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Effect of total dose infusion of iron dextran on the storage iron content of the human placenta

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SUMMARY In a series of pregnant women with iron deficiency anaemia treated by a total dose infusion of iron dextran, the non-haem iron content of the placenta at term was studied histochemically and by chemical analysis. Within a few days of the infusion the Prussian blue reaction on the placenta was very strong, but was negative by ten days after the infusion. Chemical analysis showed that both the water-insoluble fraction (haemosiderin) and the water-soluble fraction (ferritin) of the non-haem iron were increased soon after the infusion, but three weeks after the infusion they were almost the same as in untreated controls. Pinocytosis of iron dextran by the trophoblast and increased transport of transferrin-bound iron to the placenta are considered as possible causes for this large uptake of iron by the placenta.

The histochemical examination of a placenta obtained from a stillbirth which occurred three days after the mother had received a total dose infusion of iron dextran, at 32 wk gestation, revealed very large amounts of haemosiderin in the trophoblast. This is in contrast to the findings of Pathak *et al*[1] who concluded from a study of a large series of iron-deficient patients treated with total dose infusion of iron dextran that there was no resulting increase of stainable iron in the placenta. There are two features of the stillborn baby which may have contributed to the accumulation of an unusually large amount of haemosiderin in the placenta. First, the foetus was macerated, so that transfer of iron to the foetus must have ceased soon after the infusion, and secondly, the gestational age of the placenta was only 32 wk when it was examined. Further studies were therefore made to assess in more detail the effect on placental iron of infusions of iron dextran given at different stages of pregnancy. In addition to histochemical examination of the placenta, the two forms of storage iron, haemosiderin and ferritin, were estimated respectively as the water-insoluble and water-soluble fractions of the non-haem iron.[2]

Material and methods

METHOD OF STUDY

A formal experimental approach was precluded by ethical considerations. Observations were made therefore on placentas obtained during the course of normal clinical management of obstetric patients. The decision to administer iron dextran (Imferon) to anaemic patients was made by the obstetrician in charge of the case according to his usual criteria. It follows that placentas were obtained in an uncontrolled manner at various intervals after total dose infusion and after various doses of iron dextran. The latter was infused over six hours in either 5% dextrose or normal saline.

Control placentas were obtained from women delivered at full term who had not been anaemic during pregnancy and had not received parenteral iron.

EXAMINATION OF THE PLACENTA

A small piece of the placenta taken from the periphery, was fixed in 10% formol saline, and sections were stained for iron by the method of Perls.[3]

Non-haem iron

For the estimation of non-haem iron the amnion and chorion were removed from the rest of the placenta which was then blotted dry between layers of filter paper and the whole of it was homogenised. The homogenate was again blotted dry to remove released blood and a weighed aliquot was shaken for 15 min with water, which had been deionised and then glass-distilled, to extract the water-soluble iron. After centrifugation the supernatant was removed for estimation of its iron content and the deposit was extracted once more with water. The

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pooled extracts were heated with hydrochloric acid and the iron content estimated with 1,10-phenanthroline.[2]

The residue from the above procedure was ground with powdered silica which had been washed with hot hydrochloric acid to remove iron. To extract the water-insoluble iron a solution of saturated sodium pyrophosphate was added followed by trichloracetic acid and the mixture heated in a boiling water bath.[4] After cooling and centrifugation the supernatant was removed, the residue was extracted twice more and all the extracts were combined. The iron content was measured as above.

The *dry weight* was determined by drying a second weighed aliquot in an oven at 95°C.

Serum iron

Serum iron was estimated by the method of Ramsay;[5] in the presence of circulating iron dextran a 60-min period of heating was used, instead of the usual five minutes, to release the iron from the dextran.

Results

The interval between the infusion and the onset of delivery varied greatly. One patient was delivered the same day and several others went into labour within 10 days, but in most cases the infusion was given more than three weeks before delivery.

HISTOCHEMICAL EXAMINATION OF THE PLACENTA (Fig. 1)

The most striking changes were in the placenta associated with the stillbirth, in which almost every villus contained large amounts of Prussian blue-positive material in the trophoblast or immediately beneath it. No other specimen showed so much iron but in placentas obtained at four days and at six days there was still a large amount of similarly distributed Prussian blue material in the villi, though many villi contained relatively little.

In placentas obtained at eight days or longer after the infusion the findings were the same as in the controls, namely occasional specks of Prussian blue-positive material found mainly in the stroma of the villi, there being virtually none in the trophoblast itself.

WATER-INSOLUBLE NON-HAEM IRON (HAEMOSIDERIN IRON) OF THE PLACENTA

A total of 35 duplicate analyses of homogenates were made with a range of values from 6 to 108 mg iron/100 g dry weight (0·108-2·94 mmol). The largest difference between duplicates was 12%, but in 30 cases the difference was less than 6%. The mean of the duplicates was therefore taken.

