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DISTRIBUTION OF IRON IN MATERNAL AND FOETAL TISSUES FROM PREGNANT RHESUS MONKEYS TREATED WITH A SINGLE INTRAVENOUS INFUSION OF [^{59}Fe] IRON DEXTRAN

BY

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Iron dextran (Imferon) administered in one dose as an intravenous infusion has been advocated for the treatment of iron deficiency anaemia and replenishment of maternal iron stores during pregnancy. Although iron dextran given as a single large intravenous dose has been administered to a number of patients (Wallerstein, 1960; Basu, 1963; Varde, 1964; Clay, Rosenberg, Sampson & Samuels, 1965; Lane & Scott, 1965), the effect of this treatment upon placental transfer of iron to the human foetus is not known. In the pregnant rabbit, Bothwell, Pribilla, Mebust & Finch (1958) found that intramuscular injection of iron (18–22 mg iron/kg bodyweight) as an iron dextran complex resulted in a 1.7 to 1.8 fold increase in the average daily transfer of iron to each foetus.

The present paper reports a study undertaken to find out more about the fate and distribution in the mother and foetus of iron derived from an iron dextran complex (Imferon) infused or injected intravenously into the pregnant Rhesus monkey (*Macaca mulatta*). The Rhesus was selected for study since the haemochorial placenta and foetal development of man is more closely related to that of this monkey than to that of other easily available experimental animals. We treated four pregnant Rhesus monkeys with [^{59}Fe] iron dextran and subsequently studied distribution of radioactivity [^{59}Fe] and total iron and the cellular distribution of iron stores in both maternal and foetal tissues. Observations of iron transport across the placenta from mother to foetus in the monkey might be expected to give an indication of the order of transfer in the human. Nevertheless it should be noted that our experiments were carried out in normal, not iron deficient, monkeys.

METHODS

Animals. All studies were performed on healthy adult female Rhesus monkeys (*Macaca mulatta*), weight 4–13 kg, which had been maintained in the colony at the National Institute for Medical Research for at least 6 months before selection and mating for the present experiment. Additional data was also obtained from the 132–136-day foetus of one monkey (B128) which was treated with bovine hyaluronidase (4×10^5 units intravenously between 60 and 112 days' gestation), with [^{131}I]

labelled homologous serum proteins (approximately 30 mg protein and 1.3 mc injected intravenously at 111 days' gestation) and with a hysterotomy to sample foetal blood at 113 days' gestation. Animals were fed *ad lib* on cube diet 41B supplemented with fresh fruit, vegetables and brown bread. The menstrual cycles of breeding animals were recorded and 10 days after the onset of menstruation female monkeys were transferred for a few days into cages with a male. In pregnant animals the approximate duration of gestation was estimated by the size of the uterus and foetal parts; the gestation period was calculated from the dates on which mating could have occurred.

[^{59}Fe] *Iron dextran* was prepared by the Bengers Laboratories Ltd. using [^{59}Fe] labelled ferric chloride (obtained from the Radiochemical Centre, Amersham) diluted with non-radioactive ferric chloride as source of iron in small-scale runs of the process used for the manufacture of Imferon. The material fulfilled the manufacturers' usual criteria of "Imferon"; batch 1 contained 56 mg iron/ml. and at the time of its administration had a specific activity of 0.09–0.13 μc/mg iron; batch 2 contained 50 mg iron/ml. and at the time of its administration had a specific activity of 0.13 μc/mg. It was assumed that the radioactive iron [^{59}Fe] nuclide and non-radioactive iron nuclides were uniformly distributed in the complex and that the iron nuclides were metabolized in identical ways.

Administration of iron dextran. In 5 monkeys the selected dose of iron, given as [^{59}Fe] iron dextran, diluted with approximately 130 ml. (92–132 ml.) 5% glucose solution was administered by intravenous infusion over a period of approximately 4 hr to the anaesthetized monkey (pentobarbitone sodium 26.5 mg/kg bodyweight intravenously, with 30–60 mg/hr intramuscularly). In 2 monkeys (B171 and B126) the selected dose of iron was given to the anaesthetized animal by slow intravenous injection of the undiluted [^{59}Fe] Imferon. Details of animals and treatments are summarized in Table I.

Collection of samples: Pregnant and adult female control monkeys were anaesthetized with pentobarbitone sodium and live babies were delivered by Caesarean section. Adults and babies were bled out from the abdominal aorta and from cardiac puncture respectively; tissues were dissected out and weighed, and portions were taken for chemical estimation of total iron content, for counting of radioactivity [^{59}Fe] and for fixation for histological examination and electron microscopy.

Estimation of iron. All samples for chemical estimation of iron were handled in iron-free apparatus and reagents were made up using deionized distilled water. *Tissue iron:* weighed samples of tissues were digested with a mixture of concentrated sulphuric and concentrated nitric acids, clear tissue digests were diluted to known volumes with water and the iron content was estimated by the method of Ventura and Klopper (1951). Unless otherwise stated, all tabulated results are the mean of duplicate estimations (made on different portions of the same extract). Duplicate estimations agreed to within 5%. Tissues were not perfused before extraction and no correction was applied for the haemoglobin contents of tissues. No attempt was made to compare the iron contents of different regions of the brain; after taking small samples for microscopy all remaining brain tissue was extracted for estimation of iron.

Serum iron and iron binding capacity were determined by the method of Beale, Bostrom and Taylor (1962) modified to use 0.25 ml. samples of serum.

