Maternal Thyroid Function is the Major Determinant of Amniotic Fluid 3,3',5'-Triiodothyronine in the Rat

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ABSTRACT

3,3',5'-triiodothyronine, (rT3), is easily measured in human amniotic fluid (AF) during the second and third trimesters. To determine if AF rT3 levels are maintained by either maternal or fetal thyroid function, or both, models of fetal hypothyroidism (FH), maternal hypothyroidism (MH), and combined maternal and fetal hypothyroidism (MFH) were developed in pregnant rats. Hormone analyses of maternal and fetal serum and AF were performed at term. Thyroxine (T4) and 3,3',5'-triiodothyronine (T3) were not detectable in the sera and AF of term fetuses in all groups. MH rats were prepared by administration of methimazole to the dams, and in some experiments, by maternal thyroidectomy and a low iodine diet as well. In the MFH groups from the three experiments serum thyrotropin (TSH) was markedly elevated in the dams and in the fetuses. FH rats were prepared by administering T4 by various routes to dams treated according to the MFH protocols and serum TSH was elevated in fetal serum. Analysis of FH maternal serum T4, T3, and TSH concentrations suggested mild maternal hyperthyroidism or hypothyroidism depending upon the schedule of T4 administration. The MH groups were prepared by maternal thyroidectomy and in all experiments the fetuses had normal serum TSH concentrations. The degree of maternal hypothyroidism in the MH and MFH groups was equivalent. The mean concentration of AF rT3 in normal rats in three experiments was 28.4 ± 2.5 ng/dl (± SEM). In the three experiments, AF rT3 was undetectable or markedly reduced in the MH and MFH rats and was normal in the FH rats. These results in the amniotic fluid could not be explained by transfer of rT3 from fetal serum to the AF because fetal serum rT3 concentrations in these various models did not correlate with AF rT3 concentration. Furthermore, infusion of large doses of rT3 in MFH dams resulted in a 35-fold elevation in maternal serum rT3 concentration, a twofold elevation in fetal serum rT3 concentration, and only a minimal increase in AF rT3. These studies demonstrated that, in the rat, the maternal thyroid hormone has the dominant role in maintaining AF rT3, whereas little effect of fetal thyroid status on AF rT3 could be demonstrated. Transfer of maternal rT3 or of fetal rT3 derived from maternal T4 to the AF do not appear to be the mechanisms whereby the maternal thyroid maintains AF rT3.

INTRODUCTION

Patients with congenital hypothyroidism have profound retardation of physical, mental, and motor development (1). A number of workers have sought a means to diagnose congenital hypothyroidism antenataly. In 1975 Chopra and Crandell (2) observed that amniotic fluid (AF) 3,3',5'-triiodothyronine (reverse T3, rT3)1 concentrations were easily detectable during human pregnancy, in contrast to low or undetectable concentrations of thyroxine (T4) and 3,3',5'-triiodothyronine (T3). These observations have been confirmed by others (3-6) and aroused considerable interest, suggesting that AF rT3 measurements might be a useful means of diagnosing fetal hypothyroidism in utero (7, 8).

1Abbreviations used in this paper: AF, amniotic fluid; BW, body weight; C group, control group; FH group, fetal hypothyroidism group; LID, low-iodine diet; MFH group, combined maternal-fetal hypothyroidism group; MH group, maternal hypothyroidism group; MMI, methimazole; rT3, reverse T3; T3, 3,3',5'-triiodothyronine; T4, thyroxine; TSH, thyrotropin; Tx, thyroidectomized.
Since AF rT₃ could be dependent upon either maternal or fetal thyroid function or both, studies were carried out in experimental rat models of fetal hypothyroidism, maternal hypothyroidism, and combined maternal-fetal hypothyroidism. The ovine species was not chosen for these studies since term AF rT₃ concentrations are low in the sheep (8). Previously published rat (9–17) and primate (18, 19) models of fetal hypothyroidism were not used since hypothyroidism had been induced at birth rather than in the fetus or combined maternal-fetal hypothyroidism resulted from the treatment regimen. When an effort was made to prepare isolated fetal hypothyroidism, pharmacologic doses of T₄ were administered to dams receiving antithyroid drugs and/or a low-iodine diet (LID) (20). Moreover, at the time many of these studies were performed, it was not possible to characterize the hormonal status of mother and fetus by specific and sensitive measurements of T₄, T₃, and thyrotropin (TSH) concentrations.

