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THE DISTRIBUTION OF PARENTERAL IRON HAEMATINICS IN NONPREGNANT, PREGNANT AND LACTATING RATS

BY

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The distribution of ^{59}Fe has been investigated in nonpregnant, pregnant and lactating rats and their young after injection of solutions containing a high molecular weight iron-dextrin complex (iron-dextrin, intravenously), and a low molecular weight complex of iron, sorbitol and citric acid (iron-sorbitol, intramuscularly), each labelled with ^{59}Fe. At different times after injection of a dose corresponding to 1.5 mg of iron per kg of body weight, the quantity of ^{59}Fe was determined in different organs, urine and carcass, and for pregnant rats also in foetuses and placentas. In some investigations, distribution in the foetuses was also studied. Iron-dextrin was localized mostly in the livers of both pregnant and nonpregnant rats; ^{59}Fe was then redistributed from this organ and incorporated into the erythrocytes. In pregnant rats, redistribution was accompanied by a placental transfer, the degree of incorporation into the erythrocytes of the mother being diminished. About 30% of the iron-sorbitol was excreted in the urine, while the remainder was distributed throughout the whole organism. Incorporation into the erythrocytes and placental transfer began earlier with iron-sorbitol than with iron-dextrin. At 14 days from injection into nonpregnant rats, however, the degree of incorporation into the erythrocytes of iron from the two complexes was identical. The rate at which incorporation of ^{59}Fe occurred into the erythrocytes was the same for the two preparations. The degree of incorporation into the erythrocytes after injection of iron-sorbitol into pregnant rats diminished in the same pattern as for iron-dextrin. Investigations into the mechanism of the placental transfer of iron-sorbitol from mother to foetus suggested that this is essentially an active process.

The distribution of iron after intravenous administration of a colloidal solution of iron hydroxide to rabbits was studied by Polson (1928), who confirmed that the colloid was initially taken up mainly by the lungs, the Kupffer cells of the liver and the spleen. The colloid was present in the lungs as emboli, because the preparation flocculated when mixed with blood. From the lungs the iron was then transferred to the liver. Working with saccharated iron oxide solutions, Cappell showed in 1930 that, after intravenous injection into mice, this colloid was quickly absorbed by cells of the reticulo-endothelial system, and by certain blood leucocytes. The absorbed colloid was then metabolized, after which the iron was stored in the liver, spleen and lymph nodes. The rapid absorption of saccharated iron oxide in the reticulo-endothelial system was confirmed by experimental investigations carried out on animals by Nissim (1953), who used a standardized preparation of saccharated iron oxide suitable for clinical use in man. A colloidal solution of a high molecular weight iron-dextrin complex is also absorbed, according to Andersson (1950), by

the reticulo-endothelial system in rabbits, in this species particularly in the liver and spleen, after which a redistribution takes place. Pinniger & Hutt (1956), who studied the fate of saccharated iron oxide injected intravenously into rabbits, noted the same distribution as previous investigators. Parallel experiments with an iron-dextran complex showed a somewhat different distribution, however, this complex being absorbed almost immediately as stainable iron by the histiocytes in the connective tissues, while the liver cells contained iron granules before the presence of these could be established in the Kupffer cells. Goldeck & Remy (1953) investigated the distribution of saccharated iron oxide in pregnant rats after its injection at different times before parturition; no excess of histochemically detectable iron appeared in the young animals. The mother, however, had a considerably larger quantity of iron stores in the spleen and liver. The results were believed to indicate that there was no direct passage of the iron used from mother to foetus. On the other hand, Pribilla (1954) concluded that saccharated iron oxide can pass through the placental barrier in rabbits during the latter part of pregnancy and that it is then absorbed by the liver of the foetus, where it is transformed to physiological iron compounds. Regardless of the stage of pregnancy, however, most of the injected iron is stored in the mother's liver and spleen.

Wöhler (1955) observed an increased haemosiderin storage in the foetal liver after the injection into the mother of large quantities of saccharated iron oxide, although no direct passage of the injected iron complex was believed to have occurred. In studies with the same iron-dextrin complex as that used by Andersson (1950), Nylander (1954) noted that it was absorbed into the mother's reticulo-endothelial system after intravenous injection into pregnant rats. The complex was broken down there, and physiological protein-bound plasma iron in raised concentration passed through the placenta, probably resulting in an increased placental transfer of iron dependent on the foetal need.

A low molecular weight iron preparation, iron-sorbitol, contains an iron-sorbitol-citric acid complex stabilized with dextrin (Lindvall & Andersson, 1961). The molecular weight of the iron complex does not exceed 5,000 (Eriksson, unpublished). The distribution of this preparation in the organism after intramuscular injection into nonpregnant, pregnant and lactating rats has been studied, and a comparison has been made with the high molecular weight iron-dextrin preparation used by Andersson (1950) and Nylander (1954). The average molecular weight of the iron complex in the latter preparation, iron-dextrin, is about 230,000 (Eriksson, unpublished).

METHODS

Properties of the iron preparations

Iron-sorbitol contains a complex corresponding to 50 mg of iron per ml. The solution was sterilized by autoclaving and had a *p*H of 7.5 ± 0.2. The specific activity of the preparation was 50 μC/ml.

Iron-dextrin contains a complex corresponding to 20 mg of iron per ml. The solution was sterilized by autoclaving and had a *p*H of 7.6 ± 0.3. The specific activity of the preparation was 20 μC/ml.

