Acta vet. scand. 1964, 5, 265—274.

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ABSORPTION OF IRON-DEXTRIN (Fe^{59}) AND IRON-SORBITOL (Fe^{59}) FROM AN I. M. INJECTION IN PIGLETS

By

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Parenteral iron therapy for piglets has become increasingly popular during recent years. Good clinical results have been reported for two iron compounds in particular, iron-dextran (*Brag* 1957; *Lannek* 1959) and iron-dextrin (*Brag* 1957). A new iron compound intended for intramuscular injection of human beings has recently been described (*Lindvall & Andersson* 1961). The physical, chemical and pharmacological properties of this iron-sorbitol-citric-acid complex (Jectofer) have been dealt with in a series of reports (*Lindvall & Andersson* 1961; *Svärd* 1961; *Svärd & Lindvall* 1961). Metabolic studies (*Wetherley-Mein et al.* 1962) and clinical trials of iron-sorbitol on human beings (*Andersson* 1961; *Andersson & Lundin* 1961) have also been reported. This study was intended as a comparison of the absorption of iron-dextrin and iron-sorbitol after intramuscular injection of piglets.

The distribution pattern of the compounds labelled with Fe^{59} was followed by repeated examination of the blood and examination of other tissues 30 days after injection.

MATERIAL

Fe^{59}-labelled iron- dextrin and Fe^{59}-labelled iron-sorbitol. The iron-dextrin complex had an average molecular weight of 230,000 and the complex of iron, sorbitol and citric acid (iron-sorbitol)

had an average molecular weight not exceeding 5,000. The complexes were prepared on a laboratory scale by standard manufacturing techniques. The iron-dextrin contained 47 mg iron per ml and the iron-sorbitol 50 mg per ml. Iron-dextrin and iron-sorbitol were labelled during manufacture with Fe^{59} to give activities at the time of injection of 2.5 μC/ml and 2.8 μC/ml respectively.

Animals. The twelve piglets, all littermates, were ten days old at the beginning of the experiments and weighed between 2.5 and 3.2 kg. The piglets were anaemic with initial haemoglobin values ranging between 6.5 and 8.6 g/100 ml. The piglets were divided at random into two groups.

METHODS

Administration and dosage. Six of the piglets were given 2 ml labelled iron-dextrin intramuscularly (94 mg Fe and 5.0 μC Fe^{59} corresponding to a dose of 27—41 mg Fe per kg bodyweight) in the gluteal muscles. The other six piglets were injected in the same site with 2 ml labelled iron-sorbitol (100 mg Fe and 5.6 μC Fe^{59} corresponding to 29—43 Fe per kg bodyweight). The skin at the site of injection was tattooed to make it clearly visible when determining the radioactivity by surface counting.

Surface counting. For determining radioactivity at the injection site, a scintillation detector FH 421 N (Frieseke & Hoepfner) furnished with a lead collimator FH-Bl 509 was used. This was fitted with a specially-made collimator inset which was cylindrical and had an orifice 33 mm in diameter and a length of 89 mm of which 65 mm was placed within the collimator. The orifice of the collimator inset was placed against the skin of the pig. The distance between the skin and the scintillation crystal was 95 mm. The collimator orifice was always placed over the tattooed injection site and the hind leg of the pig held in the same position as when the injection was given and the site tattooed.

The radioactivity determinations naturally represent not only the activity of the iron at the injection site but also the Fe^{59} circulating in the blood below the orifice of the collimator. In order to eliminate the effect of the circulating Fe^{59}, measurements were also made on the musculature of the fore leg. A fore leg was chosen since it was evident from trials that if the musculature of the other hind leg was used the radioactivity from the injection

site influenced the measurements despite the fact that a thickleaf collimator was used.

The first measurement over the injection site was made immediately after the injection and the net value of the radioactivity in the depot obtained was then determined as 100 per cent of unabsorbed amount of Fe^{59}. At the beginning the measurements were made at short intervals and then daily. The observed activities were corrected for physical decay of Fe^{59}.

Whole — blood activity. Blood samples were taken once a week during the experimental period. The activity in two ml of heparinized whole blood was determined in a well-type scintillation counter.

Tissue samples. Fe^{59} activity was determined for various tissues taken from three pigs of each groups 30 days after injection. The pigs were killed with mebumal intraperitoneally and were not bled out. Tissue samples weighing about 10 g were wet ashed by *Ekman*'s (1961) technique. Radioactivity was measured in a well-type scintillation counter.

