

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. MFH 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 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 antenatally. 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).

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¹ *Abbreviations 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 rT_3 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 rT_3 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 T_4 were administered to dams receiving anti-thyroid 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 T_4 , T_3 , 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 rT_3 in AF. The data demonstrate that reduced AF rT_3 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 rT_3 was purchased from Washington Reference Laboratory (Washington, D. C.) and L- T_4 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 ($21 \pm 1^\circ\text{C}$) 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 thyroidectomized (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

TABLE I
Protocols Used for Inducing Maternal
and/or Fetal Hypothyroidism

| Group | Maternal treatment | | |
|-------|--------------------|---------------------------|--------------------|
| | Exp. 1 (8) | Exp. 2 (7) | Exp. 3 (11) |
| MFH | MMI* | MMI TX LID | MMI TX |
| FH | MMI T_4 | MMI Tx LID T_4 | MMI Tx T_4 |
| MH | Tx | Tx | Tx |
| C | None | None | None |

T_4 was administered in experiment 1 from day 8 to day 20 as a single s.c. injection of $1.5 \mu\text{g}/100 \text{ g BW}$; in experiment 2 via s.c. osmotic pump from day 7 to day 21 in a daily dose of $1.2 \mu\text{g}/100 \text{ g BW}$; in experiment 3 from day 11 to day 20 in s.c. doses of $0.75 \mu\text{g}/100 \text{ g BW}$ BID. Number in parentheses indicates the day maternal treatment was begun.

* 0.05% MMI in drinking water.

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 $10 \mu\text{g } rT_3/100 \text{ g body wt (BW)}$ 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 T_4 were administered to the dams. In experiment 1, the FH dams were killed 24 h after the last daily T_4 injection; in experiment 2 they were killed at the time T_4 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 T_4 . 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 T_4 and T_3 concentrations were measured by radioimmunoassay (21). rT_3 was measured by radioimmunoassay using anti- rT_3 antibody supplied by Dr. Sidney Leskowitz (Tufts University, Boston, Mass.) in a final dilution of 1/2,800. ^{125}I -labeled rT_3 was purchased from New England Nuclear (Boston, Mass.). Labeled rT_3 was displaced from anti- rT_3 antibody in a semilogarithmic fashion with the intercept at 50% displacement being 85 ng/dl. The crossreaction of $3,3',T_2,T_3$, and T_4 was 0.5, 0.03, and 0.12%, respectively. In each assay the least detectable concentration of rT_3 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-

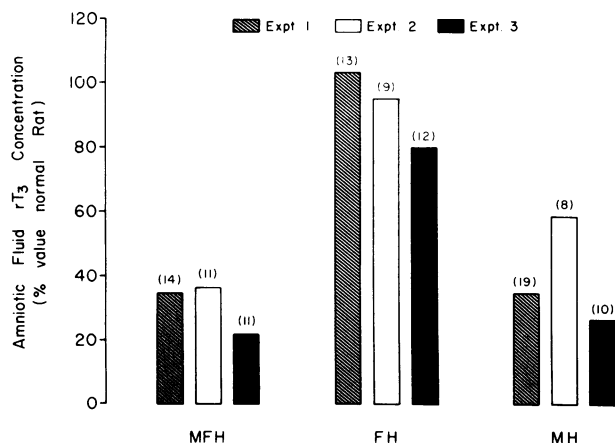


FIGURE 1 The effect of maternal and fetal thyroid status on amniotic fluid rT₃ concentrations. All values for AF rT₃ were undetectable in the MFH and MH groups in experiment 1 and in the MFH group in experiment 3. When all values in a group were undetectable, the mean value assigned to the group was the value for the lower limit of detectability of the assay. Numbers in parentheses represent number of AF specimens in each group.

tion were added to the respective standard curves in amounts identical to that used for the unknown samples. Serum and AF TSH was measured by radioimmunoassay using materials kindly supplied by the Hormone Distribution Officer, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Md. All assays were performed in duplicate and all samples from each experiment for each hormone were assayed concurrently. All results are expressed as the mean \pm SEM. In those assays in which some values were detectable and a minority of samples were below the sensitivity of the assay, the undetectable samples were assigned a value at the lower limit of sensitivity.

