115. FACTORS INFLUENCING THE ABSORPTION OF IRON AND COPPER FROM THE ALIMENTARY TRACT

By SIDNEY LIONEL TOMPSETT

*From the Biochemical Laboratory of the Institute of Pathology,
the Royal Infirmary, Glasgow*

(Received 10 April 1940)

IRON

ALTHOUGH the importance of Fe in respiratory processes has been recognized for a long period, there are still gaps in our knowledge of the many processes in which this element is involved, e.g. very little is known concerning the absorption of Fe from the alimentary tract. The work to be described is concerned with this problem.

The Wisconsin workers [Hart *et al.* 1928; Elvehjem, 1932] have produced evidence to show that "inorganic" Fe but not haematin Fe can be absorbed from the alimentary tract and subsequently converted into haemoglobin.

There has been much controversy as to whether both ferrous and ferric Fe or only the ferrous form can be absorbed as such. Heubner [1926] and Lintzel [1931] state that ferric but not ferrous Fe forms non-dialysable compounds with proteins and they believe that ferric Fe cannot be absorbed as such but must first be reduced to the ferrous state. They consider the intestine to be the site of this reduction as it contains easily oxidizable materials and has a low oxygen tension. This view is supported by Starkenstein & Weden [1930], Reimann & Fritsch [1930] and Moore *et al.* [1939]. Other workers believe that ferric Fe can be absorbed as such [Whipple & Robscheit-Robbins, 1936; McCance & Widdowson, 1937; 1938; Brock & Hunter, 1937]. These workers base their views on the following considerations: (1) in balance experiments, ferric Fe has been found to be absorbed as easily as ferrous Fe; (2) in the treatment of anaemia, ferric Fe often produces as good a response as ferrous Fe. Those who hold the first view have in no case produced any evidence that reduction of ferric Fe does take place in any part of the alimentary tract.

Tompsett [1934, 2] has shown that whereas ferric Fe could be recovered quantitatively in trichloroacetic acid extracts of materials containing simple proteins, it could not be recovered from materials containing phosphatides and phosphoproteins. Extraction of ferrous Fe was not so affected. It seems that the absorption of Fe from the alimentary tract is more likely to be hindered by phosphoproteins and phosphatides than by simple proteins.

In previous communications Tompsett [1939, 2, 3] has shown that the absorption of lead is highest on a low calcium diet and least on a high calcium diet and is increased by the addition of mineral acid to the diet. In all probability, Fe absorption is influenced by similar factors.

The dialysis of Fe salts

The object of the present experiments was to determine the factors influencing the dialysis of Fe from organic mixtures and their relation to alimentary absorption.

Parchment thimbles (Whatman) were used as dialysing membranes. In each case 5 ml. of the mixture containing an added Fe salt (1 mg. Fe) were dialysed against 15 ml. of distilled water for 4 hr. At the end of this period the Fe content of the external fluid was estimated by means of thiolacetic acid and NH_3 [Tompsett, 1934, 1, 2].

Egg yolk and egg white suspensions, containing added Fe (ferrous sulphate, iron ammonium citrate, iron alum) were subjected to dialysis. Ferrous Fe dialysed readily, whereas the dialysis of ferric Fe was nil or negligible. Acid peptic digests of egg white and egg yolk, containing added Fe (ferrous sulphate, iron ammonium citrate, iron alum), were then dialysed. Ferrous Fe dialysed readily from all digests but ferric Fe, although dialysing from egg white digests, did not do so from egg yolk digests. These experiments were repeated after neutralization of the digests with NaHCO_3 . Both ferric and ferrous Fe dialysed readily from neutralized digests of egg white but both showed negligible dialysis from neutralized digests of egg yolk. Ferric Fe was found to dialyse readily from acidified suspensions of undigested egg white.

