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STUDIES ON A NEW INTRAMUSCULAR HAEMATINIC, IRON-SORBITOL

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A new iron preparation for intramuscular use is described. It contains a complex of iron, sorbitol and citric acid. Its properties in comparison with several other complexes, particularly iron-dextran, have been studied. The preparation is stable in serum, is hypertonic, does not produce haemolysis, and affects coagulation only at very high concentrations, such as are reached only *in vitro*. Absorption from muscle takes place very rapidly; two-thirds of the iron is removed within 3 hr, and there is a very rapid increase in the serum-iron concentration. In experimental animals, the maximum level is reached after about 20 min and in man after about 2 hr. Disappearance from the serum takes place rapidly. The preparation contains a small amount of a fraction which reacts with transferrin and is dialysable. In man, about 30% of the total dose of iron is excreted through the kidneys during the first 24 hr after injection, the greater part of the excretion taking place during the first few hours.

Parenteral iron therapy has been increasingly used since Nissim (1947) discovered that saccharated oxide of iron could be used for intravenous administration. This preparation produces severe local side-reactions and cannot be administered intramuscularly (Slack, 1949). Agner, Andersson & Nordenson (1948) studied an iron preparation for intravenous use in which the iron was present as a special ferri-dextrin complex in colloidal form. Later investigations by Andersson & Bergström (1956) demonstrated that this iron compound could also be administered intramuscularly in man.

Complex compounds of the iron-dextran type (Fletcher & London, 1954), ferric disodium-N-hydroxyethyl-ethylene-diamine-triacetate (Seven & Peterson, 1958) and ferric choline citrate (Virtanen & Hartiala, 1958) have also been investigated, and of these the iron-dextran complex is used clinically (Baird & Podmore, 1954; Cappell, Hutchison, Hendry & Conway, 1954; Scott & Govan, 1954; Jennison & Ellis, 1954). This compound is said to be a low molecular dextran-iron complex and has a low toxicity. Its pharmacology has been studied by Martin, Bates, Beresford, Donaldson, McDonald, Dunlop, Sheard, London & Twigg (1955). Beresford, Golberg & Smith (1957) investigated the absorption mechanism and the local effect of the compound on muscle, and Nordén (1957), Grimes & Hutt (1957) and Karlefors & Nordén (1958) studied in man the metabolism of an intramuscularly administered iron-dextran complex labelled with ^{59}Fe.

According to these more recent investigations 50% to 80% of the dose of iron given was absorbed from the site of injection within 20 to 100 days while the residual iron remained in the muscle for a long period. A search has therefore been made for an iron preparation for intramuscular injection which can be absorbed more rapidly and completely.

Sorbitol is known to improve the absorption of oral ferrous sulphate (Herndon, Rice, Tucker, van Loon & Greenberg, 1958). The possibility of producing new iron complexes for intramuscular injection, containing sorbitol or even other substances, has been studied by Lindvall & Högberg (unpublished observation). The resulting preparations have been tested, and the product with the best properties is described here.

METHODS

Characterization of the preparations used

This is a complex of iron, sorbitol and citric acid, containing, in addition, dextrin. It is prepared by adding an aqueous solution of ferric chloride in portions to a solution of 60° C temperature containing sorbitol, citric acid and dextrin. The *p*H of the solution is adjusted to weakly alkaline after the addition of each portion. After cooling, the complex is precipitated by adding alcohol to the mixture. The iron content of the complex is 14±2%. A solution of it is sterilized by autoclaving and contains 50±2 mg/ml. elemental iron and has a *p*H of 7.5±0.2 [Jectofer, Astra]. This solution is referred to as iron-sorbitol.

Electrophoretic investigations with a paper-strip electrophoresis apparatus from L.K.B. Produkter Fabriks A.B., Sweden, in a 0.1 M phosphate buffer at *p*H 7.6 show that the compound contains at least two iron-containing components which migrate towards the anode (Fig. 1). Furthermore, about 6% of the iron is found in a more rapidly moving form in investigations with a Spinco continuous-flow paper electrophoresis using 0.07 N acetate buffer of *p*H 5.0 (Fig. 2). This part of the compound is of lower molecular weight and dialysable. Moreover, it will be seen from Fig. 2 that the dextrin can be separated from the iron-containing fractions. It is presumed that dextrin acts as a stabilizer (Eriksson, unpublished observation).

Ultracentrifugation of the poly-dispersed iron compound gives an upper limit for the sedimentation constant of 8 to 9 Swedberg units. As the density of the molecule is unknown, it is not possible to calculate the exact molecular weight. With certain assumptions, the probable average molecular weight is estimated to be below 5,000 (Eriksson, 1962).