Statistical analyses. The data was analyzed by Kendall's test with Fisher's corrections for multiple comparisons when comparisons were made between groups in which

hormone concentrations were undetectable (22). When hormone concentrations were all detectable, values were analyzed by one way analysis of variance followed by the Newman-Keuls test to detect differences of means (22).

RESULTS

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 rats 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 μ U/ml vs. MFH, 243 ± 14 μ U/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 C group 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

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

| Hormone | Specimen | Group | | | |
|-----------------------------|----------|-----------------|-----------------|-----------------|----------------|
| | | MFH (14) | FH (13) | MH (19) | C (10) |
| T ₄ , μ g/dl | MS | <0.3* | 1.2 \pm 0.1 | <0.3* | 1.8 \pm 0.3 |
| T ₃ , ng/dl | MS | 12.6 \pm 1.0 | 21.6 \pm 2.1 | 16.8 \pm 1.5* | 25.5 \pm 1.8 |
| rT ₃ , ng/dl | MS | 14.3 \pm 2.6 | 15.4 \pm 2.1 | <6.0* | 19.5 \pm 5.5 |
| | FS | 16.1 \pm 0.9* | 24.2 \pm 0.9* | 24.4 \pm 0.8* | 31.3 \pm 2.3 |
| | AF | <11.5* | 34.3 \pm 3.0 | <11.5* | 33.1 \pm 4.6 |
| TSH, μ U/ml | MS | 243 \pm 14* | <32 | 335 \pm 20* | 47.1 \pm 6.0 |
| | FS | 150 \pm 7* | 116 \pm 12* | <32 | <32 |

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

* $P < 0.01$ vs. C group.

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 μ U/ml vs. MFH, 150 ± 7 , $P < 0.05$). This may be due to the transplacental passage of small quantities of T_4 , which would be accentuated by the administration of a single daily bolus of T_4 resulting in transient supra-physiological concentrations of plasma T_4 in the dam.

Maternal serum rT_3 . Concentrations of rT_3 in maternal serum were similar in all rats except the MH group in which rT_3 was undetectable.

Fetal serum rT_3 . Fetal serum rT_3 concentrations were significantly reduced in the MFH, FH, and MH rats as compared to the C group. In MFH rats, fetal serum rT_3 was significantly lower than in the FH or MH groups ($P < 0.01$).

AF rT_3 . rT_3 was undetectable in AF in the MFH and MH groups. In contrast, AF rT_3 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 T_4 by osmotic minipumps rather than with a single daily injection of T_4 to minimize the transplacental passage of T_4 , 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 group 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, analy-

sis of maternal serum T_4 , T_3 , 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 T_3 concentration was significantly lower and the serum TSH concentration significantly elevated in the FH as compared to the C group. However, maternal serum T_4 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 rT_3 . rT_3 concentrations ranged from undetectable to 41.5 ng/dl in the C group and were undetectable in the other three groups.

Fetal serum rT_3 . As noted in experiment 1, the fetal serum rT_3 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 rT_3 concentration was not decreased in the FH group.

AF rT_3 . As in experiment 1, AF rT_3 was normal in the FH group and markedly decreased in the MH and MFH groups. Unlike experiment 1, where AF rT_3 was undetectable in these latter two groups, AF rT_3 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
Maternal Serum, Fetal Serum, and AF Concentrations of T_4 , T_3 , rT_3 , and TSH in Experiment 2

| Hormone | Specimen | Group | | | |
|--------------------|----------|------------------|------------------|------------------|----------------|
| | | MFH (11) | FH (9) | MH (8) | C (15) |
| T_4 , μ g/dl | MS | <0.5* | 3.6 ± 0.2 | <0.5* | 3.6 ± 0.3 |
| T_3 , ng/dl | MS | $6.1 \pm 0.7^*$ | $23.5 \pm 3.3^*$ | $9.5 \pm 0.9^*$ | 52.3 ± 3.6 |
| rT_3 , ng/dl | MS | <10* | <10† | <10† | 18.1 ± 2.9 |
| | FS | $13.9 \pm 1.1^*$ | 30.6 ± 1.7 | $21.1 \pm 0.8^*$ | 34.8 ± 1.8 |
| | AF | $9.2 \pm 0.5^*$ | 22.0 ± 1.4 | $13.5 \pm 0.8^*$ | 23.1 ± 1.2 |
| TSH, μ U/ml | MS | $337 \pm 23^*$ | $323 \pm 45^*$ | $279 \pm 27^*$ | 72 ± 5 |
| | FS | $186 \pm 17^*$ | $187 \pm 8^*$ | 88 ± 16 | 83 ± 3 |

