Ribosomal Protein Synthesis in 16 and 19 Day Gestation Fetuses of Hypothyroid Mothers (44063)

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Abstract. **Thyroid hormones are transported across the placenta. Thyroid hormone receptors are present in the midgestation rat fetus and fetal tissues selectively accumulate thyroid hormones prior to the onset of fetal thyroid function. It has not been demonstrated adequately that maternal thyroid hormones are essential for early fetal physiologic functions and neurological development. The present study compares the effects of maternal thyroid hormone versus fetal thyroid hormone on the regulation of rRNA and ribosomal protein synthesis in the developing fetus. This was accomplished by first comparing 16-day gestation (just prior to the onset of fetal thyroid function) fetuses of control and hypothyroid mothers. Then 19-day gestation (fetal thyroids are functional) fetuses of control and hypothyroid mothers were compared as development of fetal thyroid function lags in hypothyroid mothers. Rats made hypothyroid (Tx) by radiothyroidectomy were given replacement doses of thyroxine (T_4) until the day that they were placed with a male for mating. Control, Tx and growth hormone (GH)-treated Tx dams and their fetuses were sacrificed on either the 16th or 19th day of gestation. Ribosomes (r) were isolated from placentas and from fetal brains and livers and rRNA, total ^{14}C-leucine incorporation, and ^{14}C-leucine incorporation into ribosomal protein per microgram of rRNA were determined. On the 16th day of gestation, prior to the onset of fetal thyroid function, all three of the above metabolic parameters were reduced significantly below control levels in the placentas of Tx rats and in the brains and livers of their fetuses. This was true also for the fetal brains and livers of GH-treated Tx mothers. Development of fetal thyroid function lags in the Tx mother. rRNA, total ^{14}C-leucine incorporation, and ^{14}C-leucine incorporation into ribosomal protein per microgram of rRNA continue to be significantly depressed in these fetal tissues and placentas of Tx rats on the 19th day of gestation. In fact, brain protein synthesis falls further behind in fetuses of Tx dams at this gestational age when compared with control fetal brains. These data support the hypothesis that maternal thyroid hormones are important, by at least midgestation, for normal fetal physiologic development and support the concept that appropriate maturation of the fetal pituitary-thyroid plays a role in fetal brain protein synthesis and therefore neurological development.** [P.S.E.B.M. 1996, Vol 213]

It has been demonstrated that thyroid hormones cross the placenta in rats (1–4) and in humans (5) in physiological significant quantities. Thyroid hormone receptors are present in fetal rat tissues by midgestation (6), which is prior to the onset of fetal thyroid function. Fetal tissues also selectively accumulate thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3), by the gestational age that thyroid hormone receptors are present (7). These thyroid hormones must then be of maternal origin. Recent reviews (8, 9) and data are available that are indicative of a role for maternal thyroid hormone in early brain development. It has been demonstrated that maternal hypothyroidism (Tx) in sheep results in retarded fetal brain growth which is restored after the onset of fetal thyroid function (10). Also, fetal brain development is more severely retarded in the fetuses of ewes that are iodine deficient than in fetuses that are Tx at the time of onset

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of fetal thyroid function (11). In rats, made hypothyroid with propylthiouracil at midgestation, the data suggest a role for maternal thyroid hormones for normal brain cell development in the motor and mesencephalic nuclei of the trigeminal nerve (12). Additionally, initiating iodine therapy after the 5th month of gestation in iodine-deficient women does not prevent significant neurological damage in their offspring (13). Developmental differences exist between an altricial species such as the rat (gestation: 21–22 days) and prococial species such as sheep (gestation: 145–150 days) and the human (gestation: 280 days). Thyroid hormone receptors are present in fetal rat tissues on the 13–14th day postconception (dpc) (6, 14), which coincides with accumulation of T_4 and T_3 in fetal brain (7) but is 4 days prior to function of the fetal thyroid. In fetal lambs, brain thyroid hormone receptor increases from 50 to 80 dpc, with high saturation of the T_3 receptor by at least day 100 dpc (15–17). Thyroid hormone receptors are present in human fetal brain at 63–70 dpc, increase by 10-fold by 112 dpc, and show significant saturation at 63–91 dpc, also slightly before or coincident with development of the fetal thyroid (18, 19). It is of interest that in all three species the appearance and saturation of thyroid hormone receptors occur during the period of neuroblast multiplication and proliferation (17).

