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PLASMA IRON LEVELS AND URINARY IRON EXCRETION AFTER THE INTRAVENOUS ADMINISTRATION OF DIFFERENT IRON PREPARATIONS*

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The present study of plasma iron levels and urinary iron excretion after intravenous administration of different iron preparations was carried out to elucidate the fate of injected iron and obtain a better understanding of the mechanisms of toxicity of the different preparations. The doses used were near the maximum tolerated doses, and the compounds included both well-known and new preparations (Nissim, 1949, 1953).

METHODS

Serum and plasma iron were determined by the ortho-phenanthroline method (Laurell, 1947), modified in some details. Thus, the amount of HCl in this method (3 ml. 6N HCl to 6 ml. serum) converted only 17.5% of saccharated iron oxide into ferric chloride, whereas complete breakdown required 9 ml. HCl to 6 ml. of solution. At first estimations were carried out on blood serum, but inaccurate results were obtained with ferric glucosate, since the particles clumped and adhered to coagulating proteins during clotting. All subsequent iron estimations were made on plasma.

Ortho-phenanthroline did not dissolve in 10% absolute alcohol to give a 2% solution, and a 1% solution in 30% absolute alcohol was used instead. Analar sodium sulphite was used instead of sodium hydrosulphite : this made the solution alkaline, and 0.5N-HCl was added to get rid of the yellow paranitrophenol colour before the addition of ortho-phenanthroline. The high plasma iron values also necessitated the addition of more ortho-phenanthroline and sodium sulphite to ensure the estimation of all the iron. The amount of plasma was reduced from 6 to 0.5 ml. in order to use the steep part of the absorptiometer curve.

Effect of Haemolysis on Serum Iron Values.—High plasma iron increased the tendency of the blood to haemolyse *in vitro*. The effect of haemolysis on serum iron values was therefore investigated.

Rabbit blood (7.5 ml.) was partly haemolysed with 2.5 ml. hypotonic saline to produce a deep pink colour. Serum iron was 0.28 mg. per 100 ml. after allowing for dilution. This is not much higher than serum iron values in normal rabbits (0.10–0.25 mg. per 100 ml.).

Red cells from 4 ml. of rabbit blood were washed repeatedly with normal saline, haemolysed completely with saponin, and treated with 12 ml. conc. HCl. The iron contributed by the haemolysed cells was 0.62 mg. per 100 ml. blood, which agrees with the values of non-haemoglobin iron present in red cells (Shortland and Wall, 1936). Even conc. HCl, therefore, failed to split the haemoglobin molecule. The accidental haemolysis of even one-tenth of the red cells would introduce an error of 0.06 mg. per 100 ml., which is negligible in experiments dealing with levels of 1 to over 100 mg. per 100 ml.

Estimation of Iron in Urine.—Iron estimations were carried out as for plasma on 0.5 ml. of urine from two normal rabbits. The iron content was negligible ; it could not be estimated accurately with this method because of interference from pigments. Urinary iron excretion after iron injections was, however, high enough for comparative estimations to be made with the same simple method which was used for plasma.

Modified Phenanthroline Method used in the Present Experiments.—Blood (3–4 ml.) is mixed in a graduated centrifuge tube with 0.1 ml. of 1% heparin, and centrifuged for 15 minutes. The volume of plasma is noted, and 0.5 ml. is transferrred to a round-bottomed 50-ml. centrifuge tube ; 1.5 ml. conc. HCl is added, followed in 10 minutes by 3 ml. of 20% redistilled trichloracetic acid. The mixture is centrifuged until the supernatant fluid is clear. The latter is then poured into a 12.5-ml. volumetric flask, a drop of 1% paranitrophenol is added as indicator, followed by conc. ammonia, drop by drop, until a yellow colour is just obtained. The mixture is acidified with N/2 HCl until the colour disappears, buffered with 0.2 ml. of 1N sodium acetate, and reacidified with N/2 HCl until the colour disappears again. Fifteen drops of 1% ortho-phenanthroline are added, followed by 150 mg. of sodium sulphite. When the latter has dissolved, the solution is reacidified with N/2 HCl until all yellow colour has disappeared. Distilled water is now added to the mark, and, after mixing, the intensity of