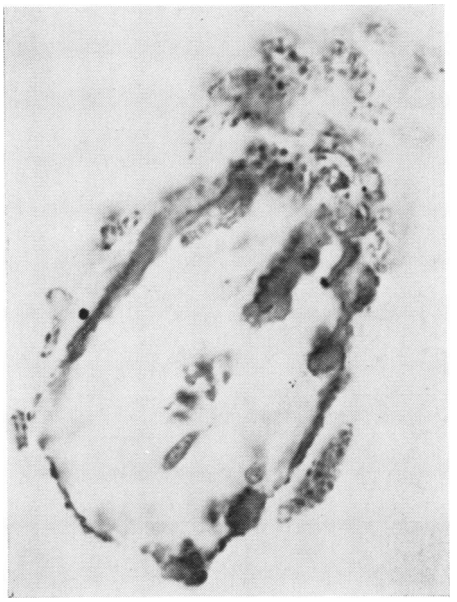

Fig. 1 *Photomicrograph of placenta obtained three days after total dose infusion of iron dextran. Villus containing irregular deposits of iron located mainly just internal to the trophoblast but also in the trophoblast. Perls's stain × 900.*

In 11 controls the placental haemosiderin iron ranged from 4·2 to 12 mg/100 g dry weight (0·076-0·216 mmol). In 26 specimens obtained after iron dextran infusion the placental haemosiderin ranged from control levels up to 128 mg (2·30 mmol)/100 g dry weight. The highest values were all found in placentas obtained within two weeks of the infusion, very much lower concentrations occurring in placentas collected between three and 20 wk after the infusion (Fig. 2).

However, the high concentrations found within two weeks of the infusion were very variable. Two placentas were obtained four days after the infusion, two others at six days and two at 12 days. There were large differences in haemosiderin iron within each of these pairs. In two of the pairs the differences could be related to differences in the dose of iron dextran, but the remaining pair came from a twin pregnancy and were hence exposed to the same maternal blood level.

The general relation between the placental haemosiderin iron concentrations and the dose of iron dextran is illustrated in Fig. 3. All but two of the highest concentrations were associated with the largest doses but they were also cases in which the

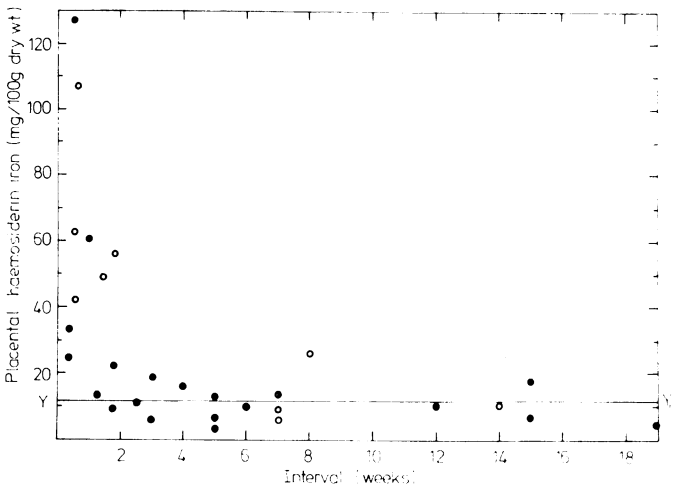

Fig. 2 *Placental haemosiderin iron and the interval between total dose infusion of iron dextran and delivery. The horizontal line YY_1 denotes the highest concentration found in control placentas.*
○ = *dose of Imferon at least 50 ml (2500 mg iron)*
● = *dose of Imferon less than 50 ml*
Conversion to SI units: 1 mg iron = 0·018 mmol.

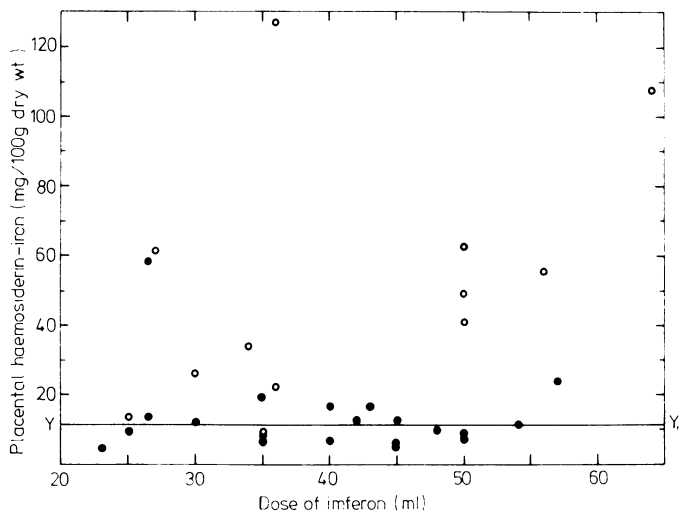

Fig. 3 *Placental haemosiderin iron in relation to the dose of iron dextran (1 ml ≡ 50 mg iron). The horizontal line YY_1 indicates the highest concentration of placental iron found in control placentas.*
○ = *patients delivered within 14 days of the infusion*
● = *patients delivered more than three weeks after the infusion*
Conversion to SI units: 1 mg iron = 0·018 mmol.

placenta was obtained within 14 days of the infusion. In the cases with a longer interval between infusion and delivery, which comprised the majority, the placental iron concentrations did not vary with the dose of iron dextran.

