Determination of Iron Toxicity in Mice

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Studies indicated that mice fasted 22 ± 2 hr. prior to treatment were consistent in the way they reacted to oral administration of iron. The maintenance of a uniformly empty intestinal tract prior to oral administration of iron made possible a linear relationship between log-dose and death. Commercial iron preparations were employed to demonstrate different toxicities due to different availabilities of iron, and LD_{50}'s were estimated where possible. The oral LD_{50} of $FeSO_4 \cdot 7H_2O$ containing 0.201 mg. of Fe/mg. was found to be 160 ± 3 mcg./Gm. (32 ± 0.6 mcg. of Fe/Gm.), using death within 24 hr. as the end point following a prior defined stated fasting period; the LD_{50} after 1.5 hr. was determined also. The symptoms of toxicity and the time and pattern of death were found similar to the form iron toxicity takes in humans. A ferrous sulfate solution was found to be a satisfactory standard iron preparation, and oral LD_{50} estimates were made in male and female Fairfield Webster and B_4BC strains of fasted mice. The B_4BC strain in both sexes was found to be more susceptible to iron toxicity than the Fairfield Webster strain. The males of the Fairfield Webster strain were found more sensitive to iron toxicity than the females. Mice exposed to feces and sawdust during the prior fasting period were found less susceptible to iron toxicity than those animals isolated from these elements during this fasting period.

THERE ARE many iron compounds available in many different forms and preparations. The toxicity varies with each, depending upon iron content and availability. This toxicity can be influenced further by a variety of factors: diet, the availability of iron from various compounds, the presence of other materials in the gastrointestinal tract, surface-active agents, sugar alcohols, and the oxidative state of the iron and pH, bile, pancreas, and oxygen tension.

That iron is a potentially toxic material is unrecognized frequently, and the literature continues to have more case histories of its lethal potentialities; its toxic manifestations are becoming better recognized, outlined, and described. Acute toxicity, sometimes with fatal outcome, is not uncommon in children who accidentally ingest large doses of ferrous sulfate. The mortality rate has been reported to be about 50% in these cases (1).

The forms of administration and the condition of animals in toxicity studies are important and have been described inadequately many times in the literature.

It was, therefore, the purpose of this investigation to provide an LD_{50} under controlled stated conditions and demonstrate some of the influences which can affect iron toxicity and might be applied also to other compounds.

EXPERIMENTAL

To obtain a well-defined LD_{50} for iron, the following routine was devised.

Mice of the B_4BC and/or Fairfield Webster strains as indicated were all fasted for at least 20 hr. and never more than 24 hr. with water *ad libitum* with room temperature kept as constant as possible. The medication was given entirely by the oral route *via* stomach intubation. The doses of all iron compounds were calculated on a milligram per kilogram weight basis. The number of animals dead after 24 hr. was observed, and the animals remaining were observed for a period of 30 days to include any possibility of latent effects.

Preliminary experiments with five commercial iron preparations were conducted on limited groups of female Fairfield Webster mice to indicate general toxicity differences between compounds. The compounds included the following: a solution containing ferrous lactate equivalent to 25 mg. of iron per milliliter,[1] referred to hereafter as ferrous lactate solution; a solution containing 125 mg. of ferrous sulfate (25 mg. of iron) per milliliter,[2] referred to hereafter as ferrous sulfate solution; a solution of iron-dextran complex containing the equivalent of 50 mg. of elemental iron per milliliter,[3] referred to hereafter as iron-dextran solution; a solution containing 20 mg. of elemental iron from saccharated iron oxide per milliliter,[4] referred to hereafter as saccharated iron solution; a suspension supplying 125 mg. of ferrous sulfate and 2 mg. of molybdenum oxide per milliliter,[5] referred to hereafter as ferrous sulfate-Mo.

The LD_{50}'s were estimated where possible using the Reed-Muench method (2).

Experiments performed to determine the oral LD_{50} of ferrous sulfate in female Fairfield Webster mice utilized a 4% aqueous solution freshly prepared. The LD_{50} was calculated after both 1.5 and 24 hr. using the Reed-Muench method (2).

The oral LD_{50} of ferrous sulfate solution, used as a standard iron preparation, was determined. The same routine was followed in all experiments. The LD_{50} was calculated using the Reed-Muench method (2) in cases of both strains and sexes.

Fasted Fairfield Webster female mice caged in a manner to exclude contact with feces and sawdust were employed to determine whether consumption of feces and/or sawdust would have a modifying effect on oral iron toxicity at a single dosage.

RESULTS

The preliminary experiments with five commercial iron preparations are summarized in Table I. The

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[1] Marketed as Ferro Drops by Parke Davis and Co.

[2] Marketed as Fer-In-Sol by Mead Johnson and Co.

[3] Marketed as Imferon by Lakeside Laboratories.

[4] Marketed as Proferrin by Merck Sharp and Dohme Research Laboratories.

[5] Marketed as Mol-Iron Drops by White Laboratories.