Labelling the iron-sorbitol and iron-dextrin complexes with ^{59}Fe was done on a laboratory scale. The identity of the preparations thus obtained with the commercial products Jectofer and Astrafer, respectively, was investigated. The labelled iron-sorbitol was checked by electrophoresis and gel-filtration, and the labelled iron-dextrin by ultracentrifugation, and both had properties identical with those of the commercial products. With iron-sorbitol, it was also shown that the ^{59}Fe was distributed, with the same specific activity per total quantity of iron, in all the fractions of the preparation obtained in electrophoresis and gel-filtration.

Rats

Not previously paired, albino female rats of Sprague-Dawley stock, with haemoglobin concentrations of 13.0 to 14.2 g/100 ml. of blood, and haematocrits of 45%, were used. Their weight ranged from 200 to 250 g. Females and males were placed in the same cage for 24 hr. The duration of pregnancy was calculated from the time after the animals had been in the same cage for 12 hr until they were killed. As a rule the pregnant rats had eight to twelve foetuses at post-mortem.

Dosage and administration of iron preparations

The dose was 1.5 mg of iron per kg of body weight. Iron-sorbitol was diluted before injection with 0.9% saline to a concentration of 5 mg/ml. of iron. Injections were given intramuscularly into the right thigh. Iron-dextrin was diluted with 0.9% saline to a concentration of 0.5 mg/ml. of iron, and the injections were given intravenously into the caudal vein.

Injections were given to nonpregnant rats at different times before they were killed. Pregnant rats received the injections at different times before death at the 19th day of pregnancy. Pregnant rats at 13 to 20 days of gestation received iron-sorbitol and iron-dextrin 3 and 24 hr, respectively, before they were killed.

Experimental procedures

Bilateral section of the carotid arteries was performed in rats anaesthetized with ether. The animals were bled to death and part of the blood was collected in heparinized tubes. After centrifugation of the blood, radioactivity in serum and in blood corpuscles was determined. To determine the ^{59}Fe content of different tissues, livers as well as all foetuses and placentas were homogenized in distilled water using an MSE homogenizer. Musculature at the site of injection, the gastrointestinal canal and its contents, the spleen, femur, sternum and the eviscerated and exsanguinated body were separately treated with hot concentrated sulphuric acid so that homogeneous masses were obtained. Kidneys, lungs, heart and brain were separately pulped in water with a glass rod so that 3 ml. of homogenate were obtained. Radioactivity was determined in aliquots of the samples.

Urine excreted by the animals after injection of a radioactive preparation was collected on filter paper, which was then burned and the radioactivity of the ash was determined.

Radioactivity determinations

The ^{59}Fe activity, in 3 ml. samples, was measured in a Baird Atomic, Single-Channel Pulse Height Analyser with a 1.75×2 in. thallium-activated sodium well-crystal shielded with 2 in. of lead. The background was stable, and about 500 counts/min were made, with an efficiency of about 25%. The results are given (as percentages) of all activity still found in the organs in proportion to the total quantity administered.

Determination of blood volumes of unmated female rats

Erythrocytes were labelled with ^{59}Fe by treating rats with [^{59}Fe]-sorbitol. At 72 hr after treatment, heparinized blood was obtained by cardiopuncture from the animals, which were first anaesthetized with ether. After centrifugation, the erythrocytes were washed twice with 0.9% saline. A known quantity of these blood corpuscles was injected intravenously into other rats, and 10 min later the animals were killed, the activity of the whole blood was determined, and the total volume of blood was calculated.

Placental transfer of iron-sorbitol

The procedure of Bothwell, Prebilla, Mebust & Finch (1958) was used. The animals were anaesthetized with ether and 0.1 ml. of 25% of chlorbutol per 100 g of body weight. The umbilical cords of half the foetuses were ligated and these foetuses were removed carefully so as not to damage the placenta. Incisions in the uterus and abdomen were then stitched, after which [^{59}Fe]-sorbitol was administered intramuscularly to the mother. The mothers were kept anaesthetized and were killed 3 hr later. After homogenization of the foetuses remaining in each animal, as well as of the placentas with normal foetal circulation and those removed from the foetuses (without foetal circulation), activity in each homogenate was determined and activity per organ calculated.

Effects on sucklings of iron-sorbitol administered to the mother

At 24 hr after parturition, lactating mothers were injected intramuscularly with labelled iron-sorbitol. Half the young animals in each litter were destroyed 24 hr, and the remainder 6 days, after injection. The young animals were anaesthetized with ether and thoroughly exsanguinated after section of both carotid arteries. Blood was retained for analysis. Intestine, liver, spleen and carcass from each litter and at each period were homogenized. Activity was determined in aliquots of the homogenates and activity was calculated per young animal.