MS, maternal serum; FS, fetal serum. Values are mean \pm SE. The following hormones were undetectable in all groups: FS $T_3 < 5.4$, AF $T_4 < 0.4$, AF $T_3 < 10$, 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.

TABLE IV
Maternal Serum, Fetal Serum, and AF Concentrations of T₄, T₃, rT₃,
and TSH in Experiment 3

| Hormone | Specimen | Group | | | |
|-------------------------|----------|-----------|-----------|-----------|----------|
| | | MFH (11) | FH (12) | MH (10) | C (9) |
| T ₄ , µg/dl | MS | <0.4* | 1.2±0.1† | <0.4* | 2.3±0.1 |
| T ₃ , ng/dl | MS | 20.4±3.2* | 48.2±8.2* | 15.2±1.8* | 87.4±4.2 |
| rT ₃ , ng/dl | MS | 7.2±2.0 | 13.7±3.0 | 5.9±2.0 | 8.7±1.8 |
| | FS | <3.5* | 11.6±0.8* | 18.6±5.8 | 19.0±1.0 |
| | AF | 6.4±1.1* | 25.4±1.8 | 8.3±1.5* | 31.9±2.8 |
| TSH, µU/ml | MS | 308±16* | 112±22 | 343±20* | <60 |
| | FS | 170±11* | 157±10* | <60 | 69±4 |

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.

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 V
Effect of rT₃ Infusion in MFH Dams on Maternal Serum,
Fetal Serum, and AF rT₃ Concentrations

| Group | n | rT ₃ | | |
|------------------------------|----|-----------------|------|------|
| | | MS | FS | AF |
| | | (ng/dl) | | |
| Normal dams | 9 | 9±2 | 19±1 | 32±3 |
| MFH dams | 11 | 7±2 | <3.5 | <7.0 |
| MFH dams + rT ₃ * | 7 | 315±26 | 35±4 | 12±2 |

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 rT_3 concentration was elevated 35 times greater and fetal serum rT_3 concentrations were increased approximately twice that observed in normal rats, AF rT_3 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 T_4 , markedly reduced T_3 , 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 T_x and LID was added to the MMI regimen and in experiment 3 dams were treated with MMI and T_x . 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 T_4 , markedly depressed T_3 , 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, T_4 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 T_4 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 $1.5 \mu\text{g } T_4/100 \text{ g BW}$ corrected maternal hypothyroidism but also decreased fetal serum TSH, although this latter value was still markedly elevated. In experiment 2, the constant infusion of $1.2 \mu\text{g } T_4/100 \text{ g BW}$, and in experiment 3, the b.i.d. administration of $0.75 \mu\text{g } T_4/100 \text{ g BW}$, did not alter fetal serum TSH concentration but maternal serum TSH remained elevated. In experiment 2, despite the fact that maternal serum T_4 con-

centration was normal in the T_4 infused hypothyroid dams, maternal serum TSH concentration was not decreased and serum T_3 concentration was only partially restored to normal. These findings in the T_4 infused rats were not unexpected for two reasons. First, Connors and Hedge (24) have reported that the constant infusion of a daily dose of T_3 in hypothyroid rats, which normalizes the serum T_3 concentration does not suppress serum TSH. The dose of infused T_3 that does suppress TSH secretion is associated with an elevated serum T_3 concentration. Because the only source of rT_3 is T_4 , it was important in the present experiment to maintain a normal serum T_4 concentration. This was achieved with the dose of $1.2 \mu\text{g } T_4/100 \text{ g BW}$. Second, in the rat, thyroid secretion of T_3 contributes far more to the serum T_3 concentration than in man and the failure to restore serum T_3 to normal with an infusion of T_4 that normalizes the serum T_4 concentration in the thyroidectomized rat reflects the absence of this thyroid contribution to circulating T_3 . Finally, the present observations support the findings of Tonooka and Greer (25) that there is a narrow range between the quantity of T_4 required to induce minimal suppression of serum TSH and that required to suppress TSH secretion to normal in thyroidectomized rats. Since T_4 and T_3 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 T_4 and T_3 remaining in fetal serum in the FH rats could not be ruled out. However, it is likely that significant depletion of T_4 and T_3 occurred since the fetal serum TSH concentration was strikingly elevated.

