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PHYSICO-CHEMICAL PROPERTIES AND TOXICITIES OF DIFFERENT IRON PREPARATIONS*

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After the preparation and standardization of saccharated iron oxide (Nissim and Robson, 1949a), a comparative study was made of this and other iron preparations with the object of gaining a clearer understanding of their pharmacological properties and of the manner in which iron compounds produce their toxic effects in the body. The iron preparations studied comprise both old and new compounds or complexes. The new forms were made during the search for other intravenous iron preparations among interaction products of iron with substances related to sucrose, including monosaccharides, disaccharides, caramelization products, and ascorbic acid. In addition, some amino-acids and protein hydrolysate were tried. Among the older preparations of iron a large number of readily available or easily prepared compounds were also investigated.

METHODS AND MATERIALS

The *p*H at which precipitation took place in aqueous solution on the addition of HCl to alkaline iron preparations and on the addition of alkali to acid preparations was determined. The effect of the addition of plasma (5 ml. to an equal volume of 0.2 g. Fe per 100 ml. of solution of the iron preparation) on the precipitation point was studied as an indication of whether the preparation was likely to precipitate when injected intravenously or not. The *p*H at which a blue colour was obtained on the addition of potassium ferrocyanide to ferric preparations, or of potassium ferricyanide to ferrous preparations, was used to indicate the ease with which the iron compound was split *in vitro*.

The toxicity of the iron preparations was tested in female white mice. With the exception of saccharated iron oxide and ferric glucosate, the toxicity figures given here are only approximate, for the toxicity tests were concerned with wide differences significant enough for the purpose of the present study. In addition to the LD50—at one week from the injection—the immediately lethal dose, i.e., the dose which causes death while the preparation is still being injected (ILD), was also determined.

Ferric Glucosate.—The interaction of iron with glucose took place more readily than with sucrose (Nissim, 1949; Nissim and Robson, 1949b). Much less heat was required for the formation of this compound. The ferric hydroxide was prepared as described for saccharated iron oxide, and was mixed with glucose and sodium hydroxide (Analar). By analogy with ferric sucrate (Mackenzie, 1913), this preparation was termed ferric glucosate. The proportions of glucose to iron could be varied, but much smaller quantities of glucose were required than of sucrose, so that samples of ferric glucosate could be made with a glucose content ranging from 0.33 g. to over 33.3 g. per g. of elemental iron. The glucose content of the sample studied here was 1.8 g. per g. of iron. Alkali was added to the mixture of ferric hydroxide and glucose to bring the *p*H to 11.7, and the amount required was found to rise with the glucose content of the sample. With the application of moderate heat (e.g., 60° C.), the *p*H gradually fell. The *p*H at which precipitation of the sample took place on the addition of dilute HCl also fell from 8.0 to as low as 2.7 with continued heat. There was also a corresponding gradual fall in the *p*H at which the Prussian-blue reaction was given on the addition of potassium ferrocyanide and HCl from 7.0 before heating to 2.0 when the precipitation point had dropped to *p*H 2.7. In other words, the binding of iron in the preparation became firmer. As with saccharated iron oxide, the progressive fall in the *p*H at which precipitation occurred and in the *p*H at which the ferrocyanide test was given was accompanied by a reduction of toxicity.

Solutions of ferric glucosate could not be autoclaved, as precipitation always took place with the fall of *p*H below 10.5.

"Ferric Hydroxide Ferrous Ascorbate."—Ascorbic acid had such a remarkable affinity for iron that it dissolved ferric hydroxide (prepared from ferric chloride and sodium carbonate) completely without the aid of alkali or heat, giving rise to a purplish-black solution. The reaction took place more rapidly at 60–70° C. The two substances were used in the proportion of 20 g. ascorbic acid to 1 g. Fe. Sodium

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hydroxide was added to the ascorbic acid/ferric hydroxide solution after 36 hours, and the *p*H brought up from 3.5 to 11.0 so that toxicity tests would be more comparable with those on saccharated iron oxide. " Ferric hydroxide ferrous ascorbate " is almost colourless at *p*H 1.5. It acquires a purplish colour when alkali is added, and the colour darkens until it becomes almost black at *p*H 11.0.

" Ferrous Chloride Ascorbate."—This was prepared by combining 1 g. of iron as $FeCl_3$ with 20 g. ascorbic acid and bringing the *p*H up to 11.0. Immediate reaction occurred between the two substances at room temperature.

" Ferric Chloride Caramelate."—According to the *B.P.C.* (1949), caramel is prepared by heating sucrose at 180–200° C. until a black viscid mass is formed. This is then mixed with sufficient hot water to produce a liquid of specific gravity 1.4. The degree of caramelization obtained with this method, and consequently the constituent products of the caramel, must vary a great deal from one preparation to another.

