Behavior of Iron-, Indium-, and Iodine-Labeled Transferrin in the Pregnant Rat[1] (34631)

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(Introduced by C. L. Conley)

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In several mammals, including the rabbit and the rat, large amounts of iron are transferred across the placenta to the fetus, particularly during the latter third of pregnancy (1, 2). We have investigated the role of transferrin in carrying iron across the rat placenta by doubly labeling transferrin with radioactive iron and indium. Indium was chosen as a second transferrin label because this metal had been shown in man, rabbit, and dog to bind to transferrin when added to whole serum (3). Furthermore, ^{113m}In is now used extensively for clinical placental scanning (4, 5), on the supposition that it behaves as a blood pool agent. Additional studies were performed using transferrin labeled with ^{125}I to compare the distribution of transferrin itself with the distributions of the metals for which it is the binding protein.

Materials and Methods. Iron and indium labeling of transferrin. Transferrin was labeled with radioactive iron and indium as follows: One milliliter of fresh rat serum was incubated with ^{59}Fe-ferrous citrate for 10 min at room temperature. A second aliquot of serum was incubated 10 min with either Indium-114m chloride or carrier-free Indium-113m chloride. The indium preparation was mixed with a neutral cation-exchange resin (Dowex-50) and passed through a 0.45-μ Millipore filter. A sample of filtrate labeled with ^{114m}In, and ^{59}Fe-labeled serum were electrophoresed on vertical starch gel using Tris–borate–EDTA buffer, pH 8.6. Good resolution of the two rat transferrin bands (6) was obtained. The ^{114m}In and ^{59}Fe portions of an unstained gel were divided transversely into 2-mm sections, counted, and histograms constructed. Binding of both nuclides appeared to be identical, the two peaks of radioactivity corresponding exactly to the two rat transferrins. In both cases more than 95% of the total radioactive counts were in the transferrin region.

Purification and iodination of transferrin. Rat serum was labeled with ^{59}Fe, and its binding capacity was saturated with excess stable iron. The serum was eluted from a 30 $\times$ 2.5-cm DEAE–Sephadex column with Tris–HCl buffer, pH 8.0, using a continuous concentration gradient from 0.075 – 0.2 *M* (6). One hundred and twelve 10-ml fractions were collected, and the five fractions containing the peak of ^{59}Fe counts were pooled and concentrated by positive pressure (45 psi) ultrafiltration through an Amicon[2] membrane. Starch-gel electrophoresis of the concentrated fractions showed only two bands, corresponding to the slow- and fast-migrating transferrins. Immunoelectrophoresis using goat anti-whole rat serum[3] also showed two bands, a single transferrin band and a small contaminating gamma globulin band. The preparation was labeled with ^{125}I using a modification of the iodine monochloride method of McFarlane (7); the iodine substitution was 0.7 – 1.5 atoms/transferrin molecule in the various preparations. After dialysis against running water overnight, more than 99% of the radioactivity in an aliquot of the labeled solution was precipitable with trichloroacetic acid. Starch-gel electrophoresis indicated that the contaminating gamma globulin was preferentially labeled and accounted for

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[2] Obtained from Amicon Corp., Lexington, Massachusetts.

[3] Obtained from Hyland Laboratories, Los Angeles, California.

up to 35% of the ^{125}I counts. To precipitate this gamma globulin, carrier serum was added, followed by saturated $(NH_4)_2SO_4$ solution to a final concentration of 40%. After filtration, immunoelectrophoresis of the supernatant fluid showed only a faint trace of gamma globulin. The remaining supernatant fluid was then dialyzed against running water. To remove any denatured transferrin, biological screening (8) was performed by injecting the iodinated preparation intravenously into a Sprague-Dawley rat whose serum was collected 20 hr later. An aliquot of this serum, labeled with ^{59}Fe, was electrophoresed on starch gel. Essentially all of the ^{125}I counts were confined to the two ^{59}Fe-labeled transferrin peaks. The remainder of the serum was used for ^{125}I-transferrin distribution experiments.

Animal experiments. ^{59}Fe- and ^{113m}In-labeled serums were mixed together, and 0.5-ml aliquots were injected into tail veins of pregnant Sprague-Dawley rats at Days 14, 16, 18, and 20 of gestation (total gestation in the rat is 21 days (9)). Animals were anesthetized with sodium pentobarbital, and the intact uterus was removed 1 hr after injection. In addition, 18-day pregnant rats were studied at various time intervals ranging from 15 min to 5 hr after injection. In a third set of experiments, ^{125}I-labeled transferrin was mixed with ^{113m}In-transferrin. Aliquots of this mixture were injected intravenously into 18-day pregnant rats, and these animals were studied 2 hr later. In all cases, at the time the uterus was removed fetuses and placentas were separated and pooled, and a maternal blood sample taken from the aorta. In a separate experiment, red cells were labeled with ^{59}Fe in a donor rat and injected into 18-day pregnant animals. The volume of maternal blood in the placentas was determined by comparing the ^{59}Fe counts in the placentas with those in 1 ml of maternal blood.