RESULTS

Non-pregnant rats

Absorption of iron-sorbitol, which had been administered to sixty-five animals at different times before they were killed, took place very rapidly. As shown in Table 1, 80 to 85% of the administered iron had already left the site of injection

TABLE 1

QUANTITY OF ^{59}Fe REMAINING AT THE SITE OF INJECTION AT DIFFERENT TIMES AFTER INTRAMUSCULAR INJECTION OF LABELLED IRON-SORBITOL INTO RATS

Mean values are percentages of doses administered

	^{59}Fe (%) at time after injection							
	3 hr	6 hr	1 day	2 days	3 days	4 days	7 days	14 days
Mean value	17·3	16·5	12·8	12·5	12·1	8·9	6·9	4·9
Standard deviation	±9·1	±4·3	±6·8	±5·5	±5·6	±5·2	±2·1	±1·9
Number of animals	9	9	12	7	11	4	6	7

3 hr after administration. Absorption of the remaining iron thereafter took place slowly and 4.9% of the injected dose remained in the muscle 14 days after administration. The rapid absorption is shown by the high content of ^{59}Fe in the serum. Based on a blood volume (mean and standard deviation) determined at 4.75± 0.26 ml./100 g of body weight, the serum content represents 4.6 and 3.1% of the quantity of [^{59}Fe]-sorbitol administered, 3 and 6 hr respectively, after the injection (Fig. 1). At 24 and 96 hr after administration the values were respectively 0.3 and 0.2% which were also present after 14 days. Iron-dextrin administered to twenty-four animals at different times before they were killed was rapidly removed from the serum. Altogether, the serum contained only 1% of the dose of radioactivity 3 and 6 hr after injection. This quantity then fell, so that the serum content 24 hr after administration was 0.6%. Radioactivity in the serum then diminished more slowly than in animals treated with iron-sorbitol and did not reach a value of 0.2% until after 7 days.

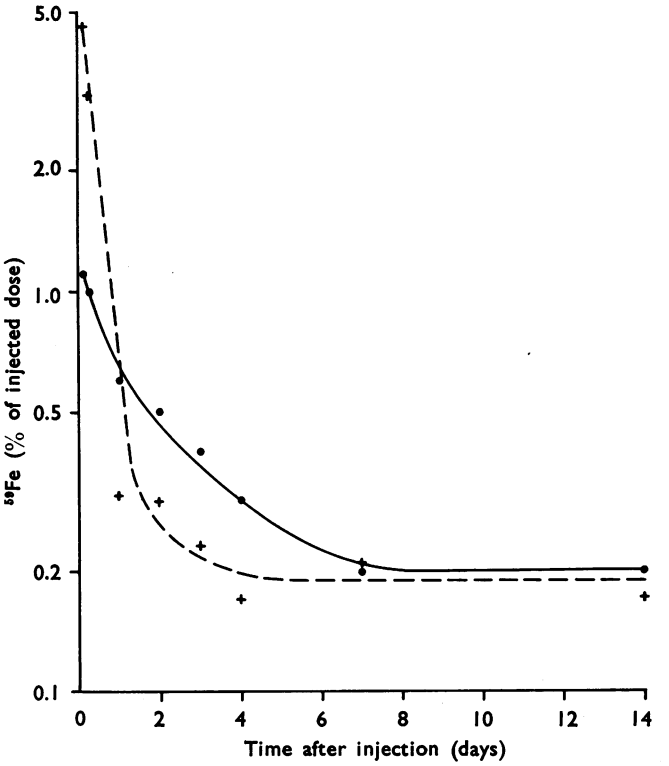

Fig. 1. Quantity of ^{59}Fe found in serum after administration of labelled iron–dextrin and labelled iron–sorbitol to rats at different times before death, given in percentages of the dose administered. ●, iron–dextrin; +, iron–sorbitol.

The high molecular weight iron-dextrin was taken up particularly in the liver; 65 to 70% of the injected dose of radioactivity was found in this organ 3 and 6 hr after administration (Fig. 2, *a*). At the same time, only some 8% of ^{59}Fe had been absorbed by the liver after injection of labelled iron-sorbitol (Fig. 2, *b*). The ^{59}Fe from iron-dextrin initially absorbed by the liver was then redistributed. The content of ^{59}Fe in the liver fell from about 65% 24 hr after administration to about 47% after 96 hr. After this period the quantity of ^{59}Fe in the liver seemed to be constant for the remainder of the observation period. On the other hand, although the quantity of iron in the liver also increased during the first few hours after injection of iron-sorbitol, only about 16% was found in this organ 72 and 96 hr after administration.

Iron-dextrin was also absorbed to an appreciable extent in the spleen (Table 2) while iron sorbitol was only slightly taken up by this organ.

Iron-sorbitol was also stored in the gastro-intestinal canal and its contents. At 6 hr after injection, 4.6% was still found here and this quantity then continued to decrease. After the same period 2.7% of the ^{59}Fe from iron-dextrin was found, and this quantity then diminished.