In this report several treatment regimens for inducing isolated maternal or fetal hypothyroidism in the rat are described. The hormonal status of these models have been characterized and they have been used to derive information on the source of the relatively large concentration of rT₃ in AF. The data demonstrate that reduced AF rT₃ in these models are indicative of maternal, rather than fetal, hypothyroidism.

METHODS

Virgin Sprague-Dawley 10-wk old female rats (Charles River Breeding Laboratories, Wilmington, Mass.) were used in these studies. They were cycled and mated in our laboratory (experiment 1) or received from the supplier during the 1st wk of pregnancy (experiments 2 and 3). Methimazole (MMI, tapazole) was a gift from Eli Lilly & Co. (Indianapolis, Ind.). LID was purchased from Teklad Test Diets (Madison, Wis.) and Alzet osmotic minipumps were obtained from Alza Corp. (Palo Alto, Calif.). Stable rT₃ was purchased from Washington Reference Laboratory (Washington, D.C.) and L-T₄ from the Sigma Chemical Co. (St. Louis, Mo.).

Treatment protocols. Pregnant rats were housed in separate cages with free access to Purina Lab Chow and water in a temperature (21±1°C)-and light (700–800)-controlled room. Specific treatment, including TX, was begun on day 8 in experiment 1, day 7 in experiment 2, and day 11 in experiment 3. Table I shows the protocols for inducing combined maternal-fetal hypothyroidism (MFH group), fetal hypothyroidism (FH group), or maternal hypothyroidism (MH group). In each experiment, untreated pregnant rats served as the control group (C group). All animals received 1% calcium lactate in the drinking water since hypocalcemia was common in the thyrodectomized (Tx) rats.

In experiment 1, the MFH group was prepared by the addition of 0.05% MMI to the drinking water. In experiment 2, the MFH group, in addition to receiving MMI in the drinking water, underwent maternal TX under light ether anesthesia and received a LID. The protocol for the MFH group in experiment 3 was identical to experiment 2 except that the LID was not used. In experiment 3 an additional group of MFH rats were studied. These rats received a subcutaneous infusion via osmotic minipump of 10 μg rT₃/100 g body wt (BW) during the last 5 d of pregnancy. In each experiment, the FH group received identical treatment as the MFH group except that physiologic doses of T₄ were administered to the dams. In experiment 1, the FH dams were killed 24 h after the last daily T₄ injection; in experiment 2 they were killed at the time T₄ was being infused via osmotic minipump; and in experiment 3 they were sacrificed 12 h after the last b.i.d. (twice daily) injection of T₄. In all experiments the MH groups were prepared by maternal TX.

Sample collection. On the 21st d of pregnancy, the dams were killed by decapitation and trunk blood was collected. The abdomen was then rapidly opened and AF was obtained by amniocentesis from the amniotic sac of each fetus and combined into one pool for each litter. The fetuses were then quickly delivered by hysterotomy, decapitated, and the trunk blood from each fetus collected and combined into one pool for each litter. Samples were stored at –20°C after centrifugation.

Hormone analyses. Serum and AF T₄ and T₃ concentrations were measured by radioimmunoassay (21). rT₃ was measured by radioimmunoassay using anti-rT₃ antibody supplied by Dr. Sidney Leskowitz (Tufts University, Boston, Mass.) in a final dilution of 1/2,800. ¹²⁵I-labeled rT₃ was purchased from New England Nuclear (Boston, Mass.). Labeled rT₃ was displaced from anti-rT₃ antibody in a semilogarithmic fashion with the intercept at 50% displacement being 85 ng/dl. The crossreaction of 3,3'2.3', and T₃ was 0.5, 0.03, and 0.12%, respectively. In each assay the least detectable concentration of rT₃ was considered to be that giving a response 2 SD away from the 0 dose. To correct for serum and amniotic fluid effects on the assay, hormone-free rat serum or amniotic fluid prepared by charcoal extrac-

