.91	2.6
ABSORPTION RATIO, with meals/between meals: Mean value 0.63				

the tablets was found to be $\pm 2.7\%$. The disintegration time was short (15 minutes — measured according to the British Pharmacopeia, 1958) and the same for all tablets.

The "between meal" schedule was chosen as to correspond to what might be performed in practice. Each subject followed the given schedule each day.

The results are given in table II. It was found that there was a greater absorption of iron when taking the tablets "between" meals than when taking them together with the meals. The reduction of the absorption was of the same relative magnitude in these subjects as in the group (A.) in which iron was given as a solution.

DISCUSSION

The daily iron doses recommended varies from about 100 mg to about 400 mg of elemental iron[8—14]. Also when the small iron doses are given with meals, a sufficient amount of iron is usually absorbed to give an adequate hemoglobin regeneration rate e.g. KERR and DAVIDSON, 1958.[17]. Because of that it is difficult or impossible to evaluate an interfering effect of meals on the amount of iron absorbed by usual clinical observations. The purpose of iron therapy in iron deficiency is not only to normalize the hemoglobin value but also to restitute tissue iron and iron stores. Therefore the real effectiveness of oral iron therapy can not be determined only from the hemoglobin regeneration rate.

With the method used in the present paper the relative amounts of iron ab-

sorbed under the two sets of condition (e.g. administration of iron with and between meals) can be quantitated. The lower absorption of iron observed, when given with or a short time after a meal, can be explained both by a chemical interaction of food components on iron (formation of insoluble or undissociated iron compounds as e.g. phosphates and phytates) and by a reduction of the concentration of iron by the bulk of the meal and by gastric and intestinal juices. All these factors can be expected to interfere with the absorption.

In a study on the effect of food factors on iron absorption by SHARPE, PEACOOK, COOKE and HARRIS[3] it was observed that the absorption of iron from a breakfast was only one fifth of that from a water solution, containing the same amount of elemental iron. It was concluded that medicinal iron should be more effective if administered between meals. However, the total amount of iron given was only 8 mg including the iron in the food. It is impossible to know to what extent the radioiron added had exchanged with the food iron. If this exchange was incomplete the absorption of iron should have been overestimated, calculated from the absorption of radioiron. Therefore, when comparing the results by SHARPE et al. with the present results, the balance of evidence suggests that the reducing effect of a meal is more pronounced when a low iron dose is given than when a higher dose is given. Probably the reducing effect of a

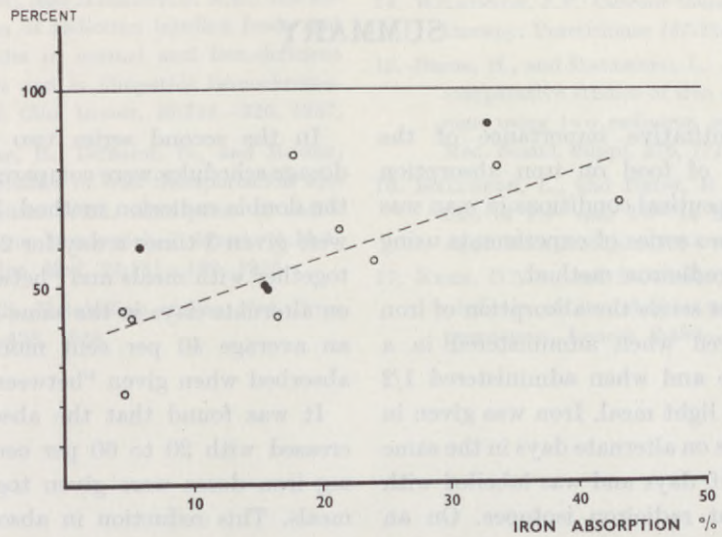

Fig. 2. Absorption ratio as a percentage (absorption after/absorption before a meal) in relation to the absorption of iron in the fasting state.

Open rings (o) denote those subjects given an iron solution before and after a standardized meal. The regression line of this group is drawn as an interrupted line. Black dots (•) denote those subjects given iron tablets with and between meals. For details see text.

meal is not only related to the size of the iron dose, but also to the size and composition of the meal.

In the present paper it was observed that the reduction of the absorption of iron, when iron was administered together with meals, seems to be less pronounced in those subjects absorbing a greater amount of iron in the fasting state. This is evident from figure 2.

The relationship between absorption decrease and absorption in the fasting state was studied statistically in those 9 subjects, in whom a solution of iron was given before and after a standardized light meal. The regression line is graphed in figure 2. The correlation coefficient (r) was 0.73, ($p<0.05$). As a comparison the results from the subjects given tablets together with and between meals are also included in figure 2 (black dots).

It seems reasonable to assume that iron can be administered together with meals in patients with iron deficiency without a substantial decrease in the amount of iron absorbed.

However, to be able to give a conclusive answer to the question of the most suitable dosage schedule in oral iron therapy, more thorough studies are needed concerning the effect of meals on the absorption of iron at various dose levels in relation to side-effects.

SUMMARY

The quantitative importance of the interference of food on iron absorption under therapeutical conditions in man was studied in two series of experiments using the double radioiron method.

In the first series the absorption of iron was compared when administered in a fasting state and when administered 1/2 hour after a light meal. Iron was given in the two ways on alternate days in the same subject for 10 days and was labelled with two different radioiron isotopes. On an average the absorption was reduced by half when the iron was given after the meal.

In the second series two therapeutic dosage schedules were compared also using the double radiorion method. Iron tablets were given 3 times a day for 24 days, and together with meals and "between" meals on alternate days in the same subject. On an average 40 per cent more iron was absorbed when given "between" meals.

It was found that the absorption decreased with 20 to 60 per cent when 30 mg iron doses were given together with meals. This reduction in absorption was found to be inversely related to the absorption of iron in the fasting state.

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FROM THE DEPARTMENT OF MEDICINE II (HEAD: PROFESSOR ERIK WASSÉN), UNIVERSITY OF GÖTEBORG, SAHLGRENSKA SJUKHUSET, GÖTEBORG, SWEDEN.

EFFECT OF SURFACE-ACTIVE AGENTS ON IRON ABSORPTION

By

HANS BRISE

INTRODUCTION

In a previous paper the absorbability of different iron compounds was compared[1]. The results indicate that it is very improbable that iron compounds can be found from which more iron is absorbed than from an easily soluble, dissociated iron salt (e.g. ferrous sulphate). The only possibility of discovering oral iron preparations, from which more iron is absorbed, seems therefor to recide in a search for substances, which in one way or another promote iron absorption as such.

Various surface-active agents have been used in pharmaceutical preparations i.a. to improve absorption. In 1954 WISSLER, BETHARD, BARKER and MORI reported that polyoxyethylene sorbitan monolaurate (Tween 20) increased the gastrointestinal absorption of iron in hamsters[2].

The present study is based on a double radioiron technic. The same amount of iron was given for 10 days and labelled with different isotopes when given with and without surface-active substance on alternate days, thus making each subject his own control as in previous study.[4]

It was found that none of the four surface-active agents studied significantly increased the absorption of iron.

METHODS AND MATERIAL

Every morning, after an overnight fast, a 25 ml solution containing 30 mg of elemental iron as ferrous sulphate was given orally for 10 days. The preparation of the solutions was the same as earlier reported[3]. Every second day the iron doses were labelled with one of the radioiron isotopes (Fe^{55} or Fe^{59}) and every second

day, when a surface-active agent also was given, iron was labelled with the other isotope.

The following surface-active agents were used:

Dioctylsodiumsulfosuccinate (*Aerosol-OT*, American Cyanamid Comp.) was administered as a powder containing 150 mg of the substance. The powder was swallowed together with the iron solution. The flasks containing the iron solution were rinsed twice and the rinse water was also taken as previously described.[4]

Polyoxyethylene sorbitan monolaurate (*Tween* 20 — Atlas Powder Comp.) was taken as a solution in a separate flask containing 400 mg Tween 20 in 15 ml. The Tween 20 solution was taken immediately after the iron solution.