A solution of iron-dextran containing Fe 50 mg/ml. and with a *p*H of 5.8 [Imferon, Benger] was used as a basis of comparison. In some of these experiments, solutions of the iron-dextrin complex containing Fe 20 mg/ml. and with a *p*H of 7.4. [Astrafer, Ferrigen, Astra], as well as solutions of saccharated oxide of iron containing Fe 20 mg/ml. and with a *p*H of 10.9 [Intrafer, Pharmacia], were also used.

Stability at different p*H*

The *p*H of the preparations in aqueous solutions was regulated within the range 1 to 8, with 0.1 and 1.0 N hydrochloric acid, in accordance with the method of Nissim & Robson (1949). After the addition of the hydrochloric acid the iron concentration in all the solutions was Fe 1 mg/ml. After the solutions had been standing for 24 hr at room temperature the precipitate was removed by centrifugation and the iron content and *p*H in the supernatant determined. The iron was estimated colorimetrically by means of ammonium thiocyanate.

Haemolytic effect

This was studied by mixing 1.0 ml. of solutions of the iron complexes containing Fe 0.04 to 20.0 mg/ml. of 0.9% sodium chloride solution with 0.43 ml. of blood corpuscle suspension consisting of 1.0 ml. of rabbit blood in 25 ml. of physiological saline. After the mixture

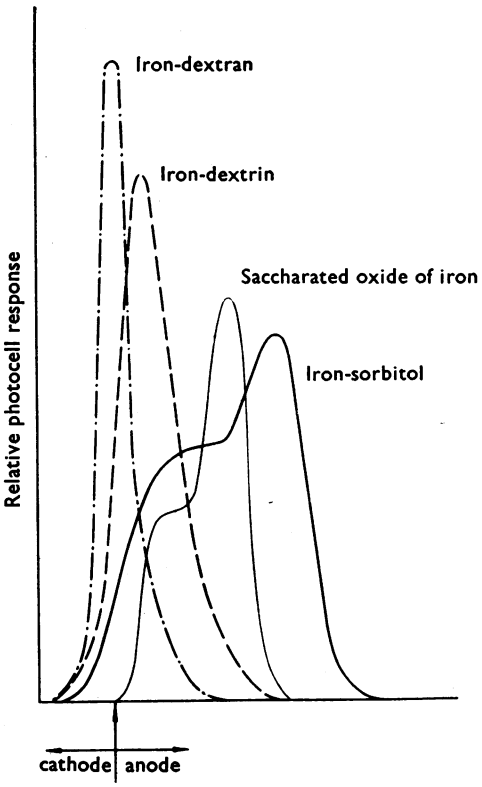

Fig. 1. Paper electrophoresis of iron-sorbitol, iron-dextran, iron-dextrin and saccharated oxide of iron in phosphate buffer at *p*H 7.6. The fractions are stained for iron by using potassium ferrocyanide in 1 N hydrochloric acid.

had been standing for 3 hr at room temperature the non-haemolysed corpuscles were removed by centrifugation. After having been washed twice with 3.5 ml. of physiological saline the blood cells were haemolysed with 5 ml. of 0.05% ammonium hydroxide solution (5 ml./100 ml. of concentrated ammonia). The extinction was read at 576 mμ.

Anticoagulant activity

This was measured *in vitro* by the method of Nissim (1954). 1 ml. of blood was taken from the carotid artery of a rabbit and transferred directly to tubes containing 0.32 ml. of iron solutions with an iron content of 0.045 to 25 mg/ml. Fe of physiological saline. After thorough mixing, the time at which coagulation occurred was measured.

Intramuscular absorption of iron from site of injection

The iron preparations were injected deep into the glutei of rabbits. (Male albino rabbits weighing 2 to 3 kg were used consistently.) The animals were killed at different time intervals after injection and the gluteal muscles were dissected away from the leg. The muscles and skin at the site of injection were wet-oxidized with sulphuric and nitric acid and the iron determined colorimetrically by means of ammonium thiocyanate. The residual iron was obtained by subtracting the iron content of normal muscle.

Iron concentration in serum

The serum-iron was estimated according to the principles of Heilmeyer & Plötner (1937). 1.0 ml. of serum was mixed with 1.5 ml. of 4 N hydrochloric acid and hydrolysed at 50° C