We have developed a rat animal model, without the use of goitrogens, that simulates mild to moderate maternal hypothyroidism (8).The rationale for the present experiments is based on the following data obtained from this animal model. The progenies of these Tx mothers have multiple metabolic and thyroid defects by the 22nd day of gestation and many of these persist into adult life (8). Among these deficits, one of the more sensitive parameters is the inability of various tissues such as liver and brain to utilize alanine in an appropriate manner (20). The fetuses and offspring of Tx mothers have significantly reduced gluconeogenesis from alanine as well as decreased ^{14}C-alanine incorporation into protein (20). Total protein levels are likewise depressed in the tissues of these progenies of Tx rats (20). This lack of protein synthesis is a contributing, if not primary, factor in the brain development deficits of these progenies that leads to their hyperactivity and inability to learn a maze (21). It has been established that learning and memory are dependent upon continuing new protein synthesis and turnover in the brain (22).

The present experiments were designed to determine the role played by each of the maternal and fetal thyroid components relative to their potential contributions in mid- to late gestational fetal development. Some dams were sacrificed at 16 days of gestation, when only maternal thyroid hormones are available for the placenta and fetal tissues. Other dams were sacrificed at 19 days of gestation. By 19 days of gestation, the fetal thyroid is functional and both maternal and fetal thyroid hormones are available (1). However, the thyroid function of fetuses of Tx mothers lags developmentally and the 19th day of gestation is prior to the time that thyroid function of these fetuses reach or surpass that of the control fetuses (1). We have shown previously that protein synthesis is compromised in the progenies of Tx mothers (20). In addition, it has been known for sometime that in adult rats early, specific, and sensitive parameters of thyroid hormone action are the regulation of nuclear and rRNA synthesis and the formation of new ribosomes (23). We have demonstrated also that the polyribosomal fraction is reduced in both brain and liver in neonates and adult offspring of Tx mothers (8, 24). We used the parameters of rRNA levels and the ability of the ribosomes to incorporate amino acid into protein as indicators of fetal responses to thyroid hormone. Our hypothesis is that the placenta and fetal tissues are dependent upon maternal thyroid hormones prior to the onset of fetal thyroid function and that late fetal development is dependent upon fetal thyroid hormone as well as maternal hormone.

Materials and Methods

Virgin Sprague-Dawley rats (140–160 g) were placed on a low iodine diet for 8 days. They were then injected ip with 300 μCi of ^{131}I, followed 8 hr later with a sc injection of 10 μg of T_4 to block the release of the ^{131}I from the thyroid. We have shown previously that this results in total radiothyroidectomy in all rats (7, 21, 24, 25). Replacement T_4 (1.0 μg/100 gbw/day) was instituted until the animals were free of the ^{131}I. Forty percent of the Tx rats received 0.5 IU of purified ovine growth hormone (GH)/day (NIADDK-OGH-12) (Tx + GH) starting on the 10th day of pregnancy because endogenous GH secretion declines rapidly following the induction of Tx. These rats served as a second control group to help distinguish between the lack of thyroid hormones and the deficiency of GH. Other animals obtained at the same time were maintained as controls. After the ^{131}I was cleared from the Tx animals, the females were placed with males for mating. T_4 replacement was discontinued on that day. In previous experiments, when we either sacrificed the animals on the 22nd day of gestation or allowed them to go through parturition and used neonates and adult offspring, T_4 administration was discontinued on Day 1 of pregnancy (1, 7, 21, 24, 25). In the present experiments, as mating is not predictable, especially in the Tx females, the length of time between the last day of T_4 replacement and the date of sacrifice was variable. This protocol was nevertheless used to make sure that a significant degree of maternal hypothyroidism was induced in these short-term experiments of 16 and 19

days. Radioimmunoassay of serum T_4 was used to determine the degree of maternal hypothyroidism.

