CCL. THE ABSORPTION AND EXCRETION OF IRON BEFORE, DURING AND AFTER A PERIOD OF VERY HIGH INTAKE

By ELSIE MAY WIDDOWSON
AND ROBERT ALEXANDER McCANCE

From the Biochemical Laboratory, King's College Hospital, S.E. 5

(Received 30 September 1937)

INTRODUCTION

FROM a study of the literature McCance & Widdowson [1937, 2] suggested that the capacity of the human body to excrete iron may be very much less than has generally been supposed. It may in fact be negligible. A series of experiments was accordingly designed to put this matter to the test and the present paper records some of the results.

In the first stage of this experiment normal human adults were placed upon a mixed diet with a reduced iron content. On this diet it was expected that they would be "in iron balance" and this was in fact found to be the case. Current theories of iron metabolism would explain this by stating that measurable quantities of iron were being absorbed every day and that the subjects were maintained in iron equilibrium by a process of regulated or controlled excretion. Assuming this to be true, the fact that they were in balance showed that they were saturated with iron up to normal capacity. Consequently, any increase in the quantity of iron in the body should have been followed by the excretion of a corresponding amount. In the second stage the subjects were given very large doses of iron by mouth. This has the effect of forcing iron into the body [Brock & Hunter, 1937; Fowler & Barer, 1937] which in itself is evidence that the processes of excretion must lag far behind those of absorption when the latter is abnormally high. In the final stage the subjects returned to a low iron intake and their iron exchanges were carefully measured. It was considered that a negative iron balance was to be expected according to current views, but that the new theory required the subjects to be in iron equilibrium.

PRESENT INVESTIGATION

The subjects. Two men and two women acted in this capacity. The two men, R. J. M. age 24 and A. W. H. age 23 were laboratory technicians, and the two women, M. M. aged 21 and H. M. S. age 29 were secretaries. They all carried out their ordinary duties throughout the course of the experiment.

(a) Stages 1 and 3. General metabolic technique

During these stages, when exact balances were being carried out at low iron intakes, food was prepared and eaten at the laboratory, care being taken to avoid the possibility of any iron contamination. Foods rich in iron such as meat, eggs, brown bread and chocolate were avoided, but no restriction was placed upon the amount of food eaten. Duplicate samples of all food and drinks were

set aside for analysis. They were then treated as described by McCance & Widdowson [1937, 1]. The urine was passed into glass vessels and stored under toluene. At the end of each period duplicate portions of 200 ml. of the mixed urine were dried, dry-ashed and analysed for iron. The faeces were marked with carmine at suitable intervals and passed directly into counterpoised glass bowls. Collections were continued in the same bowl until the end of each period. Then about 100 ml. conc. A.R. iron-free HNO_3 were added as recommended by Brock & Hunter [1937] and the whole thoroughly mixed with a stout glass rod flattened into a $\frac{3}{4}$ in. disk at the lower end. The mixture was left to stand at room temperature, with occasional stirring, for several days. By this means a uniform suspension was obtained. The contents of the bowl were then weighed and duplicate samples of 30 g. weighed out into silica crucibles, dried at 50° and dry-ashed. 10 ml. of conc. A.R. HCl were then added and the mixture heated gently for some hours on a sand-bath. Subsequent treatment of the ash was similar to that described by McCance *et al.* [1936].

The ash extracts of food, drinks, urine and faeces were analysed for iron by the thiolacetic acid method [Lyons, 1927; McCance *et al.* 1936].

(b) *Stage 2.*

It was decided to give each subject 1000 mg. of iron a day and the administration of these large doses was continued without interruption for 36–46 days.

R. J. M. and M. M. took a solution of ferric ammonium citrate by mouth. The daily supply was taken in two 20 ml. portions, one in the morning and one in the afternoon. All the doses were carefully measured with the same 20 ml. pipette which was also used to withdraw aliquots for analysis. The iron solution was diluted with water before being taken and the drinking vessel thoroughly rinsed out with water and the washings drunk. A. W. H. and H. M. S. took saccharated ferrous carbonate in 1 g. capsules. Four were taken daily in divided doses. Samples of each preparation of iron were dry-ashed and analysed by Klumpp's [1934] method which was also used for the analysis of iron in the faeces in this part of the experiment. It was found on doing so that the solution of ferric ammonium citrate contained less, and the saccharated ferrous carbonate more iron than was supposed. However, it was decided that the variations from the dosage of 1000 mg. per day, originally planned, did not matter. The large doses of iron caused no digestive disturbances, constipation or diarrhoea in any of the subjects.

