ABSORPTION OF IRON COMPOUNDS FROM THE SMALL INTESTINE IN THE RAT

by

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INTRODUCTION

Most authorities[1,2,3,4,5] agree that iron salts are absorbed from the human intestine, especially in the ferrous form. The result of investigations by GROEN and TAYLOR[6] did not seem to bear out this view. They studied iron absorption in normal human volunteers by the use of the method of intestinal intubation. A double lumen tube, with a rubber balloon attached to it, was introduced through the stomach and the duodenum into the jejunum. At this stage the balloon was inflated. This made the upper part of the intestine into a downwards closed cavity. Into this cavity solutions of respectively ferrous sulphate, ferric sulphate, or ferric ammonium citrate were introduced. The test solution was left inside the intestine for half an hour, after which what was left, was recovered by aspiration and washing. By this technique it was found that 100 % of the ferric ammonium citrate could be recovered. When ferrous sulphate or ferric sulphate had been introduced, it appeared that a considerable portion of the iron remained inside the gut. By continuing the washing, however, the intestine slowly yielded up more iron in the form of rusty coloured particles of a mucous consistency. Apparently a part of the iron salt had become *ad*sorbed to the intestinal wall and this had taken place both with ferric and ferrous solutions. After washing had been continued for three hours, all the iron that had been introduced was recovered for 100 %. The peculiar situation arose therefore that the absorption of iron, which seems definitely established by clinical and experimental experience, could not be demonstrated in a direct way. This induced us to study iron absorption in animals.

EXPERIMENTAL

The experiments were carried out on normal rats varying in age between three and fifteen months. The rats had been reared on a stock diet of wheat and milkpowder. Before the experiments they were kept for a week on a food poor in iron, consisting of skimmed milkpowder and butter. This food on the average contained 40 μG of iron per gram. The rats were housed in separate metal cages with screened bottoms as a measure against coprophagia. For external reasons it was not possible to keep the rats on a food which was completely iron free, neither did we have the opportunity to keep them in glass cages. Before the experiments the rats were kept fasting for 24 hours. Under ether-urethane anaesthesia the abdomen was opened and an empty loop of jejunum, as near as possible to the duodeno-jejunal flexure was chosen. The loop was closed at both ends by ligatures that were so applied that they did not interfere with the blood supply, i.e., they were tied round the intestine at spots which were just half way between two small vessels entering the intestine from the mesentery. The loops varied in length from 10 to 20 centimetres. One ligature was closed immediately, the other was tied over the needle of the syringe out of which the iron solution was injected into the lumen of

† Both died in 1945, W. A. VAN DEN BROEK was shot down in the street by a nazi agent; H. VELDMAN died in a German concentration camp.

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the loop. When the injection of the solution was finished, the needle was withdrawn and the ligature was tied. This little trick made escape of the iron solution through the perforation impossible. The abdomen was closed and the rats were left to themselves during a certain time, during which absorption was allowed to proceed. The animals were kept warm and sterile saline solution was injected subcutanously to guard against dehydration. After the time allowed for absorption the rat was killed by ether or bleeding. The loop containing the iron solution was excised and its outer surface rinsed with iron free water. It was then transferred to a combustion flask for destruction with iron free HCl and HNO_3. The contents of the flask were transferred to a volumetric flask and diluted with iron free distilled water to 50 ml. An aliquot part, usually 5 ml, was treated with orthophenantroline and hydroquinone at p_H 2. The resulting red colour was measured in a Zeiss Stufenphotometer[7].

At least three rats were used for every experiment. The animals stood the experimental procedure well. When the ligatures had been properly applied the loop and the rest of the intestine looked quite normal at the end of the experiment, showing that the circulation had not been interfered with. The maximum duration of an experiment was six hours.

In most experiments a quantity of iron salt equivalent to 500 μG of iron, dissolved in 0.5 ml of water, was introduced into the intestinal loop. The amount of iron introduced minus what was recovered was taken to be absorbed. This certainly was not correct as the loops themselves contained appreciable amounts of iron. In blank determinations the loops were found to contain between 18 and 82 μG of iron. Exceptionally a higher value was found. In one instance an intestinal loop of 15 cm length in a rat which had been fasting for 48 hours appeared to contain 229 μG of iron! This was quite exceptional but under these circumstances we did not feel justified in subtracting an average figure for the iron contained in the intestinal wall. We prefer to present our figures as they were obtained. It is realized that the experimental error is not inconsiderable and that value can only be attached to considerable differences in absorption found by this procedure. Fortunately nearly all the results obtained were definitely outside the experimental error.