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TABLE I

PLASMA IRON LEVELS AND URINARY IRON EXCRETION AFTER THE INTRAVENOUS INJECTION OF DIFFERENT IRON PREPARATIONS INTO RABBITS

Expt.	Compound	Weight of Rabbit in kg.	Dose mg. Fe/kg.	Fe Injected in mg.	Plasma Iron (Hours after Injection) 0	½	3	6	24	Quantity Excreted in 24 hrs., mg. Fe	% Excreted
1 a	Saccharated iron oxide (G_4) ..	2·9	45	130	125	95	70	45	5·4	—	—
b	Iron and ammonium citrate ..	2·6	45	117	110	41·5	14	7	0·5	—	—
2 a	Saccharated iron oxide (G_4) ..	2·1	45	94	120	95	41	24	1	3·0	3·2
b	Iron and ammonium citrate ..	2·7	45	122	112	32	10	5	1	46·5	38·1
3 a	Saccharated iron oxide (G_4) ..	2·05	22·5	46	62	42	28	10	—	1·15	2·5
b	Iron and ammonium citrate ..	2·4	22·5	54	45	17	7	2	—	8·7	16·1
4 a	Saccharated iron oxide (G_4) ..	2·1	180	380	340	144	56	44	—	8·25	2·2
b	,, ,, ,, ,, ..	2·3	360	828	500	375	175	—	—	16·5	2·0
5 a	Saccharated iron oxide (Sample B)	3·0	22·5	66	49	31	11·5	5	—	2	3·0
b	,, ,, ,, ,, ..	2·3	22·5	50	46	34	13	5·5	—	1·9	3·8
6 a	Ferrivenin	3·0	22·5	66	46	32·2	12·3	5·3	—	2·2	3·0
b	,, ...	2·3	22·5	50	39	29	16	4	—	1·63	3·3
7 a	Ferric glucosate	2·0	22·5	45	65	50	21·5	7.5	—	1·2	2·7
b	,, ,, ...	3·0	45	135	100	67	—	—	—	—	—
c	,, ,, ...	2·2	90	200	141	63	—	—	—	—	—
8 a	Ferric hydroxide ferrous ascorbate	2·35	22·5	51	22	8	4	2	—	14·0	27·4
b	Ferrous chloride ascorbate ..	3·15	22·5	71	41	27	—	—	—	—	—
9 a	Ferrous ascorbate	3·6	22·5	82	50	40	20	—	—	1·0	1·5
b	Neutral ferrous ascorbate ..	2·5	22·5	56	39	30	17	13	—	4·0	7·1
c	Alkaline ferrous ascorbate ..	2·5	22·5	56	36	32	19	17	—	4·1	7·3
10 a	Colloidal ferric hydroxide ..	2·05	22·5	46	11	9	1·25	0·9	—	0·125	0·27
b	Ferric chloride caramelate ..	2·8	22·5	62·4	46	25	15	8	—	11·67	18·7
11 a	Ferrous chloride pyruvate ..	2·5	22·5	56	42	23	5	2·5	—	0·16	0·29
b	Ferric chloride lactate ..	2·4	22·5	54	65	61	41	12	—	0·8	1·5
12 a	Ferric tartrate	2·58	22·5	58	45	32	17·5	10·5	—	2·04	3·5
b	Ferronascine	2·75	22·5	62	50	38	25	16	—	2·15	3·5

The concentration of Fe in the injected solutions varied between 0·5 and 2%.

the colour is estimated by the photoelectric absorptiometer, using a filter with maximum transmission of light at wavelength 490 mμ. The reading is made against a control of distilled water treated similarly.