At the time of sacrifice, the mothers were quickly anesthetized with ether and blood collected from the vena cava. The fetuses were removed from the uterus, and placentas, fetal brains, and livers were collected and weighed. All tissues were analyzed in duplicate. The placental samples each contained 1.0 g, and the fetal brains and livers were divided equally into two samples. The ribosomal extraction from these tissues was accomplished using modified methods of Dreskin and Kostyo (26). The same extraction media were used. The initial tissue homogenization was in a medium (E) composed of 50 m*M* Tris, 250 m*M* KCl, and 10 m*M* $MgCl_2$ at a pH of 7.6. The mixture was centrifuged at 13,000*g* for 15 min. The supernatant was filtered through glass wool, and Lubrol-WX (10% in 10 m*M* $MgCl_2$) and deoxycholic acid (10% in distilled H_2O) were added to give a 0.5% Lubrol and a 1.0% deoxycholic acid mixture. This mixture was placed in a teflon-glass homogenizer and gently mixed with three strokes of the pestle, while standing for 30 min. Each sample was then diluted with three volumes of Medium E and centrifuged for 2 hr at 78,000*g*. The supernatant was discarded, the pellet rinsed with Medium E and transferred to the homogenizing tube in a total volume of 2 ml of Medium E. The pellet was then resuspended by a few gentle strokes of the pestle. The resuspended pellets were then layered on 5 ml of 1 *M* sucrose in Medium E and centrifuged through this medium for 2.5 hr at 105,000*g*. The supernatant was discarded and the pellet washed with Medium G which was composed of 50 m*M* Tris, 150 m*M* KCl, and 10 m*M* $MgCl_2$ at a pH of 7.6. The pellet was suspended in Medium G, frozen with liquid nitrogen and stored in an ultra freezer. Ribonuclease inhibitor (5 units) from placenta was utilized and all procedures were carried out in an ice bath.

At the time of ribosomal assay for ^{14}C-leucine incorporation, the pellets were resuspended in Medium J composed of 50 m*M* Tris, 150 m*M* KCl, and 5 m*M* $MgCl_2$ at a pH of 7.6. The samples were allowed to stand for 5 min in an ice bath and then centrifuged at 3000*g* for 10 min. Aliquots of the diluted pellet resuspensions, based on the amount of tissue available, were added to an incubation mixture that contained a concentration of each amino acid at the level that is present in the respective tissues of control rats. We have determined the amino acid profiles in these types of animals previously (8) and although the serum amino acid levels of control fetuses and experimental fetuses of Tx mothers are not different, the tissue levels of amino acids are reduced significantly in the progenies of Tx mothers. Therefore, as a safeguard against overall amino acid availability being the only factor inhibiting protein synthesis in these animals, tissue concentrations of amino acids as determined in control fetuses were used in the incubation samples. These levels of amino acids were contained in an incubation medium that contained 2 μCi of ^{14}C-leucine, 5 mg of the respective tissue cytosolic protein, 20 m*M* ATP, 2.0 m*M* GTP, 40 m*M* phosphocreatine, 2.0 μg of creatinine phosphokinase, 50 m*M* Tris, 150 m*M* KCl, 10 m*M* $MgCl_2$, and 2 m*M* 2-mercaptoethanol at a pH of 7.6. This incubation medium differs significantly from the original (26), and the composition was decided upon only after numerous trial incubations. This medium maximized ^{14}C-leucine incorporation into ribosomal protein during a 45-min incubation period at 37°C. The reaction was terminated with 2 ml of cold 10% trichloracetic acid (TCA) which contained 1% unlabeled leucine. Five milligrams of cytosolic protein were added to cooled samples as carrier protein. Appropriate zero time samples and nonspecific binding of tracer were always determined. The newly synthesized labeled ribosomal protein was extracted and purified by the methods of Lowry *et al.* (27). The activity of ^{14}C-leucine incorporated was determined by liquid scintillation counting and the results expressed as DPM of ^{14}C-leucine incorporated per microgram of rRNA per gram of tissue.

The tissue cytosolic proteins were prepared according to the methods of Dreskin and Kostyo (26). The rRNA content of the ribosomal preparations was determined by the methods of Fleck and Monro (28) as modified by Castles and Woole (29). Yeast RNA was used as a standard, and the results are expressed as microgram of rRNA per gram of tissue.

The statistical analyses were done using ANOVA followed by Tukey's W-procedure (honestly significant difference procedure). Use of a Table U allows for the comparison of multiple groups that have unequal sample sizes (30) (Chapter 9.7, pp 225–246).