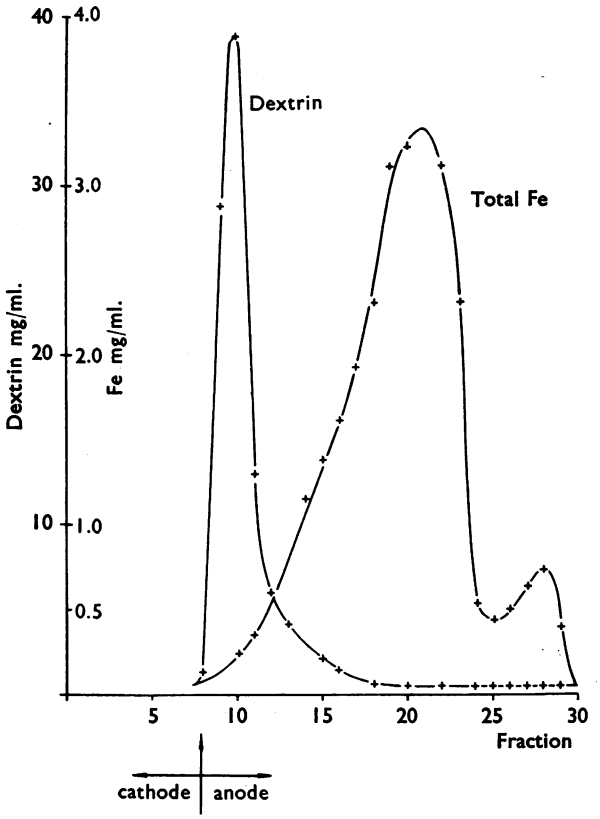

Fig. 2. Continuous-flow paper electrophoresis of iron-sorbitol in acetate buffer at *p*H 5.0.

for 20 min. After hydrolysis, the protein was precipitated with 1.5 ml. of 20% trichloroacetic acid solution. Ten minutes later, the precipitate was centrifuged off at 3,500 g for 30 min. 3.0 ml. of the clear supernatant was transferred to a 5.0 ml. volumetric flask; 5 drops of freshly prepared 0.2% ascorbic acid solution, 4 drops of 1% *ortho*-phenanthrolene in a 10% alcohol solution and 1 drop of a 0.2% 2,5-dinitrophenol solution in absolute alcohol were added with shaking. The sample was then neutralized with concentrated ammonia till the indicator turned yellow. The mixture was acidified with 0.4 N hydrochloric acid till the exact moment when the yellow colour disappeared, and the volume was adjusted to make 5.0 ml. The extinction was read in a Zeiss photometer at 515 mμ. The analytical values obtained by this method include plasma-bound iron and circulating iron preparations.

Diffusion of iron into tissue fluids

The diffusion into tissue fluids was studied by the method of Nissim (1953) with the peritoneum of the mouse as the dialysing membrane.

The mice were injected intraperitoneally with 2.0 ml. of physiological saline and immediately afterwards received iron-sorbitol and iron-dextran solutions by intravenous injection. The animals were killed with ether after different intervals, the peritoneal cavity was opened and the fluid in the abdominal cavity collected. Abdominal contents contaminated with blood were not used. The concentration of iron was determined with *ortho*-phenanthrolene in 3 ml. mixed samples from six mice according to the serum-iron method.

Iron in urine

The iron concentration was determined after the urine had been wet-oxidized with sulphuric acid and hydrogen peroxide in accordance with the *ortho*-phenanthrolene method.

RESULTS

Precipitation test

The solution of iron-sorbitol can be diluted with 0.9% sodium chloride solution to concentrations between 0.001 and Fe 25 mg/ml. without giving any visual precipitation. Nor does any precipitation take place after dilution with rabbit serum and 1% bovine fibrinogen solution to the same concentrations as above, when the solution is kept at 37° C for 2 hr.

The results from the studies about the stability of the iron preparations at different *p*H showed that iron-sorbitol precipitated within the *p*H range of 1.7 to 3.5, with maximum precipitation (40%) at *p*H 2.5. Between 3.5 and 8 there was no precipitation. Iron-dextran was stable between a *p*H of 1 and 8. Iron-dextrin showed some precipitation within the 1.0 to 2.3 range immediately after the addition of hydrochloric acid, but this disappeared after a few hours. No precipitation was observed at *p*H values exceeding 2.3. The saccharated oxide of iron used in this experiment diverged from that of Nissim. It was precipitated practically quantitatively at *p*H 4.5 to 7.0, and at *p*H 7.2 it showed 50% precipitation. At *p*H 7.6 no precipitation could be observed.

Osmotic properties

These were studied after dilution with distilled water to different concentrations. The depression of freezing point was determined with a Beckmann thermometer, and it was found that a solution, isotonic with blood, was obtained when the iron content in the diluted iron-sorbitol solution was Fe 12 mg/ml. The corresponding value for the iron-dextran preparation was Fe 35 mg/ml. The result shows that the preparations are hypertonic, especially iron-sorbitol.

Haemolytic effect

The results showed that iron-sorbitol and iron-dextrin had no haemolytic effect, while iron-dextran was haemolytic at a concentration above Fe 1.75 mg/ml. and saccharated oxide of iron had an effect at a concentration above Fe 0.44 mg/ml.

Anticoagulant activity

As may be seen from Table 1, iron-dextran as well as sorbitol alone had little effect on the coagulation. Iron-sorbitol and iron-dextrin, however, prolonged the clotting time in concentrations higher than 0.2 mg/ml. Fe ; at the highest concentrations, coagulation was completely inhibited.