RESULTS

Plasma or serum iron levels and urinary iron excretion with different iron preparations are shown in Table I. The following points call for comment.

Saccharated Iron Oxide (G_4) and Iron and Ammonium Citrate.—In each experiment two rabbits were used, one injected intravenously with saccharated iron oxide, the other with iron and ammonium citrate (both 45 mg. Fe/kg.). In Experiment 1 estimations were carried out on serum, in Experiment 2 on plasma (Table I).

A wide difference is noted in the disappearance of iron from the blood with the two preparations. Indeed, if the first sample of blood after iron and ammonium citrate had not been collected speedily, the initial high figures would have been missed. If plasma volume in rabbits is taken as 3.5–4.5% of the body weight, a dose of 45 mg./kg. would give a plasma content of 100–130 mg./100 ml., provided the whole dose remains in solution in the blood. The first figures for both preparations were within these theoretical values. With iron and ammonium citrate, however, the rate of disappearance of iron was so rapid that the figures for the first estimations (110 and 112 mg.) were very likely less than the true theoretical values. The rabbits remained alive and well after the injection. Injection of half the dose of iron gave qualitatively similar results.

Plasma Iron Levels and Urinary Iron Excretion after Intravenous Injection of Different Doses and Samples of Saccharated Iron Oxide.—Two rabbits were injected intravenously with 180 and 360 mg. Fe/kg. of saccharated iron oxide (G_4) respectively (Table I, Experiment 4). The first rabbit died accidentally eight hours after injection, as a result of a haemopericardium from a heart puncture performed for collecting the last sample of blood; the second rabbit died, shortly after collection of the three-hour sample, with pulmonary and systemic haemorrhages.

Relative to the dose injected, the immediate plasma iron levels were not as high as they should have been on theoretical calculations. By comparison with the smaller doses of 22.5 and 45 mg. Fe/kg. previously given, the first levels should have been about 480 and 960 mg. %. The actual figures of 340 and 500 obtained lead to the conclusion that from one-quarter to one-half of the injected iron was precipitated at once at the dose levels of 180 and 360 mg./kg. respectively, the proportion of immediate precipitation increasing rapidly with the dose. The rate of fall of plasma

iron after these doses is also much more rapid than with the smaller doses of 22.5 and 45 mg. Fe/kg. The plasma iron level of 500 mg. % is the highest ever recorded.

The amount of iron excreted in the urine following increasingly larger doses of saccharated iron oxide shows a steady and appreciable rise, but the actual percentage of excretion shows some decline. This is perhaps caused by iron precipitates blocking capillaries in the kidney. With truly diffusible preparations such as iron and ammonium citrate, a rise in the total amount, as well as in the percentage of iron excreted, has been obtained.

In Experiment 5, Table I, two rabbits were given intravenously a dose of 22.5 mg. Fe/kg. of sample B of saccharated iron oxide (Nissim and Robson, 1949), and another two were given the same dose of Benger's saccharated iron oxide (" Ferrivenin "). Both these samples (precipitation point at *p*H 5.7 and 5.8 respectively) gave rise to a lower plasma iron than that after sample G_4 (p. pt. at *p*H 3.7). For a quantitative comparison, the average of the two half-hour values obtained with each sample of saccharated iron oxide was studied. The half-hour values are more likely to give accurate results, since by that time the rate of fall of the plasma levels has slowed down considerably, and speed in collecting the blood samples is not so vital as with the first set of values. The half-hour averages are:

$$\text{Saccharated iron oxide } (G_4) = \frac{40 + 42}{2} = 41$$

$$\text{Saccharated iron oxide (B)} = \frac{34 + 31}{2} = 32.5$$

$$\text{Ferrivenin} = \frac{32 + 29}{2} = 30.5$$

The differences appear to be significant. Neither the histological findings nor the urinary excretion suggest that this more rapid fall is due to greater diffusibility. For this reason, and in view of the greater toxicity of sample B and ferrivenin, these lower figures are indicative of greater precipitation. The lower initial values are compatible with some degree of abrupt precipitation at this dose level, comparable to that seen with sample G_4 with doses of 180 and 360 mg. Fe/kg.

