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STUDIES ON A NEW INTRAMUSCULAR HAEMATINIC, IRON-SORBITOL

BY

S. LINDVALL AND N. S. E. ANDERSSON

From the Research Laboratories of AB Astra, Södertälje, and the Medical Department, Centrallasarettet, Danderyd, Sweden

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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

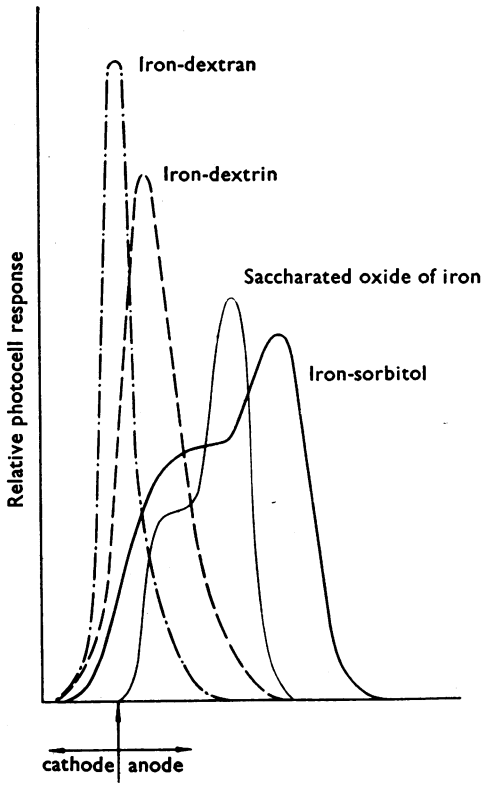

Fig. 1. Paper electrophoresis of iron-sorbitol, iron-dextran, iron-dextrin and saccharated oxide of iron in phosphate buffer at *p*H 7.6. The fractions are stained for iron by using potassium ferrocyanide in 1 N hydrochloric acid.

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

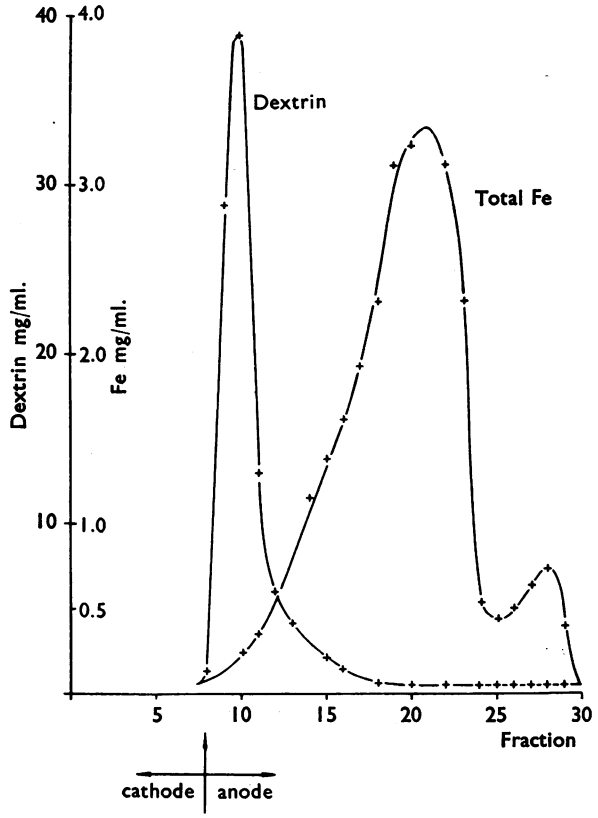

Fig. 2. Continuous-flow paper electrophoresis of iron-sorbitol in acetate buffer at *p*H 5.0.

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.

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