[^{59}Fe] *iron* was measured in scintillation type counters. Tissue [^{59}Fe] was usually estimated on a portion of the extract used for estimation of total iron content but if this contained insufficient radioactivity to give an error of less than 15% on the sample count a larger portion of unextracted tissue was taken for estimation of [^{59}Fe]. Measurements of total radioactivity in foetuses are based on counts of the whole foetus and upon recovery of radioactivity in blood, isolated organs and in an extract of the dissected carcass. It is indicated in the text whether estimates of [^{59}Fe] iron and total iron were made on the same or different portions of each tissue. From measurements of radioactivity and the specific activity of the administered iron dextran, the amount of iron in each sample derived from the administered [^{59}Fe] iron dextran was calculated. These figures are presented as quantities of "[^{59}Fe] iron" or iron derived from [^{59}Fe] iron dextran.

Electrophoresis of samples of 5 μl. serum was carried out on cellulose acetate strips using 0.06 M barbitone buffer *p*H 8.6. After electrophoresis strips were stained with 1% potassium ferrocyanide in 1% hydrochloric acid (for iron) and then with 3% Ponceau S in 5% trichloracetic acid (for

protein). Iron added to serum as iron dextran was demonstrable at a concentration >50 μg iron/ml. serum.

Tissues for histological examination: (1) *by light microscopy* were fixed in mercuric chloride-formalin or in buffered formal-saline and sections were stained with haematoxylin and eosin and with Perls' stain for iron. Histological studies were made of 4 pregnant monkeys and their foetuses, 3 non-pregnant females (B105, B125 and B132) and a female foetus (B128). Tissues examined histologically in all adults were as follows: liver, spleen, pancreas, kidney, lung, adrenal and ovary. In some cases lymph nodes, marrow, heart, brain and uterus were also seen. Foetal tissues sectioned were liver, spleen, lymph node, marrow, kidney, lung, adrenal and brain. In some, pancreas, heart, thymus and testis were also examined. Two placentae from uninjected mothers and all from injected mothers were sectioned.

(2) *by electron microscopy*, tissues were fixed in 5% glutaraldehyde in a veronal acetate buffer at *p*H 7.4. Small portions were later transferred to a 1% osmium tetroxide solution for 1 hr and the nembedded in Araldite. Thin sections (40–60 mμ) from these blocks were examined in A.E.I. E.M.6 and 6B microscopes.

RESULTS

The disappearance of radioactive iron from serum of monkeys treated with an intravenous injection of [^{59}Fe] iron dextran is shown in Fig. 1. Observed counts of radioactivity in serum are presented as μg iron derived from [^{59}Fe] iron dextran/ml. serum. Details of treatment and gestation are summarized in Table 1.

TABLE 1

TREATMENT OF ADULT FEMALE RHESUS MONKEYS WITH [^{59}Fe] IRON DEXTRAN AND RECOVERY OF [^{59}Fe] IRON FROM FOETUSES OF TREATED MOTHERS

Monkey number:	B150	B171	B153	B65	B80	B156	B126	B128
Gestation at time of treatment	Not pregnant	Not pregnant	135–138 days	127–142 days	97–102 days	100–104 days	42–55 days	—
Bodyweight at time of treatment	6·11 kg	7·43 kg	4·02 kg	8·15 kg	7·25 kg	6·57 kg	5·44 kg	11·28 kg
Treatment with [^{59}Fe] iron dextran:								
Dose (mg iron/kg bodyweight)	18	100	32	105	35·4	105	100	None
Dose (radioactivity)	12·1 μc	99 μc	12·6 μc	83·5 μc	32·8 μc	82·7 μc	72·3 μc	
Injection vol.	92 ml.	14·8 ml. (undiluted)	132 ml.	139 ml.	133 ml.	124 ml.	10·9 ml. (undiluted)	
Time taken to administer dose	5·6 hr	5 min	4.1 hr	4·0 hr	4·5 hr	4·1 hr	5 min	
Kill and study distribution of iron at:								
Interval after treatment			7 days	6 days	36 days	34 days	67 days	—
Gestation age			142–145 days	133–148 days	133–138 days	134–138 days	109–122 days	132–136 days
[^{59}Fe] Iron recovered in foetus:								
Mass (mg.)			0·95	2·4	11·5	10·1	4·3	—
% of maternal dose			0·74	0·28	4·5	1·5	0·80	—
Mass of foetus			320 g	386 g	358 g	330 g	235 g	262 g