RESULTS

In a first series of experiments iron salts were introduced into the intestinal loop which was immediately afterwards excised and combusted. These control experiments are recorded in Table I, compared with the results of another series in which ferrous

TABLE I

In the control experiments a solution of ferrous sulphate was introduced into the ligated intestinal loop. The loop was immediately excised, combusted and the iron content determined. In the absorption experiments 6 hours elapsed between the introduction of the iron solution and the excision and combustion of the loop.

Absorption experiments			Controls		
In	Rec.	Diff.	In	Rec.	Diff.
622	678	+ 56	622	658	+ 36
622	654	+ 32	622	765	+ 143
487	562	+ 75	487	576	+ 89
487	624	+ 137	487	609	+ 122
493	606	+ 113	500	580	+ 80
493	606	+ 113	500	620	+ 120
483	538	+ 55	501	551	+ 50
			560	642	+ 82

sulphate solutions were allowed to remain inside the intestine for 6 hours. It is clear from this table that no significant differences between the results of control and absorption experiments were found. Neither did we find any evidence of absorption when ferrous chloride, ferric sulphate or ferric citrate were introduced into the gut (Table II). It made no difference if these experiments were carried out in the duodenum, jejunum or ileum.

When the loop in which the iron solution had been allowed to remain for 6 hours was cut open the iron was found to be precipitated and adsorbed on the mucous membrane.

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TABLE II

ABSORPTION OF FERROUS AND FERRIC COMPOUNDS FROM THE ISOLATED LOOP OF SMALL INTESTINE

Ferrous sulphate			Ferrous chloride			Ferric sulphate			Ferric citrate		
In	Rec.	Diff.	In	Rec.	Diff.	In	Rec.	Diff.	In	Rec.	Diff.
483	538	+ 55	622	678	+ 56	1071	1240	+ 169	337	367	+ 30
492	515	+ 23	622	654	+ 32	1071	1128	+ 57	337	357	+ 20
492	535	+ 43	342	396	+ 54	1071	1143	+ 63	337	428	+ 91
492	538	+ 46	347	367	+ 20				352	502	+ 150
487	562	+ 75							352	367	+ 15
487	624	+ 137									
493	606	+ 113									
493	606	+ 113									
517	588	+ 71									
517	565	+ 48									

The mucosa was found covered with a rusty brown material resembling ferric hydroxide. This was observed in all cases where either a ferrous or ferric solution had been put in. Neither did it make any difference if the iron solutions were adjusted to p_H 1.5 or 2 by adding HCl before introducing them into the loop.

Up to this point our results were in agreement with those of GROEN and TAYLOR. Some absorption might have taken place in these experiments but then in so small amounts that we could not detect it with our technique. A definite absorption however was observed when other substances were added to the iron solutions. Table III gives

TABLE III

INFLUENCE OF ASCORBIC ACID ON THE ABSORPTION OF FERROUS AND FERRIC SULPHATE

Ferrous sulphate						Ferric sulphate					
without ascorbic acid			with ascorbic acid			without ascorbic acid			with ascorbic acid		
In	Rec.	Diff.	In	Rec.	Diff.	In	Rec.	Diff.	In	Rec.	Diff.
483	538	+ 55	495	373	— 122	1071	1240	+ 169	1071	780	— 291
492	515	+ 23	495	374	— 121	1071	1128	+ 57	1071	890	— 181
492	535	+ 43	495	408	— 87	1071	1143	+ 63	1071	838	— 233
492	538	+ 46	526	456	— 70						
487	562	+ 75	526	480	— 46						
487	624	+ 137	500	362	— 138						
493	606	+ 113	502	400	— 102						
493	606	+ 113	500	325	— 175						
517	588	+ 71	524	470	— 54						
517	565	+ 48	509	415	— 94						

the results of two series of experiments in which *ascorbic acid* was added to solutions of ferrous or ferric sulphate. A definite diminution of the iron content of the intestinal loop after 6 hours was found in all cases. The results were the same with a mixture of ferric sulphate and ascorbic acid, which is what one would expect, as the ferric salt is immediately reduced to the ferrous form when ascorbic acid is added.