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>Protocols Used for Inducing Maternal and/or Fetal Hypothyroidism</th>
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<tbody>
<tr>
<td>Group</td>
<td>Exp. 1 (8)</td>
</tr>
<tr>
<td>MFH</td>
<td>MMI*</td>
</tr>
<tr>
<td>FH</td>
<td>MMI</td>
</tr>
<tr>
<td>MH</td>
<td>Tx</td>
</tr>
<tr>
<td>C</td>
<td>None</td>
</tr>
</tbody>
</table>

T₄ was administered in experiment 1 from day 8 to day 20 as a single s.c. injection of 1.5 μg/100 g BW; in experiment 2 via s.c. osmotic pump from day 7 to day 21 in a daily dose of 1.2 μg/100 g BW; in experiment 3 from day 11 to day 20 in s.c. doses of 0.75 μg/100 g BW BID. Number in parentheses indicates the day maternal treatment was begun.

* 0.05% MMI in drinking water.


The results are summarized in Tables II-V and Fig. 1. The three experiments are discussed separately since different regimens were used in each to prepare the MFH and FH groups.

**Experiment 1 (Table II)**

**Maternal thyroid status.** Maternal hypothyroidism was induced in the appropriate groups since maternal serum TSH was significantly elevated and T4 and T3 significantly lower in the MFH and MH groups as compared with the group C rats. Maternal hypothyroidism was greater in the MH group than in the MFH group as judged by maternal serum TSH concentrations (MH, 335±20 μU/ml vs. MFH, 243±14 μU/ml, P < 0.01).

There was no evidence of maternal hypothyroidism in the FH group because maternal serum TSH was suppressed and serum T4 and T3 concentrations were not significantly different from the corresponding values in the C group.

**Fetal thyroid status.** Fetal hypothyroidism was present in the MFH and FH groups since fetal serum TSH was significantly elevated in these groups as compared to group C rats. In this and subsequent experiments, T4 and T3 were not detectable in fetal serum in any group, including the normal C group. Therefore, fetal serum T4 and T3 measurements could not be used to characterize fetal thyroid status. In the MH group there was no evidence of fetal hypothyroidism because fetal serum TSH concentrations in the MH and C groups were similar. The degree of fetal

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**TABLE II**

*Maternal Serum, Fetal Serum, and AF Concentrations of T4, T3, rT3, and TSH in Experiment 1*

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Specimen</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MFH (14)</td>
</tr>
<tr>
<td>T4, μg/dl</td>
<td>MS</td>
<td>&lt;0.3*</td>
</tr>
<tr>
<td>T3, ng/dl</td>
<td>MS</td>
<td>12.6±1.0</td>
</tr>
<tr>
<td>rT3, ng/dl</td>
<td>FS</td>
<td>14.3±2.6</td>
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<tr>
<td></td>
<td>AF</td>
<td>&lt;11.5*</td>
</tr>
<tr>
<td>TSH, μU/ml</td>
<td>MS</td>
<td>243±14*</td>
</tr>
<tr>
<td></td>
<td>FS</td>
<td>150±7*</td>
</tr>
</tbody>
</table>

MS, maternal serum; FS, fetal serum. Values are mean±SE. The following hormones were undetectable in all groups: FS T4 < 0.3, FS T3 < 0.3, AF TSH < 34, AF T4 < 10. Numbers in parentheses represent the number of dams, litters, and AF in each group.

* P < 0.01 vs. C group.

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hypothyroidism in the FH group was not as severe as fetal hypothyroidism in the MFH group since fetal serum TSH was significantly lower (FH, 116±12 μU/ml vs. MFH, 150±7, P < 0.05). This may be due to the transplacental passage of small quantities of T₄, which would be accentuated by the administration of a single daily bolus of T₄ resulting in transient supra-physiological concentrations of plasma T₄ in the dam.

Maternal serum rT₃. Concentrations of rT₃ in maternal serum were similar in all rats except the MH group in which rT₃ was undetectable.

Fetal serum rT₃. Fetal serum rT₃ concentrations were significantly reduced in the MFH, FH, and MH rats as compared to the C group. In MFH rats, fetal serum rT₃ was significantly lower than in the FH or MH groups (P < 0.01).

AF rT₃. rT₃ was undetectable in AF in the MFH and MH groups. In contrast, AF rT₃ was not reduced in the FH group (FH, 34.3±3.0 ng/dl vs. C, 33.1±4.6).