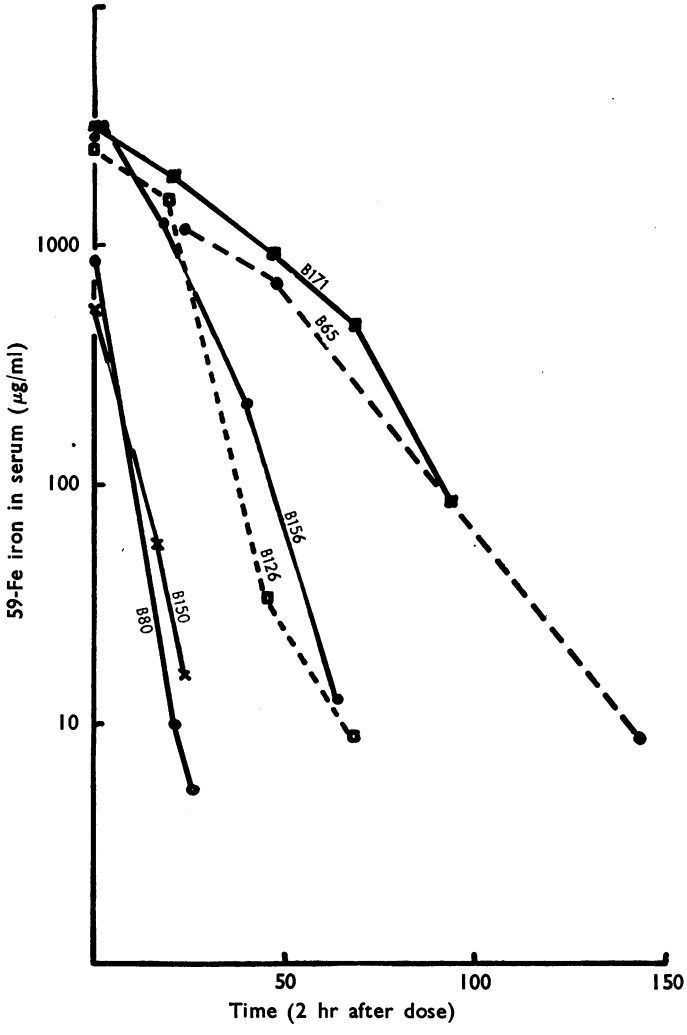

Fig. 1. Disappearance of [^{59}Fe] iron from serum after intravenous infusion of [^{59}Fe] iron dextran into Rhesus monkeys.

Serum samples from treated pregnant monkeys collected at various times after completion of the infusion of iron dextran and examined by electrophoresis on cellulose acetate showed: (1) in monkeys B153, B65, B80 and B156, at 10 min, a heavy staining iron band in the position corresponding to iron dextran; (2) in monkeys B153 and B80 (after 32–35 mg iron/kg bodyweight) at 24 hr and 21 hr respectively no demonstrable iron staining (i.e., <50 μg/ml.); (3) in monkeys B65 and B156 (after 105 mg iron/kg bodyweight) at 48 hr and 40 hr respectively iron was demonstrable in the position of the iron dextran band; at 144 hr and 48 hr respectively no iron was demonstrable.

Urinary excretion of radioactive iron from two monkeys after treatment with [^{59}Fe] iron dextran (100 mg iron/kg bodyweight injected intravenously) is shown in Table 2.

TABLE 2

URINARY EXCRETION OF [^{59}Fe] IRON IN A PREGNANT (B126) AND A NON-PREGNANT (B171) MONKEY FOLLOWING INTRAVENOUS INJECTION OF IRON DEXTRAN (100 MG [^{59}Fe] IRON/KG BODYWEIGHT)

	Monkey:	B126 (μg)	B171 (μg)
Urinary [^{59}Fe] iron	0–24 hr after treatment	654	678
	24–48 hr after treatment	76	193
	48–72 hr after treatment	51	280

During the first three days after treatment total urinary excretion of [^{59}Fe] iron represented 0.14% and 0.15% of the dose given to a pregnant and to a non-pregnant monkey respectively.

Estimates of serum iron and unsaturated iron-binding capacity of maternal and foetal sera collected 1 or 5 weeks after treatment of the mothers with an intravenous infusion of [^{59}Fe] iron dextran (as detailed in Table 1) are shown in Table 3. Values for untreated pregnant and non-pregnant female monkeys are also shown. Unsaturated iron-binding capacity and total iron-binding capacity in all pregnant monkeys was higher than in non-pregnant controls. The unsaturated iron-binding capacity in iron dextran treated monkeys was higher than in untreated pregnant controls. It can be assumed that at 5 weeks after administration of iron dextran, or 1 week after the low dose, substantially all the circulating iron is present in serum in physiological forms. In the case of monkey B65, given a high dose and measured at 1 week, the serum iron figure clearly reflects a proportion of iron dextran still present as such.

The iron content of tissues from animals treated during pregnancy and from untreated (non-pregnant) control animals is shown in Tables 4 and 5. The radioactive [^{59}Fe] iron content of tissues from treated animals is shown as a percentage of the total iron content

TABLE 3

SERUM IRON AND IRON-BINDING CAPACITY IN IRON DEXTRAN TREATED PREGNANT AND UNTREATED CONTROL MONKEYS

	Monkey	Days' gestation	Serum iron (μg/100 ml.)	Unsaturated iron-binding capacity of serum (μg/100 ml.)	Total iron-binding capacity of serum (μg/100 ml.)
Not pregnant No treatment	B12		254	250	504
	B50		249	193	442
	B64		196	302	498
	B125		160	376	536
	B132		134	236	370
	B105		140	316	456
Pregnant No treatment	B45	135–143	345	244	589
	B96	133–135	319	317	636
	B68	143–150	327	284	611
	B66	116–123	270	340	610
	B78	123–126	215	375	590
Pregnant Treated	B153	142–145	286	346	632
	B65	133–148	590	396	986
	B156	134–138	216	440	656
	B80	133–138	220	460	680
Foetus from treated	B153 foetus		126	100	226
	B65 foetus		120	188	308
	B156 foetus		88	406	494
	B80 foetus		254	220	474

TABLE 4

TISSUE IRON IN μG/G OF TISSUE AND (IN PARENTHESES) CONTENT OF [^{59}Fe] IRON AS PERCENTAGE OF TOTAL IRON

* Mean (four samples) ± S.E.M. Superscript figures indicate whether: [1] [^{59}Fe] estimated on same sample of tissue as that used to estimate total iron; [2] [^{59}Fe] estimated on different sample of tissue from that used to estimate total iron