The subjects ate their ordinary food during this stage of the experiment. Duplicate portions were not collected for analysis, but during one week all the food eaten was weighed and the amount of iron in it calculated from food analyses made in this department [McCance & Shipp, 1933; McCance *et al.* 1936; Abrahams & Widdowson, 1937]. An allowance was then made for the average daily intake of iron in the food.

Urines were not collected throughout the whole period to avoid placing unnecessary restrictions on the voluntary subjects. One 24 hr. urine, however, was collected each week and analysed for iron. In this way an approximate figure for the urinary output of iron throughout stage 2 was obtained. It was considered that these approximations with regard to food and urine were fully justified in the light of the relatively huge quantities of iron given medicinally by mouth and recovered in the faeces.

In work of this sort it is essential to deal with the faeces in an absolutely quantitative way since any conclusions about iron absorption must be based upon small differences between large amounts, and losses of any kind increase the

apparent absorption. Accordingly, the faeces were passed into the counterpoised glass bowls as before, beginning on the day following the first dose of iron, and collections were made in the same bowl for 3-7 days. Used toilet paper was burnt and the ashes mixed with the faeces. The stools were treated with nitric acid and sampled for analysis as in stages 1 and 3. After dry ashing, the residue was treated with 10 ml. conc. HCl and heated for some hours on a sand-bath. A slight modification was introduced into the method of extracting the ash since such large amounts of iron were present. The ash was boiled twice with 10 ml. of conc. HCl and the extracts filtered through an iron-free filter-paper into a 200 ml. flask. Subsequent extractions were made with boiling water up to near the 200 ml. mark. Re-ashing of the filter paper was found to be unnecessary.

The method chosen for the determination of the iron was that of Klumpp [1934] which has been used and recommended by Brock & Hunter [1937]. The method, which depends upon the oxidation of titanium salts, was tested carefully on standard solutions and found to be satisfactory. Some ash extracts, particularly those belonging to the terminal periods of stage 2 (*vide infra*), were also determined colorimetrically by thiolacetic acid after suitable dilution and the results obtained by the two methods agreed very well. In this, as in all stages of the experiment, excellent duplicates were always obtained between the 30 g. faecal aliquots, indicating that the faeces had been satisfactorily emulsified.

The Whitsun-Coronation holidays came in the middle of stage 2, and 3 of the subjects collected no faeces for 6 days. In assessing the total iron retained by these subjects, the output for these 6 days has been averaged from the preceding and following weeks. H. M. S. collected throughout without a break.

Of the two women subjects taking part in this investigation only one, H. M. S., was menstruating regularly. The preliminary and final balance studies were made when she was not menstruating, but two menstrual periods occurred during the time she was taking the iron preparation. Quantitative collections of the menstrual loss were made and the acid digestion method described by Leverton & Roberts [1937] was used to obtain a uniform sample. Aliquots of the acid digest were dried and then dry ashed and the iron extracted and estimated by thiolacetic acid in the manner already described. Blank determinations on the sanitary towels were carried out and an allowance made for the iron found in them in the calculation of the results. The losses were 18.6 and 19.0 mg. of iron during the first and second periods respectively. M. M. did not menstruate in the course of the investigation.

Results

(a) *Preliminary balances at low iron intakes (stage 1).* The experimental diets were administered for 4 days before any collections were made. The results of the balance periods of 7 days (14 days in the case of H. M. S.) are shown in Table I. The variations in intake from one subject to another are due to individual differences in appetite. It is clear that the subjects were all approximately in "iron balance" and it was concluded therefrom that they were all suitable subjects for this investigation.

(b) *Iron balances during and for 10-14 days following the administration of large doses of iron (stage 2).* The results are shown in Table II and consist for each subject in a prolonged period of high iron intake, followed by three, or in the case of A. W. H. four, short periods during which no medicinal iron was taken by mouth. The reduced food intake of iron shown by all the subjects during the last of these short periods was due to the fact that at this point they were placed upon low iron intakes in preparation for the final balance studies (stage 3).