RESULTS

Clearance from injection site. Both iron-dextrin and iron-sorbitol were rapidly absorbed after injection but iron-sorbitol was absorbed more rapidly (Fig. 1). The activity of Fe^{59} at the site of injection declined by half in about 20 minutes (range 15—22 minutes). Absorption of the corresponding amount of iron-dextrin took 60 minutes (range 53—85 minutes). By 48 hours after the injection of iron-sorbitol, less than one per cent of the initial activity remained. Accurate surface counts were impossible from that point onwards. About 30 per cent of the initial iron-dextrin activity persisted at the site of injection after 48 hours. By ten days about ten per cent had still not been absorbed (Table 1). Growth of the piglets increased the size of the original muscle depot so that accurate surface counts with the method used could not be made after ten days.

Distribution of Fe^{59}. The distribution of Fe^{59} in different tissues after the injection of the labelled iron compounds is given in Table II. The liver of the pigs given iron-dextrin contained much more iron (5.4 per cent of the dose) than the liver of pigs given iron-sorbitol (2.2 per cent of the dose). The kidneys of the pigs given iron-sorbitol, on the other hand, contained more Fe^{59}.

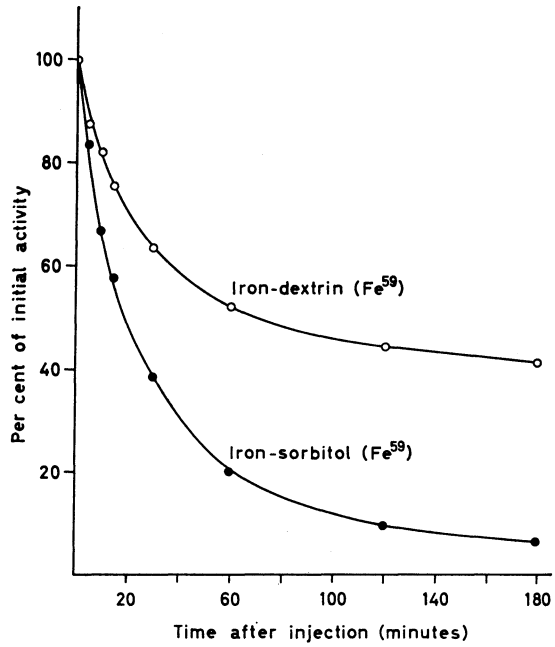

Figure 1. Clearance from injection site of Fe^{59} after intramuscular administration of labelled iron-sorbitol and iron-dextrin. Each value is the mean for six piglets.

Table I. Absorption of Fe^{59} in percentage of initial activity after intramuscular injection of piglets with iron-dextrin (Fe^{59}) and iron-sorbitol (Fe^{59}). Six pigs in each group.

Time after injection	Iron-dextrin		Iron-sorbitol	
	Mean	Range	Mean	Range
10 min.	17.9	12.0—26.4	33.4	23.4—41.3
30 „	36.7	29.0—45.9	61.5	56.3—71.0
1 h.	48.0	39.0—53.0	80.1	75.0—84.0
2 hrs.	55.6	48.0—58.6	90.4	88.0—92.5
3 „	58.7	51.8—61.5	93.6	92.0—95.2
6 „	61.8	56.5—65.0	95.9	94.8—96.5
12 „	64.4	59.0—68.0	96.9	95.6—96.4
1 day	68.4	63.0—71.5	97.8	97.2—98.5
2 days	70.5	66.5—73.0	98.5	98.1—98.9
4 „	73.2	70.0—76.8	> 99	
6 „	76.3	72.2—80.0		
8 „	80.0	75.5—82.8		
10 „	83.3	78.5—85.2		

Table II. Distribution of Fe^{59} 30 days after intramuscular injection of iron-dextrin (Fe^{59}) and iron-sorbitol (Fe^{59}). The results are expressed in per cent of dose per organ.