The effect of iron-sorbitol on coagulation was studied *in vivo* in 8 rabbits after intravenous and intramuscular doses corresponding to Fe 1.5 and 5.0 mg/kg. No effect on the coagulation was observed after 2 to 60 min ; nor was any change in the clotting time noted in man, at the same times, after injection of doses corresponding to Fe 1.5 mg/kg in 3 healthy subjects.

TABLE 1

ANTICOAGULANT ACTIVITY OF THREE IRON PREPARATIONS AND SORBITOL *IN VITRO*

Concentration of Fe or sorbitol in mg/ml.	Clotting time in min			
	Iron-sorbitol	Iron-dextran	Iron-dextrin	Sorbitol
6·0	—	17	—	7
3·0	—	15	—	7
1·5	42	12	82	7
0·75	19	12	46	7
0·37	13	12	24	5
0·18	11	7	17	5
0·09	10	7	15	5
0·045	8	7	12	5
0·022	5	7	10	5
0·011	5	7	10	5

Absorption of iron after intramuscular injection

Iron-sorbitol and iron-dextran solutions were injected into 50 rabbits in a dose of Fe 20 mg/kg (0.4 ml.). The results showed that iron-sorbitol, when administered intramuscularly, was absorbed very rapidly; after 3 hr about two-thirds of the iron had already been removed from the site of injection (Fig. 3). The corresponding

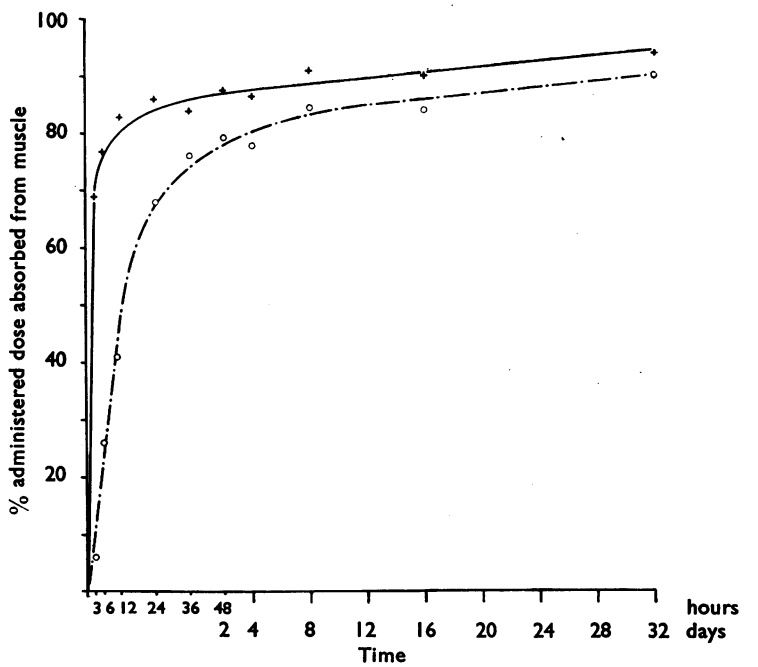

Fig. 3. Absorption of iron-sorbitol and iron-dextran after intramuscular injection in rabbit of a dose corresponding to Fe 20 mg/kg. ——— iron-sorbitol. — · — · — iron-dextran.

figure for iron-dextran after the same interval was only about 10%. For iron-sorbitol a rapid absorption phase was concluded after 12 hr, when the animals had 15 to 20% of the injected iron left at the site of administration. For iron-dextran, this phase was not completed until after 48 hr, and about 20% of the iron was then

still present in the muscle. At dissection it was seen that the muscle was free from the iron preparation after these intervals but that the fatty tissue between the muscles and the subcutaneous fat was stained with iron. The residual iron subsequently disappeared at a very slow rate; 32 days after the injection about 6% of the iron was still present in the specimens from the animals which had received iron-sorbitol and 9% in those which had received iron-dextran.

Iron content in serum after intramuscular injection

The iron content in serum was estimated in 20 rabbits at different time intervals after intramuscular injection of solutions of iron-sorbitol and iron-dextran in a dose corresponding to 1.5 Fe mg/kg. Blood samples were taken 6 to 8 times from the marginal ear vein of each rabbit. The values are given in Figs. 4 and 5; each point is the mean of 5 estimations.