When sucrose was caramelized by heat both loss in weight and fall in *p*H were noted. These changes were used as a measure of the degree of caramelization, and repeated experiments with iron and standardized caramel samples have given consistent results. The samples of caramel used in these experiments showed a loss in weight of 6% and a fall in *p*H from about 5.7, which is the *p*H of a 4% (w/v) sucrose solution before caramelization, to 3.0.

When caramel was mixed with a solution of ferric chloride it gave a dark brown solution; a precipitate formed when sodium hydroxide was added, but dissolved again when a *p*H of about 11.4 was reached. When this solution was heated at 100° C. for one hour, the resulting preparation did not precipitate at any *p*H in aqueous solution. The proportion of iron to caramel in this preparation was 1:16.6. The *p*H of the solution dropped when heated to about 9.5 and was adjusted to 11.0 with sodium hydroxide.

Ferrous Pyruvate.—Pyruvic acid and ferric hydroxide were left overnight at 50–60° C.; 25 ml. of a 57% (w/v) solution of pyruvic acid dissolved 1 g. of iron as ferric hydroxide, reducing it to the ferrous state. This ferrous pyruvate, however, precipitated when alkali was added, at *p*H 7.0, and remained precipitated at all *p*Hs on the alkaline side. It was not considered worth further investigation.

" Ferrous Chloride Pyruvate."—Pyruvic acid was mixed with ferric chloride in the same proportion of 25 ml. of 57% (w/v) pyruvic acid to 1 g. of iron and left overnight at 50–60° C. It formed a preparation which remained in solution when the *p*H was brought up from 0.9 to 11.0, but nevertheless proved more toxic than any of the preparations so far described.

" Ferric Chloride Pyruvate."—Lactic acid reacted with ferric chloride instantaneously to give a lemon yellow solution which did not give a precipitate at any *p*H. Forty ml. of lactic acid was required to keep in solution in this form 1 g. of iron.

Protein Hydrolysate and Iron.—Protein hydrolysate (Casydrol-Bengers) gave a port-wine coloured solution with ferric chloride. On the addition of alkali a precipitate formed which redissolved with further alkali at a *p*H of about 8.0. The *p*H of the solution was brought up to 11.0 as with other preparations.

Plasma and Iron.—When plasma is mixed with ferric chloride its proteins precipitate. With the addition of alkali this precipitate turns into an almost solid jelly, but redissolves at a *p*H of 11.5–12. This solution may be neutralized with dilute acid without precipitating, its precipitation point being 6.8. The proportion of iron to plasma used was 0.4 g. to 100 ml.

Ferric Tartrate.—This is a scale preparation with an iron content of 19.46%.

RESULTS

New Iron Preparations

Ferric Glucosate.—The *p*H values for the precipitation point and the ferrocyanide test as well as the *p*H at which the preparation precipitated when mixed with plasma (Table I) were all lower than those obtained with saccharated iron oxide, and on this account ferric glucosate was expected to be less

TABLE I

PHYSICO-CHEMICAL PROPERTIES AND TOXICITIES OF DIFFERENT IRON PREPARATIONS

Preparation	*p*H	*p*H of Precipitation Point	Plasma Precipitation Point	Ferrocyanide or Ferricyanide Test (*p*H)	LD50 mg. Fe/kg.	Immediately Lethal Dose mg. Fe/kg.
Saccharated iron oxide, sample A	10·7	5·7	7·2	3·5	180	450
Saccharated iron oxide, sample G_4	11·0	3·7	7·2	2·0	300	1,000
Ferric glucosate	11·0	2·7	6·8	2·0	150	600
Ferric hydroxide ferrous ascorbate ..	11·0	—	6·5	4·8	45	90
Ferrous chloride ascorbate ..	11·0	—	7·0	4·5	30	45
Ferric chloride caramelate ..	11·0	—	5·0	5·5	90	450
Ferrous chloride pyruvate ..	11·0	—	7·5	4·5	33	50
Ferric chloride lactate ..	11·0	—	—	6·5	16·5	45
Iron and protein hydrolysate	9·5	6·8	Not tested	Not tested	33	180
Ferric chloride and plasma	11·5	6·8	—	Not tested	80	180
Ferric chloride..	1·5	2·6–3·6	2·6–6·5	7·0	11	15
Ferrous sulphate	4·0	4·0–8·5	6·0–8·5	9·0	11	22·5
Colloidal $Fe(OH)_3$	8·0	2·0	8·0	2·0	25	30
Iron and ammonium citrate	7·3	2·0	7·0	4·8	16·5	360
Ferrous ascorbate (neutral) ..	7·0	—	5·5	8·0	33	90
Ferrous ascorbate (alkaline) ..	11·0	—	6·0	8·0	33	270
Ferric tartrate ..	7·3	—	7·0	6·5	16·5	90
Ferronascine ..	6·5	3·0	7·0	7·0	16·5	360

toxic. Its LD50, however, was about half that of sample G_4 of saccharated iron oxide (Nissim and Robson, 1949a), and histological sections showed abundant precipitation of the iron. Details of the post-mortem and histological findings of this and other iron preparations will be given in a later paper.