Experiment 2 (Table III)

In this experiment, several modifications of experiment 1 were made. In the FH groups, dams underwent Tx and were given a LID as well as MMI in the drinking water. They were replaced with T₄ by osmotic minipumps rather than with a single daily injection of T₄, to minimize the transplacental passage of T₄, which may have occurred in experiment 1. In addition, Tx and a LID as well as MMI were included in the treatment regimen of the MFH groups since, in experiment 1 where MMI alone was used, maternal hypothyroidism was not as great in the MFH as in the MH group.

Maternal thyroid status. As in experiment 1, analysis of maternal serum T₄, T₃, and TSH documented the presence of maternal hypothyroidism in the MFH and MH groups. In contrast to experiment 1, maternal serum TSH concentrations in these groups did not differ significantly. Maternal hypothyroidism was present in the FH group since the serum T₃ concentration was significantly lower and the serum TSH concentration significantly elevated in the FH as compared to the C group. However, maternal serum T₄ concentrations in the FH and C groups were not significantly different from each other.

Fetal thyroid status. The treatment regimen induced a similar degree of fetal hypothyroidism in the MFH and FH groups in that fetal serum TSH concentrations were equally elevated. As in experiment 1, there was no evidence of fetal hypothyroidism in the MH group in that the fetal serum TSH concentration in this group was normal.

Maternal serum rT₃. rT₃ concentrations ranged from undetectable to 41.5 ng/dl in the C group and were undetectable in the other three groups.

Fetal serum rT₃. As noted in experiment 1, the fetal serum rT₃ concentration in the MH rats was intermediate between values observed in the C group and the MFH group and was significantly lower in the MFH as compared to the MH group (P < 0.01). However, unlike experiment 1, the fetal serum rT₃ concentration was not decreased in the FH group.

AF rT₃. As in experiment 1, AF rT₃ was normal in the FH group and markedly decreased in the MH and MFH groups. Unlike experiment 1, where AF rT₃ was undetectable in these latter two groups, AF rT₃ was detectable and significantly higher in the MH as compared with the MFH rats (MH, 13.5±0.8 vs. MFH, 8.4±0.9, P < 0.05).

### Table III

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Specimen</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₄, μg/dl</td>
<td>MS</td>
<td>FH (11)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;0.5*</td>
</tr>
<tr>
<td>T₃, ng/dl</td>
<td>MS</td>
<td>6.1±0.7*</td>
</tr>
<tr>
<td>rT₃, ng/dl</td>
<td>MS</td>
<td>&lt;10*</td>
</tr>
<tr>
<td></td>
<td>FS</td>
<td>13.9±1.1*</td>
</tr>
<tr>
<td></td>
<td>AF</td>
<td>9.2±0.5*</td>
</tr>
<tr>
<td>TSH, μU/ml</td>
<td>MS</td>
<td>337±23*</td>
</tr>
<tr>
<td></td>
<td>FS</td>
<td>186±17*</td>
</tr>
</tbody>
</table>

MS, maternal serum; FS, fetal serum. Values are mean±SE. The following hormones were undetectable in all groups: FS < 0.5, FS T₄ < 5.4, AF T₄ < 0.4, AF T₃ < 10, AF TSH < 40. Numbers in parentheses represent the number of dams, litters, and AF in each group.

* P < 0.01 vs. C group.

† P < 0.02 vs. C group.
Experiment 3 (Table IV)

In experiment 1, mild maternal hyperthyroidism was present in the FH group and fetal hypothyroidism was not as marked in this group as compared to the MFH group. Modification of the treatment regimen in experiment 2 corrected these problems but resulted in some maternal hypothyroidism in the FH group as judged by an elevation in maternal serum TSH. In experiment 3, a third regimen for T4 administration was evaluated in the FH group, namely b.i.d. injections of T4.

Maternal thyroid status. As in experiments 1 and 2, analysis of maternal serum T4, T3, and TSH concentrations documented the presence of maternal hypothyroidism in the MFH and MH groups. The degree of maternal hypothyroidism in these two groups was similar since maternal serum T4, T3, and TSH concentrations did not differ significantly. Maternal serum T4 and T3 concentrations were significantly lower in the FH as compared to the C group. The elevation in maternal serum TSH in the FH group as compared to the C group only achieved significance if Fisher's correction for multiple comparisons was not used (P < 0.01).