Radioactivity measurement. All specimens were measured for radioactivity in a well-type NaI(Tl) scintillation detector coupled to a standard spectrometer. Sufficient counts were obtained to ensure counting errors of less than 2%. Dual isotope counting was performed using either the two-window method (10) or by recounting samples after decay of ^{113m}In (half-life = 1.7 hr). The percentage of the injected dose in each sample was determined by comparison with the appropriate standard prepared in an equal volume for identical counting geometry.

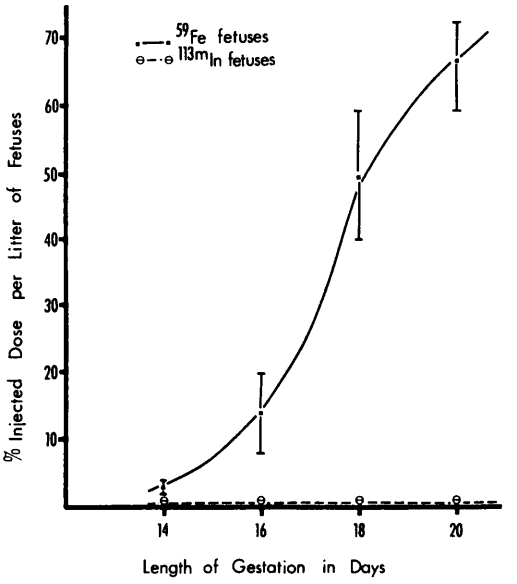

FIG. 1. Fetal uptake of ^{59}Fe and ^{113m}In, as percentage of injected maternal dose of labeled transferrin, 1 hr postinjection, at various stages of gestation. Values = mean ± 1 SD.

Results. Figure 1 shows results obtained when ^{59}Fe- and ^{113m}In-transferrins were injected into rats at various stages of gestation. Each point in the figure and in all subsequent figures represents the mean ± 1 SD of observations in three or four animals. Fetal uptake of ^{59}Fe 1 hr after injection averaged 3% of the dose on Day 14, began to increase rapidly about Day 16, and reached a peak of 70% of the dose on Day 20. Fetal ^{113m}In uptake was less than 0.3% of the dose throughout pregnancy. In Fig. 2, placental uptake of ^{59}Fe 1 hr after injection of labeled transferrin is shown. The amount of ^{59}Fe in placenta was 5–10% of the dose from Day 14 through Day 20. Placental uptake of ^{113m}In increased progressively from 5% of the dose on Day 14 to 45% on Day 20. Tagged red cell placental blood volume determinations suggested that the quantities of ^{113m}In present

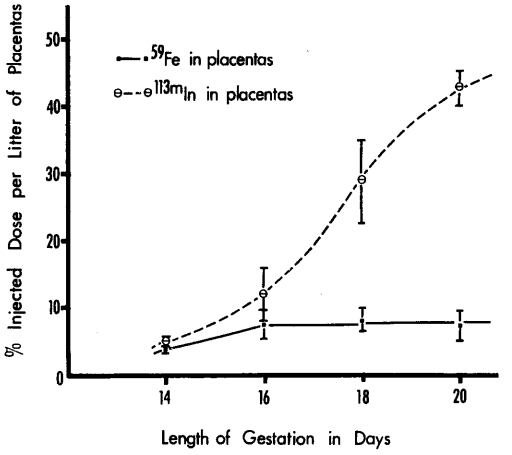

FIG. 2. Placental ^{59}Fe and ^{113m}In uptake corresponding to fetal values in Fig. 1. Values = mean ± 1 SD.

on Day 20 were 20–30 times greater than those predicted from the maternal blood present in placentas, assuming approximate equality between placental and whole body hematocrits.

Figure 3 shows fetal uptake of ^{59}Fe and ^{113m}In after injection of tagged transferrin on Day 18, as a function of time after injection. ^{59}Fe uptake reached 50% of the dose at 1 hr and remained constant thereafter, whereas ^{113m}In transfer was negligible throughout. The corresponding placental values are shown in Fig. 4. ^{59}Fe uptake plateaued by 15 min at 5–10% of the dose, while ^{113m}In accumulation increased progressively to 40% of the dose at 5 hr.

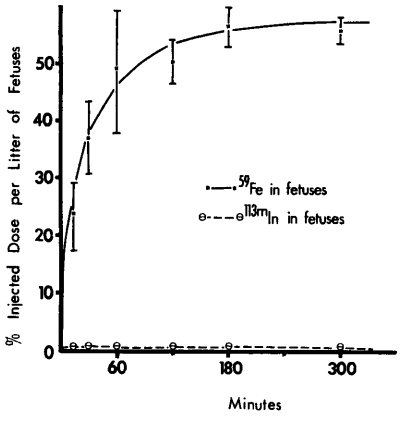

FIG. 3. Fetal uptake of ^{59}Fe and ^{113m}In, as percentage of injected maternal dose of labeled transferrin, at 18 days' gestation, at various times after injection. Values = mean ± 1 SD.