Other Sugars and Iron.—Ferric hydroxide, fructose, and sodium hydroxide gave a preparation which was the same as ferric glucosate in physico-chemical characteristics and in toxicity. The disaccharides maltose and lactose formed soluble preparations with iron possessing toxicities twice as great as that of saccharated iron oxide. These disaccharides are reducing sugars, and are acted upon by alkali to give caramelization products via the simpler monosaccharides glucose and fructose (Thorpe, 1938). They would thus be expected to have the same toxicities as ferric glucosate.

"Ferric Hydroxide Ferrous Ascorbate."—This preparation possessed the remarkable property of not precipitating at any *p*H in aqueous solution. When mixed with plasma it began to precipitate at *p*H 6.5 on the addition of HCl, and its precipitation was not complete till a *p*H of 3 was reached. It was, however, much more toxic than either saccharated iron oxide or ferric glucosate.

"Ferrous Chloride Ascorbate."—This preparation possessed different physico-chemical properties and was more toxic than "ferric hydroxide ferrous ascorbate."

"Ferric Caramelate."—The interaction product of ferric hydroxide and caramel was closely similar to ferric glucosate in all its physico-chemical characteristics and was not therefore tested for toxicity.

"Ferric Chloride Caramelate."—When mixed with plasma, solutions of "ferric chloride caramelate" did not precipitate until a *p*H of 5.0 was reached. At this *p*H the plasma proteins themselves begin to precipitate even when not mixed with iron. When injected into mice, however, "ferric chloride caramelate" proved more toxic that saccharated iron oxide as well as ferric glucosate, though it was better tolerated than "ferric hydroxide ferrous ascorbate."

Ferric Lactate.—This precipitated at a *p*H of about 5.0 on the addition of alkali, and was not studied further.

"Ferric Chloride Lactate."—This iron preparation possessed the remarkable feature, alone among all the iron preparations tested, of not precipitating at any *p*H when mixed with plasma. Even when some of the plasma proteins precipitated at a *p*H less than 5.0, the "ferric chloride lactate" itself remained in solution. It was, however, even more toxic than the pyruvate compound.

Glycine and Iron.—Glycine reacted with ferric chloride to give a yellow brown solution which did not precipitate at any *p*H. This iron complex caused immediate death in doses of 90 mg. Fe/kg., however, and was not studied further.

Plasma and Iron.—The iron binding globulin of the plasma, siderophilin, can hold about 300 μg. Fe per 100 ml., or 15 mg. for the whole of the plasma in man. It appears from the present experiments that plasma may hold iron in yet another way which reduces its toxicity a great deal. A solution of this iron-plasma complex containing 0.2 g. Fe per 100 ml. of solution was injected into mice and proved some eight times less toxic than ferric chloride.

Old Iron Preparations

Iron Preparations Precipitating in Neutral Aqueous Solution

These include compounds of both the ferric and ferrous variety. The *p*H of their solutions and their precipitation points are recorded in Table II. All gave solutions of acid reaction

TABLE II
IRON PREPARATIONS PRECIPITATING IN NEUTRAL AQUEOUS SOLUTIONS

Preparation	*p*H of Solution	*p*H of Precipitation Point
(a) Ferric group		
Ferric chloride ..	1·0–1·5	2·6
,, citrate	2·6	2·7
,, acetate	4·4	4·5
,, oxalate ..	3·3	3·4
,, formate ..	3·9	4·0
,, hypophosphite	3·7	4·0
,, pyrophosphate	4·5	7·0
,, glycerophosphate	5·7	7·0
(b) Ferrous group		
Ferrous sulphate ..	4·0	5·0
,, lactate ..	5·3	5·5
,, oxalate ..	6·0	6·5

with *p*H ranging from 2.6 to 6.0. The individual investigation of these compounds was not carried further, as it was thought that, whatever other properties they possessed, such compounds would invariably produce toxic effects due to precipitation. One example was taken, however, of the ferric and of the ferrous group for a more detailed study, so that a more accurate picture might be obtained of the type and mechanism of the toxic actions of such compounds. The two prototypes chosen were ferric chloride and ferrous sulphate.

