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THE PHARMACOLOGY OF AN IRON-DEXTRAN INTRAMUSCULAR HAEMATINIC

BY

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An iron preparation suitable for intramuscular injection should conform to the following criteria: (*a*) it should be rapidly absorbed from the muscle and the total iron content should be available for haemoglobin synthesis, (*b*) in order to avoid pain, the *p*H and tonicity should approximate to those of normal tissue fluids, (*c*) it should be stable in the presence of protein and electrolytes, (*d*) it should possess a low toxicity, and be stable on storage, (*e*) in order to keep the injection volume down to a minimum, the solution should contain at least 5% w/v of iron.

Previous workers have described the subcutaneous or intramuscular administration of compounds containing either ionic iron (Brownlee, 1942; Goldberg and Hutchison, 1953) colloidal ferric hydroxide, or saccharated oxide of iron (Slack, 1949), but preparations which contained sufficient iron to be useful in the treatment of hypochromic anaemia were found to be either toxic or painful.

Initially, we investigated two series of iron compounds. In the first series, the iron was present as a co-ordination compound, e.g., ferrous calcium ethylenediaminetetraacetate, and other ferrous or ferric alkali, alkaline earth, or organic base salts of ethylenediaminetetraacetic acid. In the second series, the preparations were based on saccharated oxide of iron, but other sugars were used in place of sucrose. On investigation in animals, compounds of the first series caused systemic haemorrhage and were rejected; none of the preparations in the second series showed satisfactory absorption from muscle, and some were, in addition, very toxic.

Iron is stored in the body as a macromolecular protein complex, ferritin, and is also transported in combination with the protein, β-globulin. It was decided, therefore, to investigate the substitution of the protein moiety by other macromolecules; a third series was therefore investigated in which the iron was combined with polysaccharides or their partial degradation products. Encouraging results were only obtained with a low molecular dextran, and, using this, an intramuscular iron preparation has been made which satisfies the foregoing criteria, in that it is well absorbed, has a low toxicity, and is a satisfactory haematinic (Fletcher and London, 1954). An account is presented of the pharmacology of this low molecular dextran-iron complex, using saccharated oxide of iron as a basis of comparison.

METHODS

1. A solution of low molecular dextran-iron complex containing 5% w/v of iron (Imferon),* with a *p*H of 6.

2. Saccharated oxide of iron. Two different preparations, each containing 2% w/v of iron, designated A (*p*H 10.76) and B (*p*H 9.6) were investigated.

In vitro *Precipitation of Ferric Hydroxide.*—This was based essentially on the work of Nissim and Robson (1949); in addition, the stability was investigated at an iron concentration (0.004% Fe) in the therapeutic range.

Paper electrophoresis was carried out on strips of 3 MM paper in an acetate buffer at *p*H 5 and a barbitone buffer at *p*H 8.6. The potential applied ranged from 100–1,000 volts, and the time from 6–24 hr. Low molecular dextran was used as an indicator of electro-osmosis.

Anticoagulant Activity.—This was measured *in vitro* by the method of Nissim (1954), but the blood was added directly to the tubes from a cannula inserted into the carotid artery. The clotting time *in vivo* was determined by the Lee and White method on samples of blood obtained by heart puncture.

Haemolytic Effect.—The method of Nissim (1954) was used, but the degree of haemolysis was determined by counting the remaining red cells.

* Patent applied for in United Kingdom and oversea territories.

Toxicity.—Albino mice were maintained on MRC diet 41 and allowed free access to water. The acute toxicity of each compound was determined after intravenous administration to groups of mice. The number of survivors in each group was counted on the tenth day after injection and the LD50 calculated by plotting the percentage mortality against the dose on logarithmic probability paper. The small size of the leg muscles of the mouse made it impracticable to administer intramuscularly more than 9.5 ml./kg. of the preparations. Doses of iron-dextran of up to 650 mg. Fe/kg. were injected intramuscularly into both hind legs of rabbits and the animals observed for six months after the injection. Chronic toxicity was studied in rats fed MRC diet 41 and rabbits fed MRC diet 18, which were given daily doses of iron-dextran over long periods.

Antigenicity.—Five guinea-pigs of 400-500 g. bodyweight were "sensitized" with 1.0 ml. of iron-dextran/kg. bodyweight intravenously and given a challenging dose of 1 ml./kg. bodyweight 14 days later. Three preparations of iron-dextran made by the same method were investigated in rabbits. Two rabbits were injected 18 times with 0.5 ml. of each preparation during a period of two months. A week later, samples of blood were taken and the sera subjected to precipitin and complement fixation tests with dextran polymer solutions over the range 10^{-3} to 10^{-6} dilution and with iron-dextran solutions over the same range of dilution.