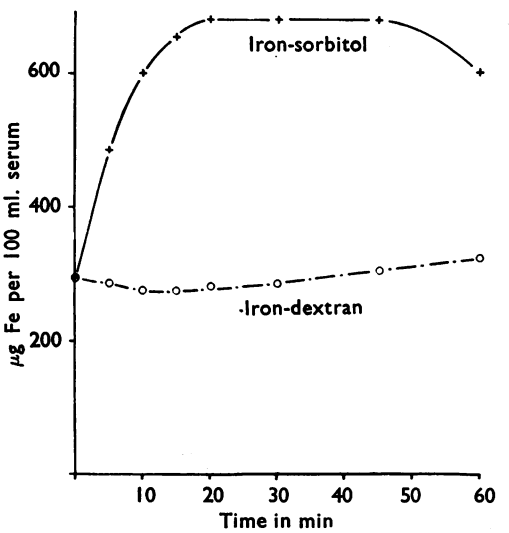

Fig. 4. Serum-iron during the first hour after intramuscular injection in rabbit of iron-sorbitol and iron-dextran in doses corresponding to Fe 1.5 mg/kg.

After iron-sorbitol there was a rapid rise in the serum-iron content; the maximum level was reached after about 20 min and was maintained for a further 30 min. With iron-dextran a definite increase was not recorded until about 3 hr after administration, and the maximum value was reached after 6 hr (Figs. 4 and 5). Similar tests were carried out with iron-sorbitol in man, doses corresponding to 25, 50 and 100 mg Fe being administered deep intramuscularly in the gluteal region in respectively 4, 4 and 5 healthy subjects. As may be seen from Fig. 6, the serum-iron began to increase immediately after the injection, reached the maximum about 2 hr later, and then decreased gradually.

Iron-binding capacity of serum

The unsaturated iron-binding capacity of serum was determined by the method of Cartwright & Wintrobe (1949) after both intravenous and intramuscular injection

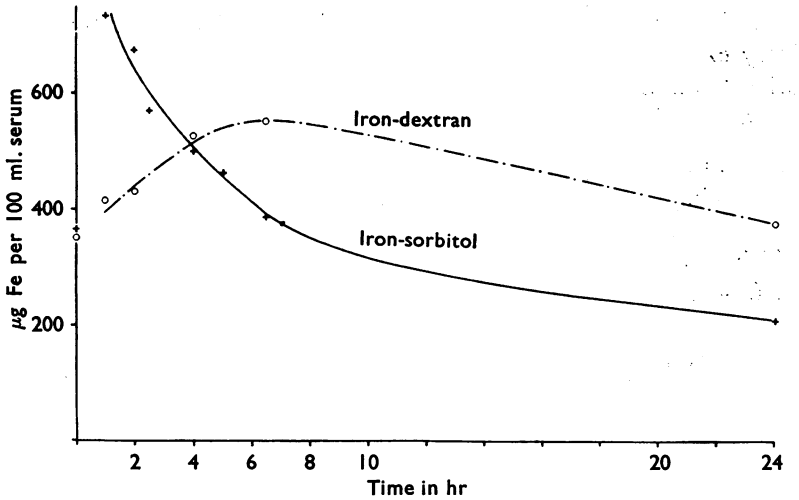

Fig. 5. Serum-iron 2 to 24 hr after intramuscular injection in rabbit of iron-sorbitol and iron-dextran in a dose corresponding to Fe 1.5 mg/kg.

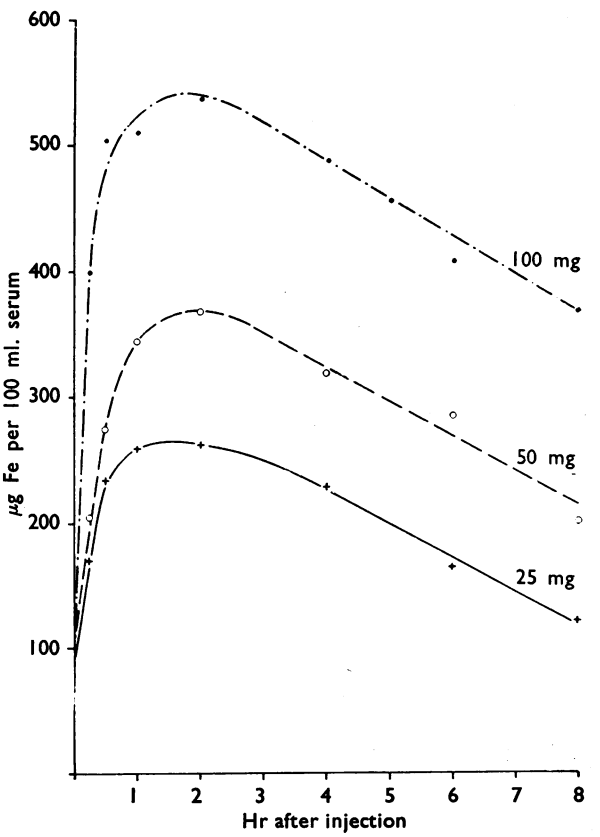

Fig. 6. Serum-iron after intramuscular injection in man of iron-sorbitol in doses corresponding to 25, 50 and 100 mg Fe.

of iron-sorbitol into rabbits in doses equivalent to Fe 1.5 mg/kg. In these preliminary experiments it was established that the transferrin was saturated with iron during the first 6 hr. After 24 hr the serum showed the same values for the unsaturated iron-binding capacity as before the injection.