Sodium lauryl sulphate (U.S.P. 16) was dissolved in the iron solutions given on alternate days as a dose of 200 mg.

Bile acids were given in gelatine capsules. Two capsules containing 146 mg cholic acid and 37 mg dehydrocholic acid (prepared from Fellesan tablets — A.B. Pharmacia, Sweden) were taken together with the iron solutions on alternate days.

A blood sample was drawn 2 weeks after the last oral dose and the ratio of the absorption of iron when given with and without a surface-active agent was calculated as previously described [3 4].

The material in this study includes 2 healthy male subjects (N). and 16 healthy blood donors (BD), who had never received any iron supplementation.

RESULTS

The results are given in table I. The effect of Tween 20 was studied in 4 subjects. No increase in absorption was noted in any of these subjects.

When dioctylsodiumsulfosuccinate was given together with iron a slight increase in absorption was observed in 3 of the 6 subjects studied. The mean absorption ratio did not significantly differ from the mean absorption ratio when only ferrous sulphate was given for 10 days in 24 subjects ($M = 1.01$, standard error of mean ± 0.02) as reported in an earlier paper[4]. It is thus probable that the somewhat higher mean absorption ratio (1.07) observed in this group is due to the normal variation of the absorption and is not an effect of dioctylsodiumsulfosuccinate on the absorption.

The same is also true for sodium laurylsulphate, in which case the mean absorption ratio also was 1.07. A mixture of cholic and dehydrocholic acid was likewise found not to increase the absorption of iron in 4 subjects.

TABLE I

Effect of surface-active agents on iron absorption.

Surface-active agent	SUBJECT	"ABSORPTION" (per cent)		ABSORPTION RATIO with/without surface-active agent	
		without surface-active agent	with surface-active agent	Individual value	Mean value
Polyoxyethylene sorbitan monolaurate (Tween 20) 400 mg	1-M-BD	15.5	16.9	1.09	
	2-M-BD	31.5	33.5	1.07	1.04
	3-M-BD	23.5	22.1	0.94	
	4-M-BD	44.8	46.5	1.04	
Dioctylsodium-sulfosuccinate (Aerosol-OT) 150 mg	5-M-BD	4.6	5.4	1.16	
	6-M-BD	15.7	19.0	1.21	
	7-M-BD	20.7	20.7	1.00	1.07
	8-M-BD	26.5	31.3	1.18	
	9-M-BD	33.5	31.1	0.93	
	10-M-BD	41.4	37.8	0.91	
Sodium lauryl sulphate 200 mg	11-M-N	5.6	6.6	1.18	
	12-M-N	6.1	7.9	1.29	1.07
	13-M-BD	16.0	16.0	1.00	
	14-M-BD	29.5	23.5	0.80	
Cholic acid 146 mg and Dehydrocholic acid 37 mg	15-M-BD	11.1	11.6	1.05	
	16-M-BD	17.3	16.7	0.97	1.03
	17-M-BD	21.5	22.2	1.03	
	18-M-BD	33.2	35.9	1.08	

DISCUSSION

The available area of absorption may considerably affect the amount of a substance absorbed. Especially, this may be true for substances, which normally are only partially absorbed. Therefore the administration of surface-active agents might theoretically be thought to increase the absorption of iron.

The amounts of surface-active agents given together with the iron solutions were as great as might be used therapeutically in iron tablets. It is evident from the results that it is not possible to increase the absorption of iron using these substances.

The observation by WISSLER et al.[2] that Tween 20 increased the absorption of iron in hamsters is not comparable with the

present findings. The animals were given large amounts of Tween 20 (5 per cent of the weight of the rations) for long time (from 8—20 weeks). EAGLE et. al. had earlier observed marked cirrhotic changes in the livers of young hamsters which were fed polyoxyethylene derivatives for longer periods of time.[5] In the study by WISSLER et al. early cirrhosis was also observed in occasional livers. It is thus probable that the increased iron absorption in the hamsters may have been due to some toxic action of Tween 20 and was not an effect directly related to a decrease of the surface-tension of the gastrointestinal content.

From these animal studies and from the present observation that Tween 20, in smaller amounts, did not increase the absorption of iron from ferrous sulphate in humans it can be concluded that there is no rational basis using Tween 20 in pharmaceutical iron preparations. This conclusion can also be extended to the other agents studied — dioctylsodium-sulfosuccinate, sodium lauryl sulphate and bile acids.

SUMMARY

The effect of surface-active agents on iron absorption was studied in 18 subjects using a double radioiron method, where each subject served as his own control. No significant increase of the absorption of iron was observed with any of the compounds studied (Tween 20, dioctylsodium-sulfosuccinate, sodium lauryl sulphate and bile acids).

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EFFECT OF ASCORBIC ACID ON IRON ABSORPTION

By

HANS BRISE AND LEIF HALLBERG

INTRODUCTION

The repeated observation that ferrous iron is better absorbed than ferric[1–4] lead to studies on the effect of reducing substances as ascorbic acid on the iron absorption. It was found that ascorbic acid increased the absorption of ferric iron and of food iron[2 5]. In studies in dogs [6] and rats [7 8] it was found that the amount of iron absorbed from ferrous sulphate was significantly increased when administered together with ascorbic acid.

In human subjects a greater plasma iron increase was observed when iron was given together with a large dose of ascorbic acid[9]. However, when a quantitative method was used to determine the absorption of ferrous iron, no effect of ascorbic acid was observed if huge amounts of ascorbic acid were not used, and it was concluded, that the addition of ascorbic acid to ferrous iron salts offered no practical advantage in iron therapy[10].

In a previous paper a method was described in which each subject served as his own control and a more exact quantitation of the effect of various substances on the absorption of iron was made possible[11]. A reevaluation of the effect of ascorbic acid on iron absorption using this method was considered to be important from both a theoretical and a practical point of view.

In the present paper it was shown that ascorbic acid, when given in sufficient amounts, increased the absorption of ferrous iron and that the absorption promoting effect increased with increasing amounts of ascorbic acid. This effect is probably mainly exerted in the gastrointestinal lumen inasmuch as intravenous administration of ascorbic acid was found to affect neither iron turnover nor iron absorption.

METHODS AND MATERIAL

The general experimental design was the same as in previous studies[11 12 13]. Unless otherwise stated, a ferrous sulphate solution containing 30 mg of elemental iron and labelled with radioiron was given orally every morning after an overnight fast for 10 days. Fe^{55} and Fe^{59} were used as labels on alternate days. Every second day ascorbic acid was given as tablets together with the iron solution. From analysis of Fe^{55} and Fe^{59} in a blood sample[14] drawn 2 weeks after the last oral iron dose, the effect of ascorbic acid on the absorption of iron could be determined, thus making each subject his own control as in previous studies.[11]

Studies of this kind were made in 42 subjects [6 healthy volunteers (N), 1 case of pernicious anemia during treatment (PA), and 35 blood donors (BD)]. The iron doses given together with ascorbic acid were labelled with Fe^{59} in 18 subjects, with Fe^{55} in 22 subjects. Twenty subjects were given ascorbic acid on the odd days of the study. The other 22 subjects were given ascorbic acid on the even days of the study.

In four additional subjects 300 mg of ascorbic acid was given intravenously instead of orally on every second day. These injections were given 5 minutes before the oral doses. These subjects were in-patients without any known hematological disorder, infection, liver or renal disease. The effect of ascorbic acid on plasma iron turnover was studied in one female and one male healthy medical student.

RESULTS

1. EFFECT OF ASCORBIC ACID ORALLY ON IRON ABSORPTION

The results are summarized in table I. The term "Absorption" means absorbed iron found in the estimated red cell mass two weeks after the last oral iron dose. The figures for absorption are only given to facilitate comparisons between individuals. The systematic errors in the estimation of the absorption do not affect the accuracy of the ratio figures as discussed in a previous paper[11].