Fetal thyroid status. As noted in experiment 2, fetal serum TSH concentrations were elevated to a similar degree in the FH and MFH groups. Fetal serum TSH, as in previous experiments, was normal in the MH group.

Maternal serum rT3. Maternal serum rT3 concentrations were frequently undetectable and no difference was observed between the four groups.

Fetal serum rT3. Fetal serum rT3 concentrations were significantly lower in the FH as compared to the C group. Serum rT3 was undetectable in the MFH group.

AF rT3. AF rT3 concentrations were low or undetectable in the MFH and MH groups. In contrast, AF rT3 was similar in the FH and C groups. Identical findings were noted in the two previous experiments.

In Fig. 1, analysis of AF rT3 data in the experimental groups is displayed as a percentage of that observed in the control group. It is evident that AF rT3 is markedly decreased in the presence of maternal hypothyroidism irrespective of fetal thyroid status (MH and MFH), but is normal when fetal hypothyroidism alone is present.

rT3 infusion in MFH dams

Table V shows that the constant infusion of large quantities of rT3 into MFH dams for 5 d resulted in a marked elevation of maternal serum rT3 concentrations (315±26 ng/dl). In spite of the fact that maternal

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Specimen</th>
<th>Group</th>
<th>T4, µg/dl</th>
<th>T3, ng/dl</th>
<th>rT3, ng/dl</th>
<th>TSH, μU/ml</th>
</tr>
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<tbody>
<tr>
<td>T4, µg/dl</td>
<td>MS</td>
<td>FH (12)</td>
<td>1.2±0.11</td>
<td>&lt;0.4*</td>
<td>2.3±0.1</td>
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</tr>
<tr>
<td>T3, ng/dl</td>
<td>MS</td>
<td>MH (10)</td>
<td>48.2±8.2*</td>
<td>15.2±1.8*</td>
<td>87.4±4.2</td>
<td></td>
</tr>
<tr>
<td>rT3, ng/dl</td>
<td>MS</td>
<td>C (9)</td>
<td>18.6±5.8*</td>
<td>19.0±1.0</td>
<td>11.6±0.8*</td>
<td></td>
</tr>
<tr>
<td>TSH, μU/ml</td>
<td>MS</td>
<td></td>
<td>434±20*</td>
<td>&lt;6</td>
<td>319.1±2.8</td>
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</tr>
<tr>
<td>TSH, μU/ml</td>
<td>FS</td>
<td></td>
<td>6.4±1.1*</td>
<td>&lt;0.01</td>
<td>69±4</td>
<td></td>
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</table>

MS, maternal serum; FS, fetal serum. All values are mean±SE. The following hormones were undetectable in all groups: FS T4 < 0.4, FS T3 < 4.0, AF T4 < 0.4, AF T3 < 10, AF TSH < 34. Numbers in parentheses represent the number of dams, litters, and AF in each group.

* P < 0.01 vs. C group.
† P < 0.02 vs. C group.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>MS (ng/dl)</th>
<th>FS (ng/dl)</th>
<th>AF (ng/dl)</th>
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<tbody>
<tr>
<td>Normal dams</td>
<td>9</td>
<td>9±2</td>
<td>19±1</td>
<td>32±3</td>
</tr>
<tr>
<td>MFH dams</td>
<td>11</td>
<td>7±2</td>
<td>&lt;3.5</td>
<td>7.0</td>
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<tr>
<td>MFH dams + rT3*</td>
<td>7</td>
<td>315±26</td>
<td>35±4</td>
<td>12±2</td>
</tr>
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</table>

Values are mean±SE. * rT3 infused via osmotic minipump in a dose of 10 μg/100 g per d for the last 5 d of pregnancy.
serum rT₃ concentration was elevated 35 times greater and fetal serum rT₃ concentrations were increased approximately twice that observed in normal rats, AF rT₃ concentration remained far below that found in normal rats.

