Acta vet. scand. 1968, *9*, 10—32.

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STUDIES ON A NEW INTRAMUSCULAR HAEMATINIC FOR PIGLET ANAEMIA

By

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Following the finding by *Nissim* (1947) that iron oxy-sacharate could be administered intravenously in adequate amounts for the treatment of anaemia due to iron deficiency in man, parenteral treatment methods have been increasingly adopted. Iron oxy-sacharate, however, could not be given intramuscularly because of its severe local side-effects (*Slack* 1949). An iron-dextrin complex for intravenous use described by *Agner et al.* (1948) was shown by *Andersson & Bergström* (1956) to be suited even for intramuscular administration in man. Also suitable were a complex of iron and low molecular dextran (*Fletcher & London* 1954) and a low molecular iron-citric acid-sorbitol complex stabilized with dextrin (*Lindvall & Andersson* 1961).

In subsequent experiments, the iron-dextran complex also proved to be useful for intramuscular treatment of iron deficiency piglet anaemia (*Barber et al.* 1955, *Brownlie* 1955). This was likewise shown to be the case with the iron-dextrin complex (*Brag* 1957). Owing to the low toxicity of these two complexes, piglets could be treated without side effects. More recently other complexes between iron and a carbohydrate component have been reported suitable for parenteral treatment of piglet anaemia (*Linkenheimer* 1959, *Zuschek et al.* 1960).

The present paper is a report of a study of a special iron compound for parenteral treatment of piglet anaemia, with reference to the influence of its physical-chemical properties on toxicity, absorption and utilization. To make these studies possible it has been necessary to carry out certain fundamental investigations into piglet anaemia.

MATERIAL AND METHODS

Iron compounds

An iron compound was prepared by addition of a solution of iron chloride to a solution containing dextrin and lactic acid. After addition the pH was adjusted to faintly alkaline reaction. The iron colloid formed was precipitated with ethanol and dried in vacuum. The iron content of the dry preparation thus obtained was 28 ± 3 %. It was dissolved in a 0.1 M citrate buffer containing sorbitol so that a solution containing 100 ± 3 mg Fe per ml at pH 6.4 was obtained after autoclave sterilization. The compound has been registered under the name of Iroject, Astra.

A solution of an iron-dextrin complex containing 20 mg Fe per ml at pH 7.4 (Astrafer, Ferrigen, Astra), a solution of an iron-sorbitol-citric acid complex — referred to below as iron-sorbitol — containing 50 mg Fe per ml at pH 7.4 (Jectofer, Astra) and a solution of iron-dextran complex containing 75 mg Fe per ml at pH 5.8 (Imposil, Pharmacia) were used for comparisons.

Animals and dosages

Six litters of piglets, crossbreeds between Swedish land race and Yorkshire breed, were used for the experiments. The experimental groups were housed in concrete stables. Equipment of low iron content was used. Hygienic conditions were good.

The sows were fed 90 % cereals (equal amounts of barley and oats) and 10 % feed concentrate. The total ration contained 14—15 % crude protein and the iron content was 465 mg per kg. The feed was mixed with water immediately before it was given to the sows. The daily allowance of feed during pregnancy and up to 2 weeks post partus was 3 kg per sow, and after that it was increased to 4 kg.

Litter 1. Five piglets were treated when 4 days old with Iroject at a dosage level corresponding to 100 mg iron per 1.5 kg body weight. The average weight at the time of treatment was 2.0 kg. Two of the piglets were sacrificed 6 hrs. and three 6 days after treatment and used for absorption studies.

Litter 2. Nine piglets were treated when 8 days old with the same dosage as used in litter 1. The average body weight at treatment was 2.7 kg. For absorption studies three of the piglets were sacrificed 6 hrs. and another three 6 days after treatment. The remaining three piglets were slaughtered 56 days after treatment.

The body weight of the latter pigs averaged 10.6 kg 28 days after treatment and 21.0 kg when sacrificed.

Litter 3. Twelve piglets were treated when 2½ days old with Iroject at a dosage level corresponding to 270 mg of iron per animal. The average body weight at treatment was 1.8 kg and increased to 7.4 kg after 4 weeks and to 8.8 kg 5 weeks later. Four of these piglets were sacrificed for absorption studies after 60 days and five 112 days after treatment with Iroject.

Litter 4. Three piglets out of a litter of ten were treated when 3 days old with Iroject at a dosage level corresponding to 270 mg of iron. They were sacrificed 4 weeks following treatment for determination of the absorption.

Litter 5. Seven piglets served as controls. Their body weight average was 1.9 kg at 3 days and 5.6 kg at 24 days of age.

Litter 6. This litter, comprising twelve piglets, likewise served as a control. The average body weight 8 hrs. after birth was 1.2 kg. Five of the piglets were blood sampled on this occasion. The treatment was given as intramuscular injection in the occipital muscles. At 4—5 weeks after parenteral iron treatment, piglets not sacrificed were given a feed supplement containing iron.

The composition of the supplementary feed was 80 % cereals (equal amounts of barley and oats) and 20 % concentrate. The ration contained 15.5 % crude protein and the iron content was 300—350 mg per kg. It was given ad libitum as dry feed. Water was given in automatic drinkers.

Before and at varying intervals after treatment, blood samples (10 ml blood) were drawn from the jugular vein for determination of Hb, serum iron and iron-binding capacity. For quantitative analysis of residual iron in muscles, all muscular tissue at and surrounding the injection site, at least 100 g, was excised.

In experiments carried out to study the absorption from rabbit muscle, 18 male albino rabbits weighing 2—3 kg were treated with Iroject deep in the gluteal muscle at a dosage level corresponding to 20 mg per kg body weight.

In experiments using rapidly growing rabbits, nine 20-day-old rabbits with an average weight of 245 g were treated with a dose corresponding to 10 mg per animal by injection in the thigh muscles. The rabbits sacrificed 7 days after injection averaged 290 g and those sacrificed 8 weeks after injection 2.600 g.

Electrophoretic investigations

A Spinco continuous-flow paper electrophoresis model CP was used with a 0.07 M acetate buffer at pH 5.0. Preparations were diluted with distilled water to an iron concentration of 20 mg per ml. The rate of addition was 0.3 ml per hr. and the residence time 4½ hrs. The current was 50 mamp and the voltage 300 v.