	Non-pregnant female monkeys			Pregnant monkeys treated with [^{59}Fe] iron dextran							
				32 mg/kg 7 days previously		105 mg/kg 6 days previously		35·4 mg/kg 36 days previously		105 mg/kg 34 days previously	
	B105	B132	B125	B153		B65		B80		B156	
Adrenal	271	360	45·4	181	(23·2)[1]	336	(69·1)[1]	150	(30·2)[1]	225	(66·3)[1]
	Tibia	Femur	Tibia					Tibia		Tibia	
Bone	13·2	8·6	21·3	—	—	—	—	18·2	(13·2)[1]	57·5	(13·9)[1]
Brain	98	46·1	51·5	48·2	(1·0)[1]	50·1	(5·0)[1]	32·0	(3·4)[1]	52·2	(4·5)[1]
Fat	50·2	—	6·69	—	—	21·0	(54·8)[2]	41·6	(38·5)[1]	62·6	(21·6)[1]
Kidney	31·4	38·2	9·7	81·1	(11·1)[2]	146	(75·5)[1]	36·6	(34·2)[1]	86·9	(71·7)[1]
Heart	36·4	63·6	6·86	—	—	—	—	50·5	(24·7)[1]	89·2	(56·0)[1]
Liver	208	68·3	64·2	*1,342 ±427	(57·5)[1]	*2,323 ±178	(88·0)[1]	*684 ±51	(77·3)[1]	*2,396 ±30	(95·5)[1]
Lung	939	444	141	118	(6·9)[1]	178	(54·6)[1]	24·6	(104)[1]	263	(18·6)[1]
Muscle	88·9	32·9	42·0	—	—	37·3	(52·9)[1]	29·4	(4·7)[1]	22·2	(<41)[1]
Ovaries	540	166	117	162	(11·4)[1]	280	(64·4)[1]	61·3	(21·1)[1]	162	(18·6)[2]
Pancreas	37·1	29·4	28·0	—	—	76·3	(48·1)[1]	27·4	—	46·5	(52·4)[1]
Skin	17·0	160	19·5	—	—	87·4	(23·9)[1]	4·7	(104)[2]	30·5	(56·7)[2]
Spleen	513	118	135	511	(34·5)[1]	1,490	(53·7)[1]	411	(50·2)[1]	2,513	(64·2)[1]

(Table 4). Livers from all treated animals and spleen and kidneys from animals treated with a high dose of iron dextran (B65 and B156) contained more iron than corresponding tissues from control animals.

In liver from all treated animals the ratio of iron derived from iron dextran (based on measurement of the tissue radioactivity and the specific activity of the dose) to the total iron content was high (Table 4) and 57–95% of the liver iron was thus calculated to be derived from the iron dextran. Livers, spleens and kidneys from animals treated with 32–35 mg iron/kg bodyweight contained less iron (a smaller proportion of which was radioactive) than corresponding tissues from animals treated with 105 mg iron/kg bodyweight. Kidneys from monkeys killed 5 weeks after treatment contained less iron than kidneys from monkeys killed 1 week after treatment.

TABLE 5

TISSUE IRON IN MG IRON/ORGAN

	Non-pregnant female monkeys			Pregnant monkeys treated with [^{59}Fe] iron dextran			
				32 mg/kg 7 days previously	105 mg/kg 6 days previously	35·4 mg/kg 36 days previously	105 mg/kg 34 days previously
	B105	B132	B125	B153	B65	B80	B156
Adrenals	0·15	0·07	0·02	0·49	0·70	0·40	0·24
Brain	6·64	3·35	3·48	—	—	2·95	3·51
Kidneys	0·78	0·84	0·34	1·12	4·36	0·59	1·84
Heart	0·97	0·70	0·27	—	—	1·41	2·05
Liver	30·4	9·2	14·2	132	464	144	431
Lungs	40·3	21·3	12·7	2·52	5·79	0·77	9·25
Ovaries	0·18	0·07	0·07	—	0·14	0·09	0·13
Pancreas	0·65	0·10	0·41	—	0·32	0·26	0·36
Spleen	2·99	0·51	0·89	1·53	10·50	2·42	10·50
Body weight (kg)	9·98	5·78	13·15	4·02	8·15	7·25	6·57

TABLE 6

TOTAL IRON AND [^{59}Fe] IRON IN TISSUES FROM FOETAL RHESUS FROM MOTHERS TREATED WITH [^{59}Fe] IRON DEXTRAN DURING PREGNANCY

[^{59}Fe]/Fe % superscript: [1] [^{59}Fe] estimated on same sample of tissue as that for total iron; [2] [^{59}Fe] estimated on different sample of tissue from that for total iron

	Untreated control	Foetuses from [^{59}Fe] iron dextran-treated mothers											
Age:	B128 foetus 132–136 days	B153 foetus 142–145 days			B65 foetus 133–148 days			B80 foetus 133–138 days			B156 foetus 134–138 days		
Body weight:	262 g	320 g			386 g			358 g			330 g		
Iron content	μgFe/g tissue	μgFe organ	μgFe/g tissue	μgFe organ	[^{59}Fe]/Fe %	μgFe/g tissue	μgFe organ	[^{59}Fe]/Fe %	μgFe/g tissue	μgFe organ	[^{59}Fe]/Fe %	μgFe/g tissue	μgFe organ	[^{59}Fe]/Fe %
Tissue:														
Adrenals	281	54	—	—	—	288	60	—	196	108	—	223	81	3·3[2]
Bone (tibia)	40·5	—	90·5	—	8·3[2]	45	—	28·8[1]	56·2	—	26·3[1]	43·6	—	33·8[1]
Brain	12·1	346	24·1	—	3·2[1]	24·7	1,334	—	9·5	461	34·2[1]	47·6	1,790	8·4[1]
Heart	48·5	82	—	—	—	—	—	—	66·9	149	29·2[1]	37·2	59	31·4[2]
Kidneys	37·2	57	79·6	119	—	56·9	86	—	77·5	178	12·0[2]	35·4	61	22·3[1]
Liver	24·5	291	342	2,950	4·0[1]	467	5,260	21·8[1]	163	1,910	41·8[1]	475	3,330	37.9[1]
Lungs	28·7	129	53·9	275	—	37·3	220	—	36·8	185	25·1[1]	30·8	145	37·6[1]
Muscle	99	—	62·0	—	—	55	—	—	53·3	—	10·3[2]	42·5	—	67·0[1]
Skin	50·5	—	44·7	—	—	53·7	—	—	53·0	—	11·5[2]	27·7	—	18·4[2]
Spleen	200	70	466	186	—	424	305	7·6[2]	344	241	96[1]	207	85	39·6[2]
Thymus	185	92	—	—	—	—	—	—	54·5	—	17·9[1]	74·6	—	32·0[1]