DISCUSSION

Models of maternal hypothyroidism, fetal hypothyroidism, and combined maternal-fetal hypothyroidism have been described in the rat. Combined maternal-fetal hypothyroidism in MMI-treated dams (experiment 1) was confirmed by the presence of undetectable T₄, markedly reduced T₃, and elevated TSH concentrations in maternal serum, and an elevated TSH concentration in fetal serum. MMI crosses the placenta and therefore blocks hormone formation in both the maternal and fetal thyroid. In experiment 2 maternal Tx and LID was added to the MMI regimen and in experiment 3 dams were treated with MMI and Tx. It might be expected that the degree of both maternal and fetal hypothyroidism in the MFH groups would have been greatest in experiment 2 because the most severe antithyroid regimen was used in this experiment. However, MFH/C ratios for serum TSH concentration in both maternal and fetal serum were lower in experiment 2 than in experiments 1 or 3.

Induction of maternal hypothyroidism was consistently achieved by thyroidectomy. In all three experiments, thyroidectomy resulted in undetectable T₄, markedly depressed T₃, and four- to eightfold elevations of TSH concentration in maternal serum. Moreover, the degree of maternal hypothyroidism achieved was similar to that in the MFH groups since maternal serum TSH concentrations in the MFH group never exceeded that in the MH group. There was no evidence of fetal hypothyroidism in the MH rats.

The greatest problems were encountered in preparing a model of isolated fetal hypothyroidism. The FH groups were treated according to the MFH protocols. In addition, T₄ was administered to the dams in a quantity similar to that reported by Gray and Galton (23) to be the physiological replacement dose. However, using the three T₄ regimens, it was not possible to both restore serum TSH to normal in the dam and leave the elevated serum TSH in the fetus unaffected. In experiment 1 the single daily dose of 1.5 µg T₄/100 g BW corrected maternal hypothyroidism but also decreased fetal serum TSH, although this latter value was still markedly elevated. In experiment 2, the constant infusion of 1.2 µg T₄/100 g BW, and in experiment 3, the b.i.d. administration of 0.75 µg T₄/100 g BW, did not alter fetal serum TSH concentration but maternal serum TSH remained elevated. In experiment 2, despite the fact that maternal serum T₄ concentration was normal in the T₄ infused hypothyroid dams, maternal serum TSH concentration was not decreased and serum T₃ concentration was only partially restored to normal. These findings in the T₄ infused rats were not unexpected for two reasons. First, Conners and Hedge (24) have reported that the constant infusion of a daily dose of T₃ in hypothyroid rats, which normalizes the serum T₃ concentration does not suppress serum TSH. The dose of infused T₄ that does suppress TSH secretion is associated with an elevated serum T₃ concentration. Because the only source of rT₃ is T₄, it was important in the present experiment to maintain a normal serum T₄ concentration. This was achieved with the dose of 1.2 µg T₄/100 g BW. Second, in the rat, thyroid secretion of T₃ contributes far more to the serum T₃ concentration than in man and the failure to restore serum T₃ to normal with an infusion of T₄ that normalizes the serum T₄ concentration in the thyroidectomized rat reflects the absence of this thyroid contribution to circulating T₃. Finally, the present observations support the findings of Tonooka and Greer (25) that there is a narrow range between the quantity of T₄ required to induce minimal suppression of serum TSH and that required to suppress TSH secretion to normal in thyroidectomized rats. Since T₄ and T₃ were undetectable in normal fetal serum, it was not possible to use these measurements to assess the degree to which fetal hypothyroidism had been induced in the FH groups. The presence of small quantities of T₄ and T₃ remaining in fetal serum in the FH rats could not be ruled out. However, it is likely that significant depletion of T₄ and T₃ occurred since the fetal serum TSH concentration was strikingly elevated.

Various estimates have been made concerning the quantity of maternal T₄ that crosses the placenta in the rat. Gray and Galton (23) concluded that both the fetal and maternal thyroid glands contribute to the maintenance of serum T₄ levels in the fetus but were not able to make a quantitative estimate of the maternal contribution (23). More recently, Dussault and Coloumbe (26) estimated that <1% of T₄ in fetal serum at term is derived from the maternal thyroid. Data in the MH groups in the present study also suggest that the amount of maternal T₄ that crosses the placenta under physiological conditions is negligible since 21-d fetal serum TSH concentrations were not affected by thyroidectomizing the dams early in pregnancy.