It is shown in table I that more iron was absorbed when given together with ascorbic acid. However, a marked effect was observed only when 200 mg or more of ascorbic acid was given together with 30 mg of iron. There was a considerable variation in the effect of ascorbic acid between individuals. Part of this variation is necessarily related to the basic variation in absorption of iron on different days[11]. Another part of the variation may be related to a varying effect of ascorbic acid in different individuals and also in the same individual on different days.

TABLE I

Effect of ascorbic acid orally on iron absorption. Different amounts of ascorbic acid were given to 30 mg of elemental iron (as ferrous sulphate).

Amount of ascorbic acid (mg)	SUBJECT	"ABSORPTION" (per cent)		ABSORPTION RATIO with / without ascorbic acid	
		without ascorbic acid	with ascorbic acid	Individual value	Mean value and standard error of mean
50	1-M-N	5.0	4.0	0.81	0.91±0.04
	2-M-N	5.5	5.7	1.03	
	3-M-BD	7.2	6.7	0.93	
	4-M-BD	10.8	8.6	0.79	
	5-M-BD	11.4	11.1	0.98	
	6-M-BD	26.2	24.4	0.93	
100	7-M-BD	3.4	3.4	0.98	1.09±0.03
	8-F-BD	5.9	7.3	1.22	
	9-M-BD	5.9	6.3	1.08	
	10-M-BD	6.8	7.4	1.08	
	11-F-BD	10.5	12.0	1.14	
	12-M-BD	21.2	21.8	1.03	
200	13-M-N	5.7	7.8	1.36	1.33±0.07
	14-M-N	5.5	6.6	1.20	
	15-M-BD	6.1	11.1	1.82	
	16-M-BD	9.4	12.8	1.37	
	17-F-N	9.6	11.6	1.21	
	18-F-BD	10.4	16.3	1.57	
	19-M-BD	11.2	14.5	1.29	
	20-M-BD	12.7	22.6	1.77	
	21-M-BD	13.9	16.6	1.20	
	22-M-BD	23.2	22.6	0.98	
	23-M-BD	23.4	29.7	1.27	
	24-M-BD	24.7	32.3	1.30	
	25-M-BD	28.6	26.7	0.93	
300	26-M-N	4.4	5.3	1.20	1.43±0.11
	27-M-BD	8.0	11.4	1.43	
	28-M-BD	9.8	20.6	2.10	
	29-M-BD	11.1	11.2	1.01	
	30-M-BD	12.9	18.5	1.43	
	31-F-BD	14.2	28.8	2.03	
	32-F-BD	18.2	20.0	1.10	
	33-F-BD	21.3	30.2	1.42	
	34-F-BD	23.6	36.1	1.53	
	35-M-BD	24.6	31.5	1.28	
	36-M-BD	24.6	23.5	0.95	
	37-F-BD	28.7	46.9	1.64	
500	38-F-BD	11.1	20.6	1.87	1.48±0.15
	39-M-BD	17.4	25.1	1.43	
	40-M-BD	18.5	23.4	1.26	
	41-M-BD	22.8	30.4	1.33	
	42-M-BD	29.4	44.1	1.50	

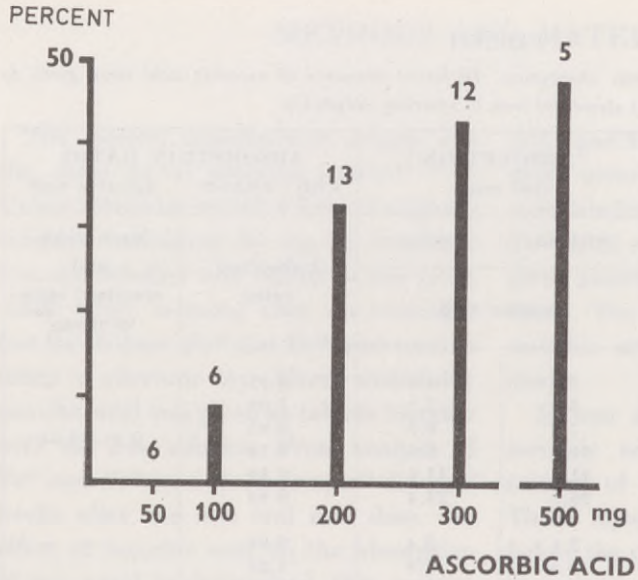

Fig. 1. Average increase of iron absorption when given together with varying amounts of ascorbic acid. The figures over the bars refer to number of subjects in each group.

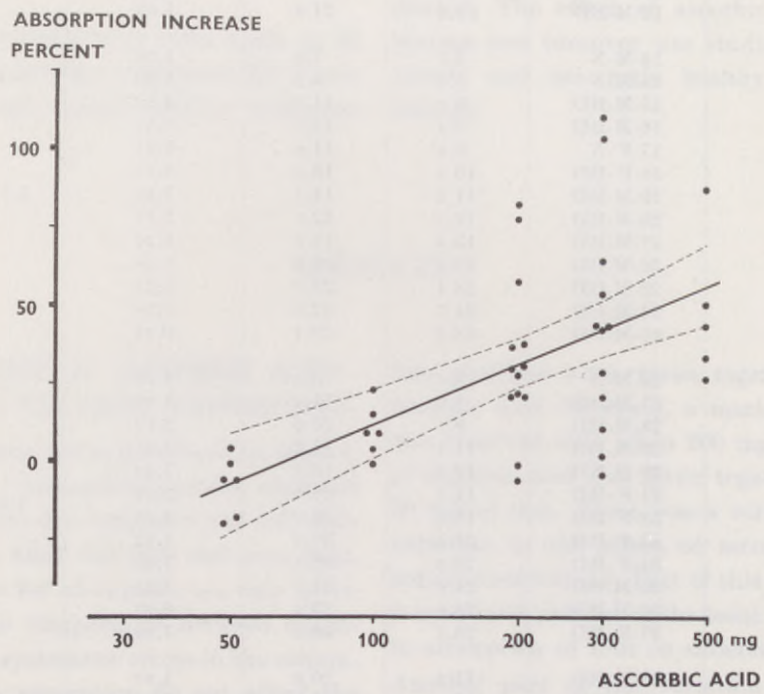

Fig. 2. Relationship between amount of ascorbic acid (logaritmic scale) given together with 30 mg of iron and absorption increase.

The regression line is drawn as a solid line and the 95 % confidence band for this line is marked by dotted lines. [The ordinate, absorption increase, is: (y—l) · 100].

The average increase of the absorption of iron, when the iron was given together with varying amounts of ascorbic acid, is shown in figure 1. The increase is expressed as the percentage of the absorption of iron when given without ascorbic acid.

A statistical analysis of the relationship between the dose of ascorbic acid and the increase of the absorption of iron is graphed in figure 2.

The following functional relationship was found within the domain studied:

$$y = 0.64 \cdot \log x - 0.17$$

where

y was the absorption ratio

and

x was the dose of ascorbic acid in milligrams.

The regression coefficient 0.64 was statistically significant from zero (t=5.07; df 40). The rest standard deviation was 0.25 and the correlation coefficient (r) was 0.62.

2. EFFECT OF ASCORBIC ACID INTRAVENOUSLY

a. Effect on iron turnover

In two healthy subjects 300 mg of ascorbic acid was given intravenously one hour after a single intravenous tracer dose of Fe^{59}. The studies were made in the morning after an overnight fast and blood samples were drawn at 10—15 minutes interval for 2 ½ hours. The technique and methods were the same as used by HALLBERG and SÖLVELL[15].