In experiments using ^{113m}In- and ^{125}I-labeled transferrin in 18-day rats, 29.8% of the ^{113m}In dose had accumulated in the placentas after 2 hr, compared to 9% of the ^{125}I dose (Table I). Only 1.2% and 0.06% of the administered ^{125}I and ^{113m}In activities were found in the fetuses, while 4.9% and 2.7% of the ^{125}I and ^{113m}In doses were present per milliliter in maternal serum. These serum values corrected for the rats' entire plasma volumes (estimated from blood volume and arterial hematocrit determinations on rats of similar weight) accounted for 63.3% of the ^{125}I and 35.4% of the ^{113m}In injected.

TABLE I. Distributions of ^{125}I and ^{113m}In 2 hr after Injection of Labeled Transferrin in 18-Day Pregnant Rats. Each Value Is the Mean of Observations in Four Animals.

	% Injected dose	
	^{125}I	^{113m}In
Placentas	9.0	29.8
Fetuses	1.2	0.06
Maternal plasma space	63.3	35.4

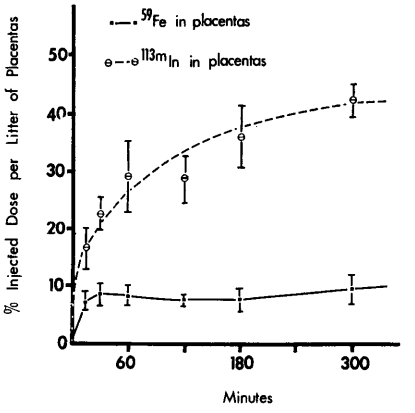

FIG. 4. Placental ^{59}Fe and ^{113m}In uptake corresponding to fetal values in Fig. 3. Values = mean ± 1 SD.

Discussion. These results confirmed the findings of others (2, 9) that, in the rat, fetal uptake of iron is large and increases rapidly during the last third of pregnancy. By contrast, virtually no indium crossed to the fetus, but large amounts accumulated in the placenta. Placental indium content was five

to ten times greater than that of iron 5 hr after injection. This accumulation of indium suggests that either (1) a large proportion of the rat's transferrin pool is associated with the placenta, or (2) the placenta removes indium from transferrin but, unlike iron, fails to transfer it to the fetus. The first of these alternatives appears unlikely in view of the small placental accumulation of ^{125}I relative to that of ^{113m}In after administration of iodinated and indium-labeled transferrin. Low iodine-to-protein substitution ratios and biological screening were used to ensure that the final iodinated transferrin preparations were not denatured and would behave in the same way as noniodinated transferrin. The small amount of ^{125}I present in the fetuses implied that significant quantities of maternal transferrin did not cross the placenta, and that the large amounts of iron transferred to the fetus were not carried across by transferrin. This is in agreement with Morgan's results (11). The disparity between the distributions of iodine and indium in both placentas and maternal blood suggests that indium is removed from plasma transferrin by such tissues as placenta, and that indium is therefore unsuitable as a tracer for plasma transferrin, at least during pregnancy.

There is inferential evidence that binding by fetal transferrin may be the rate-limiting step in placental transport of iron: (1) the time of appearance of transferrin in rat fetal blood corresponds to the time of rapid increase in placental iron transport (Day 16) (12); and (2) fetal but not maternal transferrin is almost completely iron-saturated (11). Furthermore, when indium is added to iron-saturated transferrin, electrophoretic experiments show that it no longer binds to transferrin (13). This suggests that at high percentage saturation, transferrin binds iron preferentially to indium; therefore, the failure of indium to cross the placenta may be due to inability of the near-saturated fetal transferrin to bind it. Alternatively, the block in placental indium transport might result from failure of indium to cross placental cells to the fetal side after it has detached from maternal transferrin. It seems likely that maternal transferrin is able to bind indium because it is not saturated with iron.

The rapid accumulation of indium, a toxic substance, in the placenta and its failure to be transferred to the fetus may imply both detoxifying and fetal protective functions of the placenta with respect to this metal. Moreover, if the placental accumulation of indium which we have demonstrated in the rat also occurs in humans, there are dosimetric implications involving the fetus to be considered.

Summary. Fetal and placental uptakes after administration of ^{59}Fe-, ^{113m}In-, and ^{125}I-transferrins at various stages of gestation in the rat have been studied. In the latter third of pregnancy ^{59}Fe went to the fetus in large amounts, but, in contrast, ^{113m}In accumulated in the placenta. Only small quantities of ^{125}I-transferrin reached the fetus, and considerably less ^{125}I than ^{113m}In was found in placenta. It is concluded that transferrin does not carry iron across the placenta and that indium is removed from transferrin by the placenta, but does not cross to the fetus.

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