Table IV demonstrates that the absorption promoting effect of ascorbic acid runs approximately parallel with the amount of ascorbic acid added. It is noteworthy that

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TABLE IV

THE ABSORPTION OF $FeSO_4$ ON ADDITION OF INCREASING QUANTITIES OF ASCORBIC ACID
(Absorption time 6 hours)

Fe + 0.5 mg asc.acid			Fe + 2.5 mg asc.acid			Fe + 10 mg asc.acid			Fe + 25 mg asc.acid			Fe + 50 mg asc.acid			Fe + 100 mg asc.acid		
In	Rec.	Diff.	In	Rec.	Diff.	In	Rec.	Diff.	In	Rec.	Diff.	In	Rec.	Diff.	In	Rec.	Diff.
492	509	+ 17	492	442	— 50	483	212	— 271	495	373	— 122	503	328	— 175	520	245	— 275
492	508	+ 16	492	462	— 30	483	105	— 378	495	374	— 121	503	308	— 195	520	285	— 235
499	523	+ 24	499	500	+ 1	483	425	— 58	495	408	— 87	503	240	— 263	520	220	— 300
499	511	+ 12	499	416	— 83	492	56	— 436				483	298	— 185			
499	537	+ 38	499	378	— 121	492	61	— 431									
						499	441	— 58									
						499	444	— 55									
						499	360	— 139									

when ascorbic acid had been added to the iron solution no precipitate of rusty material was found on the mucous membrane on opening the loop after six hours. These loops were somewhat distended and contained a violet fluid consisting most probably of a solution of a complex ferrous-ascorbic acid compound. In contradiction to the absorption promoting effect of ascorbic acid it was found that various indifferent substances, added in approximately isosmotic quantities, did not influence the absorption of ferrous sulphate, as shown in Table V. On the other hand, an increase in absorption was found after

TABLE V

INFLUENCE OF VARIOUS SUBSTANCES ON THE ABSORPTION OF $FeSO_4$

+ 25 mg glucose			+ 25 mg arabinose			+ 15 mg glycine		
In	Rec.	Diff.	In	Rec.	Diff.	In	Rec.	Diff.
501	636	+ 135	456	602	+ 146	441	534	+ 93
501	589	+ 88	456	569	+ 113	441	548	+ 107
495	525	+ 30	456	469	+ 13	441	497	+ 56
						541	589	+ 48
						541	558	+ 15
						541	598	+ 57
+ 5 mg NaCl			+ 10 mg urea			+ 10 mg Na_2S		
502	525	+ 23	509	577	+ 68	466	560	+ 94
502	541	+ 39	509	689	+ 180	466	541	+ 75
490	509	+ 19	502	602	+ 100	466	501	+ 35
490	544	+ 54	502	656	+ 154			
490	541	+ 51	502	602	+ 100			

adding to the ferrous solution one of the following substances:

a. organic reducing agents: cystein, glutathione, sodiumformaldehydesulfoxylate (Table VI);

b. many organic acids, among which dicarbonic acids (succinic acid), hydroxy acids (citric acid, tartaric acid (cf. Table VII), malic acid, oxybutyric acid, lactic acid), keto-acids (pyruvic acid, acetylacetic acid) and some amino acids (glutamic acid and aspartic acid).

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TABLE VI

INFLUENCE OF SOME REDUCING SUBSTANCES ON THE ABSORPTION OF $FeSO_4$

+ 25 mg asc.acid			+ 20 mg cystein			+ 40 mg glutathione			+ 25 mg sodiumformaldehydesulphoxylate		
In	Rec.	Diff.	In	Rec.	Diff.	In	Rec.	Diff.	In	Rec.	Diff.
495	373	— 122	487	276	— 211	482	460	— 22	482	500	+ 18
495	374	— 121	487	402	— 85	482	368	— 114	482	310	— 172
495	408	— 87	487	424	— 63	482	450	— 32	482	418	— 64
526	456	— 70									
526	480	— 46									
500	362	— 138									
502	400	— 102									
500	325	— 175									
524	470	— 54									
509	415	— 94									

TABLE VII

INFLUENCE OF CITRIC ACID (25 mg) AND TARTARIC ACID (25 mg) ON THE ABSORPTION OF IRON SALTS

Ferrous sulphate			Ferrous sulphate + citric acid			Ferrous sulphate + tartaric acid		
In	Rec.	Diff.	In	Rec.	Diff.	In	Rec.	Diff.
483	538	+ 55	509	170	— 339	498	398	— 100
487	562	+ 75	509	264	— 245	498	251	— 247
487	624	+ 137	509	177	— 332	498	338	— 160
493	606	+ 113						
493	606	+ 173						
517	588	+ 71						
517	565	+ 48						
Ferric sulphate			**Ferric sulphate + citric acid**			**Ferric sulphate + tartaric acid**		
1071	1240	+ 169	538	218	— 320	539	521	— 18
1071	1128	+ 57	538	288	— 250	539	561	+ 22
1071	1143	+ 63	538	190	— 348	539	530	— 9
						545	383	— 162
						545	434	— 111

TABLE VIII

INFLUENCE OF HCl AND H_3PO_4 ON ABSORPTION OF $FeSO_4$

Ferrous sulphate + HCl pH 2			Ferrous sulphate + H_3PO_4 pH 1.8		
In	Rec.	Diff.	In	Rec.	Diff.
492	515	+ 23	529	648	+ 119
492	535	+ 43	529	556	+ 27
492	538	+ 46	529	558	+ 29
509	546	+ 37			
509	599	+ 90			
509	560	+ 51			

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The addition of hydrochloric or phosphoric acid did not influence the absorption of iron salts to a measurable degree (Table VIII).