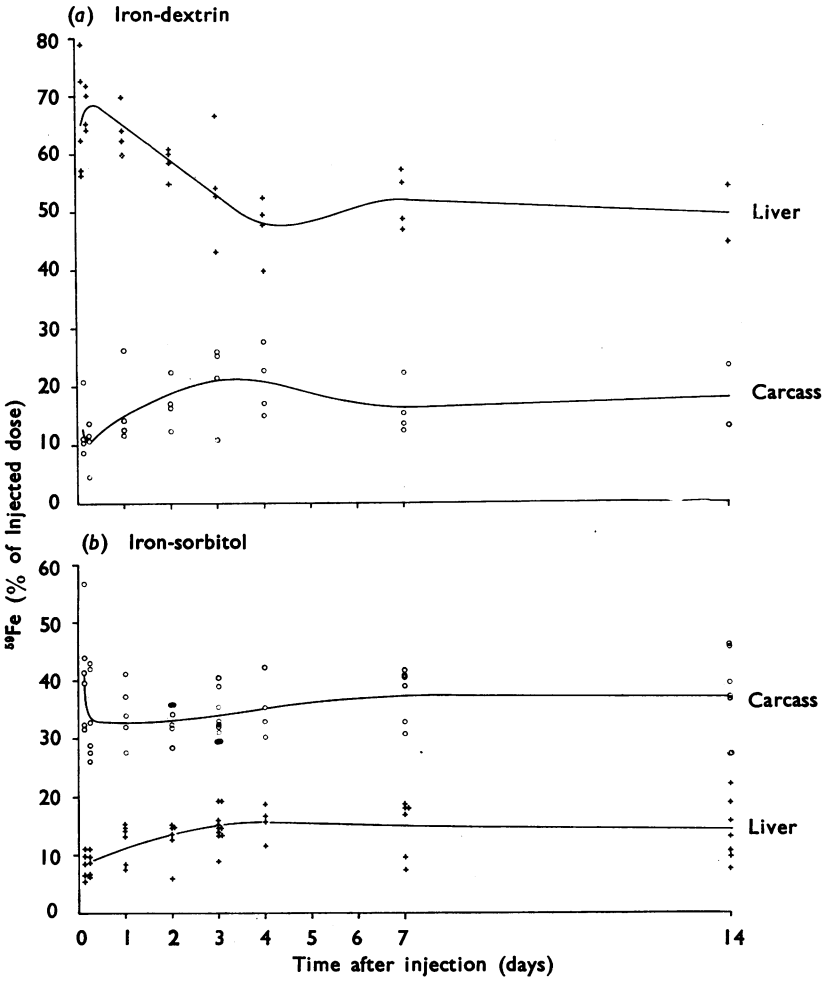

Fig. 2. Quantity of ^{59}Fe found in liver and carcass after intravenous injection of iron–dextrin (*a*) and intramuscular injection of iron–sorbitol (*b*) into rats at different times before death, given in percentages of the dose administered.

Estimation of iron excreted in the urine showed that about 30% of an injected dose of iron-sorbitol was excreted during the first 24 hr after which there was only an insignificant excretion. The kidneys themselves contained 3.5% of the administered dose 24 hr after injection and, as shown in Table 2, the quantity of ^{59}Fe in the kidneys fell only slightly. After injection of iron-dextrin only a small quantity of radioactivity was retained in these organs. Excretion in the urine was also small, 0.8% during the first 24 hr after injection. Less than 1% of the dose administered was found in other organs, for both iron preparations (Table 2).

Most of the iron-sorbitol appeared in the carcass (Fig. 2, *b*). At 3 hr after administration, 41% of the injected dose was found here, and 32% at 24 hr, after which there was a small increase. For iron-dextrin (Fig. 2, *a*) only some 10% of the injected

TABLE 2

DISTRIBUTION OF ^{59}Fe AFTER INJECTION OF LABELLED IRON-DEXTRIN AND LABELLED IRON–SORBITOL INTO RATS AT DIFFERENT TIMES BEFORE DEATH

Values are percentages of the dose administered. In the experiments with iron-dextrin, four rats were used at each time; in the experiments with iron–sorbitol, four to eleven rats were used

Compound	Organ	^{59}Fe (%) at time after injection							
		3 hr	6 hr	1 day	2 days	3 days	4 days	7 days	14 days
Iron-dextrin	Spleen	4·9	6·5	4·6	5·1	4·6	4·2	4·2	3·4
	Intestine	2·6	2·7	2·7	2·0	2·7	1·9	1·7	2·0
	Kidneys	0·5	0·5	0·5	0·6	0·7	0·7	0·8	0·9
	Lungs	0·3	0·3	0·3	0·3	0·4	0·4	0·5	0·6
	Femur	0·3	0·5	0·6	0·6	0·6	0·5	0·4	0·3
	Sternum	0·2	0·2	0·3	0·3	0·2	0·2	0·2	0·1
Iron-sorbitol	Spleen	0·3	0·5	0·7	0·5	0·4	0·4	0·5	0·5
	Intestine	3·2	4·6	4·4	4·3	3·4	3·0	2·9	2·6
	Kidneys	2·7	3·5	3·5	3·1	3·6	3·8	3·6	2·6
	Lungs	0·5	0·5	0·3	0·5	0·6	0·5	0·6	0·6
	Femur	0·3	0·5	0·7	0·5	0·4	0·3	0·3	0·3
	Sternum	0·2	0·3	0·3	0·2	0·2	0·1	0·2	0·2

dose was found in the carcass 3 and 6 hr after injection, and at 72 and 96 hr this has increased to about 21% and there was a small fall subsequently.

As shown in Fig. 3, incorporation of iron from the two complexes into the erythrocytes took place rather similarly, rapidly during the first 2 to 3 days and much more slowly subsequently. In the initial stage, however, iron-sorbitol was taken up more quickly than iron-dextrin.

