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Fetus
placental transfer
triiodothyronine (T_3)

Absence of Placental Transfer of L-Triiodothyronine (T_3) in the Rat

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Summary

To detect placental transfer of L-triiodothyronine (T_3) in pregnant rats, we injected 1 μ Ci [125 I] T_3 on the 16th, 18th, and 20th day of gestation. Three hours after the injection, which corresponds to the equilibrium time determined by a method of constant infusion, the pregnant rats and their fetuses were killed. The [125 I] T_3 was extracted from the serum or the homogenate by butanol extractions and alkaline washes. The transfer rate was calculated from the quantity of [125 I] T_3 in the serum of the fetuses after 3 hr and from the maternal metabolic clearance rate (8.19 ± 0.45 ml/hr/100 g body weight; mean $\pm$ SEM). At the 16th day of gestation, the placental transfer of T_3 was $0.82 \pm 0.11\%$ of the total maternal clearance rate/litter weight; it was $1.05 \pm 0.25\%$ at the 18th day of gestation and $0.58 \pm 0.10\%$ at the 20th day of gestation. There were no significant differences between these results. The maternal T_3 concentration was 68.27 ± 20.6 ng/100 ml and its production rate was 5.57 ± 0.31 ng/hr/100 g; with these data we calculated a maternal-fetal T_3 transfer of 46 ± 6 pg/hr. Furthermore, there was no T_3 transfer observed when the mother received 1.9 μ g unlabeled T_3 , which led to a significant rise in maternal T_3 concentration (68.27 ± 20.6 ng to 102.23 ± 7.41 ng/100 ml; $P < 0.01$); there was no detectable T_3 in the fetal serum. From these results we conclude that there is minimal or no placental transfer of T_3 in the rat and that the hypothalamic-pituitary-thyroid axis of the fetus develops autonomously.

Speculation

The present data plus the fact that the rats are hypothyroid at birth support the concept of an autonomous development of the hypothalamic-pituitary-thyroid axis. It suggests that the rat can serve as a useful model for development of this axis in the human.

Studies in the sheep (7) and guinea pig (11) have shown that

the maternal-fetal placental passage of L-triiodothyronine (T_3) is minimal, and that the fetal hypothalamic-pituitary-thyroid axis develops autonomously. However, no clear evidence is available for the autonomy of the fetal hypothalamic-pituitary-thyroid axis in the rat, a species in which this ontogenetic maturation occurs during the neonatal period (3).

Many authors have previously reported studies of placental transfer of thyroxine (T_4) and T_3 in pregnant rats. Some reported a passage of T_4 and T_3 through the placenta (9, 10), whereas others concluded that T_4 and T_3 could not reach the fetus (4, 13).

The methods utilized by these authors were indirect methods which were more qualitative than quantitative. They did not measure quantitatively the T_3 or T_4 which reached the fetus.

In order to study placental transfer of T_3 we examined T_3 kinetics in order to ascertain the presence or absence of placental transfer of T_3 in the rat.

MATERIALS AND METHODS

Virgin Sprague-Dawley rats (150-200 g) were mated at the supply house (15) and shipped 2 weeks after mating. Pregnant animals were kept in separate cages in a sound-attenuated and temperature- and light-controlled room ($24 \pm 1^\circ$; 10 hr of darkness, 14 hr of light) and had free access to rat laboratory chow and water.

EQUILIBRIUM TIME

The equilibrium time of T_3 for pregnant rats was calculated by a method of constant infusion. Purified [125 I] T_3 (9 μ Ci/hr, specific activity: 475 μ Ci/ μ g) was injected through an indwelling jugular cannula for 3 hr at a rate of 1.0 cc/hr using a model 355 syringe pump from Sage Instruments.

Blood, 0.5 ml, was collected through a carotid cannula at 0, 15, 30, 45, 60, 90, 120, and 180 min after the infusion onset and replaced with an equivalent volume of 0.9% NaCl. The blood samples were centrifuged and the serum was frozen (-20°) until extraction. The butanol extraction was performed in duplicates

by the method described by Fisher *et al.* (5) which allows recovery of over 95% of organic iodide and less than 0.5% inorganic iodide. The butanol fraction was counted in a Nuclear Chicago gamma counter, model 1185.

The percentage of injected dose per liter was then calculated for each point and plotted.

METABOLIC CLEARANCE RATE

The metabolic clearance rate (MCR), half-life T_{0.5}, volume of distribution (VD), and fractional removal rate (K) of [¹²⁵I]T₃ were calculated after an intravenous bolus of the labeled hormone was administered.