The results are shown in figure 3. It is evident that there was no effect on the iron turnover rate in these two normal subjects. Neither was any significant effect

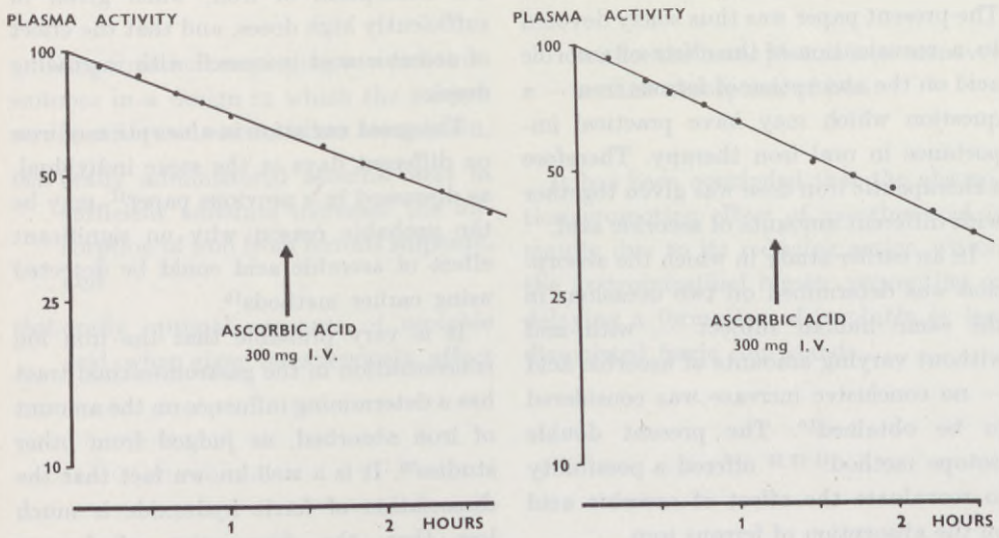

Fig. 3. Effect of ascorbic acid on plasma iron turnover rate in two subjects.

observed on the plasma iron level. The plasma iron turnover was thus not affected by ascorbic acid.

b. Effect on iron absorption

In 4 subjects iron was given for ten days, as described previously, labelled with Fe^{55} and Fe^{59} on alternate days. On every second day 300 mg of ascorbic acid was given intravenously 5 minutes before the administration of the oral dose. The results are shown in table II. It is evident that there was no significant effect of ascorbic acid on iron absorption when given intravenously.

TABLE II

Effect of ascorbic acid intravenously on iron absorption. 300 mg of ascorbic acid was given 5 minutes before the oral 30 mg iron doses (ferrous sulphate).

SUBJECT	"ABSORPTION" (per cent)		ABSORPTION RATIO with/without ascorbic acid
	without ascorbic acid	with ascorbic acid	
43-M-N	2.3	2.3	1.01
44-F-N	6.7	6.4	0.95
45-F-N	5.7	6.2	1.09
46-F-N	1.6	1.7	1.07
Absorption ratio: Mean value: 1.03			

DISCUSSION

Moore has clearly shown that ascorbic acid promotes the absorption of food iron[5]. The present paper was thus solely devoted to a reevaluation of the effect of ascorbic acid on the absorption of ferrous iron — a question which may have practical importance in oral iron therapy. Therefore a therapeutic iron dose was given together with different amounts of ascorbic acid.

In an earlier study in which the absorption was determined on two occasions in the same human subject — with and without varying amounts of ascorbic acid — no conclusive increase was considered to be obtained[10]. The present double isotope method[11 12 13] offered a possibility to reevaluate the effect of ascorbic acid on the absorption of ferrous iron.

The results presented in this paper clearly show that ascorbic acid promoted the absorption of iron, when given in sufficiently high doses, and that the effect of ascorbic acid increased with increasing doses.

The great variation in absorption of iron on different days in the same individual, as discussed in a previous paper[11], may be the probable reason why no significant effect of ascorbic acid could be detected using earlier methods[10].

It is very probable that the iron ion concentration in the gastrointestinal tract has a determining influence on the amount of iron absorbed, as judged from other studies[16]. It is a well-known fact that the dissociation of ferric hydroxide is much less than the dissociation of ferrous hydroxide at the pH existing in the

gastrointestinal tract. The same is also true for a great number of other ferric and ferrous compounds which may be formed in the gastrointestinal tract (e.g. phosphates). The reducing effect of ascorbic acid may help to keep the iron in the ferrous state and may thus prevent or delay a formation of insoluble or undissociated ferric compounds.

It is possible that, in addition to a reducing intraluminar effect, ascorbic acid promotes the absorption of iron by an action via internal transfer systems of iron. MAZUR et al.[17 18] have shown that ascorbic acid is required in addition to ATP for the incorporation reaction of transferrin bound plasma iron into ferritin. Moreover, LOCHHEAD and GOLDBERG[19] have shown that ascorbic acid also increases the transfer of iron to heme biosynthesis (protoporphyrin) From the results in this study, in which intravenous ascorbic acid influence neither the plasma iron turnover nor the absorption of iron, it can be concluded that the main effect of ascorbic acid under the conditions studied (30 mg Fe and 50—500 mg of ascorbic acid orally) is intraluminal and probably due only to its reducing action.

The absorption promoting effect of ascorbic acid observed in this study indicates that the addition of sufficient amounts of ascorbic acid to therapeutic iron doses (e.g. 200 mg of ascorbic acid to 30 mg of ferrous iron) may be of practical importance in oral iron therapy.

SUMMARY

Using a method employing two radioiron isotopes in a design in which the subject serves as his own control it has been shown,

that orally administered ascorbic acid in sufficient amounts increases the absorption of iron from ferrous sulphate, and

that orally optimal amounts of ascorbic acid, when given intravenously, affect neither the basal plasma iron turnover nor the absorption of iron.

It has been concluded that the absorption promoting effect of ascorbic acid is mainly due to its reducing action within the gastrointestinal lumen, preventing or delaying a formation of insoluble or less dissociated ferric compounds.

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EFFECT OF SUCCINIC ACID ON IRON ABSORPTION

By

HANS BRISE AND LEIF HALLBERG

INTRODUCTION

In a previous paper it was reported that there were great differences in absorbability of different iron compounds[1]. It was hypothesized that, with different iron compounds, different iron ion concentrations were obtained in the gastrointestinal tract. This hypothesis could explain the lower absorption of iron from ferric compounds and from such ferrous compounds in which a considerable part of the iron was expected to be present as complex ions in the gastrointestinal tract. However, no explanation could be given for the observation that more iron was absorbed from a solution of ferrous succinate than from solutions of quite dissociated iron compounds as e.g. ferrous sulphate.

Because of these observations it was thought that succinic ions *per se* influenced the absorption of iron. This hypothesis was tested and turned out to be correct as shown in the present paper. The present paper also includes experiments to locate and to analyse the effect of succinic ions on the absorption of iron.

METHODS AND MATERIAL

The same experimental design was used in the present study as previously described, employing two radioiron isotopes, and making each subject his own control[2]. The details of the experimental procedure and the material in different parts of the present study are described together with the results in the separate sections.

The methods for preparing solutions administered orally was the same as previously described if not otherwise stated. Determinations of Fe^{55} and Fe^{59} were also made according to a method earlier published[3].

RESULTS

The results of the present study are presented in six separate sections. The first one contains a study of the effect of different amounts of succinic acid on iron absorption. The following five sections contain experiments intended to analyze the mechanism of action of succinic acid.

I. Effect of succinic acid orally on iron absorption

Thirty milligrams of elemental iron (as ferrous sulphate) were given in a 25 ml solution also containing 10 mg ascorbic acid and 4 g sucrose. The solutions were given for 10 days in the morning after an overnight fast. Iron was labelled with Fe^{55} and Fe^{59} on alternate days. Every second day when iron was labelled with one of the isotopes the solution also contained succinic acid (pro analysi, Merck, Darmstadt) in amounts from 30 to 500 mg.