All four subjects were in positive iron balance and showed net absorptions of 1.51–5.03 g. of iron—very large amounts, considering the total amount of iron likely to be present in the body. It is important also to note that during the short after-periods there was a more or less rapid diminution in the excretion of iron so that by the last short period the subjects were actually or approximately in balance. In the light of the results to be described in stage 3 the negative

Table I. *Preliminary iron balances*

Subject	Date	No. of exp. days	Fe intake mg.	Fe excretion mg.			Balance mg.
				Urine	Faeces	Total	
A. W. H.	18–24 Mar.	7	50.2	2.8	49.0	51.8	– 1.6
R. J. M.	18–24 Mar.	7	60.2	2.7	59.5	62.2	– 2.0
M. M.	18–24 Mar.	7	44.2	1.9	40.4	42.3	+ 1.9
H. M. S.	18–31 Mar.	14	109.3	6.0	105.4	111.4	– 2.1

Table II. *The effect of a high intake of iron upon the balances*

Subject	Date	No. of exp. days	Iron intake (mg.)			Iron output (mg.)			Balance mg.
			Food (approx.)	Medicine	Total	Urine (approx.)	Faeces	Total	
A. W. H.	7 Apr.–22 May	46	932	60,500	61,432	19	55,600	55,619	+ 5,813
	22–25 May	3	61	0	61	1	2,550	2,551	– 2,490
	26–28 May	3	61	0	61	1	237	238	– 177
	29 May–1 June	4	40	0	40	2	141	143	– 103
	2–6 June	5	44	0	44	2	42	44	± 0
R. J. M.			1,138	60,500	61,638	25	58,570	58,595	+ 3,043
	7 Apr.–22 May	46	920	44,400	45,320	21	43,500	43,520	+ 1,799
	22–25 May	3	60	0	60	1	137	138	– 78
	26–28 May	3	60	0	60	1	68	69	– 9
	29 May–1 June	4	40	0	40	2	43	45	– 5
M. M.			1,080	44,400	45,480	25	43,748	43,773	+ 1,707
	7 Apr.–22 May	46	452	44,400	44,852	14	42,400	42,414	+ 2,436
	23–25 May	3	30	0	30	1	910	911	– 881
	26–28 May	3	30	0	30	1	62	63	– 33
	29 May–1 June	4	20	0	20	1	33	34	– 14
H. M. S.			532	44,400	44,932	17	43,405	43,422	+ 1,510
	17 Apr.–22 May	36	382	49,000	49,382	12	43,590	43,638*	+ 5,744
	22–25 May	3	32	0	32	1	668	669	– 637
	26–28 May	3	32	0	32	1	79	80	– 48
	29 May–1 June	4	20	0	20	1	47	48	– 28
			466	49,000	49,466	15	44,384	44,435	+ 5,031

* Includes losses of 18.6 and 19 mg. of iron during two menstrual periods.

Table III. *Final iron balances*

Subject	Date	No. of exp. days	Fe intake mg.	Fe excretion mg.			Balance mg.
				Urine	Faeces	Total	
A. W. H.	7–15 June	9	70.1	3.3	60.5	63.8	+ 6.3
R. J. M.	2–12 June	11	100.8	4.9	92.2	97.1	+ 3.7
M. M.	2–12 June	11	70.8	2.8	64.1	66.9	+ 3.9
H. M. S.	2–11 June	10	79.6	4.3	67.1	71.4	+ 8.2

balances observed during these periods were probably due to the excretion of unabsorbed iron and this is borne out by the fact that A. W. H., who was habitually more constipated than the others, showed the largest “delayed” excretion. These results are a confirmation of those of Brock & Hunter [1937] and of Fowler

& Barer [1937], who have both found that iron was absorbed and retained when large amounts were given by mouth. The percentage retention was considerably larger in Fowler & Barer's cases than in the present series, but in essentials the results are the same. It is of some interest in this connection to point out that A. W. H. and H. M. S., who took the saccharated ferrous carbonate preparation, absorbed and retained considerably more iron than either of the other two subjects, R. J. M. and M. M., who took the ferric ammonium citrate. It is not the purpose of the present article to discuss the relative merits of different iron preparations, but in this study at any rate the ferrous preparation was absorbed more readily than the ferric.

(c) *Final balance studies (stage 3)*. The last 4 days of stage 2 served as the preliminary periods for the two balance studies of 4–6 days which taken together constituted stage 3. The results are shown in Table III. All four subjects were in slightly positive iron balance.