Diffusion of iron after intravenous injection was investigated by determining the change in serum iron concentration in rabbits and the increase in the iron concentration in the peritoneal fluid in mice. Samples of blood were obtained from the marginal ear vein in rabbits. The mice were injected slowly with 200 mg. Fe/kg. of each compound and the concentration of iron in the peritoneal fluid determined (Nissim, 1953a).

Excretion of Iron in Urine and Faeces.—Rabbits were catheterized, injected intravenously with the preparation, placed in metabolism cages, and the urine collected during 24 hours, after which the animals were again catheterized and the samples added to the previously voided specimens. The faeces were collected during two 24-hour periods before injection, and at the same intervals up to four days after injection.

Determination of Intramuscular Absorption of Iron from Site of Injection.—The iron preparations were injected deep into the glutei of rabbits. The animals were sacrificed at definite time intervals after injection. The injected leg was amputated and the residual iron determined. The contralateral leg was used as a control.

Estimation of Iron.—Serum: The method of Laurell (1947) was used, but the HCl concentration was increased to 6 N in order to ensure complete breakdown of the complex prior to colorimetric estimation with either $\alpha\alpha'$dipyridyl or potassium thiocyanate (Ventura and Klopper, 1951). *Muscle, urine, and faeces:* These were wet-ashed with nitric and sulphuric acid and the iron determined colorimetrically by means of potassium thiocyanate.

Haemoglobin was estimated by the method of King (1951).

Iron utilization was studied in 140 suckling piglets. The decline in haemoglobin was followed during the first week to 10 days of life; when the haemoglobin had fallen to approximately 50%, the iron-dextran was administered in a dose of 26 mg. Fe/kg. The haemoglobin was then determined at weekly intervals.

RESULTS

In vitro *Precipitation of Protein.—Serum:* 0.05 ml. of each iron preparation was added to 1 ml. of serum and the mixture incubated for 2 hr. at 37° C. No precipitation of protein was observed with either the saccharated oxide of iron preparations or the iron-dextran. *Fibrinogen solution:* The following serial dilutions were prepared: iron-dextran, 5.0-0.125% Fe; saccharated oxide of iron, 2-0.125% Fe. To 0.3 ml. of each test solution was added 1 ml. of a 1% bovine fibrinogen solution, and the solution was kept for 2 hr. at room temperature. None of the preparations precipitated fibrinogen.

Precipitation of Ferric Hydroxide.—The *p*H at which ferric hydroxide is precipitated from a saccharated oxide of iron solution varies with the type of preparation (Table I). The *p*H of iron-dextran was reduced from 6 to 1, but no ferric hydroxide was precipitated.

TABLE I

*p*H OF PRECIPITATION OF FERRIC HYDROXIDE

Medium	Final % Fe	Iron-dextran	Saccharated Oxide of Iron A'	Saccharated Oxide of Iron B
Water ..	0·004	No precipitation down to *p*H 1	5·9	3·7
,, ..	0·1	,, ,,	6·0	3·7
Rabbit plasma	0·1	,, ,,	7·7	5·8

Paper Electrophoresis.—Saccharated oxide of iron moved towards the anode, and is, therefore, a negatively charged molecule. The iron-dextran moved in the same direction as the dextran marker, but at a slightly slower rate, and it would appear that the iron-dextran molecule is feebly negatively charged. Dr. N. J. Berridge, of the National Institute for Research in Dairying, Reading, has determined the charge of iron-dextran in a Tiselius apparatus and also found it to be negatively charged in a 0.1M phosphate buffer at *p*H 6.8.

Tonicity was determined in a modified Beckmann apparatus. The iron-dextran was dissolved in normal saline and the solution had a freezing point depression of 0.9° C.

Anticoagulant Activity.—Iron-dextran is considerably less anticoagulant *in vitro* than saccharated oxide of iron (Table II). In rabbits after intravenous doses of up to 50 mg. Fe/kg., i.e., 10 times the therapeutic dose, the clotting time was not significantly elevated.