Experiments were then carried out to determine whether pyrophosphates had any influence on the dialysis of ferric Fe. The pyrophosphate was added as the sodium salt. In acid peptic digests of egg yolk, pyrophosphates had little effect. This might have been due to the rapidity with which pyrophosphates are hydrolysed to orthophosphates by mineral acids. Pyrophosphates increased to

Table 1. *Dialysis of Fe, Cu and Pb*

Fe		Peptic digest of egg yolk neutralized with NaHCO_3	
1 mg. Fe subjected to dialysis; the figures refer to mg. Fe that have dialysed in 4 hr.		Ferrous sulphate	0.03
Undigested egg white		Iron alum	0.00
		Iron alum + sodium pyrophosphate	0.20
		Casein digested with pepsin, pH 4.2	
Ferrous sulphate	0.56	Ferrous sulphate	0.39
Iron alum	0.00	Iron alum	0.06
Iron ammonium citrate	0.01	Iron ammonium citrate	0.01
Iron alum + sodium pyrophosphate	0.28		
Cu		Cu	
Egg white digested with pepsin, pH 4.0		1 mg. Cu subjected to dialysis; the figures refer to mg. Cu that have dialysed in 4 hr.	
Ferrous sulphate	0.41	Undigested egg white	0.00
Iron alum	0.41	Acidified undigested egg white (pH 4.6)	0.22
Iron ammonium citrate	0.38	Peptic digest of egg white (pH 4.2)	0.38
Peptic digest of egg white neutralized with NaHCO_3		Peptic digest of egg white neutralized with NaHCO_3	0.05
Ferrous sulphate	0.36	Undigested egg yolk	0.04
Iron alum	0.20	Peptic digest of egg yolk (pH 4.2)	0.38
Iron ammonium citrate	0.19	Peptic digest of egg yolk neutralized with NaHCO_3	0.04
Iron alum + sodium pyrophosphate	0.19		
Undigested egg white (acidified), pH 4.0		Pb	
Iron alum	0.23	1 mg. Pb subjected to dialysis; the figures refer to mg. Pb that have dialysed in 4 hr.	
Undigested egg yolk		Undigested egg white	0.00
Ferrous sulphate	0.52	Acidified undigested egg white (pH 4.6)	0.26
Iron alum	0.00	Peptic digest of egg white (pH 4.2)	0.45
Iron ammonium citrate	0.00	Peptic digest of egg white neutralized with NaHCO_3	0.03
Iron alum + sodium pyrophosphate	0.26	Undigested egg yolk	0.03
Egg yolk digested with pepsin, pH 4.1		Peptic digest of egg yolk (pH 4.1)	0.48
Ferrous sulphate	0.56	Peptic digest of egg yolk neutralized with NaHCO_3	0.02
Iron alum	0.03		
Iron ammonium citrate	0.03		
Iron alum + sodium pyrophosphate	0.03		

a marked extent the dialysis of ferric Fe from neutralized digests of egg yolk, undigested egg yolk and egg white.

Experiments carried out with casein produced results similar to those obtained with egg yolk.

Similar dialysis experiments were carried out using lead acetate and copper sulphate (1 mg. Pb, 1 mg. Cu). The amounts of these metals in the dialysates were determined by methods that have been described already [Tompsett, 1934, 3; 1939, 1]. These indicated that under favourable conditions lead and copper dialysed as readily from egg yolk suspensions as from egg white suspensions.

Examples of the results obtained are shown in Table 1.

Animal experiments

Mice were used as experimental animals. Their total Fe contents were estimated by means of thiolacetic acid and NH_3 [Tompsett, 1934, 1, 2]. An animal was ignited in a silica dish over a Bunsen burner. The ash was dissolved in water containing 5 ml. conc. HCl and the volume made up to 50 ml.; Fe was estimated in an aliquot. A precipitate of Ca phosphate usually developed but this was removed by centrifuging prior to colorimetric analysis. This did not affect the accuracy of the results.

The effect of phosphatides and phosphoproteins upon the absorption of Fe was investigated. Young female mice aged 3 weeks were used. In general, the experiments were carried out under similar conditions to those described in a previous communication [Tompsett, 1939, 2]. Control animals were killed at the commencement of the experiments. The diet, which was of low Ca content, had the following composition: whole wheat flour, 400 g.; corn starch, 325 g.; wheat gluten, 50 g.; olive oil, 40 g.; NaCl, 20 g.; KCl, 15 g.; butter, 15 g. The animals were divided into 2 groups and placed on the following diets:

- (1) Diet + 1 ml. egg white per mouse per day.
- (2) Diet + 1 ml. egg yolk per mouse per day.