Results

Maternal body weight gain was reduced significantly in both Tx and GH-treated Tx mothers in both 16- and 19-day gestation animals (Table I). The values were a live weight recorded at the time of sacrifice and reflect the reduced conceptus of these Tx rats. The numbers of live fetuses, at sacrifice in both 16- and 19-day gestation Tx animals, were decreased significantly, and the numbers of fetal resorptions were increased significantly (Table I). The number of days between the last day of T_4 replacement and sacrifice are also shown in Table I. These values were not different, because of the high variability of mating of these animals which is induced by the decline of their serum and tissue thyroid hormone concentrations.

The placental weights of Tx rats were reduced significantly compared with controls. The administration of GH did not compensate for the maternal T_4 defi-

Table I. Reproductive Performance of Control, Hypothyroid (Tx), and Growth Hormone (GH)–Treated Tx Rats at 16 and 19 Days of Gestation

Experimental groups	Maternal body weight gain (g)	Number of live fetuses	Number of fetal resorptions	Days from end T_4 administration to sacrifice
Day 16 of gestation				
I. Control	73 ± 3.3 (17)[a]	13.0 ± 0.94	0.73 ± 0.24	—
II. Tx	29 ± 1.3 (20)	10.4 ± 0.78	2.57 ± 0.36	36.4 ± 2.8
III. Tx + GH	27 ± 2.7 (12)	8.5 ± 0.56	3.67 ± 0.67	29.0 ± 4.0
Statistics	II, III < I	II, III < I	I < II, III	
Day 19 of gastation				
I. Control	114 ± 5.4 (15)	15.0 ± 0.60	0.44 ± 0.24	—
II. Tx	52 ± 4.7 (16)	8.6 ± 1.08	3.30 ± 0.78	40.6 ± 3.7
III. Tx + GH	45 ± 3.9 (12)	8.5 ± 0.89	3.83 ± 0.53	38.2 ± 3.2
Statistics	II, III < I	II, III < I	I < II, III	

Note. Values are expressed as mean ± SEM. $P < 0.05$.
[a] Values in parentheses are the number of mothers and litters.

ciency at these gestational stages of 16 and 19 days (Table II). The brain and liver weights of fetuses of Tx mothers were also reduced significantly, and again GH therapy to the mother was ineffective in correcting brain and liver growth at these stages of gestation (Table II). Table II also contains the maternal serum T_4 levels (μg/dl). The mean serum T_4 levels of Tx and GH-treated Tx mothers were reduced to only 19%–23% of control values. In fact, no individual Tx animal had a serum T_4 level greater than 33% of the control mean.

The data on 16- and 19-day gestation brain are presented in Table III. At both gestational ages, the rRNA content per gram of brain was decreased significantly in fetuses of both groups of Tx mothers. Total ^{14}C-leucine incorporated into brain ribosomal protein of fetuses of Tx dams was significantly below that of the control fetal brains. When the data are expressed as ^{14}C-leucine incorporated into ribosomal protein per microgram of rRNA, the fetal brains of both groups of Tx mothers at both gestational ages show a significant depression of protein synthesis compared with control fetal brain (Table III). GH treatment of the Tx mother resulted in a mild beneficial effect.

The effects of maternal Tx on ^{14}C-leucine incorporation into protein in 16- and 19-day gestation fetal liver produced overall effects similar to those on the brain (Table IV). The micrograms of rRNA per gram of liver, the total ^{14}C-leucine incorporated into ribosomal protein per gram of liver, and the ^{14}C-leucine incorporated into ribosomal protein per microgram of rRNA were all reduced in the fetuses of the two groups of Tx mothers compared with control fetal livers. This was true for both 16- and 19-day gestation fetuses. GH treatment of the Tx mother again had a beneficial effect on these experimental parameters (Table IV).