Gel filtration experiments

For these experiments Sephadex G 200 was used and suspended in 0.9 % sodium chloride solution. The gel was allowed to swell for 24 hrs. Excess sodium chloride solution was removed and the thick gel was subjected to a negative pressure in order to remove air bubbles. The gel was transferred to a glass column in order to obtain a 30 cm high column of 40 mm diameter.

The different iron preparations were diluted with 0.9 % sodium chloride solution before addition to the column so that their iron concentration was 5 mg Fe per ml. Gel filtration was performed at room temperature using 10 ml (50 mg Fe) of the iron-saline solution. For elution, 0.9 % sodium chloride solution was used and the outflow time was 25 ml per hr. Fractions of 10 ml were collected in a volume controlled fraction collector.

Other methods

Iron was determined according to an ammonium thiocyanate method. Sorbitol was analysed using a modification of the method devised by *Bailey* (1959). Before the colour reaction for sorbitol, the iron is removed by extraction with iso-propyl ether from solutions strongly acidified by addition of hydrochloric acid and the acid neutralized with sodium hydroxide.

Citric acid was analysed according to the method of *MacDonald & Waterbury* (1959). Lactic acid was analysed using a method devised by *Hullin & Noble* (1953) after hydrolysis of the samples by boiling for 15 min. with 1 N-H_2SO_4. Dextrin was analysed by determination according to *Summer* (1925) of the glucose formed after hydrolysis for 45 min. with 1 N-HCl at 100 °C.

Stability test, haemolytic effect, absorption from muscle, and serum iron content were determined according to the methods described in a paper by *Lindvall & Andersson* (1961).

Serum iron-binding capacity was determined according to a method devised by *Cartwright & Wintrobe* (1949). Total haemo-

globin content was determined using a cyan-haemoglobin method according to *Crosby et al.* (1954).

RESULTS AND DISCUSSION

Electrophoretic investigations

A comparison was made between Iroject, iron-dextrin, iron-sorbitol, and iron-dextran, and as shown by Fig. 1, the iron in Iroject has a negative charge at pH 5.0. Even in iron-dextrin and iron-sorbitol the iron is negatively charged whereas the iron-dextran complex is entirely uncharged under the conditions of the present experiments. Iroject, like iron-dextrin and iron-sorbitol, contains a rapidly migrating component, though in much smaller quantities than the two other compounds.

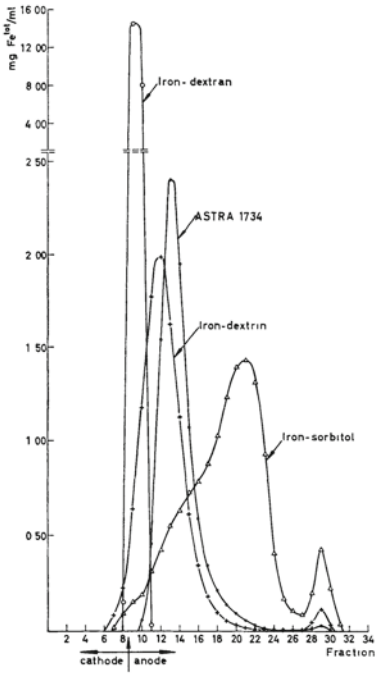


Figure 1. Continuous-flow paper electrophoresis of Iroject (ASTRA 1734), iron-dextran, iron-dextrin, and iron-sorbitol in acetate buffer at pH 5.0.

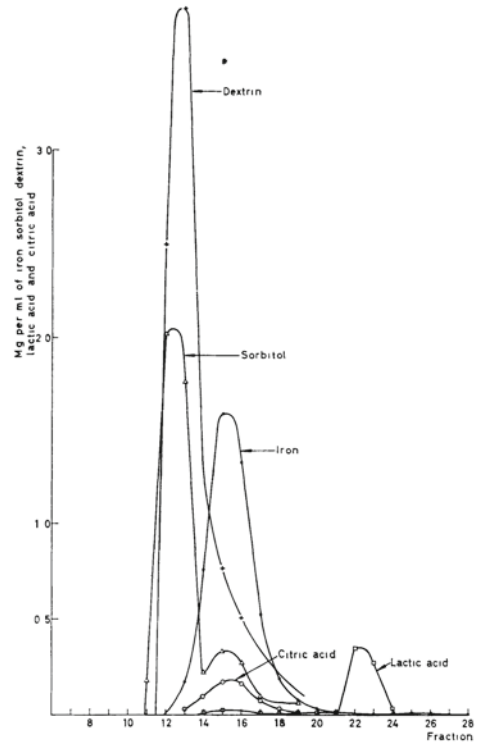
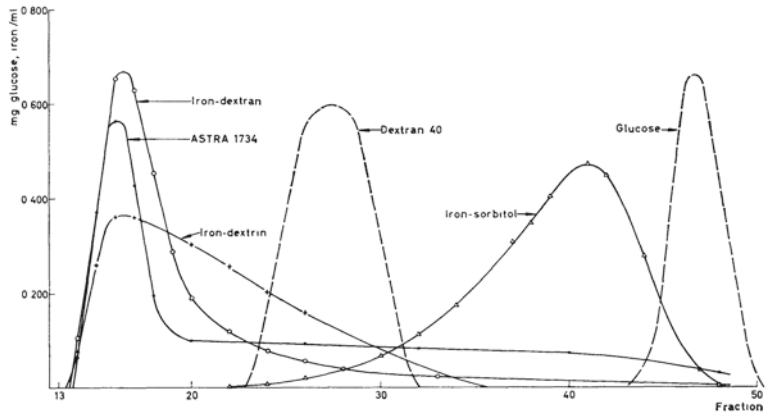


Figure 2. Separation of the different constituents of Iroject by continuous-flow paper electrophoresis in acetate buffer at pH 5.0.

In Fig. 2 the results of electrophoresis experiments with Iroject are presented, showing analysis of the different fractions for the presence of iron, sorbitol, citric acid, lactic acid, and dextrin. As shown by the figure, citric acid and part of the sorbitol as well as dextrin and lactic acid migrate at the same speed as iron under the conditions of the experiment, showing that, in Iroject, these components occur in a complex together with iron.