DISCUSSION

So far as the balances are concerned, the results on all four subjects are essentially the same so that they can be discussed as one. The crux of the whole matter is the failure to find negative balances in stage 3, following the absorption and retention of large quantities of iron during stage 2. The additional iron forced into the body during stage 2 was unwanted (if the preliminary balances in stage 1 are taken at their face value). Yet there were no signs of its being excreted during stage 3. Why not? Presumably because neither the intestine nor the kidney had any power to do so. It is concluded therefore that these results support the contention [McCance & Widdowson, 1937, 2] that the intestine has no power to regulate the amount of iron in the body by varying the amount excreted, and that in fact the intestine normally excretes little or no iron. The amount in the body must in consequence be regulated by the amount absorbed.

Fig. 1. Haemoglobin levels. N.B. H. M. S. did not commence to take iron until day 10.

Haemoglobin levels. Haemoglobin determinations were made in duplicate on each of the subjects during the experiment. The Haldane method was used in which 100 % is equivalent to 13.8 g. of haemoglobin per 100 ml. of blood. The results are shown graphically in Fig. 1, the initial level being the mean of several determinations in each case. It will be noted: (a) That the haemoglobin levels of R. J. M. and M. M. underwent no change in spite of the large quantities of iron absorbed. Initially, M. M.'s haemoglobin was unusually high for a

woman, possibly to be connected with the fact that she was not menstruating. (b) A. W. H.'s haemoglobin, which was 116 % at the beginning, rose about 10 % during the administration of iron. This was an unexpected and possibly exceptional response [Widdowson & McCance, 1936]. The level did not fall during the subsequent few weeks but reverted almost to its original level after about 4 months. (c) H. M. S.'s haemoglobin was only 84 % when the experiment began. This was rather low even for a woman and it was expected [Widdowson & McCance, 1936] that it would rise following the administration of iron. It did so, rising to 101 %, but fell again when the administration of iron was stopped, in spite of the fact that the subject had meantime stored away in her body 5 g. of additional iron—more than enough to double the haemoglobin of her blood. This is of great interest. One would not have expected this subject's haemoglobin to have fallen back as it did if its level had initially been low because of a simple iron deficiency, for the iron absorbed in this experiment ought to have satisfied the demands of this subject's body for the rest of its life. The matter is being further investigated for it is clearly most important to know more about what controls the haemoglobin level of the blood.

SUMMARY

1. Two men and two women were placed upon diets containing 7–9 mg. of iron per day and were found to be in iron balance.
2. The same subjects were then given large doses of iron by mouth (about 1000 mg. per day). Positive balances were obtained in every case and, after discontinuing the iron but allowing time for the excretion of the unabsorbed metal from the gut, net absorptions of 1.5–5 g. of iron were registered.
3. Immediately after absorbing these large amounts of iron the subjects were again found to be in iron equilibrium on low iron intakes. This supports the view that the body has little or no capacity for excreting iron.
4. In one female subject the haemoglobin rose from 84 % (Haldane) to 101 % during the administration of the large doses of iron but fell again afterwards to its original level.

The authors are very grateful to the four subjects for their steady co-operation in a rather laborious experiment. They have also to thank Miss Masters for carrying out some of the iron determinations and Miss Verdon-Roe for superintending the diets. One of them (E. M. W.) is indebted to the Medical Research Council for a part time grant.

REFERENCES

- Abrahams & Widdowson (1937). *Modern Dietary Treatment*. (London: Baillière, Tindall and Cox.)
- Brock & Hunter (1937). *Quart. J. Med.* **6** (N.S.), 5.
- Fowler & Barer (1937). *Arch. intern. Med.* **59**, 561.
- Klumpp (1934). *J. biol. Chem.* **107**, 213.
- Leverton & Roberts (1937). *J. Nutrit.* **13**, 65.
- Lyons (1927). *J. Amer. chem. Soc.* **49**, 1916.
- McCance & Shipp (1933). *Med. Res. Coun. Spec. Rep. Ser. No. 187*.
- & Widdowson (1937, 1). *Quart. J. Med.* **6** (N.S.), 277.
- & Widdowson (1937, 2). *Lancet*, ii, 680.
- Widdowson & Shackleton (1936). *Med. Res. Coun. Spec. Rep. Ser. No. 213*.
- Widdowson & McCance (1936). *J. Hyg., Camb.*, **36**, 13.