Colloidal Ferric Hydroxide.—One rabbit injected with 45 mg. Fe/kg. intravenously of 2% Fe solution of colloidal ferric hydroxide (" Colliron "-Evans) died in two minutes. Blood withdrawn by heart puncture gave a serum iron of 20 mg. %, only about a sixth of the theoretical value. Death was no doubt due to the massive precipitation known to occur with colloidal ferric hydroxide. Even the low figure of 20 mg. % could not entirely have been due to iron remaining in solution, but must have included fine floccules floating in the blood.

Ferric Glucosate.—A rabbit injected with 45 mg. Fe/kg. intravenously of 2% Fe solution of ferric glucosate died in two hours with haemoptysis. Post-mortem examination showed massive haemorrhages confined to the lungs. A dose of 22.5 mg. Fe/kg. was not fatal (Table I).

Two other rabbits were injected intravenously with 45 and 90 mg. Fe/kg. of ferric glucosate respectively. Both died before the three-hour samples could be collected (Table I), their survival times being $2\frac{1}{2}$ and $1\frac{1}{2}$ hours respectively. The theoretical values for the first samples corresponding to these doses are 120 and 240 mg. %, but from the actual figures obtained, viz., 100 and 141 mg. %, it appears that abrupt precipitation begins to occur at lower plasma levels than with saccharated iron oxide. The half-hour plasma levels show that the rate of fall of plasma iron concentration is also correspondingly more rapid.

" Ferric Hydroxide Ferrous Ascorbate."—Urinary elimination of " ferric hydroxide ferrous ascorbate " was remarkably high, and, because of the dark colour of the preparation, urine passed within the first half-hour was almost black. Most of the iron in the urine was excreted in that period as later samples were near normal in colour. The rabbit remained alive and well.

" Ferrous Chloride Ascorbate."—This rabbit died 40 minutes after the injection. Its bladder contained 6 ml. urine, and iron estimation gave a urinary excretion not higher than 0.63%. The difference between this preparation and " ferric hydroxide ferrous ascorbate " was unexpected. Although the rabbit injected with " ferrous chloride ascorbate " died within 40 minutes, its urinary excretion in 24 hours is not likely to have exceeded 2%. That this low urinary iron excretion after " ferrous chloride ascorbate " was not due to the early collapse and rapid death of the animal is shown by the fact that rabbits which were injected with large doses of " ferric hydroxide ferrous ascorbate " and which died as early as half an hour after the injection showed a urinary iron excretion as high as 20%. The two iron preparations are thus exactly opposite in this respect. Plasma iron levels of " ferrous chloride ascorbate " were higher and more maintained than those of " ferric hydroxide ferrous ascorbate." This fact is of significance, and it may be

safely concluded that the greater toxicity and lower urinary excretion of this preparation is not due to massive precipitation as with colloidal ferric hydroxide. Other more important factors are at work.

Neutral and Alkaline Ferrous Ascorbate.—One rabbit injected with freshly prepared ferrous ascorbate (Maurer and Schiedt, 1936) died in 12 hours. Post-mortem examination showed lung haemorrhages, though not as marked as with "ferrous chloride ascorbate," and considerable oedema with areas of collapse.

Urinary iron excretion did not exceed 1.5%, in contrast to the 27.4% of "ferric hydroxide ferrous ascorbate." This was not merely a fortuitous result. While carrying out toxicity studies on mice, the excretion of "ferric hydroxide ferrous ascorbate" was found to be so rapid that the urine turned black at the end of the intravenous lethal doses of 90 mg. Fe/kg., provided these were given slowly in 5–10 minutes. This finding was made use of as a rough test for urinary excretion of iron-ascorbic acid preparations. The urine of mice injected with fresh neutral ferrous ascorbate remained clear amber in colour, while samples of ferrous ascorbate kept in solution for 2–3 days showed increased urinary elimination. This was also borne out by the following experiments on rabbits.