If the mixture of ferrous sulphate with ascorbic acid, glutathione, citric acid or tartaric acid was neutralised with ammonia before bringing it into the intestine the absorption promoting influence of these substances was found to have disappeared (Table IX and X).

TABLE IX

INFLUENCE OF pH ON ABSORPTION OF FERROUS SULPHATE

Ferrous sulphate + HCl pH 2			Ferrous sulphate + asc. acid (25 mg) pH 2			Ferrous sulphate without addition pH 4.4			Ferrous sulphate + asc.acid (25 mg) pH 4.4			Ferrous sulphate + asc.acid (25 mg) pH 6.5		
In	Rec.	Diff.	In	Rec.	Diff.	In	Rec.	Diff.	In	Rec.	Diff.	In	Rec.	Diff.
492	515	+ 23	495	373	— 122	483	538	+ 55	489	482	— 7	409	523	+ 114
492	535	+ 43	495	374	— 121	487	562	+ 75	489	486	— 3	409	550	+ 141
492	538	+ 46	495	408	— 87	487	624	+ 137	444	447	+ 3	409	495	+ 86
622	678	+ 56	526	456	— 70	493	606	+ 113	444	454	+ 10	427	476	+ 49
622	654	+ 32	526	480	— 46	493	606	+ 113	444	429	— 15	427	450	+ 23
509	546	+ 37	500	362	— 138	517	588	+ 71	455	438	— 17			
509	599	+ 90	502	400	— 102	517	565	+ 48	455	398	— 57			
509	560	+ 51	500	325	— 175									
			524	470	— 54									
			509	415	— 94									

TABLE X

INFLUENCE OF NEUTRALISATION ON THE ABSORPTION PROMOTING INFLUENCE OF CITRIC ACID, TARTARIC ACID AND GLUTATHIONE

Ferrous sulphate + citric acid pH 1.7			Ferrous sulphate + citric acid pH 6		
In	Rec.	Diff.	In	Rec.	Diff.
509	170	— 339	301	268	+ 33
509	264	— 245	301	357	+ 56
509	177	— 332	301	432	+ 131
Ferrous sulphate + tartaric acid pH 1.6			**Ferrous sulphate + tartaric acid pH 6**		
In	Rec.	Diff.	In	Rec.	Diff.
498	398	— 100	444	466	+ 22
498	251	— 247	444	476	+ 32
498	338	— 160			
Ferrous sulphate + glutathione pH 1.9			**Ferrous sulphate + glutathione pH 6.5**		
In	Rec.	Diff.	In	Rec.	Diff.
482	460	— 22	421	530	+ 109
482	368	— 114	421	451	+ 30
482	450	— 32			

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The explanation we propose is this: A solution of ferrous sulphate introduced into the intestine is rapidly neutralised and precipitated as $Fe(OH)_2$ which is then transformed into $Fe(OH)_3$. This insoluble substance cannot be absorbed. Indeed if one opens the loop into which the solution of $FeSO_4$ has been introduced, the intestinal epithelium is found covered with a rusty brown material which is obviously a combination of ferric hydroxyde with mucine or some other proteinlike substance. The addition of HCl or H_3PO_4 to the iron solution in amounts as will bring the p_H down to 1.8 to 2, does not materially alter this state of affairs as the small quantities of hydrochloric and phosphoric acid which are required for this purpose are so rapidly absorbed, that the p_H of the intestinal content is again 6.8 to 6.9 within 15 minutes or less. All the organic reducing agents (all acid in character) and the organic acids, in the quantities as used in these experiments, require a much longer time before they are absorbed. Therefore they keep the p_H in the closed loop down for a longer time, and it is just this acid p_H that is necessary to keep the iron in the ferrous state and allows the absorption to proceed.