This study included 81 subjects—13 healthy volunteers (N) and 68 healthy non-anemic blood donors (BD), who had served as blood donors for varying time and who had never received any iron supplementation. In table I the letters F

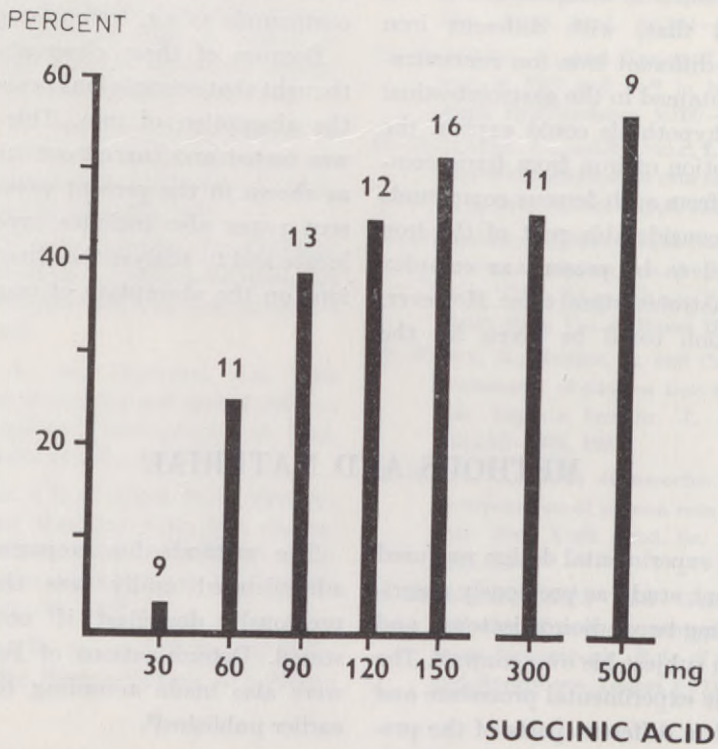

Fig. 1. Increase of iron absorption by succinic acid. Each bar shows the mean value obtained from the number of subjects given above the bar.

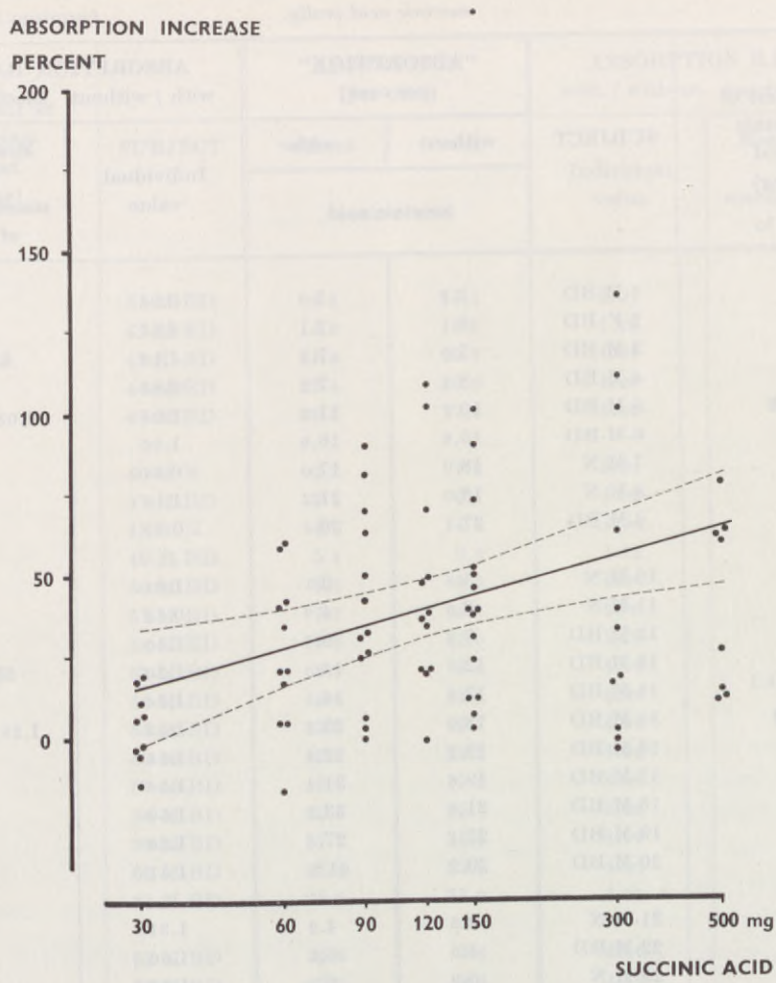

Fig. 2. Relationship between amount of succinic acid (logaritmic scale) given together with 30 mg of iron and absorption increase.

The regression line is drawn as a solid line and the 95% confidence band for this line is marked by dotted lines. [The ordinate, absorption increase, is: (y—l) · 100].

and M denote female and male subjects respectively. In 49 subjects the iron solutions containing succinic acid were labelled with Fe^{59} and in 32 subjects with Fe^{55}. In 33 subjects the solutions containing succinic acid were given on odd days. The blood sample for analyses of Fe^{55} and Fe^{59} was drawn 2 weeks after the last oral iron dose as in previous studies[1 2].

The results are given in table I. The figures given as "Absorption" are not the true absorption figures as discussed in a previous paper[2]. The figures mean per cent of administered iron in the estimated

TABLE I

Iron absorption from 30 mg of iron as ferrous sulphate with and without different amounts of succinic acid orally.

Amount of succinic acid (mg)	SUBJECT	"ABSORPTION" (per cent)		ABSORPTION RATIO with / without succinic acid	
		without succinic acid	with succinic acid	Individual value	Mean value and standard error of mean
	1-M-BD	5.2	5.0	0.96	
	2-F -BD	6.1	7.1	1.17	
	3-M-BD	7.0	7.7	1.10	
	4-M-BD	8.4	7.1	0.85	
30	5-M-BD	10.7	11.3	1.05	1.03±0.04
	6-M-BD	15.6	16.6	1.06	
	7-M-N	18.0	17.0	0.94	
	8-M-N	18.0	21.2	1.18	
	9-M-BD	27.1	26.4	0.97	
	10-M-N	5.8	6.1	1.04	
	11-M-N	6.0	4.9	0.83	
	12-M-BD	7.0	9.7	1.40	
	13-M-BD	12.3	14.8	1.20	
	14-M-BD	13.8	14.5	1.04	
60	15-M-BD	18.0	23.8	1.33	1.25±0.07
	16-M-BD	19.7	22.8	1.16	
	17-M-BD	19.8	31.4	1.58	
	18-M-BD	21.0	33.5	1.59	
	19-M-BD	23.1	27.6	1.20	
	20-M-BD	29.2	41.3	1.41	
	21-M-N	3.2	4.2	1.31	
	22-M-BD	4.5	8.2	1.80	
	23-M-N	5.2	6.5	1.25	
	24-M-N	5.3	5.5	1.05	
	25-M-BD	7.1	9.9	1.41	
	26-M-BD	7.8	12.6	1.62	
90	27-M-BD	10.0	9.9	0.99	1.39±0.08
	28-M-BD	10.2	12.6	1.24	
	29-M-BD	11.0	18.7	1.69	
	30-M-BD	11.6	21.9	1.89	
	31-M-BD	14.6	19.0	1.30	
	32-M-BD	15.9	23.9	1.49	
	33-M-BD	26.8	27.3	1.02	
	34-M-N	2.5	4.3	1.69	
	35-M-N	5.0	7.4	1.48	
	36-M-N	5.1	10.7	2.08	
120	37-M-N	6.0	7.2	1.20	1.45±0.10
	38-M-BD	8.0	10.8	1.36	
	39-M-BD	8.0	11.7	1.47	
	40-M-BD	8.4	8.2	0.98	