Two rabbits were injected with two samples of ferrous ascorbate which had been allowed to stand for a few days, one in neutral solution, the other at *p*H 11. The latter provided a control for "ferric hydroxide ferrous ascorbate" also of *p*H 11. The iron content of filtered samples of both neutral and alkaline ferrous ascorbate was estimated before injection, as some of the iron precipitated on standing. The first rabbit died on the second day with some pleural effusion, solid oedematous lungs, but no macroscopic haemorrhages. The second rabbit died on the third day with similar changes.

The increased urinary elimination of these samples of ferrous ascorbate stopped short of that shown by "ferric hydroxide ferrous ascorbate," however prolonged the period of keeping was. There is some evidence, nevertheless, that the excretion of the latter may be approached by samples of ferrous ascorbate with greater proportions of ascorbic acid.

"Ferric Chloride Lactate."—This rabbit died in six hours with copious outpouring of frothy blood-stained fluid from the mouth and nostrils. Post-mortem revealed haemorrhagic areas in the lungs with considerable oedema and some pleural effusion.

"Ferronascine" (Roche).—This rabbit died on the third day, with the same triad of patchy haemorrhage, oedema, and collapse in both lungs.

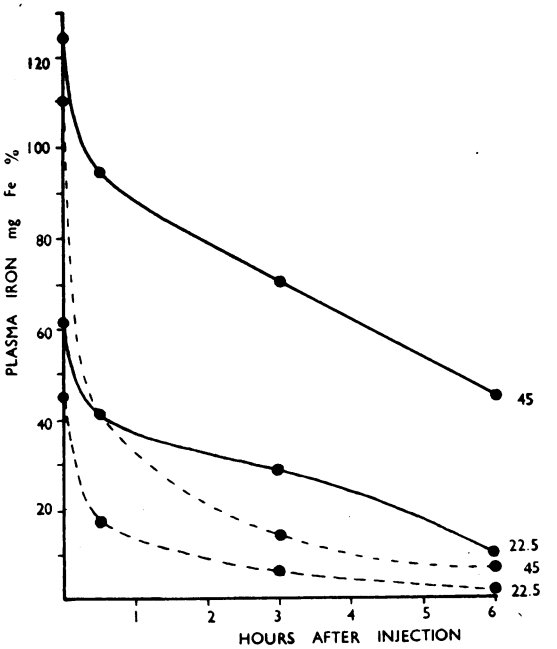

FIG. 1.—Plasma iron levels in rabbits after different iron preparations given intravenously in doses of 22.5 and 45 mg. Fe/kg.

Plasma Iron Levels in Rabbits with Ligated Renal Vessels

When the plasma iron curves of saccharated iron oxide and iron and ammonium citrate (Fig. 1) are compared, the question arises whether urinary excretion can account for the whole difference in their rates of decline. If 62 mg. Fe % is taken as the theoretical plasma level immediately after the injection of 22.5 mg. Fe/kg. of both preparations (Table I, Experiment 3), it may be calculated that the difference between their levels at the half-hour estimation is equal to 40.3% of the total dose of iron and ammonium citrate. Even if all the iron appearing in the urine were eliminated in the first half-hour, the difference between their urinary excretion could only account for 13.6%, leaving 26.7% to be accounted for. In order to obtain a definite answer, plasma iron levels were studied after ligation of renal vessels.