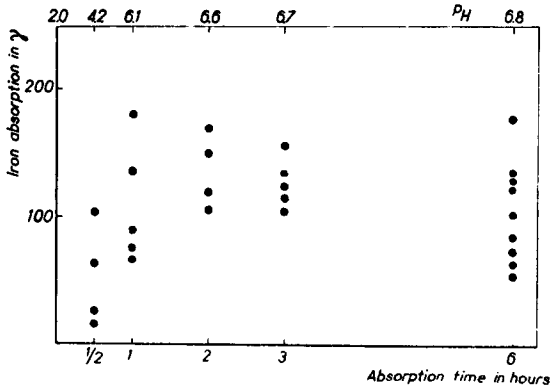

Fig. 1. Influence of addition of ascorbic acid on iron absorption and p_H inside the intestine. The absorption is seen to proceed for one hour, after which, when the p_H reached 6.1, it stopped

In Fig. 1 are plotted the results of a number of experiments in which the time, allowed for absorption of a solution containing 500 μg of iron as $FeSO_4$, with 25 mg of ascorbic acid in 0.5 ml of water, varied from ½ to 6 hours. Simultanously with the absorption experiments the p_H was determined in the intestinal content at regular intervals. It is obvious from the graph that the absorption proceeded so long as the content of the intestinal loop retained its acid action. After one hour when the p_H had reached a level of 6.1, the absorption stopped. Similar results were obtained when the absorption was studied of a solution of 500 μg iron as ferrous sulphate to which 25 mg of citric acid had been added (Fig. 2). In this case the absorption was seen to proceed for

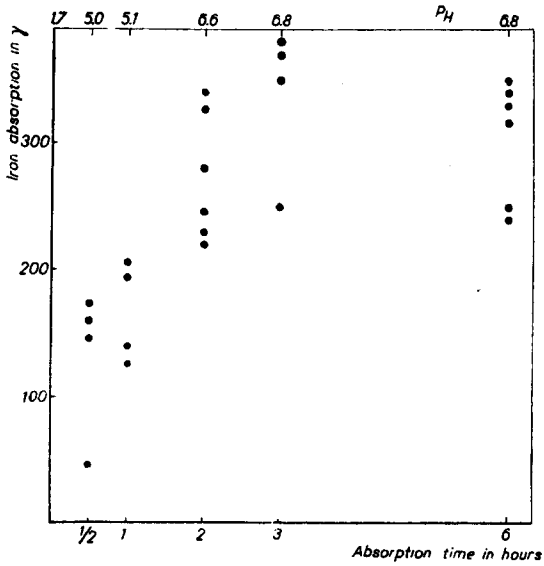

Fig. 2. Influence of addition of citric acid on iron absorption and p_H inside the intestine. The absorption proceeded for two hours, after which, when the p_H reached 6.6, it stopped

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two hours before the p_H approached neutrality at which point it came to an end.

In a third series of experiments, in which 20 mg of cystein hydrochloride was added to ferrous sulphate, no further increase in absorption occurred after one hour and this point again coincided with a rise of the p_H to 6.4 (Fig. 3). The result of these experiments supports the common conception that iron is absorbed from the intestine only in the ferrous state. Iron salts will, however, remain in the ferrous form only so long as the intestinal content is acid. At a p_H over 6 all the iron present is converted either into the complex or the ferric form and neither of these appear to be absorbed to an appreciable degree.

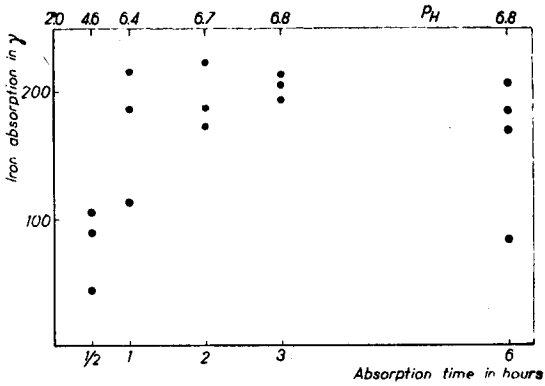

Fig. 3. Influence of addition of cystein hydrochloride on iron absorption and p_H inside the intestine. After one hour, when the p_H had reached 6.4, the absorption stopped

A further finding that seems of interest was the demonstration in these experiments that on addition of many organic acids (citric acid, tartaric acid, pyruvic acid) not only ferrous sulphate but also *ferric* sulphate was absorbed to an appreciable degree (Table VII). As an explanation it was found that these substances, which are not generally considered as reducing agents, are indeed capable of reducing ferric to ferrous iron, which is then absorbed. Acetic and butyric acid, however, promote only the absorption of ferrous sulphate, but not of ferric sulphate, as these acids are unable to reduce the ferric ion.