TABLE I.—SUMMARY OF TOXICITY STUDIES OF VARIOUS IRON COMPOUNDS IN MICE[a]

Drug	Dose of Compd. 40 mg./Kg.	80 mg./Kg.	160 mg./Kg.	320 mg./Kg.	LD_{50} Estimate, mg./Kg.
Ferrous sulfate soln.	0/5	0/5	2/5	4/5	211 ± 55
Ferrous lactate soln.	0/5	0/5	3/5	5/5	147 ± 30
Iron-dextran soln.	0/5	0/5	0/5	0/5	...
Ferrous sulfate-Mo	0/5	0/5	1/5	3/5	269 ± 72
Saccharated iron soln.	...	0/5	1/5	0/5	...

[a] Number dead/number treated.

TABLE II.—SUMMARY OF LD_{50} STUDIES OF $FeSO_4 \cdot 7H_2O$ GIVEN ORALLY TO FASTED MICE

Dose of Iron $FeSO_4 \cdot 7H_2O$ mcg. Fe/Gm.	No. Dead After 1.5 hr./Total No. Treated	No. Dead After 24 hr./Total No. Treated
26	0/10	2/10
32	5/8	5/8
40	11/20	19/20
50	2/10	7/10

40-mg./Kg. dose for saccharated iron solution was omitted because from experience with the more complexly bound iron compounds and parenteral forms of iron it was not considered toxic or lethal at this dosage. (For example, see iron-dextran solution at this dosage.) The one out of five death at the 160 mg./Kg. dose for saccharated iron solution was considered an idiosyncrasy, one death not being significant in any experiment, especially in groups containing limited numbers.

The results of the oral LD_{50} studies for ferrous sulfate solution, 1 mg. $FeSO_4 \cdot 7H_2O$ containing 0.201 mg. of iron, are shown in Table II.

The time of death appeared to follow a pattern, with approximately 50% of the animals dying 1.5 hr. after oral administration. At the end of approximately 20 to 24 hr., 90% of the total deaths had occurred. Therefore, the mortality rates have been represented in two columns. Convulsions always occurred in the mice before death.

The early symptoms observed, beginning in about 30 min. following oral administration of iron, were those of decreased activity, a flaccid weak appearance, dyspnea, progressing with the higher doses of iron to greatly decreased activity, weak muscular control, more severe dyspnea and convulsions, with the convulsions appearing only at higher doses in the terminal stages. The convulsions were clonic rather than tonic in nature, compared with mice given convulsant doses of strychnine and pentylenetetrazol.

At autopsy, the stomach and liver of the animals dying with smaller doses of iron appeared similar to controls. At higher doses of iron, the stomachs were black, indicating possible hemorrhage, and the livers appeared to be bleached at the tips.

The oral LD_{50} of $FeSO_4 \cdot 7H_2O$ calculated from the starved mice appearing in Table II, using death within 1.5 hr. as the end point, was 205 ± 5 mcg./Gm. (41 ± 1 mcg. Fe/Gm.), and using death within

TABLE III.—SUMMARY OF LD_{50} STUDIES OF FERROUS SULFATE SOLUTION GIVEN ORALLY TO FASTED MICE

Dose of Iron (Ferrous Sulfate Soln.), mcg. Fe/Gm.	No. Dead After 1.5 hr. and After 24 hr./Total No. Treated							
	Fairfield Webster Strain				B_4BC Strain			
	F		M		F		M	
	After 1.5	After 24	After 1.5	After 24	After 1.5	After 24	After 1.5	After 24
32	15/205	31/205	5/35	11/35	30/80	42/80	27/85	48/85
48	57/195	109/195	28/70	48/70	36/50	46/50	45/60	56/60

TABLE IV.—ORAL LD_{50} ESTIMATES (mcg. Fe/Gm.) FOR FERROUS SULFATE SOLUTION IN MALE AND FEMALE FAIRFIELD WEBSTER AND B_4BC STRAINS OF FASTED MICE

	Fairfield Webster		B_4BC	
	F	M	F	M
After 1.5 hr.	56 ± 1.7	51 ± 2.7	38 ± 1.7	39 ± 1.5
After 24 hr.	44 ± 1.1	41 ± 2.0	32 ± 1.6	31 ± 1.5

TABLE V.—COMPARISON OF ORAL LD_{50} ESTIMATES (mcg. Fe/Gm.) FOR FERROUS SULFATE SOLUTION IN MALE AND FEMALE FAIRFIELD WEBSTER AND B_4BC STRAINS OF FASTED MICE SEPARATELY AND COMBINED

	F	Both Sexes Combined	M	
After 1.5 hr.	56 ± 1.7	55 ± 1.4	51 ± 2.7	Fairfield Webster
	49 ± 1.1	47 ± 1.0	43 ± 1.4	Both strains combined
	38 ± 1.7	38 ± 1.1	39 ± 1.5	B_4BC
After 24 hr.	44 ± 1.1	44 ± 0.9	41 ± 2.0	Fairfield Webster
	41 ± 0.9	40 ± 0.7	35 ± 1.1	Both strains combined
	32 ± 1.6	31 ± 1.1	31 ± 1.5	B_4BC

TABLE VI.—FERROUS SULFATE SOLUTION ADMINISTERED ORALLY AT A DOSE OF 48 mcg. Fe/Gm. TO THREE GROUPS OF 20 MICE CAGED AS INDICATED[a]

Cage containing feces with sawdust	7/20 (35%)
Cage containing feces without sawdust	11/20 (55%)
Cage without feces and without sawdust	19/20 (95%)

[a] Number mice dead/number treated.