TABLE 7

RECOVERY OF RADIOACTIVE [^{59}Fe] IRON IN MATERNAL AND FOETAL TISSUES AFTER ADMINISTRATION OF [^{59}Fe] IRON DEXTRAN TO THE MOTHER

Notes: [1] Mean estimate from five samples; [2] Blood volume taken as 6% body weight from other previous estimations; [3] Mean estimate from two samples

	Monkey			
	B153	B65	B80	B156
Dose of iron dextran (mg iron/kg body weight)	32	105	35	105
Interval between dose and sampling (days)	7	6	36	34
Percentage of [^{59}Fe] dose recovered:				
Maternal liver[1]	61·2	46·9	41·9	60·1
Maternal red blood cells[2]	4·46	1·14	30·6	12·9
Maternal spleen[3]	0·38	0·79	0·38	0·76
Placenta[1]	0·58	1·74	2·09	1·53
Foetus	0·74	0·28	4·49	1·46

Extracts of brain from treated animals did not contain more iron than extracts from control animals, and from measurements of radioactivity only 1–5% of brain iron was derived from iron dextran. Pancreas from treated animals did not contain more iron than pancreas from normal control animals, although from measurements of radioactivity in tissue from two animals 48 and 52% of the pancreas iron was derived from the iron dextran.

When iron was administered to pregnant monkeys as [^{59}Fe] iron dextran, radioactive iron in maternal liver, red cells and spleen, together with iron in the placenta and foetus, accounted for 67, 76 and 79% of the dose in 3 monkeys and for 51% of the dose in the fourth monkey (B65) (Table 7).

Recovery of radioactive iron from foetuses of treated mothers ranged from 0.28–4.5% of the maternal dose. More radioactivity (equivalent to 10.1–11.5 mg iron derived from the administered iron dextran) was found in foetuses examined 5 weeks after maternal treatment at 97–104 days' gestation than in foetuses examined 1 week after treatment at 127–142 days' gestation (equivalent to 0.9–2.4 mg iron). The foetus delivered from 1 monkey (B126, Table 1) 67 days after treatment with iron dextran (100 mg iron/kg bodyweight) at 42–55 days' gestation contained less radioactivity (equivalent to 4.3 mg iron) than foetuses from mothers treated later in gestation and examined 34–36 days after treatment.

The iron content of tissues from foetuses of [^{59}Fe] iron dextran treated mothers is shown in Table 6. Control material from comparable foetuses was not available but the iron content of tissues prepared in the same manner from a 132–136-day foetus from monkey B128 (see note under " animals " on page 2) is also listed in Table 6. It may be noted that this foetus was smaller in size than the other foetuses. Figures for the percentage of the total iron in foetal tissues derived from [^{59}Fe] iron dextran are

TABLE 8

RADIOACTIVE [^{59}Fe] IRON IN RED CELLS FROM MOTHERS AND FOETUSES AFTER ADMINISTRATION OF [^{59}Fe] IRON DEXTRAN TO MOTHERS DURING PREGNANCY

	Monkey			
	B153	B65	B80	B156
Interval between treatment and sampling	7 days	6 days	36 days	34 days
Maternal red cell iron μg [^{59}Fe] iron/ml. blood	28·6	20·1	181	225
Foetal red cell iron μg [^{59}Fe] iron/ml. blood	31	40	206	248

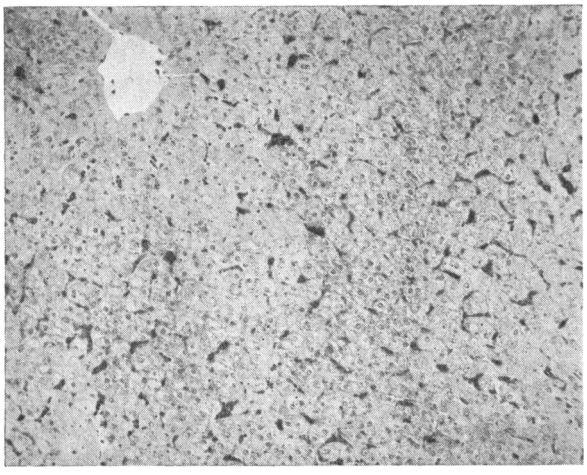

Fig. 2. Liver from iron dextran treated monkey B156. Clumps of deeply stained iron-rich Kupffer cells are scattered throughout the lobule. Perls' stain, ×108.

incomplete because of the limited amounts of tissue available and small amounts of radioactivity present in some tissues. Red cells from foetal blood contained radioactive iron (Table 8) and (like the level of iron derived from [^{59}Fe] iron dextran in maternal red cells) this appeared to be unrelated to the dose of [^{59}Fe] iron dextran but related to the interval between treatment and sampling and (in the foetus) to the time of gestation at which treatment was given (Table 1).

Live foetuses of normal body weight and appearance were obtained from iron dextran treated mothers.