TABLE II

IN VITRO ANTICOAGULANT ACTIVITY OF IRON-DEXTRAN

1 ml. of blood added to 0.32 ml. of solution of iron

Concentration of Fe %	Clotting Time (min.)		
	Saccharated Oxide of Iron	1% NaOH	Iron-dextran
1·0	45*	95*	20
0·5	40*	>240	9
0·25	28	27	9
0·125	20	13	9
0·0625	16	11	8
0·0312	11	11	7
0·0160	8	11	7
0·0080	7	11	5
Control	5	11	5

* It was not possible to obtain an accurate end-point because of haemolysis and partial clotting.

Haemolysis

The haemolysing effect of the two iron preparations was studied *in vitro*. Iron-dextran showed a gradual decline in haemolytic activity from 2 to 0.5% Fe. Below 0.25% Fe no haemolytic activity was detected. Saccharated oxide of iron caused complete haemolysis down to 0.25% Fe but no haemolysis below 0.125% Fe.

Acute Toxicity

The LD50 after intravenous injection into mice was 1,013 ± 81 mg. Fe/kg. for iron-dextran. Both preparations of saccharated oxide of iron had an LD50 of 231 ± 64 mg. Fe/kg. Both iron-dextran and saccharated oxide of iron proved more toxic after intravenous administration to rabbits than to mice. Two rabbits which were injected with iron-dextran at a dose equivalent to 500 mg. Fe/kg. died 9 days after injection. Four rabbits injected intravenously with the saccharated oxide of iron solution, preparations A and B, equivalent to 150 mg. Fe/kg., died between 6 and 24 hr. after injection. It has not been possible to reach a lethal dose-level when iron-dextran is injected intramuscularly into mice (maximum practicable dose 450 mg. Fe/kg. bodyweight); rabbits survived at 690 mg. Fe/kg. bodyweight intramuscularly.

Chronic Toxicity

Rats.—A group of 8 rats which received a total dose of iron-dextran equivalent to 100 mg. Fe/kg. bodyweight in divided doses of 2 mg. Fe/kg. over a 12-week period showed a weight gain comparable with that of normal controls and at autopsy all the organs appeared normal. The tissue/bodyweight ratios for liver, spleen, and kidneys were 11, 3, and 5% higher than those of untreated control animals. A further group of 8 rats on a higher dosage equivalent to 1,000 mg. Fe/kg., administered over a similar period, showed at autopsy enlarged livers and spleens, the tissue/bodyweight ratios in this instance being increased by 87% for liver, 50% for spleen, but only 2% for kidneys.

Rabbits.—A total dose of 1,060 mg. Fe/kg. bodyweight was given either intramuscularly or intravenously to groups of 4 rabbits in divided doses of 20 mg. Fe/kg. over a period of 3 months. Satisfactory weight gains were maintained over the first 9 weeks, but a slight falling-off compared with normal controls occurred towards the end of the period.

The rabbits were sacrificed and autopsy on those which had received the intravenous injections revealed enlarged liver, spleen, and kidneys, brown stained mesentery and excess pleural fluid, the tissue/bodyweight ratios being liver 96%, spleen 48%, and kidneys 21%, respectively, in excess of normal controls. In the rabbits which had been given iron intramuscularly, hepatomegaly was less prominent, while spleen and kidneys were reduced in weight (tissue/bodyweight ratios +41%, −33%, and −11% respectively).

A full account of the histological examination of the tissues from these animals will be published elsewhere. Suffice it to say here that the general appearance of organs such as the liver, spleen, and adrenals had much in common with the descriptions published by Cappell (1930) and Nissim (1953b). The lungs constituted a striking exception, since not only were they free from haemorrhages but iron deposition was slight, even after the heaviest and most prolonged dosage. No doubt this is a reflection of the stability of iron-dextran and is one of the reasons for its low toxicity. Such iron as was present in the lungs was confined to the connective tissue surrounding arteries and the cartilage plates of bronchi.

The appearance of the muscles at the sites of injection is of interest. With iron preparations which are poorly absorbed, such as saccharated oxide of iron, extensive brown discoloration is observed. With iron-dextran the brown staining