Table I Continued

Table I Continued

Amount of succinic acid (mg)	SUBJECT	"ABSORPTION" (per cent)		ABSORPTION RATIO with / without succinic acid	
		without succinic acid	with succinic acid	Individual value	Mean value and standard error of mean
	41-M-BD	8.5	17.1	2.01	
	42-M-BD	15.6	18.5	1.19	
120	43-F -BD	21.6	29.7	1.37	
	44-M-BD	22.4	26.9	1.20	
	45-M-BD	28.2	37.6	1.33	
	46-M-N	2.2	7.2	3.23	
	47-M-BD	3.4	6.4	1.89	
	48-F -N	4.6	4.9	1.06	
	49-M-BD	5.4	9.4	1.72	
	50-M-BD	6.5	8.9	1.37	
	51-M-BD	7.7	15.3	2.00	
	52-M-BD	8.1	12.0	1.49	
150	53-M-BD	8.1	9.0	1.11	
	54-M-BD	8.3	11.5	1.38	1.52±0.13
	55-M-BD	13.0	19.7	1.51	
	56-M-BD	16.8	23.2	1.38	
	57-M-BD	18.8	19.2	1.02	
	58-M-BD	20.9	25.9	1.23	
	59-M-BD	21.7	31.4	1.45	
	60-M-BD	25.8	33.4	1.30	
	61-M-BD	28.2	31.2	1.11	
	62-M-BD	4.6	9.3	2.00	
	63-M-BD	5.7	9.3	1.62	
	64-M-BD	5.9	8.4	1.43	
	65-M-BD	9.0	21.1	2.35	
	66-M-BD	9.8	20.6	2.10	
300	67-M-BD	11.1	11.2	1.01	1.46±0.15
	68-M-BD	13.3	17.5	1.32	
	69-M-BD	15.2	17.7	1.16	
	70-M-BD	15.8	15.5	0.98	
	71-M-BD	16.1	18.9	1.17	
	72-M-BD	24.6	23.5	0.95	
	73-M-BD	5.9	17.2	2.91	
	74-M-BD	6.7	10.8	1.61	
	75-M-BD	10.3	16.7	1.62	
	76-M-BD	10.9	12.1	1.11	
500	77-M-BD	11.1	19.7	1.77	1.57±0.19
	78-M-BD	11.8	18.8	1.59	
	79-M-BD	12.7	14.0	1.10	
	80-M-BD	20.2	22.8	1.13	
	81-M-BD	20.8	26.1	1.25	

red cell mass 2 weeks after the last oral iron dose. The systematic errors affecting these figures do not influence the absorption ratio figures. The figures for "Absorption" are only given to characterize the subjects and to facilitate comparisons between individuals.

A significant effect of succinic acid was observed when 60 mg or more were added to the solutions. The mean values graphed in figure 1 indicate that with increasing amounts of succinic acid more iron was absorbed.

The following functional relationship was found within the domain studied:

$$y = 0.4 \cdot \log x + 0.56$$

where

y was the absorption ratio, and
x was the dose of succinic acid in milligrams.

The regression coefficient 0.4 was statistically significant different from zero ($t = 3.10$; df 79). The rest standard deviation was 0.40 and the correlation coefficient (r) was 0.30.

The observed data and the regression line are shown in figure 2.

II. Effect of succinic acid intravenously on iron absorption

In an attempt to locate the effect of succinic acid on iron absorption the acid was given intravenously, instead of orally, together with the oral iron doses on the alternate days.

In 5 normal subjects a ferrous sulphate solution containing 30 mg of elemental iron labelled with Fe^{55} and Fe^{59} on alternate days was given orally for 10 days as described in section I. On alternate days, when the iron was labelled with one of the isotopes, 150 mg of succinic acid was given slowly intravenously starting 5 minutes before the administration of the oral iron dose.

TABLE II

Iron absorption from 30 mg of iron as ferrous sulphate with and without 150 mg of succinic acid administered intravenously.

SUBJECT	"ABSORPTION" (per cent)		ABSORPTION RATIO with/without succinic acid
	without succinic acid	with succinic acid	
82-F-N	3.3	4.0	1.22
83-F-N	4.7	7.0	1.51
84-F-N	5.1	5.4	1.06
85-M-N	11.3	18.8	1.66
86-M-N	13.8	16.5	1.20
Mean value: 1.33			

The pH of the solution given intravenously was adjusted to 7 with sodium hydroxide and the solution contained 30 mg succinic acid and 6 mg sodium chloride per ml.

The results are shown in table II. In 4 of the 5 subjects a significantly higher absorption of iron was observed when succinic acid was administered intravenously.

III. Effect of succinic acid orally on iron turnover

It has been observed that there is a close relationship between iron absorption and iron turnover. Any factor increasing the

turnover of iron, e.g. increased erythropoiesis, may also increase the absorption of iron[4 5].

In order to be able to analyze further the effect of succinic acid on iron absorption, it is thus necessary to know its effect on iron turnover.

In two healthy volunteers a tracer dose of Fe^{59} (3—4 μC Fe^{59}) was given intravenously after an overnight fast. The details of the method were the same as those described by HALLBERG and SÖLVELL[6]. Blood samples were drawn at intervals of about 15 minutes. After one hour 200 mg of succinic acid was given orally in a 25 ml solution.

The results were identical in both subjects. As shown in figure 3 there was no effect on the iron turnover rate. Four plasma iron determinations were made during the study in each individual. Since there was no change in the plasma iron level during the study, it can be concluded that oral administration of succinic acid does not influence plasma iron turnover.

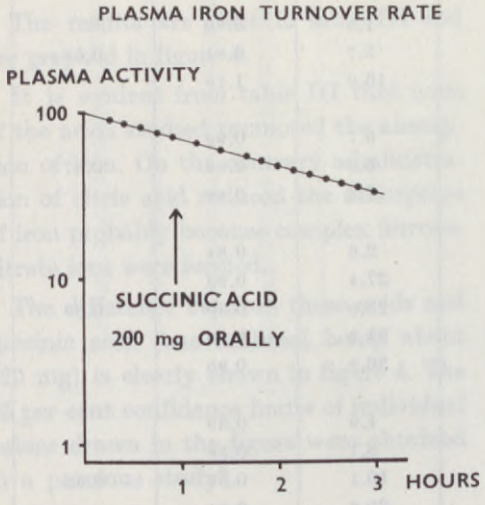

Fig. 3. Plasma iron turnover rate, before and after 200 mg of succinic acid orally.

IV. Effect of some related organic acids on iron absorption

In theory one possible mechanism of action of orally administered succinic acid on iron absorption might be a buffering action on the gastrointestinal content. With this in mind the effects of some related acids were studied. Most of the acids studied were among those which are integral parts of intracellular metabolic processes. The reason for this selection will be discussed later.

The general experimental design was the same as in previous sections. A solution of ferrous sulphate containing 30 mg of elemental iron, labelled with Fe^{55} and Fe^{59} on alternate days, was given every morning for 10 days. On alternate days, when the iron was labelled with one of the isotopes one millimol of acid was given in the solution (e.g. 146 mg α-ketoglutaric acid).

Thirtyfive subjects were included in this study. Twenty of these subjects were blood donors.

TABLE III

Iron absorption from 30 mg of iron as ferrous sulphate with and without some organic acids in amounts of one millimol.