Six pregnant rats were studied. One microcurie of purified [¹²⁵I]T₃ was injected in a bolus through a jugular cannula and each rat received Lugol intraperitoneally. Blood samples (0.5 ml) were collected 1.5, 3, 5, 7, 12, and 24 hr after injection through the same cannula and replaced with 0.5 ml physiologic saline. The blood was centrifuged and the serum frozen (-20°) until butanol extraction (5). The percentage of injected dose per liter was then calculated for each time and plotted.

PLACENTAL TRANSFER

Six pregnant rats were studied at 16, 18, and 20 days of gestation. Each rat received 2 μCi purified [¹²⁵I]T₃ intrajugularly and Lugol intraperitoneally. The controls received a saline injection.

Three hours after injection, the time which corresponds to the equilibrium time, the rats were killed and serum collected. The fetuses were delivered by hysterotomy and decapitated. The serum from fetuses of a single litter was pooled. The maternal and fetal sera were frozen (-20°) until butanol extraction (5).

In addition, six pregnant rats at each stage of gestation received 1.9 μg unlabeled T₃ intrajugularly and controls received a saline injection. Three hours after injection the mothers and their fetuses were killed by decapitation and the serum frozen until assay. The maternal and fetal T₃ concentrations were measured by the radioimmunoassay method of Chopra *et al.* (1)

CALCULATIONS

The T₃ half-life, its volume of distribution, disappearance rate, and MCR were calculated from the T₃ disappearance curve (Fig. 1) by the single compartment method as described by Sterling *et al.* (13).

$$VD = \frac{100}{\% \text{ injected dose/liter (t = 0)}} \times 1,000 \text{ ml/liter} \quad (1)$$

$$T_{0.5} = (y - b)/m \quad (2)$$

$$y = [\% \text{ injected dose/liter (t = 0)}]/2$$

$$b = \% \text{ injected dose/liter (t = 0)}$$

$$m = \text{curve slope}$$

$$K = \frac{\ln 2}{T_{0.5}} \quad (3)$$

$$MCR = VD \times K \quad (4)$$

The T₃ placental transfer rate (K_f) was calculated from T₃ radioactivity found in the fetal serum in relation to maternal MCR and was expressed as percentage of maternal clearance/litter weight (grams) using the following equation

$$\% K_f = \frac{[^{125}\text{I}]T_3 \text{ in fetal serum}}{MCR_m \times 3 \text{ hr} \times [^{125}\text{I}]T_3 \text{ in maternal serum}} \times 100$$

where: % K_f = T₃ transfer rate (in percentage of maternal clearance/litter weight) grams and MCR_m = maternal MCR. The data were analyzed statistically using Student's *t* test (12).

RESULTS

Figure 1 shows the [¹²⁵I]T₃ disappearance curve obtained from six pregnant rats. Each point comprises the mean ± SEM. From

this curve we calculated values for T_{0.5}, VD, K, and maternal MCR.

The T₃ half-life in pregnant rats was 9.74 ± 1.19 hr (0.406 ± 0.05 days). The volume of distribution was 126.8 ± 18.5 ml/100 g body weight; the disappearance rate (K), 0.0711 ± 0.0073 hr⁻¹ (1.7063 ± 0.176 day⁻¹) and the maternal MCR, 8.19 ± 0.449 ml/hr/100 g body weight (196.54 ± 10.8 ml/day/100 g body weight).

The equilibrium time, as calculated by a constant infusion technique, was 3.0 hr. The percentage of injected dose per liter rose gradually and reached a plateau 3 hr after infusion onset.

As seen in Table 1, the maternal-fetal T₃ transfer was 0.82 ± 0.11% of maternal clearance per litter weight at 16 days of gestation; 1.05 ± 0.25% at 18 days of gestation, and 0.58 ±

Fig. 1. Curve of the disappearance of L-[¹²⁵I]triiodothyronine (¹²⁵I-T₃) in serum of pregnant rats (n = 6). Each point is expressed as the mean ± SEM.

Table 1. Placental transfer of ¹²⁵I-labeled L-triiodothyronine([¹²⁵I]T₃) in rats at different periods of gestation

Maternal-fetal T ₃ transfer					
16 days		18 days		20 days	
% of maternal clearance	T ₃ , pg	% of maternal clearance	T ₃ , pg	% of maternal clearance	T ₃ , pg
0.912	51	0.531	29	0.634	35
0.603	34	0.683	38	0.575	32
0.731	41	1.621	128	0.128	7
1.226	68	1.669	93	0.642	36
0.944	52	0.760	42	0.604	34
0.528	29			0.889	49
0.824	46	1.053	66	0.579	32
±0.105 ¹	±6	±0.245	±19	±0.101	±6

¹ Mean ± SEM.