Histological examination showed that in tissues from iron dextran treated monkeys

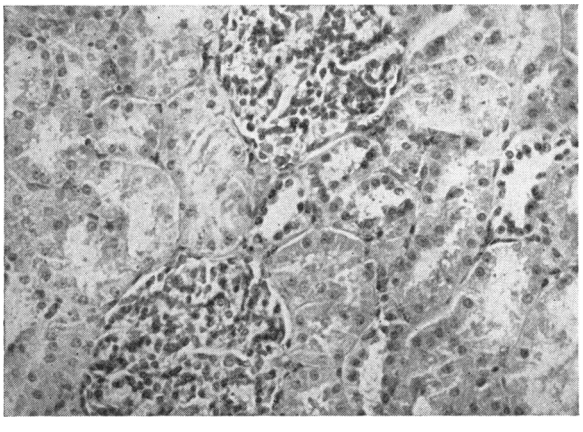

Fig. 3. Kidney from iron dextran treated monkey B65. Iron-positive granules in glomeruli and tubules are very scanty. Perls' stain, ×295.

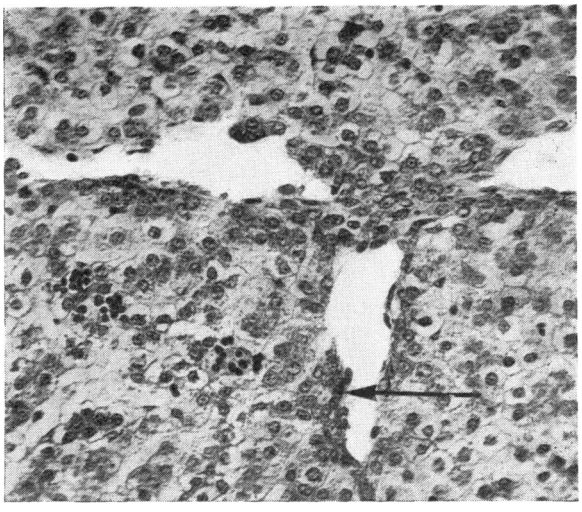

Fig. 4. Liver from foetus, B80, of iron dextran injected monkey. Iron-positive granules (arrow) are very scanty, and confined to periportal parenchymal liver cells. Perls' stain, ×295.

and their foetuses an increase in iron staining was observed, as compared with small amounts in control tissues from non-pregnant uninjected animals and one foetus.

In the mothers, siderosis was predominantly reticuloendothelial. Hepatic Kupffer cells contained large quantities of iron, and in one case (B156) were aggregated into clumps (Fig. 2). Smaller amounts of iron were detected in spleen, bone-marrow, lymph nodes and phagocytic cells of other organs. Elsewhere iron staining was slight. It was increased in liver cells in all 4 animals. Renal tubular epithelium was just positive in 2 animals, whereas gomeruli contained stainable iron in 3 (Fig. 3). Adrenal cortical cells were

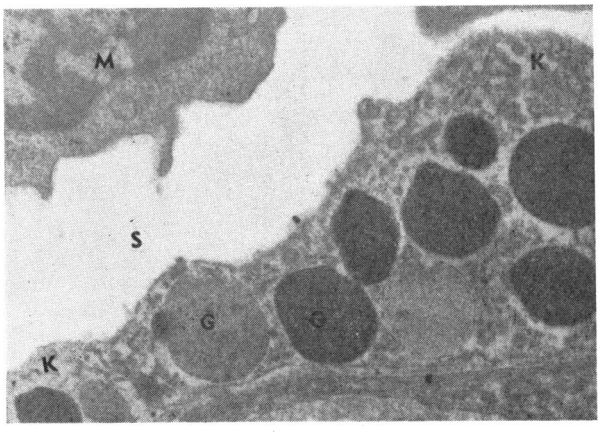

Fig. 5. Liver from iron dextran treated monkey B153. A hepatic sinusoid (S) containing a monocyte (M) is lined by a Kupffer cell (K). The Kupffer cell cytoplasm is laden with granules (G) of varying electron density. ×36,000.

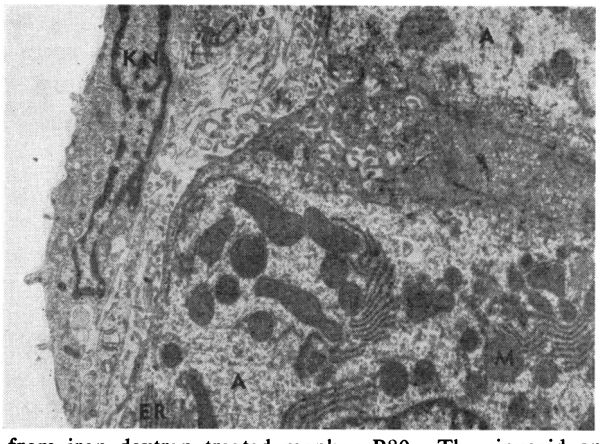

Fig. 6. Foetal liver from iron dextran treated monkey B80. The sinusoid on the left is lined by an extended Kupffer cell containing its nucleus (KN). The parenchymal cells on the right contain endoplasmic reticulum (ER), mitochondria (M) and clearer glycogen storage areas (A). ×20,000.