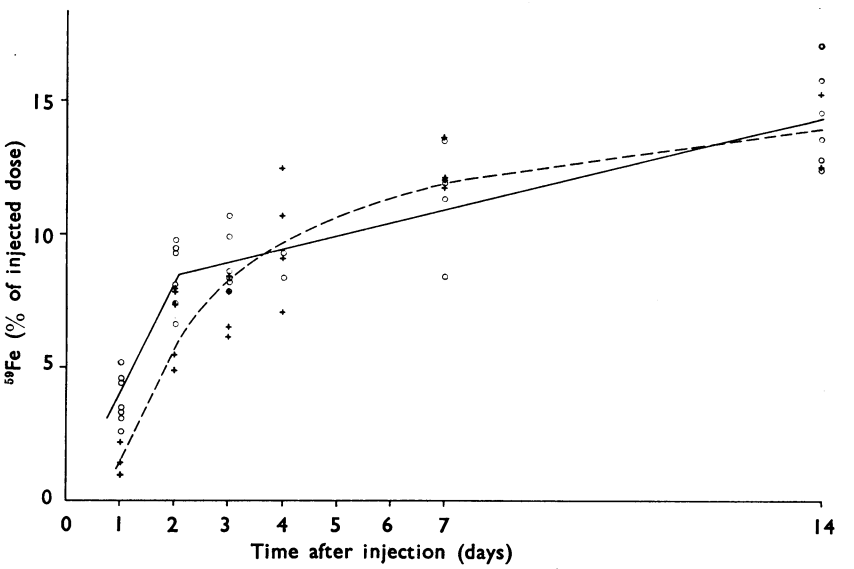

Fig. 3. Quantity of ^{59}Fe found in erythrocytes after injection of iron–dextrin and iron–sorbitol into rats at different times before death, given in percentages of the dose administered. +, iron–dextrin; ○, iron–sorbitol.

Pregnant rats

Fifty rats on the 19th day of pregnancy were used. As Table 3 shows, 1.12% of a dose of iron-sorbitol was already in each foetus 3 hr after administration. By that time, 0.16% of the ^{59}Fe in iron-dextrin had passed the placental barrier. With longer intervals the transfer of ^{59}Fe in iron-sorbitol reached a maximum at 24 hr, after which it decreased. The quantity of iron from iron-dextrin passing the placental barrier reached a maximum at 48 hr. With iron-sorbitol a rather higher activity than with iron-dextrin was noted in the placenta 3 hr after administration, 0.19 and 0.12% respectively (Table 3). After injection of iron-sorbitol, the maximal quantity

TABLE 3

QUANTITY OF ^{59}Fe FOUND IN EACH FOETUS AND PLACENTA AFTER ADMINISTRATION OF LABELLED IRON-DEXTRIN AND LABELLED IRON-SORBITOL TO PREGNANT RATS AT DIFFERENT TIMES BEFORE THE 19TH DAY OF PREGNANCY

The quantity of ^{59}Fe is given in percentages of the dose administered to the mother. Values in parentheses refer to the numbers of rats used

Compound	Organ	^{59}Fe (%) at time after injection						
		3 hr	6 hr	16 hr	1 day	2 days	3 days	4 days
Iron-dextrin	Foetus	0·16 (2)	0·54 (3)	1·22 (2)	1·35 (7)	1·85 (3)	1·77 (2)	— —
	Placenta	0·12	0·18	0·27	0·32	0·44	0·65	—
Iron-sorbitol	Foetus	1·12 (10)	1·11 (3)	1·30 (3)	1·37 (4)	1·17 (4)	1·08 (3)	0·93 (4)
	Placenta	0·19	0·19	0·25	0·37	0·36	0·27	0·28

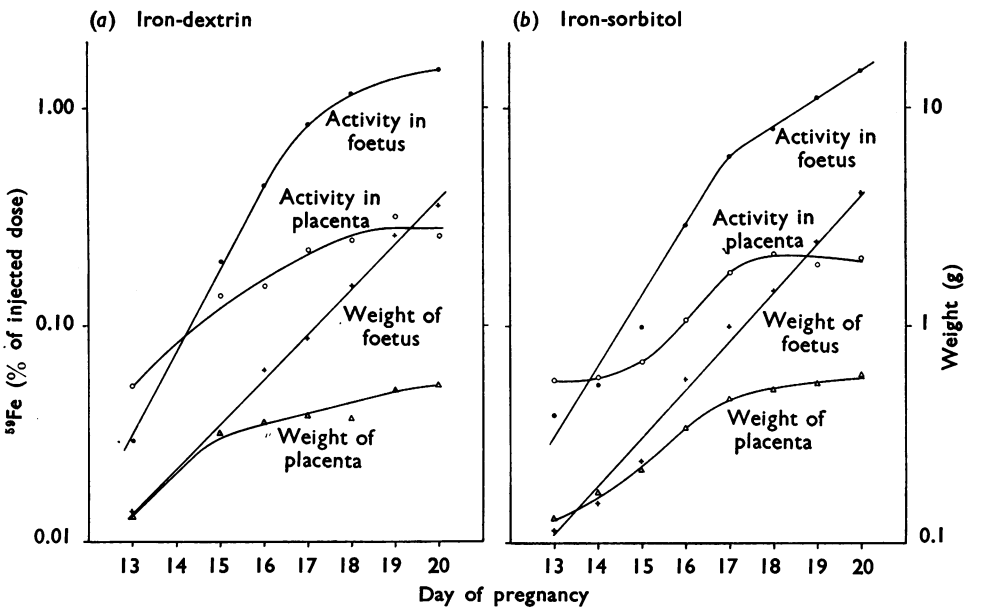

Fig. 4. Quantity of ^{59}Fe found in foetus and placenta after injection of iron–dextrin (*a*) and iron–sorbitol (*b*) into pregnant rats, given in percentages of the dose administered (left-hand ordinates, log scale). Iron–dextrin was administered 24 hr, and iron–sorbitol 3 hr, before death. Foetal and placental weights are given (right-hand ordinates, log scale.)