GH treatment of the Tx mother resulted in a restoration of placental rRNA levels to values within the range of control placentas, whereas the placentas of nontreated Tx mothers had a significant reduction of placental rRNA (Table V). However, the total ^{14}C-leucine incorporated into placental ribosomal protein

Table II. Placental and Brain and Liver Weights of 16- and 19-Day Gestation Fetuses and Serum T_4 of Control, Hypothyroid (Tx), and Growth Hormone (GH)–Treated Tx Mothers

Experimental groups	Placental weights (g)	Brain weights (mg)	Liver weights (mg)	Maternal serum T_4 (μg/dl)
Day 16 of gestation				
I. Control	0.33 ± 0.007 (17)[a]	94.3 ± 5.12	27.8 ± 1.32	3.00 ± 0.06
II. Tx	0.26 ± 0.008 (20)	79.8 ± 3.97	16.7 ± 0.68	0.68 ± 0.03
III. Tx + GH	0.23 ± 0.003 (12)	70.5 ± 2.20	18.2 ± 0.48	0.64 ± 0.03
Statistics	II, III < I	II, III < I	II, III < I	II, III < I
Day 19 of gestation				
I. Control	0.54 ± 0.012 (15)	129.9 ± 4.27	160.0 ± 8.24	2.98 ± 0.07
II. Tx	0.47 ± 0.011 (16)	97.9 ± 2.94	117.4 ± 3.12	0.58 ± 0.03
III. Tx + GH	0.45 ± 0.010 (12)	100.8 ± 3.26	101.7 ± 4.80	0.56 ± 0.04
Statistics	II, III < I	II, III < I	II, III < I	II, III < I

Note. Values are expressed as mean ± SEM $P < 0.05$.
[a] Values in parentheses are the number of mothers and litters.

Table III. Ribosomal (r) RNA Levels and ^{14}C-Leucine Incoporation into Protein by Brain Ribosomes at 16 and 19 Days of Gestation in Fetuses of Control, Hypothyroid (Tx), and Growth Hormone (GH)–Treated Tx Mothers

Experimental groups	μg rRNA/g brain	^{14}C-leucine incorporated/g brain (DPM × 10^6/g)	DPM ^{14}C-leucine incorporated by ribosomes/μg rRNA/g brain
Day 16 of gestation			
I. Control	1357 ± 44 (34)[a]	2.93 ± 0.03	2133 ± 39
II. Tx	1055 ± 27 (40)	1.62 ± 0.03	1503 ± 12
III. Tx + GH	1128 ± 36 (24)	2.00 ± 0.06	1751 ± 40
Statistics	II, III < I	II < III < I	II < III < I
Day 19 of gestation			
I. Control	1493 ± 27 (30)	5.07 ± 0.06	3417 ± 44
II. Tx	1139 ± 26 (32)	1.95 ± 0.08	1776 ± 46
III. Tx + GH	1249 ± 35 (24)	3.20 ± 0.09	2560 ± 47
Statistics	II, III < I	II < III < I	II < III < I

Note. Values are expressed as means ± SEM $P < 0.05$.
[a] Values in parentheses are the number of observations.

Table IV. Ribosomal (r) RNA Levels and ^{14}C-Leucine Incorporation into Protein by Liver Ribosomes at 16 and 19 Days of Gestation in Fetuses of Control, Hypothyroid (Tx), and Growth Hormone (GH)–Treated Tx Mothers

Experimental groups	μg rRNA/g liver	^{14}C-leucine incorporated/g liver (DPM × 10^6/g)	DPM ^{14}C-leucine incorporated by ribosomes/μg rRNA/g liver
Day 16 of gestation			
I. Control	3150 ± 42 (34)[a]	7.51 ± 0.16	2382 ± 31
II. Tx	2444 ± 52 (40)	4.11 ± 0.09	1695 ± 29
III. Tx + GH	2718 ± 70 (24)	5.60 ± 0.12	2072 ± 35
Statistics	II < III < I	II < III < I	II < III < I
Day 19 of gestation			
I. Control	3657 ± 41 (30)	5.16 ± 0.08	1413 ± 17
II. Tx	2843 ± 68 (32)	2.71 ± 0.05	962 ± 19
III. Tx + GH	2995 ± 65 (24)	3.59 ± 0.09	1202 ± 12
Statistics	II, III < I	II < III < I	II < III < I

Note. Values are expressed as mean ± SEM. $P < 0.05$.
[a] Values in parentheses are the number of observations.

was depressed significantly in both groups of Tx mothers at both gestational periods. This resulted in a decrease of ^{14}C-leucine incorporated into placental ribosomal protein per microgram of rRNA in both groups of Tx mothers at both gestational times (Table V).