In addition the diets contained supplements of added Fe, at the rate of 0.8 mg. Fe (iron alum or ferrous sulphate) per mouse per day. The duration of the experiments was 14 days. At the end of this period the animals were placed on the diets without added Fe for 4 days to remove unabsorbed Fe from their alimentary tracts; they were then killed and their total Fe contents estimated.

The results are shown in Table 2. It will be seen that phosphatides and phosphoproteins inhibit the absorption of Fe from the alimentary tract, and that similar results were obtained irrespective of whether ferric or ferrous Fe was fed.

Table 2. *Young female mice (aged 3 weeks). Time of experiment 14 days. Fe added: 0.8 mg./mouse/day*

Diet	Form of Fe added	No. of mice in group	Fe mg./100 g./mouse		
			Highest	Lowest	Mean
Control	Iron alum	14	6.76	5.00	6.11
Control + egg yolk	"	12	4.71	3.37	3.85
Control + egg white	"	12	8.16	5.88	6.80
Control	FeSO_4	6	7.57	4.78	5.72
Control + egg yolk	"	6	5.41	3.94	4.58
Control + egg white	"	6	7.54	6.56	7.01

Experiments were carried out in which iron pyrophosphate was the source of Fe. The results, which are not reported in detail, agreed with those obtained with iron alum and ferrous sulphate. This was due probably to the hydrolysis of the pyrophosphate by the acid of the gastric juice.

The influence of the Ca content of the diet upon the absorption of Fe has been investigated. The diets used had the following compositions:

Low Ca diet		High Ca diet	
	g.		g.
Whole wheat flour	400	Whole wheat flour	700
Casein	100	Whole milk powder	300
Corn starch	325	Marmite	50
Wheat gluten	50		
Olive oil	40		
NaCl	20		
KCl	15		
Butter	15		

The diets, to which were added supplements of Fe (as iron ammonium citrate), were fed at the rate of 2.5 g. per mouse per day. In the first experiments adult male mice were used; the experiments lasted 21 days. At the end of this period the animals were placed on the diets without added Fe for 4 days to remove unabsorbed Fe from their alimentary tracts; they were then killed and their total Fe contents estimated. The experiments were repeated using young female mice aged 3 weeks; the duration of these experiments was 14 days. The results indicate that Fe is absorbed more easily on a low Ca than upon a high Ca diet. The effect of the addition of HCl to the diet upon the absorption of Fe was then investigated. Young female mice aged 3 weeks were used and the duration of the experiment was 14 days. The results indicated that Fe absorption was increased by this means.

In an animal experiment, the subject usually consumes a diet every mouthful of which has the same composition. In man this does not occur. This difference is often neglected when results obtained from animal experiments are interpreted in terms of human nutrition. In view of the fact that the absorption of essential constituents of the diet may be hindered by their mutual inhibition, it seems important for a daily diet to be arranged so that such inhibition is reduced to a minimum. The following experiment was carried out with this aim in view. Young female mice aged 3 weeks were placed on high and low Ca diets on alternate days, together with added Fe (as iron ammonium citrate), for 14 days. At the end of this period, the animals were placed on a high Ca diet alone for 4 days to remove unabsorbed Fe from their alimentary tracts; they were then killed

Table 3. *Influence of Ca content of diet on absorption of Fe given as iron ammonium citrate*

Sex and age	Time in days	Diet	Amount of Fe added mg.	No. of mice in group	Fe, mg./100 g. mouse		
					Highest	Lowest	Mean
Adult male	21	Control	2	5	6.67	6.00	6.35
	21	High Ca	2	4	6.60	5.83	6.22
	21	Low Ca	2	4	10.22	8.29	8.92
Female, 3 weeks	14	Control	0.8	8	6.80	5.04	5.53
	14	High Ca	0.8	8	7.77	5.58	7.27
	14	Low Ca	0.8	8	17.25	8.54	12.04
	14	High Ca + HCl	0.8	8	13.25	8.75	11.04
	14	High and low Ca alternate days	0.8	8	5.99	4.38	5.17

and their total Fe contents estimated. The results show that the absorption of Fe was very good, but in addition it should be noted that their increases in weight were very much better than in the other experiments.

The results of the above experiments are shown in Table 3.