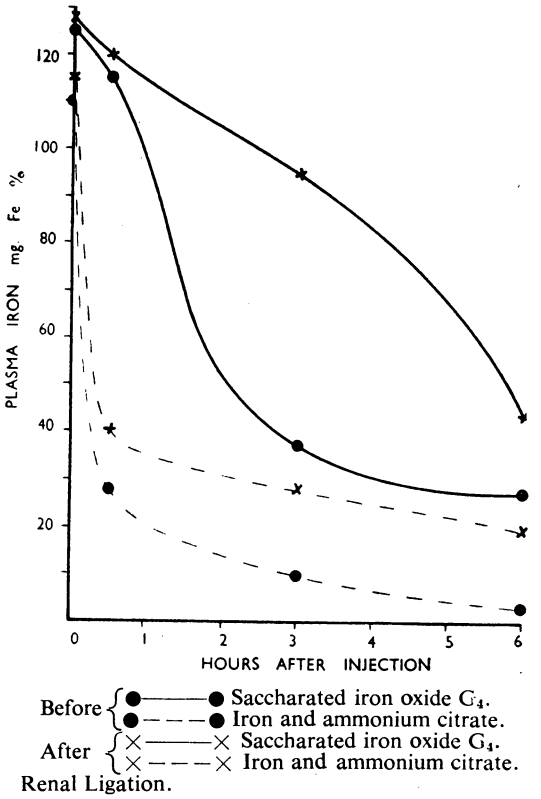

FIG. 2.—Plasma iron levels in rabbits before and after ligation of renal vessels, following saccharated iron oxide (G_4) and iron and ammonium citrate given intravenously in doses of 45 mg. Fe/kg.

Two rabbits were injected intravenously with 45 mg. Fe/kg. of saccharated iron oxide and iron and ammonium citrate respectively, shortly after their renal arteries and veins had been ligated on both sides; the plasma iron levels are shown in Table II, Experiment 1. Both animals were killed at the end of the experiment. Their renal vessels were found to be well ligated and their bladders empty. As expected, the fall of plasma iron was more rapid after iron and ammonium citrate. An unexpected feature was the slowing in the fall of plasma iron in both rabbits.

Two pairs of rabbits were injected intravenously with 45 and 22.5 mg. Fe/kg. of saccharated iron oxide and iron and ammonium citrate respectively, and their plasma iron levels and urinary iron excretion estimated. A fortnight later bilateral ligation of the renal vessels was performed, and shortly after recovery of the animals from anaesthesia they were injected with the same dose of their respective preparations. Plasma iron levels were estimated as before, the animals were killed at 24 hours, and faulty renal ligation was excluded. The whole of the alimentary tract was removed after ligating both ends, and rinsed free from blood. Its contents were added to the faeces passed in the 24 hours by the corresponding rabbit, and the mixture dried and powdered. An aliquot portion was ashed, and iron estimated by the phenanthroline method (Table II).

The considerable slowing in the fall of plasma iron after the ligation operation is more than can be accounted for by retention of iron previously excreted in the urine. Iron uptake by the kidneys was excluded by histological examination, as well as by a control experiment with saccharated iron oxide, in which the ureters were ligated instead of the renal vessels; the slowing was again evident. This slowing may be presumed, therefore, to be due to depression of the activities of the reticulo-endothelial system, and the metabolic processes in general, as a result of uraemia, the anaesthetic (ether), or both.

The iron present in the faeces plus alimentary tract contents was almost the same for both rabbits in both experiments, and any excretion of

TABLE II

PLASMA IRON LEVELS AND IRON EXCRETION IN RABBITS AFTER INTRAVENOUS INJECTION OF SACCHARATED IRON OXIDE (S.I.O.) AND IRON AND AMMONIUM CITRATE (I.A.C.) BEFORE AND AFTER LIGATION OF RENAL VESSELS