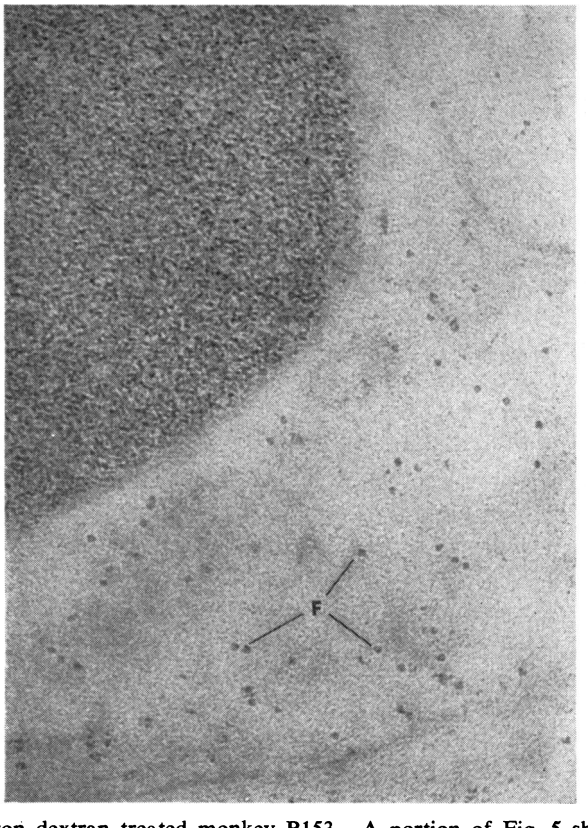

Fig. 7. Liver from iron dextran treated monkey B153. A portion of Fig. 5 showing the edge of a granule in a Kupffer cell and the surrounding cytoplasm. The fine particles in the granule resemble iron dextran, ferritin images (F) are seen outside the granule. ×200,000.

positive in one case only. In the placentae, iron was seen in phagocytes in the decidua, and there was slight staining of trophoblast and mesenchymal cores of villi. Siderosis was accentuated in " infarcted " areas. In animal B65 alone iron-positive granules were seen in phagocytic cells of the foetal part of the placenta. In general, more iron was apparent in those animals receiving the higher doses, but the precise distribution of the stainable iron between organs varied.

Other changes in adult tissues were minor. They included fatty liver and focal necrosis and inflammation (B65), slight renal tubular degeneration and scarring (B80), fatty infiltration of the pancreas (B156) and a lesion in a mesenteric lymph node resembling human infectious mononucleosis (B156). None of these changes was directly attributable to iron overload. In B156 there was more variation in size of myocardial fibres and nuclei than in controls, and iron-positive granules were present in scattered phagocytes in the myocardium. Parasitic lesions were found in B153 and B65 as well as in a control monkey.

Foetal siderosis was of a much smaller degree, and involved phagocytes in spleen, lymph nodes, marrow, thymus and testis as well as liver cells (Fig. 4). Minute quantities of iron were also demonstrated in the liver of the single control foetus examined. In one foetus (B156) an axillary lymph node was the seat of massive siderosis, together with slight fibrosis and fatty infiltration. Unfortunately this node was not examined for radioactivity. Other nodes examined were not affected. No other foetal abnormalities were demonstrated.

Electron microscopic examination of the maternal livers, from the infused animals, confirmed the extensive storage of iron in these organs, which was demonstrated by analysis and iron staining for light microscopy. In all the maternal livers from the infused specimens, the Kupffer cells contained large electron dense granules (Fig. 5), these varied in density and at very high magnification (Fig. 7) were seen to be composed of closely packed fine spherical particles with a diameter of 10–20 Å. These particles closely resembled the structure of isolated iron-dextran, hence the granules can be considered as ingestion vacuoles. The surrounding Kupffer cell cytoplasm contained numerous images of ferritin iron, which were probably synthesized from the ingested iron dextran (Richter, 1959 ; Muir & Golberg, 1961).

Ingestion vacuoles were not seen in the hepatic parenchymal cells and any change in the concentration of lysosomes and ferritin molecules in these cells was concealed by the variation between individual cells.

None of the foetal livers from the infused specimens contained any ingestion vacuoles in the Kupffer cells similar to those present in their mothers. The foetal parenchymal cells had areas of glycogen storage (Fig. 6), but, again, any overall increase in ferritin content was not detectable by electron microscopy.

DISCUSSION

The present experiments were undertaken as part of a study of the selective transfer of macromolecules across the placenta in the Rhesus monkey (Bangham, Hobbs & Tee, 1960 ; Bangham, 1960) and specifically to investigate the placental transfer of iron

(administered as in current clinical practice as an intravenous infusion of an iron dextran complex) from mother to foetus.

The fate of an iron complex, injected intravenously into a primate during pregnancy, is likely to depend upon: (1) the dose of iron, the nature of the complex and its mode and rate of breakdown; (2) the recipient: state of iron stores, stage of gestation (which may affect selective transfer of iron at the placenta as well as the rate of utilization of iron by the foetus) and renal handling of the iron complex and its breakdown products.

The size of the doses administered should be considered in relation to the iron requirements of the pregnant animal. From analysis of the normal Rhesus monkey foetus delivered stillborn at term, it was calculated that a 350 g foetus might contain approximately 30 mg iron, together with approximately 12 mg iron in the placenta. Mean total liver iron in three normal adult Rhesus monkeys was 18 mg. Thus an iron depleted pregnant monkey might be expected to require 60 mg extra iron (without taking into account conservation of iron from cessation of menstrual blood loss during the latter part of pregnancy). In the present studies, in normal (not anaemic) monkeys, the doses of iron administered (32 and 35 mg iron/kg bodyweight or 128–256 mg iron and 100 and 105 mg iron/kg bodyweight or 544 to 860 mg iron) were greatly in excess of the theoretical requirements of an anaemic monkey. We found that in the Rhesus monkey treated at various stages of gestation with these large amounts of iron dextran (administered to the mother by intravenous injection or infusion) only a small proportion of the administered iron accumulated in the foetus.

The exact nature of the iron dextran complex Imferon is not known. Serum clearance of [^{59}Fe] iron after intravenous injection of [^{59}Fe] iron dextran was rapid (Fig. 2) and an insignificant proportion of the administered iron was excreted in urine of the two monkeys studied. This does not exclude the possibility that renal loss of iron dextran might be increased in toxaemia of pregnancy. (We have been unable to find any record of the occurrence of this condition in the Rhesus monkey.)