0.10% at 20 days of gestation. There were no significant differences among these results. The maternal serum concentration of T_3 was 68.27 ± 20.6 ng/100 ml; the maternal production rate for T_3 was therefore 5.57 ± 0.31 ng/hr/100 g body weight. From this production rate we calculated the net maternal-fetal transfer of T_3 : 46 ± 6 pg T_3 /hr at 16 days of gestation, 66 ± 19 pg/hr at 18 days of gestation, and 32 ± 6 pg/hr at 20 days of gestation. Since the number of fetuses per litter was 12.3 ± 1.56 at 16 days of gestation, 13.8 ± 0.66 at 18 days of gestation, and 8.5 ± 1.3 at 20 days of gestation, each 16-day-old fetus received 3.7 pg T_3 /hr from the mother, the 18 day-old fetuses received 4.8 pg T_3 /hr, and at 20 days of gestation each fetus received 3.8 pg T_3 /hr.

In the experiment in which we injected $1.9 \mu\text{g}$ unlabeled T_3 into the mother, the T_3 maternal concentration raised from normal values of 68.27 ± 20.6 ng/ml to 102.23 ± 7.41 ng/100 ml ($P < 0.01$). Despite this significant elevation of T_3 in the mother, we found no detectable T_3 in the fetal serum (Table 2). These data indicate that there is minimal or no placental transfer of T_3 and that this transfer does not increase toward the end of gestation.

DISCUSSION

The thyroid gland becomes functional in the rat between the 16th and 17th day of gestation, since at this stage, it has been shown to synthesize labeled T_4 from tracer doses of ^{131}I (8). For this reason we elected to study placental transfer at the 16th and subsequent days of gestation.

The elements of T_3 turnover, i.e., $T_{0.5}$, VD, K, and MCR in pregnant rats were not significantly different from the same parameters in normal adults (unpublished data), in agreement with the results of Dussault *et al.* (2) in the sheep; we were unable to measure these parameters in the fetuses.

Our equilibrium time (3 hr) is in agreement with the data of Nataf *et al.* (10), who found an equilibrium of T_3 between mother and fetuses 2 hr after the injection of labeled T_3 .

We found no significant differences in the maternal-fetal T_3 transfer at 16, 18, and 20 days of gestation; therefore, the hypothesis advanced by many authors (8, 10) that thyroid hormones cross the placental barrier more intensely at the end of gestation is not verified by our experiments.

To study T_3 kinetics in the mother-fetus system, we assumed a simplified two compartment model (see Appendix). The total quantity of T_3 cleared by the mother (8.19 ml/hr/100 g body weight) is divided in two ways: a fractional irreversible disappearance of T_3 (99.2%) and a fractional transfer rate from mother to fetus (0.8%). Thus, 8.12 ml/hr/100 g body weight is irreversibly cleared by the mother and 0.07 ml/hr/100 g body weight is transferred to the fetuses. With a maternal T_3 concentration of 68.2 ng/100 ml, we calculated that 46 pg T_3 is transferred to the fetuses per hour. The number of fetuses per litter was 11.4 ± 0.89 ; each fetus received approximately 4 pg T_3 /hr.

No data is available for T_3 kinetics in the fetal rat. For simplification we have assumed that the fetal turnover of T_3 is zero since T_3 is undetectable in the serum of fetuses. However, it must be noted that the estimated placental T_3 transfer in these experiments is maximal since we have used in our calculations the minimal quantity of ^{125}I T_3 recovered in maternal serum, that is, 3 hr.

Furthermore, verification for the minimal placental T_3 transfer is found in the last experiment. Despite the injection of unlabeled T_3 to the mothers who attained hyperthyroid levels, there is no rise in the fetal serum T_3 concentration; it is still below the limits of detection of the assay (< 5 ng/100 ml).

Our findings are in disagreement with previous reports which indicate a placental transfer of T_3 . These differences may be related to methodology. In contrast to the indirect methods previously described, we have used a direct method which indicates that there is little or no placental transfer of T_3 in the rat.

The present data indicate a minimal placental transfer of T_3 in the rat, in agreement with Varma *et al.* (14) and Fisher *et al.* (6), and clearly indicate that the fetal pituitary-thyroid axis is functioning autonomously of the maternal system.

CONCLUSION

Studies of T_3 kinetics and placental transfer were conducted in pregnant rats during the last days of gestation, using radioiodine labeled and unlabeled triiodothyronine (T_3). The maternal clearance rate of T_3 was 8.19 ± 0.45 ml/hr/100 g body weight. The placental transfer rate was $0.81 \pm 0.11\%$ of the maternal clearance/litter weight at the 16th day of gestation, $1.05 \pm 0.25\%$ at the 18th day of gestation, and $0.58 \pm 0.10\%$ at the 20th day of gestation. These results are not statistically different. We calculated that 47 pg T_3 /hr crossed the placenta and was distributed to the 11.4 ± 0.89 fetuses in each litter; that is, 4 pg T_3 /hr/fetus. There was no detectable T_3 in sera of the fetuses when the mother received unlabeled T_3 injection. Since little or no placental transfer of T_3 occurred during these studies, we conclude that the fetal rat hypothalamic-pituitary-thyroid axis develops autonomously.