The reduction of ferric Fe in the alimentary tract and its significance in absorption

The effect of various foodstuffs upon ferric Fe has been examined by means of the $\alpha\alpha'$ -dipyridyl reaction [cf. Hill, 1931].

1 mg. ferric Fe (as iron alum) was added to about 1 g. of foodstuff suspended in about 5 ml. of water. After allowing the mixture to stand for 10 min., a little $\alpha\alpha'$ -dipyridyl was added. In acid solution (pH 4-5) a number of these substances produced a marked reduction of ferric Fe as evidenced by the production of a red colour. That this was not due to food Fe was indicated by the negative results obtained with controls. At neutral reactions the results were indefinite. Of the substances examined the following were found to be capable of producing a rapid reduction of ferric Fe in acid solutions: whole wheat flour, whole milk powder, wheat gluten, egg white (boiled and unboiled), cows' milk (boiled and unboiled), commercial egg albumin. Casein, glucose, lactose, egg yolk (boiled and unboiled) were found to be without effect upon ferric Fe. Peptic digests of egg white and egg yolk (pH 4-5) were then examined. The former had a marked reducing effect on ferric Fe but the latter was without effect.

The effect on ferrous Fe of those substances which had no reducing action on ferric Fe was then examined. Except in the case of egg yolk no effect was observed. Ferrous Fe allowed to stand in contact with egg yolk became oxidized to the ferric state. This was shown by the fact that when 1 mg. of ferrous Fe (as ferrous sulphate) was added to 5 ml. of egg yolk, the mixture gave no reaction for ferrous iron with $\alpha\alpha'$ -dipyridyl at the end of 1 hr. This phenomenon is considered to be due to autoxidation in which the phosphatides play a part.

Ferric Fe has been shown to dialyse readily from acid peptic digests of egg white and from acidified, but not from neutral, egg white. The state of the iron in the dialysates was examined with $\alpha\alpha'$ -dipyridyl. It was found that approximately 66% of the Fe was in the ferrous state and the remainder in the ferric state. The duration of the experiments was long, viz. 4 hr., so that some re-oxidation could have taken place. That reduction had taken place was to be expected. It is quite possible, however, that ferric Fe did dialyse under the conditions in which these experiments were carried out. Ferrous Fe was observed to dialyse readily from egg yolk and from acid peptic digests of egg yolk. That this was possible is due to the fact that autoxidation takes time. It is considered that the inability of ferrous Fe to dialyse from neutralized peptic digests of egg yolk is due primarily to its precipitation as phosphate. Dialysis of ferric Fe from acid peptic digests of casein was almost negligible. This was due to the formation of stable undissociated ferric complexes and the inability of the mixture to reduce ferric Fe.

In an earlier communication it was shown that added ferric Fe could be almost completely recovered in trichloroacetic acid extracts of simple proteins, e.g. egg white [Tompsett, 1934, 1]. These experiments were repeated, the extracts being examined for the presence of ferrous Fe; only traces could be detected, the greater part of the Fe being in the ferric state. This indicates that reduction was not necessary for these extractions. Complexes of ferric Fe with simple proteins are not as stable as those with phosphatides and phosphoproteins.

The evidence that has been presented suggests that ferric Fe as such has very little chance of absorption from the alimentary tract but that ferrous Fe should be absorbed easily. It would appear that ferric Fe can be reduced in the alimentary tract, not in the small intestine as some workers have suggested, but in the stomach. This has been confirmed *in vivo*. Two rats were fed on (a) boiled egg white + iron alum, (b) boiled egg yolk + iron alum. At the end of 2 hr. they were killed and their stomach and intestinal contents examined for the presence of ferrous Fe with $\alpha\alpha'$ -dipyridyl. In the former animal the stomach and intestinal contents gave an immediate and strong reaction for ferrous Fe; no trace of ferrous Fe could be observed in the latter animal.

A clearer insight into the results obtained from the animal experiments can now be obtained. The low absorption of Fe by mice on a diet containing egg yolk was no doubt due to the predominating autoxidation properties of the diet. With egg white in the diet Fe absorption was much higher, owing to the predominating reducing properties of the diet. It also becomes apparent why similar results were obtained when ferrous and ferric Fe were fed with diets containing egg yolk and egg white.