The main portion of the dextrin, and also the greater part of the sorbitol, are found in the fractions unaffected by the electric field, whereas the main part of the lactic acid is moving anodically, faster than the iron towards the anode.



F i g u r e 3. Gel filtration of Iroject (ASTRA 1734), iron-dextran, iron-dextrin and iron-sorbitol on Sephadex 200. Dextran 40 and glucose are references.

Gel filtration experiments

The results of gel filtrations of Iroject, iron-dextrin, iron-sorbitol, and iron-dextran are presented in Fig. 3. As shown by the figure, the iron in Iroject is present partly in one fraction passing the gel faster than dextran with molecular weight 41,000 and partly in other fractions passing at the same time as this dextran fraction or after it. Of these latter fractions, some coincide with fractions in which the iron-sorbitol is found by gel filtration. In contrast, the fractions with higher molecular weight than dextran 40 coincide with the fractions in which iron-dextrin and iron-dextran are found. The latter compounds, however, do not contain any appreciable amounts of fractions comparable in molecular weight with iron-sorbitol.

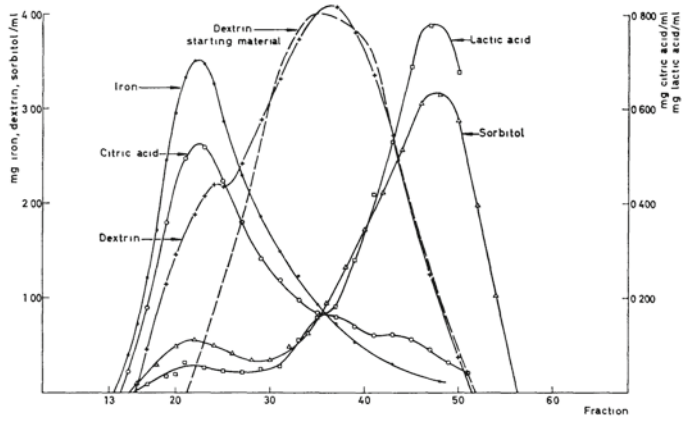


Figure 4. Separation of the different constituents of Iroject by gel filtration on Sephadex G 200.

Fig. 4 presents the results of gel filtration experiments with Iroject, where the different fractions were analysed for iron, sorbitol, citric acid, lactic acid, and dextrin. To obtain measurable amounts of the different constituents in the fractions, 10 ml solution containing 50 mg Fe per ml was added to the columns in these experiments. As shown by the figure, the different components, on passing through the gel, are split in a way to make the citric acid and part of the sorbitol, as well as lactic acid and dextrin, appear in the fractions where the major part of the iron is found. This fact shows that the different constituents in Iroject are present in iron-bound form, and is in agreement with the results obtained in the electrophoresis experiments. The major part of the sorbitol, lactic acid, and dextrin, though, seems to occur free in relation to iron under the conditions of the present experiments. Sorbitol and lactic acid are found in the same fractions as glucose when the latter substance is used for calibration of the gel. The major part of the dextrin appears in the same fractions as the dextrin used as raw material for preparation of the iron complex.

Stability test

The new haematinic can be diluted with 0.9 % sodium chloride solutions or serum from piglet to concentrations between 0.001 and 50 mg Fe per ml and kept in 37°C for 2 hrs. without giving any visual precipitation. The results of the studies on the

stability at different pH show that Iroject is precipitated within the pH range 1.0—2.9 with optimal precipitation at pH 2.5. These results agree with those obtained earlier for iron-sorbitol. No precipitation is observed within the pH range 3.2—8.0. Iron dextran is stable at pH 1—8, whereas for iron-dextrin a certain precipitation occurs within the pH range 1.0—2.5 immediately following adjustment of the pH. This precipitate, however, soon dissolves again.

Osmotic properties

The freezing point depression was determined using a Beckman thermometer after dilution of the different compounds with distilled water to different iron concentrations. The results indicate that a solution with the same freezing point depression as pig serum is obtained when the iron content of a diluted solution of Iroject is 23 mg per ml. Corresponding figures are, for iron-dextrin 20 mg Fe per ml, for iron-sorbitol 16 mg Fe per ml, and for iron-dextran 35 mg Fe per ml. These findings mean that the iron-dextrin compound, which is available in solutions containing 20 mg Fe per ml, is isotonic in relation to pig serum, whereas the other two compounds are hypertonic.

Haemolytic effect

Pig erythrocytes were used for these studies. The results show that iron-dextrin and iron-sorbitol have no haemolytic effect on pig blood cells within the concentration range used. Iroject has a low haemolytic effect, and only 3—5 % of the blood cells are haemolyzed in solutions containing 17.5 mg Fe per ml. The same degree of haemolysis is obtained with iron-dextran at a concentration of 1.75 mg Fe per ml. Above this concentration the haemolytic effect increases successively in relation to the concentration of iron-dextran.

Acute toxicity

The intra-peritoneal toxicity of Iroject was determined by administering 9 dosages within the range of 260—1100 mg Fe per kg mouse, distributed as a geometrical progression and to 40 mice per dosage level. LD50 was thus determined to be 570 mg Fe per kg body weight.

For comparison the toxicity of iron-sorbitol was determined by administering within the range 32.5—165.0 mg Fe per mouse.

To 40 mice per dosage level LD50 was found to be 72 mg Fe per kg body weight for this complex.

Absorption of iron after intramuscular injection

As shown by Table 1 piglets were sacrificed 6 hrs., 6 days and 4, 8, and 16 weeks, respectively, after administration of Iroject.

Table 1. Resorption of Iroject after intramuscular administration to piglets.