ORGANIC ACID	SUBJECT	"ABSORPTION" (per cent)		ABSORPTION RATIO with/without organic acid	
		without organic acid	with organic acid	Individual value	Mean value
α-Ketoglutaric acid (146 mg)	95-M-BD	5.7	6.0	1.06	1.11
	96-M-BD	10.7	11.2	1.05	
	97-F-BD	12.6	13.6	1.08	
	98-F-BD	19.1	21.3	1.12	
	99-F-BD	24.1	30.1	1.25	
Fumaric acid (116 mg)	100-M-N	3.5	3.7	1.06	1.08
	101-M-BD	4.3	5.5	1.29	
	102-M-N	4.8	5.1	1.07	
	103-M-BD	6.5	7.8	1.20	
	104-M-BD	7.2	7.6	1.06	
	105-M-N	8.0	7.6	0.94	
	106-M-BD	19.4	17.7	0.91	
	107-M-BD	22.3	24.7	1.11	
	108-F-BD	22.7	23.3	1.03	
	109-M-BD	23.1	26.8	1.16	
	110-M-BD	23.2	24.7	1.06	
l-Malic acid (134 mg)	111-M-N	4.8	4.3	0.88	0.89
	112-M-N	7.0	7.0	1.00	
	113-M-N	7.4	5.8	0.79	
d-Malic acid (134 mg)	114-M-N	2.7	1.8	0.66	0.88
	115-F-N	7.0	5.7	0.81	
	116-M-BD	13.5	16.0	1.18	
dl-Isocitric acid (192 mg)	117-M-N	7.0	6.7	0.96	0.87
	118-F-N	7.3	6.3	0.86	
	119-F-N	9.4	7.6	0.80	
Oxalacetic acid (132 mg)	120-M-N	3.1	2.6	0.84	0.89
	121-M-BD	29.6	27.4	0.93	
	122-M-BD	31.6	28.0	0.89	
	123-M-BD	34.9	31.3	0.90	
	124-M-BD	40.3	36.2	0.90	
Citric acid (192 mg)	125-M-N	4.9	1.9	0.39	0.62
	126-F-N	15.2	8.7	0.58	
	127-F-N	18.1	10.1	0.56	
	128-M-BD	25.2	20.2	0.81	
	129-M-BD	35.7	27.3	0.76	

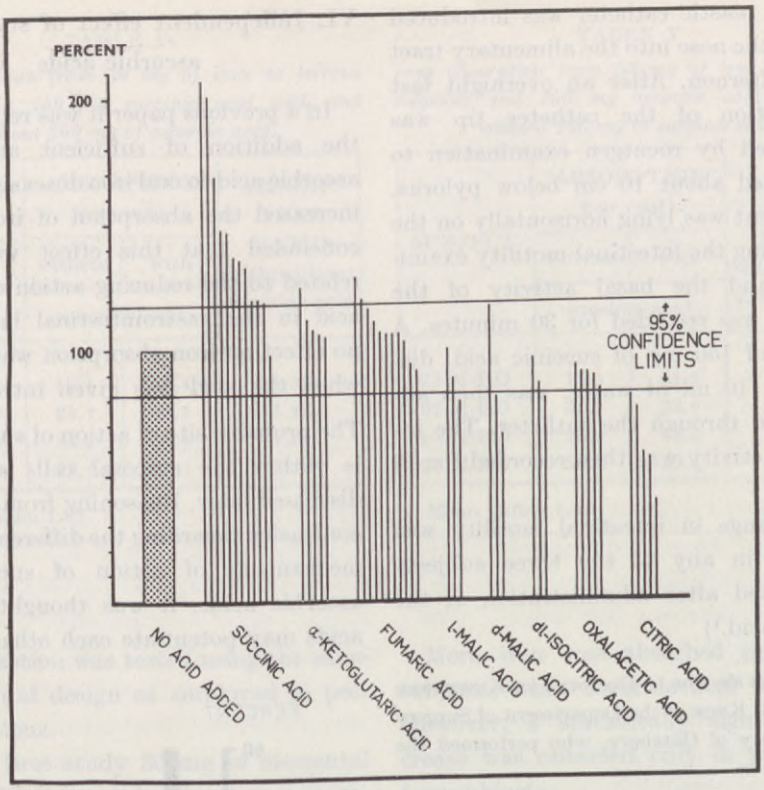

Fig. 4. Effect of some organic acids orally on iron absorption. Each acid is given in an amount of one millimol.

The results are given in table III and are graphed in figure 4.

It is evident from table III that none of the acids studied promoted the absorption of iron. On the contrary administration of citric acid reduced the absorption of iron probably because complex ferrous-citrate ions were formed.

The difference between these acids and succinic acid (one millimol being about 120 mg) is clearly shown in figure 4. The 95 per cent confidence limits of individual values drawn in the figure were obtained in a previous study[2].

V. Effect of succinic acid on intestinal motility

Changes in intestinal motility may change the absorption from the gastrointestinal tract. Because of this the effect of succinic acid was tested on intestinal motility.

This investigation was carried out with a method devised by KEWENTER and KOCK[8]. (For details see reference).

Three patients without current gastrointestinal disorders were investigated, one of them had been subjected to a Bilroth II resection some years previously.

A soft plastic catheter was introduced through the nose into the alimentary tract in the afternon. After an overnight fast the position of the catheter tip was determined by roentgen examination to be situated about 10 cm below pylorus. The patient was lying horizontally on the back during the intestinal motility examination, and the basal activity of the intestine was recorded for 30 minutes. A solution of 150 mg of succinic acid, dissolved in 10 ml of water, was then administered through the catheter. The intestinal activity was then recorded for 15 minutes.

No change in intestinal motility was observed in any of the three subjects investigated after administration of the succinic acid.[1])

[1]) Thanks are due to Doctors JAN KEWENTER and NILS G. KOCK of the Department of Surgery I, University of Göteborg, who performed the motility tests.

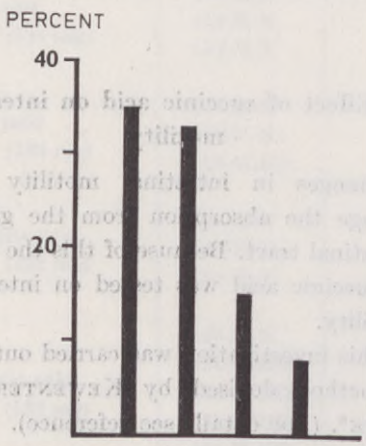

Fig. 5. Independent effect of succinic and ascorbic acid on iron absorption. Effect of ascorbic acid (200 mg) in the precense of succinic acid (150 mg).

VI. Independent effect of succinic and ascorbic acids

In a previous paper it was reported that the addition of sufficient amounts of ascorbic acid to oral iron doses significantly increased the absorption of iron. It was concluded that this effect was mainly related to the reducing action of ascorbic acid in the gastrointestinal lumen since no effect on iron absorption was observed when the acid was given intravenously.

The probable site of action of succinic acid is within the mucosal cells as will be discussed later. Reasoning from the above conclusion regarding the different sites and mechanisms of action of succinic and ascorbic acids, it was thought that the acids may potentiate each other's effects.

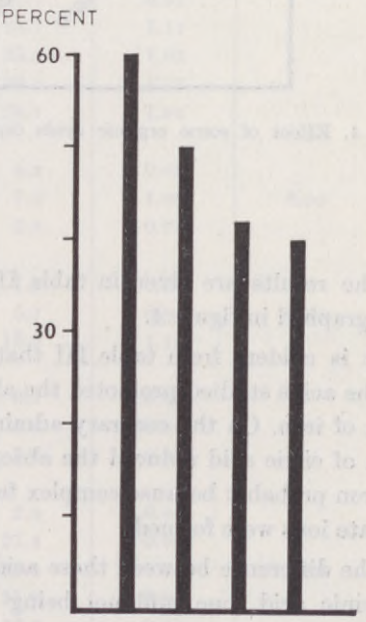

Fig. 6. Independent effect of succinic and ascorbic acid on iron absorption. Effect of succinic acid (150 mg) in the presence of ascorbic acid (200 mg).

TABLE IV

Iron absorption from 30 mg of iron as ferrous sulphate and 150 mg succinic acid with and without 200 mg of ascorbic acid.

SUBJECT	"ABSORPTION" (per cent)		ABSORPTION RATIO with/without ascorbic acid
	without	with	
	ascorbic acid		
87-M-BD	12.1	16.4	1.35
88-M-BD	21.7	28.7	1.33
89-M-BD	24.4	26.4	1.08
90-M-BD	33.1	38.1	1.15
Mean value: 1.23			

TABLE V

Iron absorption from 30 mg of iron as ferrous sulphate and 200 mg ascorbic acid with and without 150 mg of succinic acid.