DISCUSSION

It is well known that under normal circumstances only a small proportion of any iron compound given by mouth is absorbed. This has necessitated the administration of relatively large doses of iron salts to patients suffering from iron deficiency anaemia. The daily administration of an iron salt equivalent to 500 mg of iron in the ferrous form may be required to produce an absorption of about 25 mg per day as estimated by the output of haemoglobin. If one administers metallic iron, complex iron salts or ferric salts, the amounts required to obtain an appreciable absorption are even higher[3, 8, 9]. As an explanation of this small absorption McCance and Widdowsen[10] have suggested that the absorption of iron is conditioned mainly by the relative concentration of free ionised iron in the lumen of the intestine and in its epithelial cells. Moore, Arrowsmith, Welsh and Minnich[3] concluded from their experiments that the ease of ionisation of ferrous salts, under those conditions of acidity or alcalinity as are present in the gastro-intestinal tract, is the important factor in determining the relative rates of absorption of different iron salts. The present investigation furnishes further evidence for this concept.

The limiting factor in the absorption of iron ions appears to be, first of all, the simple law of inorganic chemistry by which iron is precipitated from the solution of its salts when the p_H approaches neutrality[11]. This law, together with the tendency of the in-

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testine to preserve the neutrality of its content, is responsible for the fact that ferrous ions introduced into the intestine in simple solution or formed from other iron compounds, remain only for a short time in the ionised state. It is only during this short period that iron can be absorbed from the upper part of the intestine and this is why normally iron absorption is limited. Any influence that will keep the p_H of the intestinal contents at an acid level for a longer time, will promote absorption of ferrous ions. Because ferrous iron is oxidised to ferric as soon as the p_H approaches neutrality, reducing substances also promote absorption. It happens that most of the substances (ascorbic acid, citric, cystein hydrochloride, etc.) that increase iron absorption, are at the same time acids with a slow absorption rate and reducing agents for ferric. This is why these substances also promote the absorption of ferric salts.

Thus most of the facts that are known about iron absorption are explained by the simple chemical properties of the metal itself. METTIER and MINOT[12], e.g., demonstrated that small doses of ferric ammonium citrate given by mouth in a strongly acid buffered medium, so designed that it kept the reaction inside the duodenum acid for a long time, were more effective in the treatment of hypochromic anaemia than when given without acid or in an alcaline buffered medium. MOORE and his coworkers[3] have demonstrated that the iron tolerance curve in the serum rose to a higher level when ferrous than when ferric salts were administered by mouth. Moreover, a clear cut increase in the iron tolerance curve was found when ascorbic acid or sodium formaldehyde-sulfoxylate were given together with ferrous sulphate. POWELL[13] has shown that the addition of ascorbic acid to ferrous sulphate will greatly increase the effectiveness of the iron compound in the treatment of iron deficiency anaemia. The high rate of absorption of hydrochloric acid makes this acid less fit to keep the p_H in the intestine down long enough. This explains why MOORE and his associates were unable to demonstrate any difference in iron absorption between subjects who received ferrous sulphate with or without hydrochloric acid.

In the present investigation no iron absorption could be demonstrated unless some organic acid or reducing agent was added. This does not mean that no absorption took place, but only that the degree of absorption if present was too small to be detected by the method used by us. Actually we are forced to admit that under normal circumstances some iron is always absorbed. The bulk of this absorption must take place during the short time when the iron is in the ferrous state in the duodenum, where the reaction is still acid. Lower down in the jejunum, where the reaction approaches neutrality, the iron is presumably precipitated in the ferric form or transformed into a complex anion, neither of which are absorbed. It cannot be excluded, however, that some iron is absorbed from the lower part of the intestine, for we know that the intestinal content reduces the iron when it passes through the ileum, because one finds all the iron transformed into ferrous sulphide before it reaches the caecum. There are bacteria in the normal human intestinal tract that are able to reduce ferric to ferrous iron[14]. In how far these bacteria play a part in normal iron absorption, should be further investigated. Reduction by bacterial action might explain the ease with which dogs absorb iron irrespective of the form in which it is administered[5,15,16,17].

The theory of the mechanism of iron absorption presented above, is evidently in contrast to the views expressed by LINTZEL[2], HEILMEYER and KOCH[18], and HAHN, WHIPPLE, and their coworkers[15–17, 19–23]. According to them the mechanism of acceptance or refusal of iron by the mucosa of the gastro-intestinal tract is not a matter determined

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first of all by the concentration of ferrous ions inside the gut, but a part of a physiological barrier. HAHN, BALE, LAWRENCE, and WHIPPLE[20–23] base this view on experiments with iron isotopes, in which they claim to have demonstrated that anaemic dogs absorb iron at a higher rate than normal or plethoric dogs. The first objection against their technique is the appreciable loss of radio iron that occurs during the experiments[20]. In the second place it is not proved that all the radio iron that has been absorbed, appears as haemoglobin in the bloodstream as these authors assume. In this respect there exists a difference between the normal and the anaemic individual, as shown by DUBACH, MOORE, and MINNICH[19] in the human. If the amount of radio iron present in the bloodstream is assumed to represent the total iron which has been absorbed, one might erronously conclude that iron absorption proceeds at a higher rate in the anaemic than in the normal individual[23]. We feel this has not yet been established beyond doubt.