Litter no.	Age at treatment (days)	When killed after treatment (days)	Dose mg Fe	Fe in muscle mg	Resorption %
1	4	1/4	140	90.1	35.6
	4	1/4	135	82.1	39.2
2	8	1/4	183	115.5	36.9
	8	1/4	147	95.5	35.0
	8	1/4	187	103.5	44.7
					$\bar{x}$ 38.3
1	4	6	135	58.5	56.7
	4	6	100	51.5	48.5
	4	6	140	63.0	55.0
2	8	6	180	67.5	62.5
	8	6	187	86.5	53.7
	8	6	200	86.5	56.8
					$\bar{x}$ 55.5
4	3	28	270	38.0	85.9
	3	28	270	37.0	86.3
	3	28	270	42.0	84.4
					$\bar{x}$ 85.5
2	8	56	193	7.1	96.3
	8	56	166	5.9	96.5
	8	56	180	3.2	98.2
					$\bar{x}$ 97.0
3	2½	60	270	1.2	99.6
	2½	60	270	3.6	98.7
	2½	60	270	1.9	99.3
	2½	60	270	1.3	99.5
					$\bar{x}$ 99.3
3	2½	112	270	1.5	99.8
	2½	112	270	0.9	99.7
	2½	112	270	1.0	99.6
	2½	112	270	1.0	99.6
	2½	112	270	0.5	99.8
					$\bar{x}$ 99.6

It is evident that absorption of the iron given starts immediately and that 38.3 % of it has been removed from the site of injection as early as 6 hrs. after injection. Following this rapid phase, absorption occurs more slowly but continuously, so that 55.5 % has been absorbed after 6 days, and 97.0—99.3 % of the injected dose has been removed from the injection site in the muscle after about 8 weeks. Less than 0.5 % of the amount of iron administered is found to remain at the injection site 16 weeks after injection. It is also seen from the table that the amount injected does not appreciably influence the degree of absorption 8 weeks after treatment.

The experiments on adult rabbits show that 25.5 % of the given amount of Iroject has been absorbed from the muscles 7 days after injection, and 60.0 % after 8 weeks. When given to rapidly growing rabbits, 23.3 % of Iroject has been absorbed 7 days after injection, and in contrast to the finding in adult rabbits, as much as 89.0 % has been removed from the injection site 8 weeks after administration.

Iron and iron-binding capacity in serum after intramuscular injection into piglets

The results of these investigations are summarized in Fig. 5 and Table 2. As seen from the first part (A) of the figure, the piglets have a serum iron value of 47 μg per 100 ml and an unsaturated iron-binding capacity in serum of 122 μg Fe per 100 ml. In non-treated piglets, with increasing age, a decrease occurs in serum iron and a simultaneous increase in their unsaturated iron-binding capacity, so that, at 24 days of age, the serum iron value is as low as 9 μg Fe per 100 ml and UIBC as high as 925 μg Fe per 100 ml serum.

The rapid absorption phase following treatment of piglets with Iroject, as observed in the absorption experiments, is reflected in Table 2 in high values for serum iron even 1 hr. after administration. The values found exceed the total iron-binding capacity observed before treatment, but nevertheless serum from the piglets has still the capacity to bind iron. This is an indication that the iron in Iroject is absorbed and transported mainly in the non-ionized form. Three hrs. after administration, however, the unsaturated iron-binding capacity of the piglets is completely saturated. At the same time the iron content of the serum has started to decrease, and this decrease continues afterwards so

Table 2. Iron in serum before and at various times after intramuscular administration of Iroject to piglets at a dose corresponding to 100 mg Fe per 1.5 kg body weight.

	Age at treatment (days)	Before treatment			Hours after treatment																
					1			3			6			24			72			144		
		SJ	UIBC	TIBC	SJ	UIBC	TIBC	SJ	UIBC	TIBC	SJ	UIBC	TIBC	SJ	UIBC	TIBC	SJ	UIBC	TIBC	SJ	UIBC	TIBC
Litter 1	4	27	465	492	1065	216	1281	902	0	902	665	0	665									
	4	31	340	371	1425	0	1425	1036	0	1036	609	0	609									
	4	29	405	434																138	388	526
	4	35	350	385																154	313	467
	4	20	408	428																120	318	438
	Average	28	394	422	1245	108	1353	969	0	969	637	0	637							137	340	477
Litter 2	8	15	712	727	1775	392	2167	1644	0	1644	783	0	783									
	8	10	670	680	1464	340	1804	1269	0	1269	681	0	681									
	8	13	685	698	2263	320	2583	1809	0	1809	786	0	786									
	8	21	892	913										430	455	885	233	535	768	80	435	515
	8	19	682	701										467	280	747	255	340	595	122	290	412
	8	16	800	816										415	462	877	155	535	690	123	410	533
	Average	16	740	756	1834	351	2185	1574	0	1574	750	0	750	437	399	836	214	470	684	108	378	486

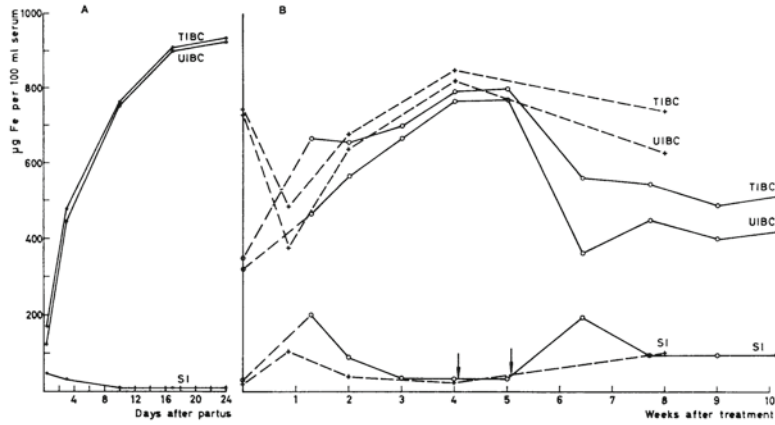


Figure 5. A. Serum-iron (SI), unsaturated iron-binding capacity (UIBC) and total iron-binding capacity (TIBC) in serum of untreated piglets at different intervals after partus. Each dot represents the mean value for 5 animals from litter 6 (8 hrs.) and for 7 animals from litter 5 (3, 10 and 24 days).