The values for the unsaturated iron-binding capacity of the human subjects used for the determination of the iron content in serum were also examined. The values decreased following the injections. The decrease was most marked after the 100 mg dose, which produced complete saturation of the transferrin 0.5 to 6 hr after the injection (Fig. 7). The levels of the total iron-binding capacities of the respective

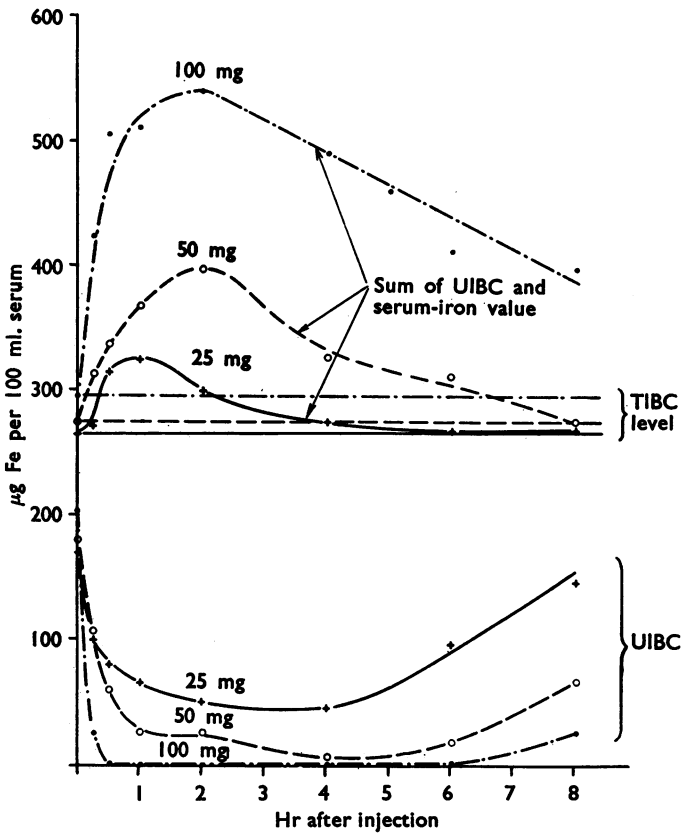

Fig. 7. Unsaturated iron-binding capacity (UIBC) and the sum of UIBC and serum-iron value after intramuscular injection in man of iron-sorbitol in doses corresponding to 25, 50 and 100 mg Fe. The levels for total iron-binding capacity (TIBC) are also shown.

groups are also indicated in the figure. These levels have been determined by adding the values of the unsaturated iron-binding capacity to the serum-iron values of the treated subjects prior to the injection of iron-sorbitol. The sum of the unsaturated iron-binding capacity and the serum-iron value has also been calculated at different times after the injection of iron-sorbitol.

During the first 6 hr this sum, as can be seen from Fig. 7, exceeds the total iron-binding capacity as found before the injection. The reason for this is that the value of the iron content of serum after the injection includes, as mentioned above, both iron bound to plasma and circulating iron-sorbitol constituents not directly bound to plasma proteins. An attempt to remove this surplus iron, by chromatography with alumina by the method of Laurell, showed that the surplus was not adsorbable as is the case with iron-dextran (Laurell, 1958).

In *in vitro* experiments, it was found that the iron-sorbitol solution could react with, and saturate, transferrin. Closer study established that this property was bound to the low-molecular and dialysable part of iron-sorbitol. The dialysate, however, needed a larger amount of iron in order to saturate the transferrin than a standard solution containing ferrous nitrate. The results are given in Fig. 8.

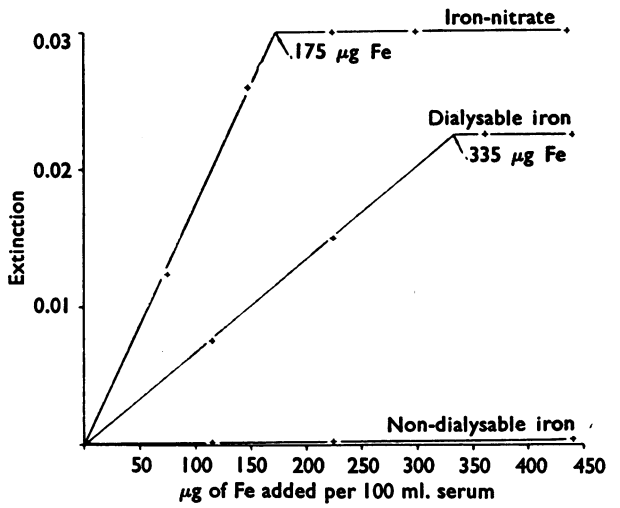

Fig. 8. Saturation of transferrin *in vitro* by adding iron-nitrate, dialysable and non-dialysable parts of iron-sorbitol to rabbit serum.