(0.37%) in the placenta occurred 24 hr after administration. Maximal activity after iron-dextrin occurred at 72 hr when 0.65% of the dose was found in each placenta.

For studying the passage of the two iron preparations from mother to foetus in pregnancies of between 13 and 20 days, eighty-one rats were used. As shown in Fig. 4, *a* and *b*, the passage of radioactivity from the two iron preparations through placentas to foetuses was related to the weight of the foetuses. The transfer became considerable after 15 days of gestation, when the weight of the foetuses was increasing rapidly. From this time until the 18th day of pregnancy, there was a relationship between the growth of the foetuses and their absorption of iron. During the last few days of gestation, the weight of the foetuses continued to increase, while the placental transfer of iron showed only a little rise. Fig. 4 also shows that the weight of the placenta was related to the amount of ^{59}Fe in this organ.

The qualitative distribution of the two iron preparations in rats an the 19th day of pregnancy was the same as in the nonpregnant animals. The considerable transfer of ^{59}Fe to the foetuses became evident in the mothers, however. As Table 4 shows,

TABLE 4

QUANTITY OF ^{59}Fe IN 3 ML. OF SERUM AFTER ADMINISTRATION OF LABELLED IRON-DEXTRIN AND LABELLED IRON-SORBITOL AT DIFFERENT TIMES BEFORE DEATH OF RATS

The quantity of ^{59}Fe is given in percentages of the administered dose. Values in parentheses refer to the numbers of rats used

Compound	Rats	^{59}Fe (%) at time after injection					
		3 hr	6 hr	1 day	2 days	3 days	4 days
Iron-dextrin	Nonpregnant	0·58 (4)	0·54 (4)	0·30 (4)	0·23 (4)	0·19 (4)	0·13 (4)
	19 days pregnant	0·17 (2)	0·12 (3)	0·12 (7)	0·10 (3)	0·09 (2)	— —
Iron-sorbitol	Nonpregnant	2·51 (6)	1·60 (6)	0·17 (7)	0·16 (7)	0·13 (11)	0·09 (4)
	19 days pregnant	1·10 (10)	0·31 (3)	0·06 (4)	0·04 (4)	0·07 (3)	0·04 (4)

the radioactive iron disappeared much more rapidly from the serum of pregnant animals treated with both iron-dextrin and iron-sorbitol than it did from the serum of the nonpregnant animals. At 3 hr after injection of iron-dextrin or iron-sorbitol, the iron content in the serum of pregnant animals was one-third of that of virgin rats. At 24 hr after administration, serum from the pregnant animals showed only slight radioactivity, which later fell only insignificantly. The foetus's need of iron is shown by different organs in the mother. Most of the values for the radioactive content of the liver, spleen, kidneys and intestine in pregnant rats were lower than the corresponding values for virgin animals (Tables 5 and 2, Fig. 2). An exception, however, was the liver and spleen of the animals treated with iron-dextrin, in which the content of ^{59}Fe in pregnant animals 3 and 6 hr after administration was the same as in nonpregnant animals. At 24 hr and more after injection of the high molecular weight complex, however, values in these organs in the pregnant animals were lower than in the corresponding virgins.

TABLE 5

DISTRIBUTION OF ^{59}Fe AFTER INJECTION OF LABELLED IRON–DEXTRIN AND LABELLED IRON–SORBITOL INTO PREGNANT RATS AT DIFFERENT TIMES BEFORE THE 19TH DAY OF PREGNANCY

Values are percentages of the administered dose. In the experiment with iron–dextrin, two to seven rats were used at each time, and in the experiment with iron–sorbitol three to ten rats

Compound	Organ	^{59}Fe (%) at time after injection					
		3 hr	6 hr	1 day	2 days	3 days	4 days
Iron–dextrin	Liver	67·6	63·6	58·4	44·8	41·0	—
	Spleen	6·5	3·2	4·1	2·2	3·0	—
	Kidney	0·24	0·27	0·29	0·34	0·46	—
	Intestine	1·0	1·6	2·3	3·3	3·5	—
	Carcass	5·6	6·7	15·6	18·5	21·9	—
Iron–sorbitol	Liver	3·8	4·4	4·3	5·7	5·9	6·7
	Spleen	0·43	0·64	0·36	0·25	0·28	0·26
	Kidney	2·3	2·1	2·0	2·1	1·9	2·2
	Intestine	2·3	2·2	1·4	1·5	1·6	1·5
	Carcass	29·3	33·2	15·5	24·5	22·3	16·8

TABLE 6

QUANTITY OF ^{59}Fe FOUND IN 3 ML. OF PACKED ERYTHROCYTES AFTER ADMINISTRATION OF LABELLED IRON–DEXTRIN AND LABELLED IRON–SORBITOL AT DIFFERENT TIMES BEFORE DEATH OF RATS

The quantities of ^{59}Fe are given in percentages of the dose administered. Values in parentheses refer to the numbers of rats used

Compound	Animals	^{59}Fe (%) at time after injection					
		3 hr	6 hr	1 day	2 days	3 days	4 days
Iron–dextrin	Nonpregnant	0·21 (4)	0·56 (4)	0·99 (4)	4·07 (4)	4·59 (4)	6·08 (4)
	19 days pregnant	0·23 (2)	0·19 (3)	1·12 (7)	2·62 (3)	2·97 (2)	—
Iron–sorbitol	Nonpregnant	0·57 (6)	0·71 (6)	2·48 (7)	5·63 (7)	5·65 (11)	6·40 (4)
	19 days pregnant	0·51 (10)	0·51 (3)	1·65 (4)	3·82 (4)	3·29 (3)	3·12 (4)

Table 6 shows that incorporation of the added iron complex into the erythrocytes was also different in pregnant and nonpregnant rats. This became evident 48 hr after administration of iron-dextrin, when the quantity of ^{59}Fe incorporated into the erythrocytes of the pregnant animals was about 65% of that incorporated into the blood cells of the virgin rats. With iron-sorbitol, a similar difference was noticeable as early as 24 hr after administration.