Concerning the application of our results to the treatment of iron deficiency anaemia, it might be pointed out that the addition of ascorbic acid to ferrous iron seems a rational procedure. We would venture to suggest, however, that the amount of ascorbic acid added to the iron should be higher than usually prescribed, certainly not less than equal amounts (by weight) of ascorbic acid and ferrous sulphate. Citric acid, tartaric acid, etc. might serve the purpose equally well. It would seem useless to prescribe hydrochloric or phosphoric acid with ferrous salts, unless in continual intraduodenal drips!

Finally, our results may well have a bearing upon the absorption of iron from naturel food substances. It was shown that the absorption of iron is markedly influenced by other substances that are present at the same time in the intestine. It seems significant that many of the substances that promote iron absorbtion occur in natural food stuffs. Thus it may well be that substances like citric acid, tartaric acid, lactic acid, etc., the nutritional significance of which is on the whole still unknown, have an important function in aiding the absorption of iron and possibly other nutrients. This insight throws new light on the old question of the "availability" of iron in various foods. It appears as if availability of food iron is determined not so much by the nature of the iron compounds contained in the food, as by concomittant substances which may either be present in the same food or in other food-stuffs taken with the same meal. A similar view was put forward a few years ago by TOMPSETT[24]. Among the substances that would render iron more "available", some amino acids and many organic acids that are contained in fruits and vegetables, seem to be the most important. The function of hydrochloric acid in the gastric juice in the absorption of food iron may also be viewed in a different light. By itself it cannot keep the reaction inside the gut acid enough to play an important part. But hydrochloric acid probably liberates the organic acids, plant acids, and aminoacid-complexes in the foods from their salts. These acids then acidify the chyme sufficiently to allow absorption of iron in physiological quantities.

SUMMARY

The absorption of different iron salts was studied after they had been introduced into a closed loop of small intestine in the anaesthetised rat. The absorption of iron from solutions of various salts (ferrous, ferric or complexes) in these loops was so small that it could not be demonstrated. A definite absorption was found when to the iron salts were added either an organic reducing agent (ascorbic acid, cystein, glutathione, formaldehyde sulphoxylate) or some organic acid. Especially plant acids (citric ,tartaric, succinic, malic acid), lactic acid, pyruvic acid and some amino acids (glutamic, aspartic acid) were active in this respect. It appeared that the absorption under these circumstances

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proceeded only so long as the p_H inside the intestine remained acid. The addition of hydrochloric or phosphoric acid did not promote the absorption. The author presents the following explanation for these findings:

1. Iron is absorbed as ferrous ion only.
2. Iron can persist in the ionised ferrous form only in acid medium.
3. The normal p_H of the small intestine is about 6. At this p_H, almost all the iron is transformed in the complex or ferric form, neither of which is absorbed to a measurable degree. This explains the poor absorption of iron from a simple solution introduced into the gut.
4. The intestine tends to reestablish its neutral reaction if acids are introduced together with the iron salt. Hydrochloric and phosphoric acid are very quickly absorbed. For this reason these acids cannot keep the p_H inside the gut down for a long time and therefore do not promote iron absorption to a measurable degree.
5. The organic acids and reducing agents (all acid in character) that promote iron absorption, appear to do so by keeping the p_H of the intestine down for a longer time. As soon as the intestinal content becomes neutral ,all the iron is transformed into the complex or ferric form and the absorption stops.

The importance of these facts for the understanding of the mechanism by which iron is absorbed, for the choice of the best preparation for iron therapy and for the elucidation of the problem of iron availability is discussed.