APPENDIX

A simplified two compartment model of T_3 kinetics in the rat maternal-fetal system has been assumed (Fig. 2). The symbols in Figure 2 are defined as: Y_M = T_3 plasma concentration in the mother (percentage of injected dose per liter); Y_F = T_3 plasma concentration in the fetus (percentage of injected dose per liter); K_f = fractional transfer rate from mother to fetus (milliliters per hr per 100 g body weight); K_r = fractional transfer rate from fetus to mother; K_{MO} = fractional irreversible disappearance of T_3 from mother (milliliters per hr per 100 g body weight); K_{FO} = fractional irreversible disappearance of T_3 from fetus; VM = T_3 distribution volume in the mother (ml/100 g body weight); VF = T_3 distribution volume in the fetus (ml/100 g body weight).

Y_M and Y_F were calculated after extraction and were used to

Fig. 2. A simplified two compartment model for the transplacental passage of L-triiodothyronine in the rat. For explanation of symbols, see Appendix.

Table 2. Placental transfer of unlabeled L-triiodothyronine (T_3) in pregnant rats at 20 days of gestation

Group	No. of rats	Maternal		Fetal		
		Weight, g	Serum T_3 , ng/100 ml	No. of fetuses	Weight, g	Serum T_3 , ng/100 ml
Control (1 cc 0.9% saline)	10	359.9 $\pm 13.2^1$	68.27 ± 20.6	11.10 ± 0.7	46.55 ± 7.5	< 5
T_3 (1.90 μg)	13	399.2 ± 15.2	102.22 ± 7.4	11.38 ± 1.1	30.00 ± 4.7	< 5

¹ Mean $\pm$ SEM.

² $P < 0.01$ versus control.

calculate the percentage of maternal clearance which passed to fetuses.

$$\frac{Y}{MCR_M \times 3 \text{ hr} \times Y_M} \times 100 \quad MCR_M = K_{M0} + K_f$$

We calculated that K_f was $0.81 \pm 0.10\%$ of maternal clearance/litter weight (mean $\pm$ SEM). Therefore: $K_f = 0.066 \pm 0.007$ ml/hr/100 g body weight (0.045 ± 0.005 ng T_3 /hr/100 g body weight); $K_{M0} = 8.12 \pm 0.80$ ml/hr/100 g body weight (5.54 ± 0.54 ng T_3 /hr/100 g body weight); K_r , K_{F0} , and VF are unknown, but we may assume that they are equal to zero since there is no T_3 in the fetal serum.

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Amniotic fluid respiratory distress syndrome
lung surfactant serum plasminogen

Serum Plasminogen and Lung Surfactant in the Respiratory Distress Syndrome

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Summary

Plasminogen, total protein, and surface-active material were measured in amniotic fluid in 112 pregnancies at 11-42 weeks' gestation. In 65 of these pregnancies, cord blood was also analyzed for serum plasminogen and total protein. Plasminogen was detected in 25 of 114 amniotic fluid samples, and 23 came from pregnancies of less than 37 weeks' gestation. Plasminogen was found in 15 of 32 amniotic fluid samples from pregnancies with complications, but only in 10 of 80 "uncomplicated" pregnancies. The mean cord serum plasminogen was relatively constant in births or abortions of 17 to 30 weeks' gestation, but was present in increasing amounts in births of gestational ages from 30 to 40 weeks. The concentration of plasminogen in cord serum was directly related to the cord total protein ($r = 0.7513$, $P < 0.001$). The cord plasminogen concentration was significantly higher in infants with a positive foam stability test (5.6 ± 0.3 mg/100 ml) than in the combined group of infants with negative and intermediate tests (4.3 ± 0.16 , $P < 0.005$). However, infants with a positive foam stability also had a significantly greater

gestational age than infants with a negative or intermediate foam stability test. With one exception, infants with a low cord plasminogen (below 4 mg/100 ml) developed respiratory distress syndrome (RDS) only if amniotic fluid surfactant was low. The data suggest that low levels of serum plasminogen are correlated with severe lung disease only in the presence of surfactant deficiency.

Speculation

In infants that develop the respiratory distress syndrome, many serum proteins are found in diminished concentration in the umbilical cord blood. Interest has naturally focused on the antiproteins because a functional deficiency in these could contribute to fibrin deposition, a characteristic finding in hyaline membranes. However, it has been difficult to determine whether antiproteins are low because of increased consumption or as a result of nonspecific leakage of many proteins from the blood. Even the evidence of a selectively greater depletion of α_1 -antitrypsin than the larger molecule, plasminogen, or other cord