Experiment	Compound	Weight of Rabbit in kg.	Fe Injected in mg.	Plasma Iron (Hours after Injection)					Quantity Excreted in Urine in 24 hrs., mg. Fe	Quantity Excreted in Faeces in 24 hrs., mg. Fe
				0	½	3	6	24		
1. After renal ligation ..	S.I.O.	3·3	74	125	110	100	95	5	—	—
	I.A.C.	3·5	79	120	72	45	20	—	—	—
2. Before renal ligation ..	S.I.O.	2·4	108	125	115	37	27·5	1·5	4·2	—
	I.A.C.	2·1	94	110	28	9·5	3	0·5	36	—
After renal ligation ..	S.I.O.	2·4	108	128	120	95	44	—	—	29·5
	I.A.C.	2·1	94	115	40	28	19	—	—	31·2
3. Before renal ligation ..	S.I.O.	2·2	50	64	40	25	8	—	1·9	—
	I.A.C.	2·05	46	39	14	6	2	—	7·6	—
After renal ligation ..	S.I.O.	2·2	50	66	61	31·5	8·7	—	—	25·8
	I.A.C.	2·05	46	44·5	23·5	9	4	—	—	25·6

In Experiments 1 and 2 a dose of 45 mg. Fe/kg., and in Experiment 3 22.5 mg. Fe/kg. was used. The concentration of the solutions injected was 2 g. Fe/100 ml.

iron and ammonium citrate via the bowel could not adequately explain the rapid fall of plasma iron with this preparation. If excretion by this route were to explain the difference in plasma iron at the half-hour estimations, the figure for the iron and ammonium citrate rabbit would have to be 58.8 mg. in the first experiment and 25.8 mg. in the second.

The total iron of the faeces plus alimentary tract contents in all four rabbits was high. As no data were available on faecal iron in rabbits, control estimations were carried out on two normal rabbits of the same weight, their 24-hour faeces and alimentary tract contents collected, and the iron estimated as before. One rabbit gave an iron content of 28.5, the other 32.6 mg. It was realized that the iron in the diet of the rabbits (rabbit diet 18) was high, and in fact proved to be 13 mg. % of its dry weight. Faecal iron in normal rabbits on this diet was 57 mg. % even without ashing. For exact determination of percentage iron excretion via the bowels, rabbits should be kept on iron-free diet, or radio-iron used. The above experiments show, however, that iron excretion via the alimentary tract must be very small even with the massive doses of intravenous iron preparations used here.

Diffusion of Different Iron Preparations into Tissue Fluids

The easy passage of iron through the kidneys with preparations showing a high urinary iron excretion suggested that diffusion of the injected iron into tissue fluids might also take place. The diffusibility of some iron preparations was therefore studied, using the live peritoneum as a dialysing membrane. Mice were injected with 2.0 ml. physiological saline intraperitoneally, and then, immediately afterwards, with the iron preparation intravenously. The latter was given over a period of ten minutes; in this way larger doses of the more toxic preparations could be administered. At the end of the injection the mice were killed, the peritoneal cavity was opened, and 1.0 ml. of fluid withdrawn. Great care was taken to avoid the contamination of this fluid with blood. The iron content was estimated with the phenanthroline method (Table III).

TABLE III

DIFFUSION INTO THE PERITONEAL CAVITY OF DIFFERENT IRON PREPARATIONS INJECTED INTRAVENOUSLY

Iron Preparation	Dose Injected (mg. Fe/kg.)	Iron Concentration in Peritoneal Fluid (mg. Fe %)
1. Saccharated iron oxide (G_4) ..	180	0·25, 0·18
2. Iron and ammonium citrate ..	180	12·5, 7·5
3. Ferric glucosate	180	0·35
1. Saccharated iron oxide (G_4) ..	90	0·2, 0·15
2. Iron and ammonium citrate ..	90	4·3, 6·5
3. Ferric hydroxide ferrous ascorbate	90	7·2, 7·3, 8·6, 4·5
4. Ferric chloride caramelate ..	90	4·0, 5·5, 4·5
5. ,, tartrate	90	2·0, 2·0
6. Ferronascine	90	2·0, 1·5
7. Ferric chloride lactate ..	90	0·4, 0·3
8. ,, glucosate	90	0·24, 0·16

The three most diffusible preparations are those that showed the highest renal elimination, headed by "ferric hydroxide ferrous ascorbate." The diffusibility of saccharated iron oxide, ferric glucosate, and "ferric chloride lactate" is negligible. Ferric tartrate and Ferronascine occupy an intermediate position, which is not matched, however, by their relative rates of renal elimination.