The question naturally arises as to whether the Fe of the egg is available at all, i.e. whether the effect of the white predominates over that of the yolk or *vice versa*. Experiments were carried out to decide this point. Peptic digests of boiled and unboiled whole egg were prepared. At pH 4-5, a marked reducing effect was observed. This was confirmed *in vivo* on a rat. These results indicate that the effect of the white predominates over that of the yolk.

COPPER

The study of the alimentary absorption of Cu seems to have been neglected. The writer has shown that the absorption of both Fe and Pb from the alimentary tract is influenced by the Ca content of the diet. The addition of acid to the diet increased the absorption both of Fe and of Pb. It seemed very probable that the absorption of Cu would be governed by similar factors and the following experiments were undertaken to investigate this question. Dialysis experiments indicated that the absorption of Cu would probably not be greatly inhibited by the presence of phosphoproteins and phosphatides in the diet as was found to be the case with ferric Fe.

The experiments were carried out in a similar manner to those with Fe. The diets had the same composition and both adult and young female mice were used.

The total Cu contents of the animals were estimated in the ash with sodium diethyldithiocarbamate as described for urine and faeces in a previous communication [Tompsett, 1934, 3].

Table 4. *Influence of diet on absorption of Cu given as Cu sulphate*

Sex and age	Time in days	Diet	Amount of Cu added mg.	No. of mice in group	Cu, mg./100 g. mouse		
					Highest	Lowest	Mean
Adult male	21	Control	2	8	0.17	0.10	0.14
	21	Low Ca	2	8	0.33	0.21	0.27
	21	High Ca	2	7	0.18	0.14	0.16
	21	High Ca + HCl	2	8	0.37	0.21	0.28
Female, 3 weeks	14	Control	0.8	5	0.29	0.21	0.25
	14	Low Ca	0.8	8	0.69	0.30	0.38
	14	High Ca	0.8	6	0.22	0.18	0.20
	14	High Ca + HCl	0.8	7	0.42	0.30	0.36

The results are shown in Table 4. It will be seen that the absorption of Cu is higher on a low Ca diet than on a high Ca diet and is increased by the addition of acid to the diet. The latter observation indicates the importance of the gastric acidity for the absorption of Cu.

DISCUSSION

It has been shown that certain foodstuffs are capable of reducing ferric Fe to the ferrous state especially at an acid reaction. It is of interest to speculate as to the nature of the substances responsible for this reducing action. Certain vitamins, e.g. ascorbic acid, might prove to be responsible but the writer is inclined to the view that protein is chiefly responsible. More work upon these problems is necessary.

The results obtained in this work suggest that ferric Fe as such stands very little chance of absorption from the alimentary tract. Its absorption would be hindered by the presence of phosphatides and phosphoproteins. Evidence has been produced which shows that ferric Fe can be reduced in the alimentary tract, not in the small intestine as has been suggested, but in the stomach. This reduction is brought about by substances which are common constituents of the diet. On the other hand, the diet may contain substances which produce an opposite effect, i.e. the oxidation of ferrous Fe to the ferric state. Ferrous Fe appears to be the only form to be absorbed from the alimentary tract.

The following factors have been shown to influence the absorption of food Fe from the alimentary tract:

- (1) The gastric acidity.
- (2) The Ca content of the diet.
- (3) The oxidation-reduction potential of the stomach contents.

The gastric acidity is important in two respects: (1) it aids the solution of the Fe; (2) it appears to be necessary for the reducing action of the diet, if any, to take its full effect. The Ca content of the diet has a marked effect. Similar results have been obtained with Pb and suggestions put forward in the report dealing with Pb [Tompsett, 1939, 2] probably apply also to Fe. The composition of the diet determines the state of the ingested Fe, i.e. whether it is in the ferric or ferrous state. In a diet which is predominantly reducing, then, irrespective of whether the Fe was initially in the ferric or ferrous state, its final state will be ferrous and as a result it should be absorbed easily. In a diet which is predominantly oxidizing, the final state of the Fe will be ferric and it is presumably not absorbed. Only in an inert diet will the state of the Fe remain unchanged.

Lack of gastric acidity has been held responsible for the hypochromic anaemia that often occurs in achlorhydria. Anaemia does not always occur in achlorhydria. Even in this condition, the gastric contents have an acid reaction, although this is weak. A certain amount of Fe should pass into solution and it appears that a reducing action might occur. Whether or not the amount of Fe absorbed under such conditions is sufficient to prevent anaemia will depend upon the requirements.