Discussion

As these studies were several days shorter in duration than most of our previous experiments, we altered the experimental protocol. When T_4 replacement was maintained until Day 1 of pregnancy in Tx rats, the mean maternal serum T_4 level was still 39% of the control value on day 22 of gestation (1). Discontinuing T_4 replacement on the day that females were placed with males for mating induced a variable period between the last day of T_4 replacement therapy and the day of sacrifice. All Tx rats did eventually mate and carry some viable fetuses to the 16th and 19th days of gestation even though the mean maternal serum T_4 levels of the two groups of Tx rats were decreased to only approximately 20% of the control level. We did accomplish our purpose of inducing a more severe maternal hypothyroxinemia without the use of a goitrogen or iodine deficiency, which would have involved more direct effects on the fetuses.

When considering the general data on maternal body weight gain, etc., the question arises as to what degree these effects of maternal Tx are due to undernutrition. In full gestational term experiments, we have measured the nutritional level of the Tx mothers in metabolic cages, then food-restricted control mothers to this nutritional level. The food restriction of control mothers had no significant effects on any of the reproductive parameters, on fetal-neonatal tissue weights, on tissue protein content, or on the behavioral patterns of their adult offspring (21). GH treatment

Table V. Ribosomal (r) RNA Levels and ^{14}C-Leucine Incorporation into Protein by Placental Ribosomes at 16 and 19 Days of Gestation in Control, Hypothyroid (Tx), and Growth Hormone (GH)–Treated Tx Rats

Experimental groups	μg rRNA/g placenta	^{14}C-leucine incorporated/ g placenta (DPM × 10^6/g)	DPM ^{14}C-leucine incorporated by ribosomes/μg rRNA/g placenta
Day 16 of gestation			
I. Control	1446 ± 25 (34)[a]	5.21 ± 0.12	3612 ± 68
II. Tx	1053 ± 20 (40)	2.49 ± 0.05	2380 ± 37
III. Tx + GH	1428 ± 32 (24)	3.48 ± 0.07	2455 ± 64
Statistics	II < III, I	II < III < I	II, III < I
Day 19 of gestation			
I. Control	1439 ± 23 (30)	4.90 ± 0.09	3440 ± 62
II. Tx	1109 ± 30 (32)	2.54 ± 0.07	2293 ± 36
III. Tx + GH	1413 ± 47 (24)	3.58 ± 0.11	2538 ± 34
Statistics	II < III, I	II < III < I	II, III < I

Note. Values are expressed as mean ± SEM. $P < 0.05$.
[a] Values in parentheses are the number of observations.

of the Tx mother neither alleviated any of the effects of this level of maternal hypothyroxinemia on reproductive performance nor protected the fetuses of these Tx mothers from deficits in growth of brain and liver. When one considers also that the number of fetuses per litter is reduced significantly in the Tx rats, the dependence upon thyroid hormone is magnified. These data in rats are supported by previous studies in rats (31–40) and sheep (10, 11) and demonstrate a role for maternal thyroid hormone in the general development of fetal physiological systems. It has been demonstrated that maternal hypothyroxinemia induced by either surgical Tx or by iodine deficiency results in thyroid hormone deficiency in their fetuses both before the onset of fetal thyroid function and up to the 21st dpc (31, 32). The induction of a more severe maternal hypothyroidism resulted in T_4 and T_3 levels in embryotrophoblasts that were either barely or nondetectable (31, 32). Using a somewhat more sensitive HPLC method for measuring iodothyronines in tissues and body fluids (33), we were able to show depressed but selective accumulation of T_4 and T_3 in the brains of 13 dpc fetuses (7). It has been shown also that even in late gestation (21 dpc) 17.5% of the T_4 in fetal tissues is of maternal origin (4). These investigators (34–36) have determined also that T_4, not T_3, is preferentially taken up by the fetal brain and therefore adequate transfer of maternal T_4 is essential to protect the brain from thyroid hormone deficiency. However, both the fetuses (37) and the progenies (38) of Tx mothers have tissue deficiencies of iodothyronine 5′ deiodinase. Also, we have shown that, although the brain concentrations of T_4 and T_3 are dramatically reduced in the 10- to 60-day-old offspring of Tx rats, they have brain rT_3 concentrations that are increased by 200%–300% over those of control progenies (39) indicating that maternal Tx results in an abnormal metabolism of iodothyronines in the tissues of their offspring. This in turn may result in altered brain maturation (40) and learning impairment (21).