If the placenta was freely permeable to iron dextran we should expect the greatest exchange of iron between mother and foetus to occur soon after iron dextran treatment, when the concentration in maternal serum was at its highest level. We might expect to see some persistent evidence of this initial exchange even in tissues examined 1 or 5 weeks later. This did not appear to occur for the following reasons: (1) Kupffer cells in liver from foetuses from iron dextran treated mothers did not show large vacuoles containing colloidal, non-ferritin iron, as were seen in maternal livers. In contrast, maternal livers contained large amounts of phagocytosed material, which was presumably derived directly from the infused iron dextran. (2) In monkeys treated at 97–104 days' gestation the foetus from monkey B156 (treated with 105 mg iron/kg bodyweight) did not contain appreciably more iron than the foetus from monkey B80 (treated with 35 mg iron/kg bodyweight). Nevertheless it was noted that one lymph node (out of three examined) from the foetus of B156 showed massive siderosis and it is not clear what was the source of this iron. (3) We examined foetal blood from another monkey (B94) in which the mother, at 111–118 days' gestation was injected intravenously with [^{59}Fe] iron dextran (89 mg iron/kg bodyweight). Twenty-four hr after completion of the iron dextran drip, foetal blood contained radioactivity equivalent to 10 μg [^{59}Fe] iron/ml., which was 2% of the level found in corresponding maternal blood. (4) In foetuses from

monkeys treated later in gestation (127–142 days) more radioactivity was found in the foetus of monkey B65 (treated with 100 mg iron/kg bodyweight) than in the foetus of monkey B153 (treated with 32 mg iron/kg bodyweight). It is possible that at this later stage of gestation the placental metabolism of iron dextran changes or that a high proportion of the maternal serum iron crosses the placenta.

The proportion of the iron dose recovered in each foetus cannot easily be correlated with the maternal dose of iron. Nor do our observations indicate whether the total iron store of each foetus was increased by maternal treatment. Maternal doses were graded in proportion to bodyweight: the dose of iron administered to monkey B65 (105 mg/kg bodyweight) was 860 mg and to monkey B153 (32 mg/kg bodyweight) was 128 mg. Thus the ratio of iron dose given to these monkeys may be regarded as 3.2:1 or as 6.7:1. Approximately two and a half times as much radioactivity was recovered in the foetus from B65 as in the foetus from B153, which was treated and examined at a comparable stage of gestation. In the pair of foetuses from monkeys B156 and B80 (treated earlier in gestation with 689 and with 256 mg iron respectively) recovery of radioactivity in the two foetuses was almost identical. Bothwell *et al.* (1958) showed that in the rabbit, placental iron transport was increased by an increased supply of iron in maternal plasma. From our results it is not clear how far the supply of iron directly available to the foetus was influenced by infusion of iron dextran into the maternal circulation.

Our observations suggest that at or near term in the pregnant Rhesus monkey (as in man) total iron-binding capacity in serum is higher than in the non-pregnant animal. After iron dextran treatment of pregnant animals, unsaturated iron-binding capacity was higher than in untreated animals. In the foetuses of iron-treated animals serum iron levels were usually lower than in corresponding maternal sera and sera from untreated pregnant controls. These serum iron levels may be low because blood from the foetal monkey contains large numbers of circulating normoblasts still able to take up iron from transferrin and it is possible that, before separation of serum from foetal blood samples, iron may have been removed from serum and incorporated into red cells. In man, Sturgeon (1954) found the plasma iron level at birth to be 193 μg/100 ml., falling to 46 μg/100 ml. during the first few hours of life. During the same time there was also a drop in total iron-binding capacity. A similar phenomenon may be responsible for the different levels of serum iron found in foetuses from treated monkeys.

Parenteral iron therapy is advocated when iron by mouth is not tolerated or absorbed, when the patient cannot be relied upon to take it or when time is too short to decide whether oral iron is effective. Thus a young woman might receive several "total dose infusions" of iron dextran for treatment of anaemia or replenishment of iron stores during successive pregnancies. In the present series of experiments maternal liver was the main site of deposition of iron derived from administered iron dextran. In the treated Rhesus iron was abundant in Kupffer cells, whereas neither ferritin nor histochemically demonstrable iron was present in greatly increased quantities in the parenchymal cells. Examination of livers 1 week and 5 weeks after infusion revealed no evidence of progressive hepatic damage or fibrosis. Nevertheless 5 weeks after treatment iron was still present in large amounts, and we have not examined animals after longer intervals.

Treated monkeys were not examined for signs of local reactions at the site of injection of the iron dextran. No systemic reactions were observed.

Thus in pregnant Rhesus monkeys, treated with iron dextran, a small proportion of the administered iron was transferred across the placenta to the foetus and most of the iron dose was stored in maternal liver and later appeared in circulating maternal red cells.

SUMMARY

1. Six pregnant Rhesus monkeys were given a single intravenous infusion or injection of [^{59}Fe] iron dextran (approximately 32 or 100 mg iron/kg bodyweight) in an experiment planned to simulate the clinical use of iron dextran for treatment of anaemia and replenishment of iron stores during pregnancy.

2. Distribution of radioactive iron in maternal and foetal tissues was studied in 4 monkeys killed at 133–148 days' gestation and 1 or 5 weeks after treatment.

3. Measurement of radioactivity in foetuses showed that 0.25–4.5% of the maternal dose of iron from iron dextran (0.9–11.5 mg iron) was transferred to each foetus. More radioactive [^{59}Fe] iron was recovered in foetuses killed 5 weeks after treatment than in foetuses killed 1 week after treatment.

4. In histological sections from tissues of treated monkeys increased iron staining was predominantly in the reticuloendothelial system. Staining in foetal tissue was slight. Kupffer cells of maternal livers contained large ingestion vacuoles which, in electron micrographs, appeared to contain a colloidal iron with structure similar to the infused material. Such vacuoles were not observed in foetal livers.

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