Various estimates have been made concerning the quantity of maternal T_4 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 T_4 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 T_4 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 T_4 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 rT_3 could predict fetal hypothyroidism in the rat and to determine the source of rT_3 in the AF. The present data indicates that at term the majority of AF rT_3 is derived from maternal rather than fetal sources and does not provide evidence that AF rT_3 can be used to predict fetal thyroid function in the rat. In the presence

of maternal hypothyroidism, AF rT_3 concentrations were strikingly reduced irrespective of fetal thyroid function. Moreover, AF rT_3 was not decreased in the presence of fetal hypothyroidism alone, offering further evidence that the fetal thyroid contributes little to AF rT_3 . Because the extent of the decrease in serum T_4 concentration in the hypothyroid fetus could not be determined, complete absence of T_4 in the fetus might have resulted in a decrease in AF rT_3 . Although absolute fetal thyroid agenesis in the rat may, therefore, be associated with reduced AF rT_3 concentrations, the above data suggests that this reduction would be small.

In contrast to the findings in amniotic fluid, fetal serum rT_3 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 rT_3 concentrations were even lower or undetectable. These data suggest an approximately equal contribution to serum rT_3 concentration by maternal and fetal sources of T_4 . It is unlikely, therefore, that AF rT_3 concentration is directly dependent upon fetal serum rT_3 .

In view of these complex findings, it is most difficult at the present time to define the mechanism(s) whereby maternal thyroid secretion of T_4 directly affects the concentration of rT_3 in amniotic fluid. Direct transfer of rT_3 from maternal serum or by way of the fetus to the AF is unlikely, in that the constant infusion of large quantities of rT_3 to MFH rats resulted in a 35-fold increase in maternal serum rT_3 concentration, a twofold increase in fetal serum rT_3 concentration, and only a minimal effect on AF rT_3 . These findings also tend to rule out a contribution to AF of fetal serum rT_3 , which could conceivably arise from the placental conversion of maternal T_4 to rT_3 . Placental transfer of T_4 from dam to fetus under physiologic conditions is minimal (26), therefore it is unlikely that sufficient maternal T_4 would reach the fetus and be a major source of AF rT_3 generated in fetal liver or kidney. Two other possibilities remain. Direct transfer of T_4 from the dam to the amniotic fluid and the conversion of this T_4 or rT_3 by amniotic fluid cells in the amniotic fluid, or the amniotic sac. In the sheep, evidence for in vivo and in vitro conversion of T_4 to rT_3 in amniotic fluid has been reported (27). This might be possible because, in the sheep, it has been suggested that a small quantity of T_4 is directly transferred from mother to the amniotic fluid (28). The final, and most attractive hypothesis, is that maternal T_4 is deiodinated to rT_3 at a tissue site in the region of the maternal compartment and the amniotic sac with direct transfer of this rT_3 into the amniotic fluid. A possible site for this conversion to rT_3 and its subsequent transfer into the amniotic fluid is the chorion. This tissue, as demonstrated in a re-

cent 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 T_4 to rT_3 but not to T_3 as determined by incubation of chorion homogenates with ^{125}I - T_4 and analysis of the labeled products by descending paper chromatography in hexane-tertiary amyl alcohol-2N NH_3 (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. rT_3 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 rT_3 in rat amniotic fluid. It seems evident that, in the rat, AF rT_3 derives primarily from maternal sources of T_4 . In view of these results in the rat and the recent report that AF rT_3 values were not useful in predicting fetal hypothyroidism in two patients (31), the possibility must be considered that, in man, AF rT_3 does not reflect fetal thyroid status.

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