DISCUSSION

The importance of using adequate amounts of the reagents, and particularly of HCl, in the estimation of saccharated iron oxide cannot be over-emphasized. Established methods for the estimation of serum iron use too little HCl to split this compound completely. The comparatively low figures (about a quarter of the expected values) obtained by Cameron, Bensley, and Wood (1951), who used the method of Barker and Walker (1940) for the estimation of serum iron, are evidently due to the small amounts of HCl added (1 ml. of 1.2% HCl to 2 ml. of plasma).

The present work represents the first comparative study of plasma iron and urinary iron excretion after the intravenous administration of different iron preparations at such high dose levels. It was carried out as part of an investigation into the different mechanisms of toxicity of iron preparations. The investigation by McCance and Widdowson (1938) of urinary and faecal iron excretion after repeated daily intravenous iron and ammonium citrate in man bears little relation to the present work in aim, method of administration, and dosage. It is of interest, however, that both in these and in the present experiments iron excretion was mainly renal, practically no iron being eliminated through the bowel.

The experiments described here demonstrate the wide differences in the rate of disappearance of iron from the plasma after the intravenous administration of different iron preparations. The plasma iron level immediately after injection may correspond to the theoretical value based on the assumption that there is complete and even distribution in plasma. This is so with saccharated iron oxide, ferric glucosate, and "ferric chloride

lactate." The subsequent rate of fall is slow, and is presumably due, in the main, to the activity of the reticulo-endothelial system. With other iron preparations a rapid fall in plasma iron occurs. This may be due to rapid flocculation, as is known to occur with colloidal ferric hydroxide. A third group of preparations show a rapid fall in plasma iron associated with high urinary iron excretion, e.g. iron and ammonium citrate, " ferric chloride caramelate," and " ferric hydroxide ferrous ascorbate."

The three experiments on rabbits with ligated renal vessels gave consistent results and established that the rapid fall of plasma iron after iron and ammonium citrate as compared with saccharated iron oxide was not due merely to greater urinary elimination. The rapid disappearance of iron from the plasma after iron and ammonium citrate and the other preparations which showed a high urinary iron excretion can therefore only partly be accounted for by the quantity of iron in the urine. The estimation of iron in the faeces plus alimentary tract contents has shown that excretion via the alimentary tract must be negligible if it occurs at all. The difference in diffusibility between the different iron preparations, on the other hand, is indeed striking, those with high urinary iron excretion also having the greatest diffusibility.

This interesting correlation between iron preparations in urinary iron excretion and diffusibility does not appear absolute, however, since ferric tartrate and ferronascine, which occupy an intermediate position regarding diffusibility, show rather poor renal elimination. The present diffusibility studies provide ample explanation for the very rapid fall in plasma iron levels which occurs with " ferric hydroxide ferrous ascorbate," " ferric chloride caramelate," and iron and ammonium citrate—a fall which could not be fully accounted for by their high urinary elimination.

Summary

Iron preparations fall into one of three groups. The first includes compounds like saccharated iron oxide, ferric glucosate, and " ferric chloride lactate," which exhibit a slow rate of disappearance from the blood associated with poor urinary excretion. Compounds of the second group, such as colloidal ferric hydroxide, show rapid fall of plasma levels associated with rapid precipitation of the iron and poor urinary excretion. In the third group are such compounds as iron and ammonium citrate, " ferric chloride caramelate," and " ferric hydroxide ferrous ascorbate," which show a rapid fall in plasma iron associated with high urinary excretion.

The highest percentage of urinary excretion was obtained with " ferric hydroxide ferrous ascorbate " (27.4%), the lowest with colloidal ferric hydroxide (0.27%). The three compounds showing the highest renal elimination also possess the highest diffusibility. This accounts for the rapid fall in their plasma levels even after renal ligation.

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