Iron-sorbitol in foetal tissues

Localization of radioactivity in foetal tissues after 19 days of gestation was studied for iron-sorbitol only. Nine pregnant animals were injected at different times before the samples were taken. Table 7 shows the distribution in liver, blood and carcass of the foetus. As can be seen, 3 hr after injection 14.1% of the foetal activity was in the blood (and practically all of this was in the erythrocytes), 44% in the liver, and 42% in the carcass. At 72 hr, 31% of the activity was in the blood, while the decrease in activity was somewhat more pronounced in the liver than

TABLE 7

DISTRIBUTION OF ^{59}Fe TRANSFERRED TO FOETUS AFTER INJECTION OF LABELLED IRON–SORBITOL INTO PREGNANT RATS AT DIFFERENT TIMES BEFORE THE 19TH DAY OF PREGNANCY

Foetuses from five females were used for determinations 3 hr after the injection, and foetuses from two females at the other times. Values are ranges of percentages of the dose injected

	^{59}Fe (%) at time after injection		
Organ	3 hr	24 hr	72 hr
Liver	43·9	26·9–32·0	31·2–33·7
Blood	14·1	20·3–24·9	29·3–32·0
Carcass	41·9	47·8–48·1	34·2–39·4

in the carcass. Table 8 shows that the rate of iron incorporation was considerably higher into the foetal erythrocytes than into the maternal ones. Activity was more than ten-times higher 24 hr after the injection, and 7.4% of the injected iron was found in 3 ml. of packed foetal cells, compared with 0.5% in 3 ml. of the maternal erythrocytes. Except for the most active phase of absorption after intramuscular injection, the activity was higher in the foetal than in the maternal serum.

TABLE 8

QUANTITY OF ^{59}Fe IN 3 ML. OF PACKED ERYTHROCYTES AND 3 ML. OF SERUM FROM MOTHER AND FOETUS AFTER INJECTION OF LABELLED IRON–SORBITOL INTO PREGNANT RATS AT DIFFERENT TIMES BEFORE THE 19TH DAY OF PREGNANCY

Values are percentages of administered dose. Four rats and their foetuses were used for determinations 3 hr after injection (mean values); two rats and their foetuses were used at other times

Time after injection (hr)	^{59}Fe (%) in			
	Mother		Foetus	
	Erythrocytes	Serum	Erythrocytes	Serum
3	0·19	0·33	2·35	0·06
24	0·46, 0·55	0·02, 0·01	7·27, 7·51	0·30, 0·32
72	1·53, 1·61	0·02, 0·02	7·04, 6·18	0·14, 0·08

The placental transfer of iron-sorbitol

The foetus and placenta with normal foetal circulation from the operated animals contained somewhat less ^{59}Fe than corresponding organs from the animals referred to in Fig. 4, *b* (Table 9). The distribution between placenta and foetus of the total quantity of ^{59}Fe in the intact foetus-placenta unit was, however, more or less the same as in the animals which had not been operated on. The lower content of ^{59}Fe in the foetus and placenta of the operated animals was probably due to absorption from the site of injection, being lower in anaesthetized than in unanaesthetized rats. The placenta without foetal circulation contained two- to five-times more ^{59}Fe from iron-sorbitol than did that with normal foetal circulation (Table 9), which suggests that the placenta separated from the foetus can store iron from the iron-sorbitol administered.

Effect on sucklings of iron-sorbitol administered to the mother

Five lactating mothers with ten to twelve young per litter were used. It was confirmed that 0.98% of the iron-sorbitol given to the mother had been transferred

TABLE 9

QUANTITY OF ^{59}Fe IN PLACENTAS WITHOUT FOETAL CIRCULATION, PLACENTAS WITH FOETAL CIRCULATION AND FOETUSES AFTER INJECTION OF LABELLED IRON–SORBITOL INTO PREGNANT RATS

Values are percentages of doses administered to the mother. Placenta I: without foetal circulation; placenta II: with normal foetal circulation

Day of pregnancy	Organ	^{59}Fe (%)	
18	Placenta I	0·38	
	Placenta II	0·18	0·74
	Foetus	0·56	
19	Placenta I	0·68	
	Placenta II	0·12	0·86
	Foetus	0·74	
20	Placenta I	0·71	
	Placenta II	0·15	1·07
	Foetus	0·92	
20	Placenta I	0·29	
	Placenta II	0·12	0·57
	Foetus	0·45	

to each of the young animals within 24 hr after administration. At 6 days after injection, the quantity of ^{59}Fe transferred to the young animals had increased to 1.9% per animal. The distribution of the ^{59}Fe from iron-sorbitol transferred to the young animals is shown in Table 10. The quantity of iron in the intestine and its contents were 36.8 and 11.6% respectively after 24 hr and 6 days, and in the blood and carcass after 24 hr were 21.2 and 32.6% respectively, with considerable increase during the next few days. The radioactive contents of liver and spleen were low compared with that in the remaining organs.