B. Ditto in piglets at different intervals after treatment with Iroject.
+ - - + - - + Mean values for 3 piglets from litter 2 treated 8 days after partus with a dose corresponding to 180 mg Fe.
o —— o —— o Mean values for 7 piglets from litter 3 treated 2½ days after partus with a dose corresponding to 270 mg Fe.
The arrows indicate the time when the feed supplement was given.

that, 1 week after injection of the dosage level used in these experiments, the serum iron value is 108 µg Fe per 100 ml serum.

The iron-binding capacity is completely utilized even 6 hrs. after administration of a dose of 100 mg Fe per 1.5 kg body weight. On determining the UIBC 24 hrs. after treatment, however, it is evident that serum again has an unutilized iron-binding capacity showing a further increase 72 hrs. after treatment and again decreasing after 144 hrs.

In contrast, the total iron-binding capacity continuously decreases after 24 hrs. and up to 144 hrs. after treatment, no increase having been demonstrable as for UIBC. It is assumed that the increase in UIBC 72 hrs. after treatment is due to the fact that the absorbed iron is used in haematopoiesis with a decrease in serum iron as a consequence. The continuous decrease of the total iron-binding capacity is due to the fact that the animals' acute demand for iron has been temporarily met by dosing with Iroject.

The subsequent changes in serum iron and iron-binding capacity are seen from the latter part (B) of Fig. 5. This part also illustrates the influence of the size of the given dose. When 100

mg Fe per kg body weight (= 180 g Fe) is given, the decrease in serum iron observed during the first week following treatment continues so that a low value is found after 2 weeks. The serum iron decreases further and is 20 µg per 100 ml serum 4 weeks after treatment. A large increase in both unsaturated and total iron-binding capacity of the serum coincides with this decrease in serum iron.

Similarly a successive decrease in serum iron occurs after injection of a larger dose, namely 270 mg per piglet. There is a lag in time, however, so that the low value observed 2 weeks after treatment with the lower dose does not appear until after 3 weeks using the higher dose. Coinciding with the decrease in serum iron, there is an increase in unsaturated iron-binding capacity and later also in total iron-binding capacity. Even in this respect there is a lag of about 1 week.

When piglets treated with a lower or higher dose are fed with a supplement containing iron after 29 and 36 days, respectively, there is an increase in serum iron and a decrease in both unsaturated and total iron-binding capacity.

Haemoglobin changes

Piglets not receiving supplementary iron show, as seen from the first part of Fig. 6, a successive decrease in the initially high haemoglobin content of their blood — 10.4 g haemoglobin per 100 ml blood. At 24 days of age they show a severe anaemia with not more than 4.7 g haemoglobin per 100 ml blood. The latter part of the figure illustrates the pattern in piglets treated with 100 mg Fe per 1.5 kg body weight (= 180 mg Fe) and 270 mg Fe per piglet, respectively. When injecting the lower dose, the haemoglobin content of the blood increases during the first week, but even 2 weeks after treatment, the rapid growth and the consequent haemoglobin formation contribute to a small decrease of the haemoglobin value. This decrease is later accentuated so that the piglets show a blood haemoglobin value of 7.55 g Hb per 100 ml blood 4 weeks after treatment.

Use of the higher dose of Iroject results in a successive increase of the haemoglobin content during the first 3 weeks after treatment, in spite of the high demands due to growth and haemoglobin formation. At the end of this period the value is about 12 g haemoglobin per 100 ml blood. The amount of iron administered is not able to compensate completely for the high demand for

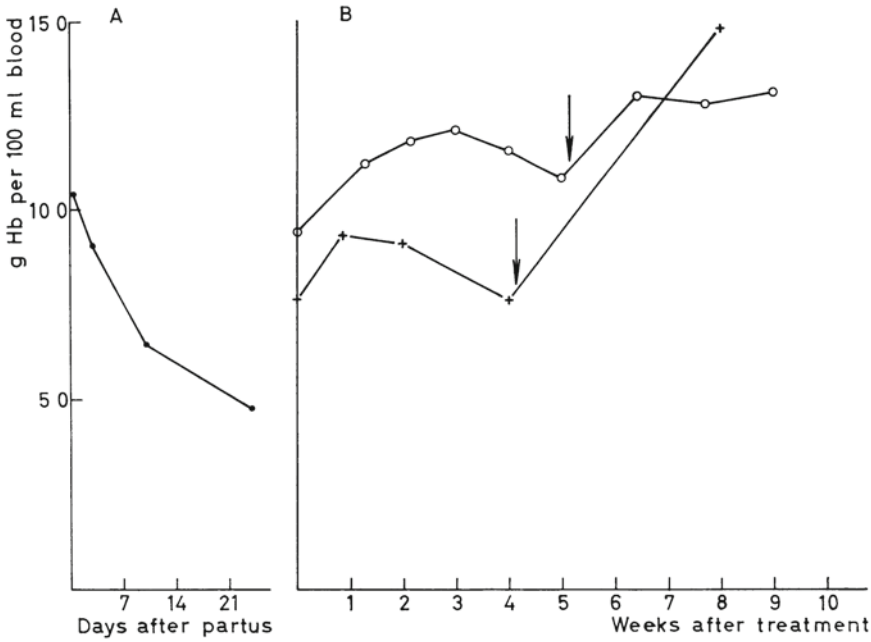


Figure 6. A. Haemoglobin values in untreated piglets at different intervals after partus. Each dot represents mean value for 5 animals from litter 6 (8 hrs.) and for 7 animals from litter 5 (3, 10 and 24 days). B. Haemoglobin values in piglets at different intervals after treatment with Iroject.

+ — + — + Mean values for 3 piglets from litter 2 treated 8 days after partus with a dose corresponding to 180 mg Fe.

o —— o —— o Mean values for 7 piglets from litter 3 treated 2½ days after partus with a dose corresponding to 270 mg Fe.

The arrows indicate the time when the feed supplement was given.

iron in the rapidly growing piglets. Therefore, a decrease of the haemoglobin values is observed 4 weeks after treatment. After 5 weeks the values have decreased to about 11 g haemoglobin per 100 ml blood. Feeding a supplement containing iron results in an increase of the haemoglobin content of the blood in the piglets treated with Iroject, and at 9 weeks of age they show values of about 13—14 g haemoglobin per 100 ml blood.