Organ	Iron-dextrin				Iron-sorbitol			
	Pig no. 80	Pig no. 81	Pig no. 83	Mean	Pig no. 84	Pig no. 85	Pig no. 87	Mean
Heart	0.34	0.55	0.43	0.44	0.37	0.24	0.43	0.35
Liver	5.28	5.30	5.65	5.41	1.67	3.41	1.63	2.24
Lungs	1.54	0.98	0.92	1.15	2.00	0.56	1.01	1.19
Spleen	0.53	0.95	0.87	0.87	0.36	0.32	0.67	0.45
Kidneys	0.43	0.68	0.62	0.58	0.75	0.83	0.63	0.74

Fe^{59} activity in the different tissues 30 days after injection is given in Table III. The blood followed by the spleen had the highest levels of activity. More Fe^{59} was accumulated in the bone marrow by the pigs given iron-dextrin than by the pigs given iron-sorbitol. Except for the kidneys, all tissues examined had accumulated more Fe^{59} activity after the injection of iron-dextrin than after the injection of iron-sorbitol.

Subcutaneous and intramuscular discolouration at the site of deposition was observed in all the piglets injected with iron-dextrin. There was no tissue staining in the pigs given iron-

Table III. Distribution of Fe^{59} 30 days after intramuscular injection of iron-dextrin (Fe^{59}) and iron-sorbitol (Fe^{59}). The concentration of Fe^{59} is expressed in per cent of dose per gram wet weight. All data are adjusted to a constant body weight (8.00 kg).

Tissue	Iron-dextrin				Iron-sorbitol			
	Pig no. 80	Pig no. 81	Pig no. 83	Mean	Pig no. 84	Pig no. 85	Pig no. 87	Mean
Injection site	0.010	0.030	0.155	0.065	0.006	0.005	0.005	0.005
Muscle	0.002	0.003	0.002	0.002	0.002	0.003	0.003	0.003
Heart	0.009	0.013	0.011	0.011	0.009	0.006	0.011	0.009
Liver	0.016	0.010	0.017	0.015	0.007	0.011	0.006	0.008
Spleen	0.045	0.058	0.059	0.054	0.009	0.017	0.037	0.021
Kidney	0.012	0.014	0.015	0.014	0.016	0.018	0.014	0.016
Lung	0.016	0.012	0.010	0.013	0.020	0.006	0.013	0.013
Intestine	0.003	0.003	0.004	0.003	0.004	0.003	0.007	0.005
Bone marrow	0.016	0.025	0.019	0.020	0.001	0.006	0.010	0.006
Whole blood	0.121	0.093	0.092	0.102	0.067	0.057	0.070	0.065

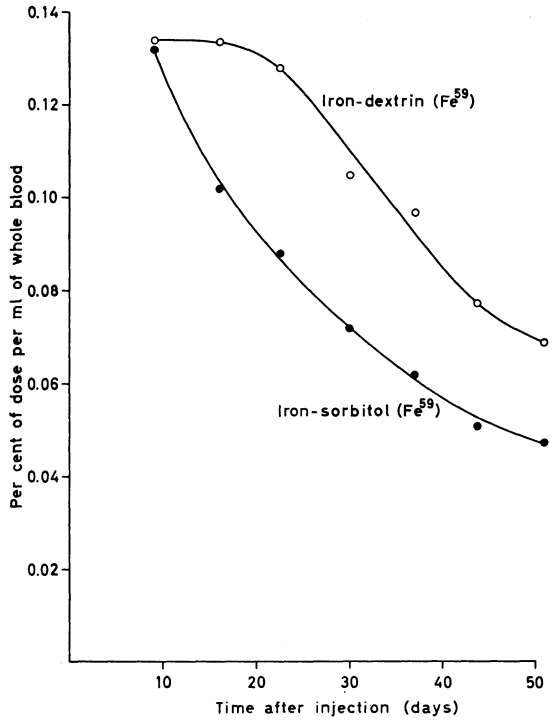

Figure 2. Blood concentration of Fe^{59} after the intramuscular injection of labelled iron-dextrin and iron-sorbitol. Up to day 30 each value represents the mean for six pigs and after this period, for three pigs.

sorbitol. Fe^{59} activity was determined at the site of injection. There were no significant differences in activity levels between the site of injection of iron-sorbitol and muscle tissue from the opposite leg (Table III). There was much more activity at the site of injection of iron-dextrin than in other muscles.

Whole-blood activity. In the first samples taken nine days after injection, Fe^{59} activity in the blood was of much the same magnitude for both groups (Fig. 2). During the next two weeks, blood activity declined much more rapidly for the pigs given iron-sorbitol than for the pigs injected with iron-dextrin. From 22 days until the end of the experiment (51 days after administration) the rate of decline in Fe^{59} activity was much the same for both groups.