At 6 days after administration of iron-sorbitol, the distribution of ^{59}Fe in the lactating mother was very similar to that in normal animals, except for the carcasses in which the content was 20% lower. This value agrees quite well with that (20.9%) found in a litter of young.

TABLE 10

DISTRIBUTION OF ^{59}Fe TRANSFERRED TO SUCKLINGS AFTER INJECTION OF LABELLED IRON–SORBITOL INTO FIVE LACTATING MOTHER RATS

Values are percentages of administered dose

Time after treatment (days)	Transferred ^{59}Fe (%) in				
	Blood	Intestine	Liver	Spleen	Carcass
1	21·3	36·8	7·4	1·9	32·6
6	33·9	11·6	4·2	1·8	48·6

DISCUSSION

Incorporation into erythrocytes of ^{59}Fe from labelled iron-sorbitol begins earlier than with iron-dextrin, despite the less direct method of administration (intramuscular compared to intravenous). This difference is due to the fact that higher serum iron levels are maintained for longer periods (Fig. 1). According to Lindvall & Andersson (1961), there is a temporary saturation of the iron-binding capacity of the serum immediately after an intramuscular injection of iron-sorbitol corresponding to 1.5 mg/kg of body weight. Of the large quantity of ^{59}Fe found in the

serum shortly after the injection some may therefore be assumed to consist of transferrin-bound iron, which is immediately accessible for haematopoiesis. During the time when the animals treated with iron-sorbitol have a high content of ^{59}Fe in the serum, there is also a considerable incorporation of ^{59}Fe into the erythrocytes. At 2 days after administration, the high content of ^{59}Fe has fallen and at the same time the initial rapid incorporation of ^{59}Fe into the erythrocytes has begun to fall off. A slower phase of incorporation of iron from iron-sorbitol then begins. Initially, labelled iron-dextrin is removed from the serum much more quickly than is iron-sorbitol. The content of ^{59}Fe in the serum is, however, higher 2 days after administration of iron-dextrin than of iron-sorbitol ; at this time incorporation of iron from iron-dextrin into the erythrocytes is also considerable. The first, rapid phase of incorporation ends about 3 days after injection, and a slower rate then begins. In spite of the difference in character between the two preparations, incorporation of radioactivity from them into the erythrocytes takes place at the same rate both during the first, rapid phase, and during the second, slower one. The somewhat delayed incorporation of ^{59}Fe from iron-dextrin compared with iron-sorbitol into the erythrocytes is due to the fact that iron-dextrin is absorbed chiefly by the liver and spleen, a fact which Andersson noted in 1950. The present investigation shows that the ^{59}Fe stored in the liver is redistributed during the period when rapid incorporation into the erythrocytes takes place. This result indicates that the high molecular weight iron-dextrin, which was absorbed in the liver, is metabolized there and then transferred by transferrin to the haematopoietic organs for incorporation into the erythrocytes. Cappell (1930) and Nissim (1953) believe that the same process applies to saccharated iron oxide.

The transfer of iron-dextrin iron from mother to foetus in pregnant rats also takes place after some delay. This result confirms the occurrence of an initial storage of the preparation in reticulo-endothelial cells where metabolism takes place with the iron being later redistributed in a physiological form. There is thus no direct transfer of the high molecular weight iron-dextrin preparation. This conclusion agrees with those of Goldeck & Remy (1953) with regard to saccharated iron oxide, and of Nylander (1954) for the placental transfer of iron-dextrin.

Transfer of ^{59}Fe from iron-sorbitol, on the other hand, starts immediately after its administration. This immediate transfer can be due to two mechanisms: either the active transfer shown by Wöhler (1955, 1957, 1959), or simple diffusion such as Pribilla (1954) concludes for saccharated iron oxide. The results of our investigations, by analogy with those of Bothwell *et al.* (1958), indicate that transfer of iron from iron-sorbitol from mother to foetus can be regarded as mainly an active process, in accordance with Wöhler's views. The similarity between the storage after injection of iron-sorbitol in the placentas from which foetuses have been removed, and the results of Bothwell *et al.* (1958) after injection of transferrin-bound iron, support this conclusion. Moreover, there is a striking resemblance between the distribution of ^{59}Fe in the foetus after injection of iron-sorbitol and the distribution in the foetus of transferrin-bound iron administered to the mother (Bothwell, *et al.*, 1958).

Our investigations have also shown that, at the time when absorption from the site of injection is extremely rapid and when there is a high content of ^{59}Fe in the

mother's serum, only minor activity is present in the serum of the foetus. When, however, most of the absorption is over and the content of ^{59}Fe in the mother's serum is low, the serum of the foetus has a high content. These facts also suggest that the mechanism for the transfer of iron in iron-sorbitol from mother to foetus is an active process.

It appears that, with the injection of iron-dextrin and iron-sorbitol in the same doses, more iron from the former than from the latter preparation passes the placental barrier, in spite of a slower rate of transfer. If it is borne in mind that about 30% of the iron from iron-sorbitol is excreted by the kidneys, 1.93% of the iron remaining in the rat has been transferred to each foetus, and this value may be compared with the 1.85% for iron-dextrin.

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