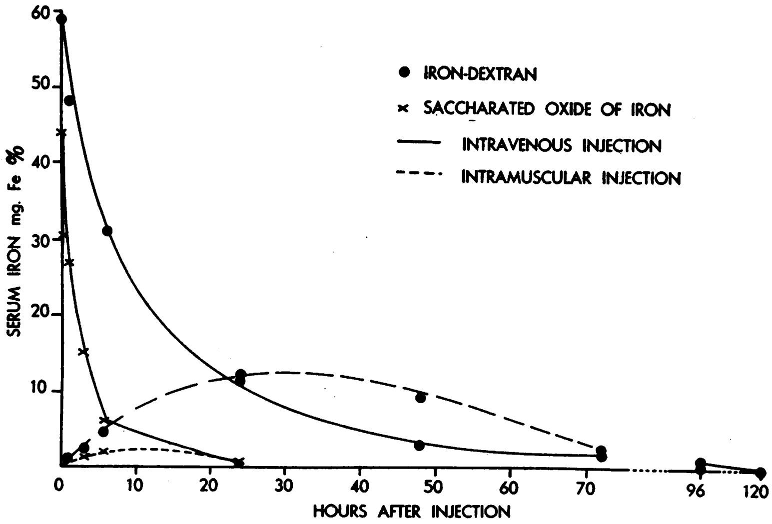

FIG. 1.—Serum iron levels in rabbits after different iron preparations administered i.v. and i.m. in a dose equivalent to 20 mg. Fe/kg.

FIG. 2.—Serum iron levels in rabbits after different iron preparations administered i.v. and i.m. at varying dose levels.

was minimal and often wholly absent. In section the muscle fibres appeared normal, but considerable iron deposition could be demonstrated in the connective tissue between the fibres and in the endothelial cells of some capillaries.

Antigenicity

There were no signs of anaphylaxis in guinea-pigs when the challenging dose was administered 14 days after sensitization. In no rabbit, after injecting each 18 times with 0.5 ml. of iron-dextran during two months, has it been possible to demonstrate any antigenic properties for the preparation in that no serum antibodies were produced which would react with either dextran or iron-dextran.

Diffusion of Iron after Intravenous Injection

From Fig. 1 it can be seen that iron-dextran is much more slowly removed from the blood stream than an equivalent dose of saccharated oxide of iron. When the dose level was increased to 150 mg. Fe/kg. (Fig. 2), the serum iron concentration in the 5 min. sample of saccharated oxide of iron preparation A was only 51 mg.% (average 2 rabbits), with preparation B it was 308 mg.%, whereas after iron-dextran it was 431 mg.% (average 2 rabbits). The rabbits on both saccharated oxide of iron preparations died shortly after the 6 hr. sample was collected. Serum iron concentrations were determined in rabbits which received a dose intravenously of iron-dextran equivalent to 500 mg. Fe/kg. bodyweight (Fig. 2). All three animals which received this dose died between the 8th and 9th day after injection. Saccharated oxide of iron and iron-dextran were administered to mice by slow intravenous injection in doses of 200 mg. Fe/kg. In each case the iron concentration of the peritoneal fluid was estimated, and similar values were obtained (Table III).

TABLE III

DIFFUSION OF IRON INTO THE PERITONEAL CAVITY OF MICE AFTER I.V. INJECTION

Preparation Injected	Dose in Terms of Iron	Fe Concentration mg./100 ml. in Peritoneal Fluid 10 min. after Injection
Iron dextran ..	200 mg./kg.	0·556 mg.
Saccharated oxide of iron	200 ,,	0·374
Saline control ..	Nil	0·227

Excretion of Iron into the Urine and Faeces

The urinary excretion of iron after the administration of varying doses of iron-dextran is comparable with that of saccharated oxide of iron (Table IV). The mean faecal iron of the 2 rabbits which received iron-dextran at a dose equivalent to 500 mg. Fe/kg. was 21 mg. Fe/24 hr. before injection and 17.5 mg. during the 24 hr. period after the injection. During the 24–48 hr. period the animals developed diarrhoea and the faecal iron rose to 40.1 mg., at 48–72 hr. it was 36.9 mg., i.e., 2.6% of the total dose of iron was excreted during the first 72 hr. after injection. The diarrhoea increased in severity until death occurred and it was impossible to collect satisfactory faecal samples after the third day. Animals which received 20 mg. Fe/kg. of iron-dextran showed no increase in faecal iron during the four days after injection.

TABLE IV

EXCRETION OF IRON INTO THE URINE AFTER INTRAVENOUS INJECTION

Compound	Dose (mg. Fe/kg.)	mg. Fe Excreted in 24 hr.	% of Dose Excreted
Iron-dextran ..	20	0·48	0·5
	50	0·5	0·5
	500	1·52	0·12
Saccharated oxide of iron A ..	50	1·3	1·3

Control 24 hr. urine. 0.032 mg. Fe/24 hr.