Iron content in serum after intravenous injection

The iron content in serum has been determined after intravenous injection of Iroject, iron-dextrin, iron-sorbitol, and iron-dextran at a dosage level corresponding to 1.5 mg per kg body weight, three rabbits being used for each compound. The injections were given in the left auricular vein and then blood

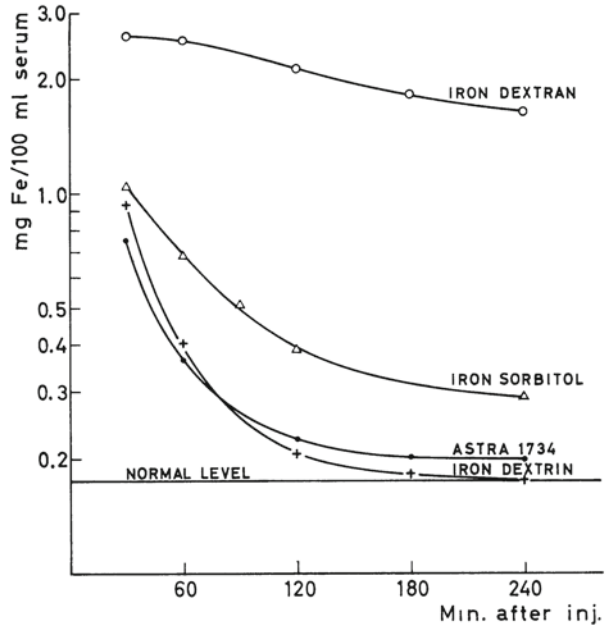


Figure 7. Iron in serum after intravenous injection in rabbit of iron-sorbitol, iron-dextran, iron-dextrin, and Iroject (ASTRA 1734) at a dose corresponding to 1.5 mg Fe per kg body weight.

samples were drawn at different intervals from the right auricular vein.

The elimination of the iron compounds injected is seen from Fig. 7, showing that Iroject is eliminated very rapidly from the blood. The iron content of serum is almost normalized 3—4 hrs. after injection. A similar elimination pattern is shown by iron-dextrin and iron-sorbitol, whereas iron-dextran is eliminated more slowly than the other compounds. Iron-dextrin is eliminated more completely than Iroject within the period of time studied.

GENERAL DISCUSSION

The influence on toxicity and absorption rate of the molecular weights of intramuscularly injectable iron complexes is evident from studies by *Andersson* (1950), *Svärd* (1962), and *Brag et al.* (1964). It was shown by these workers that an iron complex with a high molecular weight is lower in toxicity and slower in absorption than one with a low molecular weight.

By using oxy-acids such as lactic and citric acids, which form complexes with iron, and sorbitol and dextrin, which also tend to be bound to iron complexes, it was possible to produce a com-

plex with a high molar weight suitable for obtaining solutions with a high iron content. Owing to the high molar weight and the consequently slower absorption rate, the acute toxicity is low, allowing adequate dosing of piglets with no risk for side effects. *Högberg & Lindvall* (unpublished data) studied the absorption rate of the high molar iron-dextrin complex. They found that 70—80 % of the iron given as an intramuscular deposit of that compound to adult rabbit is left at the injection site 7 days after administration. In contrast, *Brag et al.*, using the same compound and administration route in piglets, found 70—80 % to be absorbed after the same lapse of time. This difference might be due to animal species or to age. The influence of the age of the animal is illustrated by the absorption of Iroject following intramuscular injection in rabbits of different age. There is no difference in the degree of absorption 7 days after treatment. The difference is not evident until after 8 weeks when the young rabbits have increased their body weights approximately tenfold, but the adult rabbits have gained very little.

The influence on absorption of animal species is seen from the experiments with young rabbits and piglets. Comparison of the absorption rates from the injection site during the first 7 days after injection shows that absorption occurs faster in pigs than in rabbits, suggesting more favourable conditions for absorption in the former. It cannot be concluded, whether this fact is primarily due to differences in permeability or cell metabolism in the muscles of the injection site or to the difference existing between the physical activities of the two species and the lymphatic absorption related to this activity. As for cell metabolism it should be pointed out that the young rabbits gained approximately 20 % in body weight during the first 7 days after treatment, whereas the piglets doubled their body weights during the same time. It is therefore probable that the differences in absorption rates between the two species are due to factors pertaining to cell multiplication and growth rather than to other factors. This assumption is also supported by the results obtained comparing absorption rates in rapidly growing and adult rabbits respectively.

The influence of the rapid growth on the general iron metabolism of piglets is clearly shown by the values for their serum iron, iron-binding capacity and haemoglobin content (Fig. 5 A). As shown by this investigation, control pigs had low values for

serum iron 8 hrs. after birth under the conditions of the present experiments. The values found are approximately one third of those given by *Kolb* (1963) for newborn piglets. This difference may be due to sampling at different intervals after birth, "newborn" not being clearly defined by *Kolb* (1963). The decrease in serum iron demonstrated for the piglets during their earliest life is in agreement with the findings of *Kolb* (1963). When no supplementary iron is available for the piglets in the present experiment, this decrease is very marked. The iron-binding capacity in the 8-hour-old piglets is low in relation to data given for older animals (*Kolb* 1959, *Planas & de Castro* 1960). With increasing age and growth rate there is an extremely accentuated increase in iron-binding capacity. This increase is an expression of the demand for iron which is aroused in the piglets by the high growth rate and the consequently increasing blood volume. Increase of the latter without any corresponding iron supplementation results in a decrease in haemoglobin values.

Assuming that the blood volume corresponds to 1/13 of the body weight, one can calculate that the newborn piglets have approximately 40 mg Fe incorporated in haemoglobin. This amount is in good agreement with the value given by *Venn et al.* (1947) for newborn pigs. A similar calculation for 24-day-old piglets reared in an iron deficient environment and given no supplementary iron except for that present in the sow's milk, shows the amount of iron in the form of haemoglobin to be approximately 70 mg. As the analyses for serum iron and UIBC indicate a severe depletion of the iron deposits, the amount of iron detected in the blood-stream of 24-day-old piglets should be composed of the haemoglobin iron of the newborn, their deposit iron, if any, plus the amount of iron — 1 mg per day — which the suckling piglets receive via the sow's milk according to *Venn et al.* *Ullrey et al.* (1960) points out that the iron content of the sceletal muscles is much lower in pigs with iron deficiency anaemia than in non-anaemic pigs. Similar findings are reported by *Gubler et al.* (1957), showing the myoglobin content of pigs with iron deficiency to be considerably lower than that of healthy pigs. The amounts of iron calculated in the present experiments indicate that the total amount of iron in the muscles can be assumed to be constant in spite of the gains in weight. It thus decreases in relation to weight in agreement with the results of the authors referred to above.