Iron content in serum after intravenous injection

The iron content of the serum was estimated at different intervals after intravenous injection of the iron preparations into rabbits in doses corresponding to 1.5 mg/kg. Iron-sorbitol, iron-dextran, iron-dextrin and saccharated oxide of iron were injected into the left ear of respectively 16, 8, 3 and 3 animals; blood specimens were taken from the right ear.

The results have been assembled in Fig. 9, which shows that, about 4 hr after the injection of iron-sorbitol, iron-dextrin and saccharated oxide of iron, the iron concentration in the serum was in the neighbourhood of the normal. Iron-dextran, however, was removed from the blood stream at a very slow rate.

Diffusion into tissue fluids

360 male albino mice weighing 20 g received iron preparations intravenously in doses corresponding to Fe 5, 10 and 25 mg/kg.

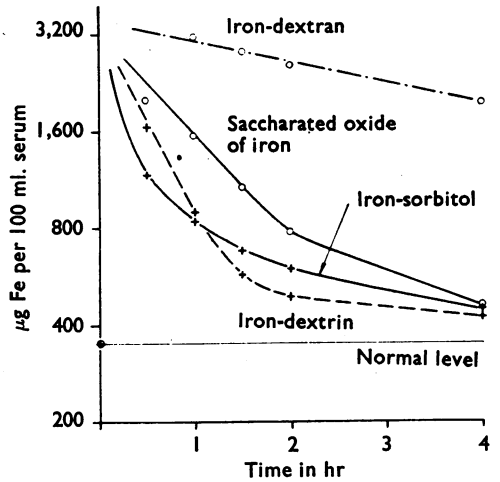

Fig. 9. Iron in serum after intravenous injection in rabbit of iron-sorbitol, iron-dextran, iron-dextrin and saccharated oxide of iron in a dose corresponding to Fe 1.5 mg/kg.

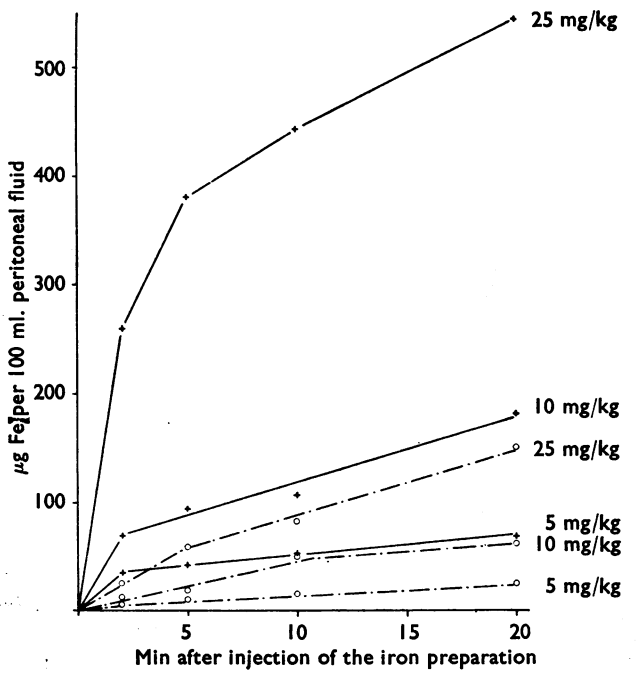

Fig. 10. Diffusion into the peritoneal cavity of iron-sorbitol and iron-dextran after intravenous injection in mice in doses corresponding to Fe 5, 10 and 25 mg/kg. ——— iron-sorbitol. —·—·— iron-dextran.

In Fig. 10 each point represents the mean of 3 determinations in the case of iron-sorbitol and 2 in the case of iron-dextran. It will be seen that both preparations diffused into the peritoneum in amounts to some extent proportional to the dose injected, though the diffusion of iron-dextran was much less.

Renal excretion

The renal excretion of iron-sorbitol following intramuscular injection into 4 rats (Sprague-Dawley) of doses corresponding to Fe 1.5 mg/kg was studied in preliminary experiments. The animals were placed in metabolism cages and the urine was collected during 24 hr. It was found that the rats excreted respectively 24.7, 26.1, 20.5 and 34.5% of the dose of iron injected within 24 hr.