Attempts have been made to determine the minimum amount of Fe necessary in the human diet [Sherman, 1933]. The results obtained have not been particularly convincing. The writer considers that it is impossible to state any particular quantity of Fe as being sufficient since the degree of Fe absorption is dependent on so many factors.

Workers on nutrition usually consider human requirements in terms of the daily intake. The writer has shown that absorption of essential substances may

result in mutual inhibition. A daily diet should be so arranged into meals that mutual inhibition is reduced to a minimum. One could even go a stage further and plan a meal into courses since it is well known that food tends to layer in the stomach so that the digestion and absorption of one course is independent of another. This is of particular importance in children where the requirements are large.

A large number of Fe salts, both ferric and ferrous, have been used in the treatment of hypochromic anaemias. Large doses, especially of the ferric salts, are now usually given. They may be administered on an empty stomach or after a meal. When medicinal Fe is administered after a meal its absorption will be subject to the same factors as that of food Fe. When administered on an empty stomach the conditions of absorption will be different, the constituents of the secretions of the alimentary tract being the only limiting factor. So far these have been neglected, but they must have some influence.

Although ferric Fe appears to be capable of dialysing as the pyrophosphate, this fact appears to be of little physiological importance.

The value of large doses of therapeutic iron, especially of ferric salts, is probably due to the fact that only a part is converted into non-dissociated colloidal complexes. Under such conditions the possibility should not be overlooked that ferric Fe may be absorbed as such.

Absorption of Fe and Cu probably occurs in the upper part of the small intestine where the contents still have a pronounced acid reaction. The reaction of the intestinal contents must exert a very marked influence and this must depend on a number of factors such as the gastric acidity, the base content of the diet and the character of the intestinal secretions. Much more work is still required before we can obtain a clear understanding of Fe absorption.

SUMMARY

1. The dialysis of ferric Fe, but not of ferrous Fe, is inhibited by phosphatides and phosphoproteins.
2. Ferric Fe may be reduced by certain foodstuffs.
3. Ferrous Fe is oxidized readily to the ferric state in the presence of egg yolk, presumably by a process of autoxidation.
4. Ferric Fe does not appear to be capable of absorption from the alimentary tract. Fe, to be absorbed, must be in the ferrous state.
5. Reduction of ferric Fe can occur in the stomach, this being effected by the diet.
6. The absorption of both Fe and Cu from the alimentary tract is influenced by the gastric acidity and the Ca content of the diet.
7. The relation of these factors to human nutrition has been discussed.

I wish to thank Dr A. B. Anderson for his helpful criticism and advice.

REFERENCES

- Brock & Hunter (1937). *Quart. J. Med.* **6**, 5.
Elvehjem (1932). *J. Amer. med. Ass.* **98**, 309.
Hart, Steenbock, Waddell & Elvehjem (1928). *J. biol. Chem.* **77**, 797.
Heubner (1926). *Klin. Wschr.* **5**, 588.
Hill (1931). *Proc. Roy. Soc. B*, **107**, 205.
Lintzel (1931). *Ergebn. Physiol.* **31**, 844.
McCance & Widdowson (1937). *Lancet*, **2**, 680.
——— (1938). *J. Physiol.* **94**, 148.
Moore, Minnich & Welch (1939). *J. clin. Invest.* **16**, 613.
Reimann & Fritsch (1930). *Z. klin. Med.* **115**, 13.
Sherman (1933). *Chemistry of Food and Nutrition*. London.
Starkenstein & Weden (1930). *Arch. exp. Path. Pharmacol.* **134**, 278.
Tompsett (1934, 1). *Biochem. J.* **28**, 1536.
——— (1934, 2). *Biochem. J.* **28**, 1802.
——— (1934, 3). *Biochem. J.* **28**, 2088.
——— (1939, 1). *Biochem. J.* **33**, 1231.
——— (1939, 2). *Biochem. J.* **33**, 1240.
——— (1939, 3). *Brit. J. exp. Path.* **20**, 512.
Whipple & Robschey-Robbins (1936). *Amer. J. med. Sci.* **191**, 11.