Iroject has been given to piglets 2½ and 8 days after birth in order to counteract this iron deficiency anaemia. Two different dosage levels were used in order to elucidate the implication of the dose level, and alimentary iron supplementation was postponed until the piglets reached 5 weeks of age. The absorption of the complex is reflected by increased values for serum iron above the total iron-binding capacity of the serum during the first few hours. This increase was not accompanied by any signs of intoxication (Table 2). An iron-binding capacity can be demonstrated again 24 hrs. after treatment, but the value for serum iron is still high. It remains higher than before treatment until 1 week after treatment, especially in piglets receiving the higher dose. During this first week the haemoglobin content increases independently of the dosage level, suggesting that the iron given was sufficient for optimal haemoglobin synthesis in both experiments. The piglets given 180 mg Fe show a low value for serum iron 2 weeks after treatment and at the same time the unsaturated iron-binding capacity is strongly increased, suggesting a certain iron deficiency. In these piglets, the increase in haemoglobin content has also been arrested. The serum iron value remains at the same level 4 weeks after treatment as it was at 2 weeks after treatment, but UIBC and TIBC show further increases. Determination of haemoglobin at this time clearly shows the amount of 180 mg iron to have been too low, as the haemoglobin value has decreased to the same level as before treatment. A calculation of the amount of iron circulating in the form of haemoglobin suggests that the piglets had approximately 55 mg Fe before treatment. Four weeks later the iron in the blood stream had increased to approximately 220 mg Fe (three 10 ml blood samples included). The increase found — 165 mg — corresponds to the amount of iron absorbed from the injection site. Besides parenteral iron the piglets received approximately a further 30 mg of iron via the sow's milk. Any amount of iron ingested via the sow's faeces has not been considered. *Ullrey et al.* have shown that pigs with severe iron deficiency have 6.6 µg Fe per g sceletal muscle. The corresponding value found for pigs with 7.9 g % Hb was 10.5 µg/g. As the piglets of the litter treated with 180 mg iron had 7.9 g% Hb both before and after treatment, it can be assumed that these piglets had approximately 10 µg Fe per g muscle on these occasions. The remaining haemoglobin iron, which amounts to approximately 20 % of the total iron content in muscular

tissue according to the findings of *Lintzel* (1931) in different animals, has not been considered in this connection. The sceletal muscles comprise ⅓—½ of the body weight. Thus, the piglets should have increased their sceletal muscles by 3—4 kg during the 4 weeks which have elapsed since the administration of iron. Consequently 30—40 mg Fe could be estimated to be present as muscular iron, 20 % of which consists of haemoglobin iron. The amount of non-haemoglobin iron in the muscles is in good agreement with the amount received by the suckling piglets via the sow's milk.

Two weeks after administration of the higher dose — 270 mg — the value for serum iron is still above that before treatment. UIBC, though, is increasing and so is haemoglobin. The two latter findings are similar 3 weeks after treatment, but on this occasion the value for serum iron has decreased to the low level found at 2 weeks after treatment with a 180 mg dose. The haemoglobin value shows a coincidental slight increase. Four weeks after treatment, the iron deficiency reflected 1 week earlier in the values for serum iron and UIBC, also begins to show in the haemoglobin values. The decrease found in the latter is not great enough, though, to allow a haemoglobin value of over 11 g Hb per 100 ml blood. During these 4 weeks the amount of iron circulating in the form of haemoglobin, when calculated as above, has increased by 195 mg. Judging from the absorption studies, 230 mg of the iron administered should have been removed from the injection site 4 weeks after treatment. The discrepancy found between the amount of iron absorbed, and the amount calculated in the form of haemoglobin can be due to, inter alia, a temporary decrease in absorption from the gastro-intestinal tract, storing of iron in liver and spleen, and a high iron content in the muscles of the non-anaemic piglets. In order to meet with the high demand for iron of the rapidly growing piglets, it is necessary to administer such a large dose of iron as to temporarily overload them with iron. According to *Conrad & Crosby* (1963) and *Conrad et al.* (1964) a decrease occurs in the absorption of iron from the gastro-intestinal tract in overloaded animals. *Greenberger & Ruppert* (1966), by treating rats with a high dose of intramuscular iron, demonstrated a decrease in the mucosal uptake and a marked reduction in the transfer of iron 2 weeks after administration. *Obel* (1967) observed that the gastro-intestinal tract of the experimental animals treated with Iroject

contained large amounts of iron, which can be assumed to influence the absorption.

The results obtained indicate that the animals utilized the iron compound employed for synthesis of haemoglobin, myoglobin and other iron-containing compounds. To prevent the Hb values of the piglets from sinking below 11 g Hb per 100 ml blood at 4 weeks of age, an administration of 250—300 mg of iron is required. These results are in full agreement with earlier findings by *Venn et al.* Administration of up to 300 mg of iron in single doses might cause toxicity problems. Results from clinical studies, which will soon be published by *Brag & Thafvelin* (1968) show, however, that Iroject is well tolerated by such a high dosage.

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SUMMARY

In order to study some effects of a new haematinic for treatment of piglet anaemia, some basic studies on untreated animals have been performed. It has been shown that during the first weeks of their life, the piglets developed iron deficiency manifested in decreased haemoglobin and serum iron value and an increase of total and unsaturated iron-binding capacity of the serum. This increase was observed very soon after birth.

The haematinic used in the study contains a complex of iron, dextrin, sorbitol, citric and lactic acid. The preparation is stable be-

tween pH 3.2—8.0 and in serum, it is hypertonic, does not produce haemolysis and has an intraperitoneal toxicity in mice of 570 mg Fe per kg body weight.