An important reason for developing these models was to determine whether alterations in AF rT₃ could predict fetal hypothyroidism in the rat and to determine the source of rT₃ in the AF. The present data indicates that at term the majority of AF rT₃ is derived from maternal rather than fetal sources and does not provide evidence that AF rT₃ can be used to predict fetal thyroid function in the rat. In the presence
of maternal hypothyroidism, AF rT₃ concentrations were strikingly reduced irrespective of fetal thyroid function. Moreover, AF rT₃ was not decreased in the presence of fetal hypothyroidism alone, offering further evidence that the fetal thyroid contributes little to AF rT₃. Because the extent of the decrease in serum T₄ concentration in the hypothyroid fetus could not be determined, complete absence of T₄ in the fetus might have resulted in a decrease in AF rT₃. Although absolute fetal thyroid agenesis in the rat may, therefore, be associated with reduced AF rT₃ concentrations, the above data suggests that this reduction would be small.

In contrast to the findings in amniotic fluid, fetal serum rT₃ concentrations were, in general, reduced and to the same degree in the presence of either maternal or fetal hypothyroidism. When both maternal and fetal hypothyroidism were present, fetal serum rT₃ concentrations were even lower or undetectable. These data suggest an approximately equal contribution to serum rT₃ concentration by maternal and fetal sources of T₄. It is unlikely, therefore, that AF rT₃ concentration is directly dependent upon fetal serum rT₃.

In view of these complex findings, it is most difficult at the present time to define the mechanism(s) whereby maternal thyroid secretion of T₄ directly affects the concentration of rT₃ in amniotic fluid. Direct transfer of rT₃ from maternal serum or by way of the fetus to the AF is unlikely, in that the constant infusion of large quantities of rT₃ to MFH rats resulted in a 35-fold increase in maternal serum rT₃ concentration, a twofold increase in fetal serum rT₃ concentration, and only a minimal effect on AF rT₃. These findings also tend to rule out a contribution to AF of fetal serum rT₃, which could conceivably arise from the placental conversion of maternal T₂ to rT₃. Placental transfer of T₄ from dam to fetus under physiologic conditions is minimal (26), therefore it is unlikely that sufficient maternal T₂ would reach the fetus and be a major source of AF rT₃ generated in fetal liver or kidney. Two other possibilities remain. Direct transfer of T₄ from the dam to the amniotic fluid and the conversion of this T₄ to rT₃ by amniotic fluid cells in the amniotic fluid, or the amniotic sac. In the sheep, evidence for in vivo and in vitro conversion of T₄ to rT₃ in amniotic fluid has been reported (27). This might be possible because, in the sheep, it has been suggested that a small quantity of T₂ is directly transferred from mother to the amniotic fluid (28). The final, and most attractive hypothesis, is that maternal T₂ is deiodinated to rT₃ at a tissue site in the region of the maternal compartment and the amniotic sac with direct transfer of this rT₃ into the amniotic fluid. A possible site for this conversion to rT₃ and its subsequent transfer into the amniotic fluid is the chorion. This tissue, as demonstrated in a recent study by Murphy (29), is metabolically active, has different enzyme systems than the placental cotyledons, is frequently adherent to the maternal decidua, and is separated from the amniotic cavity only by the thin amnion which is permeable to other hormones such as the adrenal steroids. Very preliminary data do suggest that rat and human chorion convert T₄ to rT₃ but not to T₃ as determined by incubation of chorion homogenates with I⁻⁴I-T₄ and analysis of the labeled products by descending paper chromatography in hexane-tertiary amyl alcohol-2N NH₃ (1:5:6, vol/vol/vol).

In summary, the present studies demonstrate that models of maternal-fetal hypothyroidism, maternal hypothyroidism, and fetal hypothyroidism, that approximate the ideal have been developed in the rat. rT₃ is present in fetal rat serum and AF. The concentration is approximately fivefold lower in fetal rat serum at term than that reported in human cord blood (30) and about threefold lower in rat AF than that reported in the human at term (2–6). We are unaware of any other reports demonstrating rT₃ in rat amniotic fluid. It seems evident that, in the rat, AF rT₃ derives primarily from maternal sources of T₄. In view of these results in the rat and the recent report that AF rT₃ values were not useful in predicting fetal hypothyroidism in two patients (31), the possibility must be considered that, in man, AF rT₃ does not reflect fetal thyroid status.

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