More detailed studies were made of the urinary excretion pattern in man. Nine healthy subjects received 100 mg Fe by intramuscular injection and during the first 24 hr samples of urine were taken at different intervals after the injection. On an average, a total of 30% of the dose of iron given was excreted. As may be seen

TABLE 2

RENAL EXCRETION OF IRON AFTER INTRAMUSCULAR INJECTION IN MAN OF IRON-SORBITOL IN A DOSE EQUIVALENT TO 100 MG Fe

	Renal excretion as %				
	Hr after injection				
Patient	0–3	3–6	6–9	Over 9	Total
G. A., female	12·8	11·1	6·7	1·4	32·0
H. J., male	20·8	4·0	3·7	1·6	30·1
I. M., female	15·2	7·5	4·5	6·3	33·5
I. J., female	13·2	9·8	2·0	2·6	27·6
L. M., female	13·0	7·5	2·1	1·9	24·5
E. H., female	9·7	12·7	4·8	2·4	29·6
K. P., female	15·0	12·5	2·9	6·5	36·9
S. L., male	21·2	11·0	3·8	1·0	38·0
H. H., male	9·4	4·5	3·3	1·2	18·4
Mean value	14·5	9·0	3·8	2·8	30·1

from Table 2, the greater part of the excretion took place during the hours immediately following the injection, and in general it had been completed within 24 hr. The same percentage excretion was obtained after doses corresponding to 50 and 25 mg Fe.

DISCUSSION

According to Golberg (1958), absorption of the iron-dextran complex from the site of administration after an intramuscular injection takes place mainly during the first 72 hr. The absorption rate mentioned by Golberg for the iron-dextran complex has in the main been verified in the present investigation ; about 80% of the iron was absorbed within 48 hr of an injection of iron-dextran. For iron-sorbitol, the first absorption phase was very short ; two-thirds of the injected iron had already been cleared from the site of administration after 3 hr, and 12 hr after the injection an 80 to 85% absorption from the muscle was recorded. The rapid absorption of this complex is rendered possible partly because of its low mean molecular weight, which does not exceed 5,000, and also because, like iron-dextran, it is stable in tissue fluids and at a physiological *p*H.

The residual iron remaining at the site of injection after the first absorption phase is removed very slowly. Golberg (1958) observed that in the case of iron-dextran about 10% was still unabsorbed 15 days after administration into rabbits. In the present investigation, 9% of the iron was still present at the site of administration

32 days after injection of the iron-dextran complex. For iron-sorbitol about 6% of the iron remained uncleared at the end of the same period. A probable reason why some of the iron from both of the preparations was retained at the site of injection is that the iron complex may have spread to the subcutaneous fat and to the fatty tissues between the muscles where clearance is more difficult. The large dose of iron given to the experimental animals is also a factor which may have contributed towards producing a more extensive spread of the iron from muscle to fatty tissue.

The rapid absorption of iron-sorbitol from the site of injection was also apparent from the iron concentration in serum which, in the animal experiments, had already reached the maximum level after 20 min. Iron-dextran, on the other hand, produced no appreciable elevation of the iron content in serum during the first few hours. The difference between the behaviour of the two complexes in serum is due to the feature demonstrated by Svärd & Lindvall (1961), that iron-sorbitol is absorbed into the blood stream both directly and through lymphatic pathways, while iron-dextran is absorbed only via the lymphatic system.

When iron-sorbitol appears in the serum, after absorption, it has no effect on the clotting mechanism of the blood. *In vitro* experiments demonstrated, however, that iron-sorbitol as well as iron-dextrin had a certain inhibitory effect on coagulation. This inhibition, however, occurred only at concentrations considerably higher than those reached in clinical use. The citric acid content of the preparations is the cause of this *in vitro* inhibition.

Despite the rapid absorption that follows intramuscular injection of iron-sorbitol, the iron content of the serum was not as high as was expected, taking into consideration the blood volume and the amount of iron given. This is due to the fact that the absorbed iron disappears rapidly from the circulation, as was seen in the investigations on the clearance of intravenously administered iron preparations. Iron-sorbitol and iron-dextrin preparations disappeared from the blood stream at approximately the same rate while iron-dextran circulates for a long period. According to Andersson (1950), the uptake of iron-dextrin by the reticulo-endothelial system runs parallel to the disappearance from the blood. The iron-sorbitol is probably also taken up by these organs. The initial clearance of the iron-sorbitol preparation takes place more rapidly, however, than that of iron-dextrin, and this suggests that the elimination of the former is influenced by other factors as well. Filtration of the iron through glomeruli, which is made possible by the low molecular weight of iron-sorbitol, is a contributing factor in the rapid initial clearance. About 15% of the dose of iron injected in man was excreted into the urine within the first 3 hr of injection. Iron-sorbitol can, furthermore, diffuse into the tissue fluids.

Following intramuscular administration of the new preparation, and when the doses are large, a temporary saturation of the iron-binding capacity of the serum occurs both in man and in animals shortly after the injection. This property of the preparation is bound to the low-molecular, dialysable fraction. Owing to the fact that this fraction reacts with transferrin it is presumably immediately available for erythropoeisis while the other part of the preparation is taken up by the reticulo-endothelial system for further transformation and metabolism.

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Jectofer, Astra, is pending British patent application number 5791/61.

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