24 hrs. as the end point, was 160 ± 3 mcg./Gm. (32 ± 0.6 mcg. Fe/Gm.).

Experience with aqueous ferrous sulfate solutions, the need for freshly prepared solutions, indicated their relative instability under ordinary conditions so that impurities of ferric salts usually were present unless rather extreme measures were taken to prevent oxidation. As a substitute, a commercially available form of ferrous sulfate solution[2] was found to be more satisfactory as a standard iron preparation with stability, uniform potency, and compatible pH.

The results of the oral LD_{50} studies for ferrous sulfate solution recorded are in Table III. The LD_{50} estimates for each strain and sex are listed in Table IV. A comparison of LD_{50} estimates in male and female Fairfield Webster and B_4BC strains of fasted mice calculated separately and combined is shown in Table V.

The results of the oral LD_{50} studies indicate that the B_4BC strain, in both males and females, is more sensitive to the toxicity of iron as ferrous sulfate solution than the Fairfield Webster strain. Also, the Fairfield Webster males are more sensitive to the toxicity of ferrous sulfate solution than the females.

The data from the studies of oral iron toxicity in mice fasted under special conditions are recorded in Table VI.

Mice exposed to feces and sawdust were less susceptible to iron toxicity than those isolated from these elements during the 22 ± 2 hr. fasting period. This demonstrates the importance of absence of food and other materials in oral toxicity studies.

DISCUSSION

The time of death in mice following oral administration of iron took a pattern with approximately 50% dying after 1.5 hr. and 90% of the total deaths occurring after 20 to 24 hr. In the oral LD_{50} studies for aqueous ferrous sulfate solution, 4%, all tests conformed to this pattern, suggesting that the same mechanism of toxicity seemed in operation and would further suggest the possibility of more than one mechanism of toxic action. Death never occurred earlier than 30 min., which would seem to rule out local action of iron on the gut as the principal cause of death. Convulsions always preceded death, a pattern similar to the usual fatal symptoms in human cases (1). It should be noted that in humans, iron toxicity takes the form of rapid death within 1 or 2 hr., delayed death after 24 hr., and apparent recovery (1), similar to the pattern illustrated by the mice in Table II.

Initially, some unfasted mice were given iron orally, and it was not possible to obtain a clear linear relationship between log-dose and death, a demonstration of the influence that food had in protecting the animals from the toxicity of iron. This was possible, however, when the animals were fasted. A linear log dosage-response curve could be obtained when micrograms per gram of iron administered orally was plotted against per cent mortality, therefore demonstrating that the success of this method depended upon having empty gastrointestinal tracts as a result of uniform fasting periods prior to treatment.

Also initially, other unfasted animals were given iron intraperitoneally for comparison of toxicities under different conditions and modes of administration, and the ferrous sulfate solution appeared to be less toxic *via* this route than when an equivalent dose was administered orally in fasted animals.

The oral LD_{50} of $FeSO_4 \cdot 7H_2O$ was calculated to be 32 ± 0.6 mcg. of Fe/Gm. after 24 hrs. The LD_{50} estimate reported by Weaver *et al.* (3) for male Swiss Webster mice was 225.6 mcg. of Fe/Gm. after 24 hr. Since no reference was made to fasting, it could be assumed that the mice were unfasted, which could account for the sevenfold difference in the LD_{50} estimate compared with the data appearing in Table II. This further emphasized the importance of maintaining a uniform empty condition of the gastrointestinal tract in order to make valid comparisons using death as the end point.

The oral LD_{50} of $FeSO_4 \cdot 7H_2O$ calculated to be 32 ± 0.6 mcg. of Fe/Gm. after 24 hr. using the Reed-Muench method (2) was found to agree with the LD_{50} calculated using the method by Finney (4), and the expanded tables of Fisher and Yates (5).

The oral LD_{50} of ferrous sulfate solution in fasted female Fairfield Webster mice using death within 24 hr. as the end point was 44 ± 1.1 mcg. of Fe/Gm. The difference in LD_{50} figures between the aqueous ferrous sulfate solution and the ferrous sulfate solution is undoubtedly due to the added preservatives and/or flavoring agents present in the ferrous sulfate solution.

An important refinement possibly would be to remove sawdust and feces in the prior fasting period to insure uniformity of conditions of the intestinal tract within groups and between groups. It was noted that cannibalism, which is always a problem in starvation experiments with mice, was more apparent in the absence of sawdust and feces. This is another factor which must be taken into consideration, since any group showing evidence of cannibalism had to be excluded from the study. The literature concerning iron absorption studies seldom mentions a preliminary fasting period, which would seem to make it difficult to obtain consistent results and make valid comparisons. A sevenfold difference in LD_{50} estimates in this laboratory compared with Weaver *et al.* (3) would illustrate this point.

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