The major thrust of the present study was to determine the possible role of maternal thyroid hormones on a specific function in the fetus that results in the diffuse alterations of fetal development that are seen in the offspring of Tx mothers and especially their neurological development by evaluating the thyroid hormone effects on rRNA activity in a cell free system. The brains of the fetuses of the Tx mothers show such sensitivity as the micrograms of rRNA are decreased significantly below that of the control fetuses at 16 days of gestation. This occurs prior to the onset of fetal thyroid function. The total ^{14}C-leucine incorporated into ribosomal protein and incorporated into ribosomal protein per microgram of rRNA is reduced even more drastically in the fetuses of Tx mothers. We have found previously that, although the polyribosomal fraction is reduced significantly in neonates and adult offspring of Tx mothers (8, 24), there are other factors that result in a lack of ribosomal participation in protein synthesis. We have not as yet identified these factors.

Following the onset of fetal thyroid function (the data on 19-day gestation fetuses), the brains of fetuses of Tx mothers continue to show this sensitivity to thyroid hormone deficiency. Although the fetuses of Tx mothers hypersecrete T_4 by term, at 19 days of gestation they have reduced serum levels of T_4 compared with control fetuses (1). As concerns fetal brain, this mid- to late gestation lag in the development of fetal thyroid function further exacerbates the ability of brain ribosomes to incorporate ^{14}C-leucine into protein. ^{14}C-leucine incorporated into ribosomal protein fell from 70% to 52% of the control value at 16 and 19 days of gestation, respectively. This is indicative of a

further lack of participation of ribosomes in protein synthetic activity as the actual μg of rRNA showed a mild increase between 16 and 19 days of gestation. GH replacement to the Tx mother has a mildly beneficial effect on fetal brain as concerns these effects but the protein synthetic activity of brain ribosomes continues to be depressed below that of control fetuses.

The data obtained on fetal liver in the present study are in some aspects similar to that of the brain. Livers of fetuses of Tx mothers had a significantly reduced capacity for ribosomal protein synthesis. However, the degree of the protein synthetic deficit was similar at the two gestational ages and showed no further decline from control levels in the 19-day gestation fetuses. The developmental pattern of rRNA and the ribosomal protein synthetic activity was considerably different between the two tissues. There was a significant increase in the micrograms of rRNA in fetal liver between the 16th and 19th days of gestation. However, the synthesis of ribosomal protein per microgram of rRNA actually declined. This cannot be explained on the basis of some rate-limiting factor in the incubation media, as multiple trial experiments were conducted to ensure optimal conditions for the maximal incorporation of ^{14}C-leucine into ribosomal protein.

We have shown previously that the tissue concentration and content of T_4 and T_3 in 13- and 16-day gestation fetuses of Tx mothers are reduced significantly and that there is selective accumulation of hormone in brain and liver at these gestational ages (7). The presence of tissue T_3 receptors has been demonstrated in the rat fetal tissues even prior to these fetal ages (6). These data coupled with the present studies suggest that there are midgestation actions of maternal T_4 and T_3 in fetal brain development and that fetal T_4 and T_3 participate as well in this brain development during the latter stage of gestation.

It could be argued that the effects of maternal Tx on fetal development are mediated *via* poor placental development and perhaps decreased placental blood-flow and transport. Placental development in both groups of Tx mothers is impaired (Tables II and V). In untreated Tx mothers, placental rRNA concentration is decreased and ribosomal protein synthesis is impaired. GH treatment of the Tx mother corrects only the parameter of placental rRNA concentration. The placentas are less well developed and there are still significant deficits of placental ribosomal protein synthesis in these GH-treated Tx mothers. Nevertheless, it seems unlikely that these effects observed in the fetuses of Tx mothers would be solely due to decreased substrate supply to the fetus because of poor placental development as the present studies were conducted on a cell free system where substrate availability would no longer be a limiting factor.

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