WATER-SOLUBLE NON-HAEM IRON (FERRITIN IRON) OF THE PLACENTA

The water-soluble non-haem iron was determined in 17 treated cases. No estimation was made in any of the controls, but the results after treatment fall into two groups: in three out of six cases with increased concentrations of haemosiderin iron the ferritin iron was higher than in the remaining 14 cases, in most of which the concentrations of haemosiderin iron were within the control range.

MATERNAL SERUM IRON

In some patients the maternal serum iron was measured at the time of delivery. The results are shown in the Table. In most cases the concentrations were high due to the presence of circulating iron-dextran.[6]

Discussion

It is clear from the results for the water-insoluble iron that the storage iron in the placenta may be markedly increased after total dose infusion of iron dextran, especially within a few days of a large dose. Thereafter, if pregnancy continues long enough, it seems that the concentrations are likely to return to normal. The small number of data

Maternal serum iron and placental storage iron at term

Case	*Interval between infusion and delivery (days)*	*Maternal serum iron (total) at delivery (μg/100 ml)**	*Placental non-haem iron (mg/100 g dry weight)**	
			Haemosiderin	*Ferritin*
1	2	17840	25	19
2	4	17600	107	—
3 twin I	3	9416	63	63
" twin II	"	"	42	61
4	7	3050	50	—
5	6	333	61	59
6	12	172	56	—
7	12	103	22	—

*Conversion to SI units: 1 μg/100 ml = 0·18 μmol/l.

for the water-soluble iron content are in accord with this, although they are less satisfactory as an index of the ferritin content because the washing of the placenta after delivery may have led to falsely low results.

These results are in agreement with those on several other species[7 8] but not with another study on the human placenta in which it was concluded that there was no increase of Prussian blue-positive material after a therapeutic infusion of Imferon.[1] In fact, all but a small number of our cases were similarly negative for haemosiderin histochemically, the increased iron content being shown only by chemical analysis.

The mechanism by which placental iron increases is uncertain but two processes seem likely to contribute. The first is the increased transport to the placenta of iron bound to transferrin. The amount of iron available to be carried to the fetus in this way increases as the iron is split from the dextran moiety in the reticuloendothelial system; this probably occurs quite rapidly, as the half-life of iron dextran in the plasma of the patients was about 42 h.[6] Increased amounts of iron may therefore be taken up by the placenta via specific transferrin receptors[9] and reconverted temporarily into storage forms.

Another possibility is that Imferon itself is taken up and degraded by the trophoblast. Electron microscopic evidence for this was adduced by Muir[10] who found, in the human trophoblast, pinocytotic vesicles containing electron-dense material identical with vesicles found in cells of the reticuloendothelial system of monkeys after total dose infusion of iron dextran; such vesicles were never seen in controls. The rate of accumulation of storage iron in the placenta is consistent with this as it has been shown in both pregnant and non-pregnant humans that the accumulation of iron in the reticuloendothelial system reaches a maximum about 4-6 days after infusion.[11-13]

The relative contributions of these two processes to the total iron uptake is speculative. However, it was found in women that after infusion of ^{59}Fe-labelled iron dextran counts of radioactivity over the uterus reached a maximum at 10 min and never exceeded counts over the heart.[13] This suggests that the total uptake by the feto-placental unit is small, though it must be remembered that the placenta contains a relatively large amount of blood.

Wöhler[8] found that the storage iron content of the human placenta was related to the maternal serum iron concentration in that placental concentrations were slightly lower in mothers who were iron-deficient than in those who were not. It is of interest therefore that in the present study increased amounts of iron in the placenta were usually associated with abnormally high concentrations of maternal serum iron. The latter were due to incomplete clearance of iron dextran from the circulation by the time of delivery[6] which makes it likely that the maternal iron stores would still have been replete with iron, available to be transported to the placenta by transferrin.

There appears to be no information available on what happens to the iron when it disappears from the placenta. The presumption is that most of it will be transferred to the fetus, and there is evidence that the total iron transferred across the placenta after total dose infusion of iron dextran is, at least in some patients, greater than normal.[14]

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