DISCUSSION

Absorption of iron-sorbitol from an intramuscular depot has previously been studied on rabbits (*Lindvall et al.* 1961). The rabbits were injected with iron-sorbitol in doses corresponding to 20 mg Fe/kg bodyweight, i. e. less than the dose used for these pig experiments. Through determination of the amount of stable iron remaining at the site of injection *Lindvall et al.* concluded that about 85 per cent had been absorbed by 48 hours. In the case of the piglets, absorption appeared to be practically complete by this time. The difference in rates of absorption could depend upon the piglets so young and anaemic. Absorption of iron-sorbitol after intramuscular injection is known to be more rapid in anaemic than in non-anaemic human beings (*Wetherley-Mein et al.* 1962).

Absorption of iron-dextrin, used in this experiment, can be compared with that of iron-dextran. *Stewart* (1958) reported that 85 per cent of iron-dextran is absorbed within 72 hours. We recorded an absorption of about 72 per cent of the injected iron-dextrin for the corresponding period. By ten days, however, about 83 per cent had been absorbed and absorption undoubtedly continued although our method did not permit us to follow absorption for longer than ten days. The results listed in Table III and the staining of the site of injection indicate that some of the iron-dextrin remains unabsorbed.

Molecule size of a compound is a major factor determining the path of absorption and to some extent the rate of absorption. Snake venom, for example, with a molecular weight of 5000 was absorbed directly by the blood but toxins and venom with molecular weights greater than 20,000 were absorbed and carried by the lymph (*Barnes & Truete* 1941). Iron-sorbitol with its molecular weight of less than 5000 is directly absorbed by the blood but some lymphatic transport also occurs (*Svärd & Lindvall* 1961).

In spite of the more compete absorption of iron-sorbitol, tissue levels 30 days after injection were less than those for iron-dextrin. The differences in accumulation by the liver, spleen and bone marrow were particularly great. The major explanation is undoubtedly the greater excretion of iron-sorbitol in the urine. *Andersson* (1961) and *Wetherley-Main et al.* (1962) have demonstrated that some 30 per cent of labelled iron is excreted by human beings in the urine within 48 hours after the administra-

tion of iron-sorbitol. A few determinations made on the pigs in this experiment confirmed the greater and more rapid excretion of Fe^{59} in the urine of the pigs given iron-sorbitol.

The rapid absorption of iron-sorbitol does not give as high serum concentration as could be expected from calculation of the blood volume in relation to the amount of iron injected (*Lindvall & Andersson* 1961). Rapid withdrawel of iron from the blood is the explanation. This has also been shown to apply to pigs (*Ekman, Thafvelin & Thorell* unpublished). Accumulation of the iron of iron-dextrin by the reticuloendothelial system parallels the decline in the blood level (*Andersson* 1950). *Lindvall & Andersson* (1961), while recognizing RE uptake, consider other factors to be involved as well glomerulifiltration and diffusion to tissue fluids.

In the pig experiment described here, blood levels of Fe^{59} were first determined nine days after the injection of iron-sorbitol and iron-dextrin. At this time nearly all the Fe^{59} has been incorporated into haemoglobin. When given as iron-dextrin the dose of iron was sufficient to maintain a relatively constant concentration of Fe^{59} in the haemoglobin for about two more weeks. The concentration of Fe^{59} declined steadily when the iron had been given as iron-sorbitol.

Clinical experience of the prophylactic and therapeutic treatment of piglets with about 100 mg Fe as iron-dextrin suggests that a single injection is sufficient for the maintainance of satisfactory haemoglobin levels for three to four weeks (*Brag* 1957).

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SUMMARY

Absorption of iron has been followed after the intramuscular injection of 12 piglets with iron-dextrin and iron-sorbitol, labelled with Fe^{59}. Disappearance from the site of injection was followed by a method developed for external radioactivity measurements. Blood levels of Fe^{59} were measured at intervals during a period of 51 days. The tissue distribution pattern of Fe^{59} was examined in six of the piglets after 30 days.

Both iron-dextrin and iron-sorbitol were rapidly absorbed, iron-sorbitol more rapidly. After 20 minutes, 50 per cent of the iron-sorbitol had been absorbed and less than one per cent remained at the site of injection after 48 hours. The amount of Fe^{59} remaining at the site of injection of iron-dextrin had decreased by half by 60 minutes but some ten per cent had still not been absorbed after ten days. When given as iron-dextrin (94 mg Fe per piglet), the level of Fe^{59} in the blood remained fairly constant for 14 days. On piglets given iron-sorbitol, (100 mg Fe each) the blood level declined steadily. The amount of Fe^{59} in the liver, spleen and bone marrow was much greater 30 days after the injection of iron-dextrin than after iron-sorbitol. The relation was reversed in the kidneys, presumably because of the greater excretion of iron-sorbitol in the urine.