RÉSUMÉ

L'auteur étudie l'absorption de différents sels de fer après leur introduction dans une anse isolée de l'intestin grêle du rat anesthésié. L'absorption du fer des solutions de différents sels (ferreux, ferriques ou complexes) est pratiquement nulle. Une absorption notable n'a lieu que lorsque les sels de fer sont en présence soit d'un agent organique réducteur (acide ascorbique, cystéine, glutathion, aldéhyde formique, sulfoxylate) soit d'un acide organique. Les acides végétaux (citrique, tartrique, succinique, malique), l'acide lactique, l'acide pyruvique et quelques acides aminés (glutamique, aspartique), sont particulièrement actifs à ce point de vue. Dans ces conditions, l'absorption n'a lieu qu'autant que le p_H à l'intérieur de l'intestin reste acide. L'acide chlorhydrique ou l'acide phosphorique ne déclenchent pas d'absorption. L'auteur explique ces observations de la façon suivante:

1. Le fer est absorbé uniquement sous forme d'ion ferreux.
2. Le fer ne peut se maintenir sous la forme ferreuse ionisée qu'en milieu acide.
3. Le p_H normal de l'intestin grêle est de 6 environ. A ce p_H, le fer est presque totalement transformé en forme complexe ou ferrique qui ne sont absorbées ni l'un ni l'autre en quantités mesurables. Ainsi s'explique la médiocrité de l'absorption du fer à partir d'une solution simple introduite dans le tube digestif.
4. L'intestin tend à rétablir sa réaction neutre lorsque des acides sont introduits en même temps que le sel de fer. L'acide chlorhydrique et l'acide phosphorique étant absorbés très rapidement, ils ne peuvent maintenir le p_H acide suffisamment longtemps à l'intérieur du tube digestif, et ne peuvent par conséquent faciliter l'absorption du fer d'une façon notable.
5. Les acides organiques et les substances réductrices (toutes de caractère acide), déclenchant l'absorption du fer, semblent agir en maintenant le p_H de l'intestin suffisamment bas pendant une période suffisamment longue. Dès que le contenu intestinal redevient neutre, tout le fer est transformé soit en complexe soit en sel ferrique et l'absorption s'arrête.

L'auteur discute l'importance de ces faits pour la compréhension du mécanisme par lequel le fer est absorbé, pour le choix de la meilleure préparation dans la thérapeutique du fer, et la solution du problème de l'utilisation du fer.

ZUSAMMENFASSUNG

Die Absorption verschiedener Eisensalze wurde untersucht, nachdem diese Salze in eine geschlossene Dünndarmschlinge in der betäubten Ratte eingeführt waren. Die Eisenabsorption aus Lösungen verschiedener Salze (Ferro-, Ferri- oder Komplexsalze) in diesen Schlingen war so gering, dass sie nicht nachgewiesen werden konnte. Eine nachweisbare Absorption wurde festgestellt, wenn zu den Eisensalzen entweder ein organisches Reduktionsmittel (Ascorbinsäure, Cysttin, Glutathion, Formaldehydsulfoxylat) oder gewisse organische Säuren hinzugefügt wurden. Besonders Pflanzensäuren (Zitronen-, Wein-, Bernstein-, Apfelsäure), Milchsäure, Brenztraubensäure und gewisse Aminosäuren (Glutamin-, Asparaginsäure) waren in dieser Hinsicht aktiv. Anscheinend vollzieht die Absorption unter diesen Umständen sich nur so lange, wie der p_H innerhalb des Darmes Sauer blieb. Zufügen von Salz- oder Phosphorsäure beförderte die Absorption nicht. Der Autor schlägt folgende Erklärung für diese Funde vor:

1. Eisen wird nur als Ferro-Ion absorbiert.
2. Eisen kann in der ionisierten Ferro-Form nur in saurem Milieu bestehen bleiben.
3. Der normale p_H des Dünndarms ist ungefähr 6. Bei diesem p_H wird fast alles Eisen in die

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komplexe oder die Ferri-Form übergeführt, welche nur in unmessbaren Mengen absorbiert werden. Das erklärt die geringe Absorption von Eisen aus einfachen, in den Darm eingeführten, Lösungen.

4. Der Darm strebt danach seine normale Reaktion wiederherzustellen, wenn Säuren zusammen mit dem Eisensalz eingeführt werden. Salz- und Phosphorsäure werden sehr schnell absorbiert. Aus diesem Grunde können diese Säuren den p_H innerhalb des Darms nicht für längere Zeit niedrighalten und befördern daher die Eisenabsorption nicht in nachweisbarem Masse.

5. Die organischen Säuren und Reduktionsmittel (alle mit saurem Charakter), die die Eisenabsorption befördern, tun dies anscheinend dadurch, dass sie den p_H des Darmes längere Zeit niedrig halten. Sobald der Darminhalt neutral wird, wird alles Eisen in die Komplex-oder Ferri-Form umgesetzt und die Absorption hört auf.

Die Bedeutung dieser Tatsachen für das Verständnis des Mechanismus, durch den Eisen absorbiert wird, für die Wahl des besten Eisenpräparates für die Eisentherapie und für die Aufklärung des Problems der Eisenbenutzung, wird diskutiert.

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