Ferric Chloride.—Readily ionized ferric compounds are noted for their precipitation of proteins, the iron itself precipitating in the process. The *p*H of 2 g. Fe per 100 ml. of solution of ferric chloride was 1–1.5. In aqueous solution it precipitated between *p*H 2.5 and 3.5. The precipitate of ferric hydroxide did not, of course, redissolve with the addition of more alkali as when the ferric chloride was mixed with plasma. The precipitation of ferric chloride in plasma also began at *p*H 2.5, but was not complete till *p*H 6.5.

Because of its rapid protein precipitating effect, ferric chloride proved the most toxic of all iron compounds, especially when injected in a relatively concentrated form. Even as little as 9 mg. Fe/kg. resulted in immediate death, when the dose was given in a concentration of 0.2 g. Fe per 100 ml. of solution. To cause immediate death with 0.02 g. Fe per 100 ml. of solution, however, some 15 mg. Fe/kg. had to be given. The LD50 of 11 mg. shown in the Table was that of 0.02 g. Fe per 100 ml. of solution.

Ferrous Sulphate.—In contrast to ferric ions, ferrous ions do not precipitate proteins. The *p*H of 2 g. Fe per 100 ml. of solution of ferrous sulphate was about 4.0. The precipitation of ferrous sulphate in aqueous solution began at a *p*H just exceeding 4.0, and was not complete till *p*H 8.5. This agreed with the greater solubility of ferrous as compared with ferric ions. When ferrous sulphate was mixed with plasma, precipitation occurred gradually between *p*H 6.0 and 8.5. The immediate toxicity was less than that of ferric chloride, for its immediately lethal dose was about 22 mg. Fe/kg. Its toxic effects in smaller doses manifested themselves gradually, however, so that its LD50 was about the same as that of ferric chloride.

Iron Preparations Remaining in Solution at a Neutral pH

Colloidal Ferric Hydroxide.—The sample used was "Colliron" (Evans Medical Supplies). Its ILD was very close to its LD50 (Table I). Mice receiving 22.5 mg. Fe/kg. showed acute dyspnoea and convulsions immediately on injection. Those which survived this stage tended to recover completely. This was in contrast to the gradually developing toxic effects seen after administration of saccharated iron oxide, ferric glucosate, or iron and ammonium citrate; the acute onset of the toxic effects must have been caused by the sudden flocculation of colloidal ferric hydroxide in the blood.

Iron and Ammonium Citrate.—Iron and ammonium citrate (*B.P.*) contains 20.5–22.5% iron. It differed from other iron preparations so far tested in showing an ILD which was some 20 times greater than its LD50.

Ferrous Ascorbate.—A sample of ferrous ascorbate prepared according to the method of Maurer and Schiedt (1936) was divided into two portions; one was kept neutral and the *p*H of the other was brought up to 11.0 to render possible a strict comparison with "ferric hydroxide ferrous ascorbate." Both the neutral and the alkaline ferrous ascorbate proved more toxic than "ferric hydroxide ferrous ascorbate," and though both showed the same LD50 their ILD differed markedly.

Ferronascine (Roche).—This is the sodium salt of di(α:γ-dihydroxy-β:β-dimethyl-butyrate)-ferric acid. Solutions had a *p*H of 6.5, and did not precipitate at any *p*H, but with plasma it began to precipitate at a *p*H of about 7.0. On standing overnight at this *p*H, it converted the plasma to a soft gel. This effect was not observed with any of the other iron compounds.

It is seen from the account given here that iron preparations differ widely in their toxicity, their LD50s ranging from 11 to 300 mg. Fe/kg. The gap between the LD50s of the older iron preparations and the new high level of 300 mg. for saccharated iron oxide has been bridged by the other new iron preparations which have been recently investigated, e.g., "ferric hydroxide ferrous ascorbate," "ferric chloride carame'ate," and ferric glucosate.

Summary

The physico-chemical properties of a number of iron preparations have been investigated, and their toxicities compared by tests on mice. Iron compounds exist with almost evenly spaced LD50s ranging from 11 to 300 mg. Fe/kg.

References

British Pharmaceutical Codex, 1949.

Mackenzie, J. E. (1913). *The Sugars and their Simple Derivatives*, Gurney and Jackson, London.

Maurer, K., and Schiedt, B. (1936). *Biochem. Z.*, **285**, 67.

Nissim, J A. (1949). M.D. thesis, University of London.

—— and Robson, J. M. (1949a). *Lancet*, **1**, 686.

—— —— (1949b). Ibid., **1**, 326.

Thorpe, J. F. (1938). *Dictionary of Applied Chemistry*, Longmans, London.