ZUSAMMENFASSUNG

Absorption von Eisen-Dextrin (Fe^{59}) und Eisen-Sorbitol (Fe^{59}) bei Ferkeln.

Die Absorption von Eisen von einem i. m. Depot wurde bei 12 Ferkeln studiert. Die Untersuchung wurde mit radioaktiv (Fe^{59}) gemerktem Eisen-Dextrin und Eisen-Sorbitol durchgeführt. Die Absorp-

tion vom Injektionsdepot wurde durch äussere Messungen der Radioaktivität über der Injektionsstelle verfolgt. Die Konzentration von Fe^{59} im Blut wurde durch 51 Tage hindurch studiert und die Verteilung von Fe^{59} in den Geweben wurde nach 30 Tagen bei sechs Ferkeln untersucht.

Sowohl Eisen-Dextrin (Fe^{59}) als auch Eisen-Sorbitol (Fe^{59}) werden rasch absorbiert, doch wies Eisen-Sorbitol eine raschere Absorption auf als Eisen-Dextrin. Nach 20 Min. waren 50 % des injizierten Eisen-Sorbitols absorbiert und nach 48 Stunden fand man weniger als 1 % der injizierten Menge an der Injektionsstelle. Bei Eisen-Dextrin zeigte sich, dass die Menge Fe^{59} am Injektionsdepot nach 60 Min. auf 50 % abgesunken war, während noch nach 10 Tagen etwa 10 % der injizierten Menge nicht absorbiert waren. Nach der Injektion von Eisen-Dextrin (94 mg Fe pro Ferkel) wurde durch 14 Tage hindurch ein verhältnismässig konstanter Eisenspiegel im Blute festgestellt, während die Blutkonzentration nach der Injektion von Eisen-Sorbitol (100 mg Fe pro Ferkel) kontinuierlich absank. 30 Tage nach der Injektion von Eisen-Dextrin fand man wesentlich höhere Konzentration von Fe^{59} in der Leber, Milz und dem Knochenmark als nach der Injektion von Eisen-Sorbitol. In den Nieren war das Verhältnis umgekehrt, was wahrscheinlich auf die höhere Ausscheidung von Eisen-Sorbitol im Urin zurückzuführen ist.

SAMMANFATTNING

Absorptionen av järn-dextrin (Fe^{59}) och järn-sorbitol (Fe^{59}) hos smågrisar.

Absorptionen av järn från en intramuskulär depå efter injektionen av järn-dextrin och järn-sorbitol har studerats på tolv smågrisar. Järnpreparaten hade märkts med radioaktivt järn (Fe^{59}). Försvinnandet från injektionsdepån följdes genom externmätningar över injektionsstället. En redogörelse för den utarbetade mätmetodiken gives. Koncentrationen av Fe^{59} i blodet följdes under 51 dagar. Vävnadsdistributionen av Fe^{59} undersöktes på sex av grisarna efter 30 dagar.

Både järn-dextrin (Fe^{59}) och järn-sorbitol (Fe^{59}) absorberas snabbt men järn-sorbitol dock snabbast. Efter 20 minuter hade 50 procent av järn-sorbitolen absorberats och 48 timmar efter injektionen återfanns mindre än en procent i injektionsdepån. Mängden Fe^{59} i den intramuskulära depån efter injektion av märkt järn-dextrin sjönk till hälften på 60 minuter medan ännu efter 10 dagar ca 10 procent ej hade absorberats. När järnet gavs som järn-dextrin (94 mg Fe per gris) uppehölls relativt konstant koncentration av Fe^{59} i blodet under ca 14 dagar medan koncentrationen sjönk kontinuerligt hos de grisar, som erhöll järn som järn-sorbitol (100 mg Fe per gris). Koncentrationen av Fe^{59} i lever, mjälte och benmärg var betydligt större 30 dagar efter injektion av järn-dextrin än efter tillförsel av järn-sorbitol. Motsatt förhållande var rådande i njurarna sannolikt beroende på den större urinutsöndringen av järn-sorbitol.

(Received November 3. 1963).