Absorption from muscle takes place in two stages. Six hrs. after an intramuscular administration, 38.3 % of the administered dose is absorbed. Then a continuous slow absorption takes place and 6 days after administration 55.5 % has been removed from the injection site. After 8 weeks 97.0—99.3 % has been absorbed.

During the first hour after administration there is an increase of iron in the serum exceeding the total iron-binding capacity of serum without saturation of the iron-binding capacity of the serum. Three hrs. after injection the animals' iron-binding capacity is saturated, but at that moment the value for iron in serum is decreasing.

The utilization has been studied using two different dosages, namely 180 and 270 mg Fe per animal. It has been found that the lower dose increases the haemaglobin value for the animal during the first week after treatment, but after 2 weeks there will be a tendency towards a lower value. With the higher dosage there is an increase up to 3 weeks after administration. After that time there is a slight decrease during the following 2 weeks. Before detecting any decrease in the animals' haemoglobin value, a decrease in serum iron value and an increase in the total iron binding capacity is observed. Absorption, utilization and dosage in piglets is discussed.

ZUSAMMENFASSUNG

Studien an einem neuen intramuskulären Eisenpräparat gegen Ferkelanämie.

Um den Effekt eines neuen parenteralen Eisenpräparates gegen Eisenmangelanämie bei Ferkeln zu studieren, wurden einige grundlegende Untersuchungen mit nichtbehandelten Tieren vorgenommen. Es zeigte sich, dass die Versuchstiere im Laufe der ersten Lebewoche einen Eisenmangel entwickelten mit niedrigen Serum-Eisen- und Hämoglobinwerten und einer erhöhten totalen und ungesättigten eisenbindenden Kapazität im Serum. Diese Steigerung setzte sehr kurz nach der Geburt ein.

Das Eisenpräparat, das in den Versuchen benutzt wurde, enthält eine komplexe Verbindung von Eisen, Dextrin, Sorbitol samt Zitronen- und Milchsäure. Die Verbindung ist stabil bei pH zwischen 3,2 und 8,0 und in Serum, ist hypertonisch und verursacht nicht Hämolyse. Die intraperitoneale Toxizität an Mäusen ist 570 mg Fe pro kg Körpergewicht. Die Resorption von den Muskeln erfolgt in zwei Phasen. Sechs Stunden nach intramuskulärer Applikation sind 38,3 % der Dosis resorbiert. Danach findet eine langsame kontinuierliche Resorption statt, nach 6 Tagen sind 55,5 % und nach 8 Tagen 97,0—99,3 % von der Injektionsstelle verschwunden.

Bereits eine Stunde nach der Injektion des Eisens wurden hohe Serum-Eisen-Werte festgestellt. Diese überstiegen die totale eisenbindende Kapazität im Serum trotzdem die ungesättigte eisenbindende Kapazität nicht völlig ausgenutzt wurde. Drei Stunden nach der In-

jektion war die ungesättigte eisenbindende Kapazität der Tiere völlig ausgenutzt, jetzt war die Serum-Eisen-Konzentration jedoch abnehmend. Die Verwertung des Eisens wurde untersucht indem man zwei verschiedene Dosen, und zwar 180 bzw. 270 mg Fe pro Tier benutzte. Es zeigte sich, dass die kleinere Dosis den Hämoglobinwert während der ersten Woche erhöhte, jedoch schien schon 2 Wochen nach der Behandlung eine Tendenz zur Senkung einzutreten. Nach der grösseren Dosis erfolgte eine sukzessive Steigerung im Hämoglobingehalt bis 3 Wochen nach der Injektion, wonach ein leichter Rückgang während der folgenden 2 Wochen eintrat.

Bevor ein Rückgang im Hämoglobinwert der Tiere nachgewiesen werden konnte, wurde ein Rückgang im Serum-Eisen-Wert und eine Steigerung in der totalen eisenbindenden Kapazität festgestellt. Resorption, Verwertung und zweckmässige Dozierung werden diskutiert.

SAMMANFATTNING

Studier av ett nytt intramuskulärt järnpreparat mot smågrisanemi.

För att studera effekten av ett nytt parenteralt järnpreparat mot järnbristanemi hos smågrisar har vissa grundläggande undersökningar företagits av obehandlade djur. Det visades att försöksdjuren under första levnadsveckorna utvecklade en järnbrist med sänkta serumjärn- och hemoglobinvärden och ökad total och omättad järnbindande kapacitet i serum. Denna ökning inträdde mycket snart efter födelsen.

Det järnpreparat som använts i försöken innehåller en komplex förening av järn, dextrin, sorbitol samt citron- och mjölksyra. Föreningen är stabil vid pH mellan 3,2—8,0 och i serum, är hypertonisk och framkallar ej hemolys. Den intraperitoneala toxiciteten på möss är 570 mg Fe per kg kroppsvikt. Resorptionen från muskeln sker i två faser. Sex timmar efter intramuskulär applikation är 38,3 % av dosen resorberad. Därefter sker en långsam kontinuerlig resorption och efter sex dygn har 55,5 % och efter åtta dygn 97,0—99,3 % avlägsnats från injektionsstället.

Redan en timme efter administrering av järnet konstaterades höga serumjärnvärden överstigande den totala järnbindande kapaciteten i serum trots att den omättade järnbindande kapaciteten inte helt utnyttjats. Tre timmar efter injektion var djurens omättade järnbindande kapacitet helt utnyttjad, men vid det tillfället var serumjärnet sjunkande. Järnets utnyttjande har studerats genom att använda två olika doseringar, nämligen 180 resp. 270 mg Fe per djur. Det framkom att den lägre dosen höjde hemoglobinvärdet under den första veckan men redan två veckor efter behandlingen syntes en tendens till sänkning. Med den högre doseringen skedde en successiv höjning av hemoglobinhalten fram till tre veckor efter administreringen, varefter en lätt sänkning inträdde under de följande två veckorna.

Innan sänkning av djurens hemoglobinvärde kunde fastslås påvisades en sänkning av serumjärnvärdet och en ökning av den totala järnbindande kapaciteten. Resorption, utnyttjande och lämplig dosering diskuteras.

(Received November 20, 1967).