Absorption of Injected Iron from Site of Administration

The percentage absorption of each of the compounds studied when administered intramuscularly at a dose of iron equivalent to 20 mg. Fe/kg. is given in Table V. In rabbits which received iron-dextran at a dose equivalent to 690 mg. Fe/kg., the residual iron in the muscle 42 days after the injection was 2%—i.e., 98% of the dose of iron administered had been absorbed. The absorption of iron-dextran from the muscle can be followed by the resulting increase in serum iron concentration.

TABLE V

ABSORPTION OF IRON AFTER INTRAMUSCULAR INJECTION

Dose: 20 mg. Fe/kg.

Compound	Time after Injection (Days)	
	2	56
Iron-dextran	87·5% (6)	98% (4)
Saccharated oxide of iron ..	10% (2)	47% (7)

No. of rabbits shown in parentheses.

Elevation of Serum Iron after Intramuscular Injection

The increments in serum iron obtained after doses of each of the preparations equivalent to

20 mg. Fe/kg. bodyweight are compared in Fig. 1. Saccharated oxide of iron, which is only poorly absorbed, produces a slight initial elevation of the serum iron which is not maintained, whereas the serum iron concentration a few hours after administration of iron-dextran is elevated well above that of the iron binding capacity of the plasma protein, and the serum colour becomes progressively darker, making it likely that the iron is being absorbed as iron-dextran. The serum iron concentration continues to increase, reaching a maximum during the 24–48 hr. period. In rabbits which received intramuscularly a dose of iron-dextran equivalent to 660 mg. Fe/kg. the serum iron concentration reached a maximum on the second day after injection, when it was of the same order as that obtained immediately after an intravenous dose of 100 mg. Fe/kg. of iron-dextran. The level remained constant until the fifth day; presumably during this period the rate of absorption of iron into the plasma equalled the rate of removal by the tissues.

Iron Utilization

Litters of domestic piglets, which suffer from a naturally occurring hypochromic anaemia during the first weeks of life, showed a remarkably rapid response to intramuscular injections of iron-dextran. A typical litter of 8 piglets had an average haemoglobin level of 5.1 g.% with a total circulating content of 21.6 g. before treatment. Following an average dose of 140 mg. of iron-dextran, the haemoglobin level rose to 9.6 g.% (48.3 g. circulating) within 7 days and 9.8 g.% (60.8 g. circulating) at 14 days—that is, 63.5% and 93.5% utilization respectively (McDonald, 1955).

Discussion

Iron-dextran has one-third of the acute toxicity of the best type of saccharated oxide of iron preparation; the latter was the least toxic iron preparation previously described. In animals, iron-dextran has been shown to be innocuous when given intravenously or intramuscularly, but in man its prime use is as an intramuscular preparation. The mechanisms by which iron exerts its toxic effect are complex, but the difference in toxicity between the two types of preparation can be correlated with their different stability, anticoagulant activity, and haemolytic effect. The stability of a colloid is a function of its charge (ζ potential) and degree of solvation.

Electrophoretic measurements indicate that iron-dextran is a feebly negatively-charged molecule which does not depend for its stability on an adsorbed layer of strongly charged ions, as does saccharated oxide of iron. In this manner the stability of iron-dextran in the presence of ions would be accounted for. Thus the low molecular dextran appears to act as a protective lyophilic colloid. Saccharated oxide of iron contains 1% of NaOH, and the colloid is stabilized by hydroxyl ions so that neutralization of the negative charge causes precipitation of ferric hydroxide. The *p*H at which precipitation takes place varies with the type of preparation; of the two which were tested, the more stable preparation B precipitated from aqueous solution at *p*H 3.7, and from plasma at *p*H 5.8. The difference in stability of the two preparations observed *in vitro* is also found *in vivo*.

When saccharated oxide of iron *p*H 9.6–11 is administered intravenously the resulting reduction in *p*H lowers the stability of the colloid; this may be the reason for the pulmonary haemorrhage, and precipitation of iron, described by Cappell (1930) and by Nissim (1935b) after intravenous doses of saccharated oxide of iron greater than 45 mg. Fe/kg. Iron-dextran does not precipitate with change of *p*H, and therefore the buffering action of the blood does not appear to affect its stability.

Saccharated oxide of iron preparations administered intravenously are rapidly removed from the circulation (Figs. 1, 2). There is a negligible increment in peritoneal fluid iron content, and only 1–2% of the iron is excreted into the urine; the iron is largely removed by direct uptake by cells of the reticulo-endothelial system. When the dose of saccharated oxide of iron is increased to 150 mg. Fe/kg., the iron concentration immediately after injection is lower than that calculated from the plasma volume of the animal (Nissim, 1953). All the rabbits which received this dose of iron died; pulmonary and systemic haemorrhages were noted at autopsy.