SUBJECT	"ABSORPTION" (per cent)		ABSORPTION RATIO with/without succinic acid
	without	with	
	succinic acid		
91-M-BD	18.4	24.2	1.32
92-M-BD	22.0	32.9	1.50
93-M-BD	26.9	43.0	1.60
94-M-BD	30.3	39.4	1.30
Mean value: 1.43			

This suggestion was tested using the same experimental design as employed in previous sections.

In the first study 30 mg of elemental iron (as ferrous sulphate) was given together with 150 mg of succinic acid in a solution every morning for 10 days. This amount of succinic acid was found to have an optimal absorption promoting effect. (Section I in the present paper.) On alternate days, when the iron was labelled with one of the isotopes, 200 mg of ascorbic acid was also given. In this way the effect of ascorbic acid could be tested when the absorption of iron was probably optimally increased by succinic acid. The results are shown in table IV and fig. 5.

More iron was absorbed in all four subjects, when ascorbic acid was given. However, a statistically significant increase was obtained only in two of the four subjects.

In the second study the iron was given together with 200 mg of ascorbic acid on all 10 days. On alternate days 150 mg of succinic acid were given.

The results are given in table V and figure 6. A significant increase in the absorption of iron was observed in all four subjects. This increase was of the same magnitude as that observed when succinic acid alone was given as an absorption promoter (section I).

DISCUSSION

The starting point for the present study was the unexplained observation that more iron was absorbed from a solution of ferrous succinate than from a solution of ferrous sulphate. The results in the first section of the present paper showed, that the addition of succinic acid to a solution of ferrous sulphate increased the absorption of iron. These results indicate that succinic ions *per se* promote the absorption of iron, because the increase was related to the amount of succinic acid added, even when more than equivalent amounts (in relation to ferrous iron) were given. The practical importance of this marked promoting effect of succinic acid on iron absorption was not studied in the present paper. However, preliminary studies indicate that the use of succinic acid in oral iron preparations may have great practical advantages in iron therapy[7].

In contradistinction to solutions, no increased absorption of iron was observed from tablets containing ferrous succinate in relation to tablets containing ferrous sulphate as reported in a previous paper[1]. As shown in table VI the solubility and rate of dissolution of ferrous succinate are considerably less than those of ferrous sulphate.

At the lowest pH usually existing in the stomach (ca. pH 1) both the solubility and the rate of dissolution of ferrous succinate are less than one tenth of the corresponding values for ferrous sulphate. At the higher pH of the upper part of the small intestine (pH ca. 5—6) the corresponding ratios are only 1:20. Table VI shows the amounts (in mg of elemental iron per ml) of ferrous succinate and ferrous sulphate which have gone into solution after different times of shaking 40 g ferrous sul-

TABLE VI

Rate of dissolution of ferrous sulphate and ferrous succinate at 37° C at various pH levels expressed as g Fe dissolved in 1 ml solvent after different times. See text.

IRON COMPOUND	Time (minutes)	pH 1 (0.1 N HCl)	pH 5.5 (1/20 M bicarbonate-HCl buffer)	pH 7 (water)
Ferrous sulphate ($7H_2O$)	2	0.054	0.057	0.053
	5	0.063	0.057	0.068
	10	0.095	0.071	0.095
Ferrous succinate ($3H_2O$)	2	0.004	0.002	0.002
	5	0.005	0.004	0.004
	10	0.008	0.004	0.004
	20	0.009	0.004	0.004

phate ($7H_2O$) and 20 g ferrous succinate ($3H_2O$) respectively in 50 ml solvent at 37° C at various pH levels.

Because the conditions for the absorption of iron are more favourable in the upper part of the gastrointestinal tract (e.g. lower pH, greater area for absorption), the time factor will be of importance for the absorption of iron. The time for the disintegration of the tablets and the dissolution of the iron compound can thus be expected to influence the amount of iron absorbed. The great difference in rate of dissolution of ferrou succinate and ferrous sulphate will thus be a probable explanation for the absence of the expected increased absorption of iron from ferrous succinate tablets.

The main part of the present paper consisted of studies designed to analyze the mechanism of action of succinic acid on iron absorption.

Roughly, the absorption of iron will be increased:

(a) if the concentration of iron ions in the gastrointestinal lumen is increased (or more exactly if the product of the concentration and area of absorption is increased),

(b) if the transfer of iron across the mucosal cells is stimulated or

(c) if the elimination of absorbed iron from plasma to other sites of the body is enhanced.

The first alternative explanation for the action of succinic acid — increasing the concentration of iron ions in the gastrointestinal tract — was studied in various ways.

The absorption promoting effect of succinic acid can not be a simple acid effect, because other acids related to succinic acid did not increase the absorption of iron (section IV). Neither is it probable that succinic acid acts as a reducing agent in the gastrointestinal lumen, inasmuch as the effect of succinic acid was not less when almost optimal amounts of ascorbic acid as a reducing agent were also given together with the iron (section VI).

The result in section II that the absorption of iron was increased also when succinic acid was administered intravenously indicate that the absorption promoting effect of succinic acid cannot be due to an action on the gastrointestinal content (the iron ion concentration). When given intravenously the concentration of succinic acid in the gastrointestinal content must be much lower than when the acid is given orally. In section I it was found that no effect of succinic acid was observed when doses lower than 60 mg were given orally. Only 150 mg succinic acid was injected intravenously and an absorption promoting effect was evident in most cases. An increased area of absorption is not a probable explanation for the effect of succinic acid, because no change in intestinal motility was observed as a result of oral administration of 150 mg succinic acid (section V). It can thus be concluded, that succinic acid does not exert its action through an increased iron ion concentration in the gastrointestinal content or through distribution of iron over a greater gastrointestinal surface.

The two main remaining alternatives attempting to explain the promoting effect of succinic acid on iron absorption are therefore (a) an increased transfer

across the mucosal cells and (b) an increased elimination of iron from plasma. The latter was studied in section III, and no effect of succinic acid on iron turnover could be observed.

By a process of elimination the probable explanation for the action of succinic acid on iron absorption, will then be a direct stimulation of the transfer of iron through the mucosal cells.

This interpretation of the present data fits in with recent observations suggesting that the absorption of iron is an active process dependent upon oxidative metabolism and the generation of phosphate-bound energy[9].

Succinic acid is an integral part of the citric acid cycle. Addition of succinic acid can thus be expected to increase the energy available for the intracellular mucosal transfer of iron. However, the observation in section IV that other acids comprised in the Krebs' cycle did not increase the absorption of iron, makes it probable that succinic acid exerts its action in some other step in the intracellular metabolism. Succinic acid is for instance also linked with the cytochrome system. There may also be other steps in which the amount (or concentration) of succinic acid has a determining influence on the rate of cellular metabolism. However, a further analysis necessitates the use of other methods than those employed in the present investigation.

The fact that succinic acid increases the active transport of iron across the mucosal cells suggests that the absorption of other substances may also be increased by succinic acid. It is also possible that succinic acid is a rate limiting factor for the active transfer of some substances through other cells besides those of the gastrointestinal mucosa because the energy metabolism of all cells in the body follows the same general pathways.

SUMMARY

Succinic acid was found to increase the absorption of iron. The increase was related to the amount of succinic acid added to the oral iron dose, (81 subjects).

The mechanism of action of succinic acid on iron absorption was studied in a series of experiments in 48 subjects.

It was concluded that the promoting effect on iron absorption was due to a direct action on the transfer of iron across the mucosal cells, for the following reasons:

(1) succinic acid did not affect iron turnover or intestinal motility;

(2) intravenously administered succinic acid also increased the absorption of iron;

(3) other organic acids related to succinic acid (when given in equivalent amounts — one millimol) did not increase iron absorption.

It is suggested that succinic acid exerts its action by increasing the intracellular mucosal metabolism. It is possible that the absorption of other substances may also be promoted by succinic acid.

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