Iron-dextran is more slowly removed from the plasma than saccharated oxide of iron, and animals which received 150 mg. Fe/kg. survived. The immediate serum iron concentration after doses of up to 500 mg. Fe/kg. was of the order expected from the plasma volume of the rabbit. The rate of diffusion of the iron into the peritoneal fluid, and the urinary and faecal iron excretion, are of the same order as those found after administration of saccharated oxide of iron.

The *in vitro* anticoagulant activity of iron-dextran, as determined by the method of Nissim (1954), is considerably less than that of saccharated oxide of iron. The sodium hydroxide present in

the latter shows marked *in vitro* anticoagulant activity. Hence, it is difficult to assess what contribution is made by the iron sucrose combination. The alkali is rapidly neutralized when the preparation is administered to animals, and it would not therefore contribute to *in vivo* anticoagulability. Ten times the therapeutic dose of iron-dextran given intravenously to rabbits has not elevated the clotting time.

The formation of antigens by absorbing polysaccharides on colloids has been reported (Zosaya, 1932). With iron-dextran no evidence of antibody production was found in rabbits, nor was it possible to produce anaphylactic shock in guinea-pigs with the preparation.

Brownlee (1942) investigated the absorption, after subcutaneous injection to rats, of a series of iron compounds and reported that iron triethanolamine chelidamate was a satisfactory haematinic. Slack (1949) administered this compound intramuscularly in doses of the equivalent of 7–14 mg. Fe per patient. The patients experienced severe pain and treatment had to be discontinued. Reports (Thomas, 1954 ; Munro, 1954) have been published that saccharated oxide of iron can be administered intramuscularly, but studies in rabbits have revealed that it is only partially absorbed. This may be due to neutralization of the alkali by the tissue fluid, with the resultant precipitation of ferric hydroxide which is not absorbed.

Iron-dextran is stable in the presence of tissue fluids, and is well absorbed after intramuscular injection. The complex is apparently absorbed unchanged, and intramuscular injection of this compound is perhaps comparable with a slow intravenous injection. Up to the equivalent of 1.3 g. Fe has been absorbed in rabbits after intramuscular injection. The complex is only slowly removed from the circulation, and is taken up by the reticulo-endothelial system, where the iron is made available for haemoglobin synthesis. Piglets suffering from iron deficiency anaemia utilized, during the first 14 days after intramuscular injection, 93% of the dose of iron administered. In cases of iron deficiency anaemia in man, the utilization of the iron after intramuscular injection has been of the same order as that described after the intravenous administration of saccharated oxide of iron (Fletcher and Fee, 1953 ; Baird and Podmore, 1954 ; Scott and Govan, 1954 ; Cappell, Hutchison, Hendry, and Conway, 1954 ; Jennison and Ellis, 1954).

Summary

1. A study has been made of the pharmacology of an iron-dextran complex (" Imferon ") and a comparison drawn with saccharated oxide of iron.

2. Iron-dextran is more slowly removed from the blood than is saccharated oxide of iron. The serum iron immediately after a dose of iron-dextran equivalent to 500 mg. Fe/kg. corresponds to the theoretical value based on even distribution in the plasma. With doses of saccharated oxide of iron equivalent to 150 mg. Fe/kg. the serum iron concentration immediately after injection was lower than the theoretical value.

3. Section of lungs from animals which received doses of saccharated oxide of iron equivalent to 150 mg. Fe/kg. revealed heavy iron precipitation and haemorrhages. Only slight deposition of iron was observed in the lungs from animals which received up to 500 mg. Fe/kg. of iron-dextran, and the lungs were free from haemorrhage.

4. The acute toxicity in mice of iron-dextran is only of the order of one-third that of saccharated oxide of iron.

5. The urinary and faecal iron excretions are of the same order as those described for saccharated oxide of iron.

6. After intramuscular injection, iron-dextran is rapidly absorbed, the iron being taken up by the reticulo-endothelial system. Muscle absorption studies on saccharated oxide of iron, up to two months after injection, revealed that it was only partially absorbed, and pronounced brown staining of the muscle persisted.

7. Anaemic piglets utilized, during the first 14 days after intramuscular injection, 93% of the dose of iron administered.

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