Maternal Diabetes Mellitus, a Rat Model for Nonthyroidal Illness: Correction of Hypothyroxinemia with Thyroxine Treatment Does Not Improve Fetal Thyroid Hormone Status

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ABSTRACT

Maintenance of normal maternal thyroxinemia prevents severe triiodothyronine (T₃) deficiency of the fetus with primary thyroid failure (1). We have studied whether thyroxine (T₄) would also protect the fetal brain when maternal hypothyroxinemia is caused by nonthyroidal illnesses. We have used the streptozotocin-induced diabetes mellitus pregnant rat as a model of maternal nonthyroidal illness. We measured the effects of diabetes mellitus, and of correction of the ensuing maternal hypothyroxinemia with T₄ as compared to insulin, on maternal body weight, the outcome of pregnancy, glucose, insulin, T₄, T₃, reverse T₃, and thyrotropin levels in the maternal and fetal circulation, as well as T₄ and T₃ concentrations in tissues, and iodothyronine deiodinases in liver, lung, and brain. The diabetic mothers showed changes in thyroid hormone status typical of nonthyroidal illnesses. Thyroid hormone status of the fetuses was severely affected: the total T₄ and T₃ pools decreased to one-third of normal values. T₄ and T₃ concentrations in the fetal brain were lower than normal and the expected increase in 5'-deiodinase activity was not observed. Although insulin treatment avoided or mitigated these changes, the low cerebral T₃ did not improve with T₄ treatment of the maternal hypothyroxinemia. Several findings indicated that treatment of the severely ill dams with T₄ was actually harmful for the outcome of pregnancy. These negative effects were observed without the expected increase in the maternal or fetal T₃ pools.

INTRODUCTION

THYROXINE (T₄) and 3,5,3'-triiodothyronine (T₃) concentrations are very low in all tissues of rat fetuses from dams with methimazole (MMI)-induced primary thyroid failure. If the maternal hypothyroxinemia is avoided by treatment with T₄, the brain is preferentially protected against the deficiency of T₃ until birth (1): fetal brain T₃ reaches normal concentrations, despite lower than normal fetal serum T₄ and increased TSH levels. T₄ is by far the main source of cerebral T₃ in the rat fetus (1,2); changes in circulating T₃ hardly affect cerebral T₃ within a physiological range of serum T₃ levels. Both the capacity of cerebral type II 5'-iiodothyronine deiodinase (5'D-II) to respond to low fetal serum T₄ with a marked increase in its activity and the amount of T₄ transferred from the mother to the fetus play crucial roles in maintaining T₃ homeostasis in the brain of the hypothyroid fetus. This protective effect of the maternal T₄ would explain the lack of major brain damage of most hypothyroid fetuses at birth, in contrast with the irreversible central nervous system damage observed in the neurological cretin. The mother of the latter is severely hypothyroxinemic, and does not offer any protection to the fetal brain, which is therefore markedly T₃ deficient before birth, despite the expected increase in 5'D-II activity (3). Both in the neurological cretin (4) and in the experimental model of rats fed a diet with a very low iodine content (3), maternal circulating T₃ is normal, but this does not improve fetal brain T₃ concentrations unless the maternal hypothyroxinemia is also corrected.

These findings have drawn attention to the importance of maintaining normal maternal T₄ levels during pregnancy, even when normal T₃ levels might prevent the appearance of clinical hypothyroidism. We already cautioned (1), however, against extrapolation of this conclusion to other situations where changes in maternal and fetal thy-

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TSH may be decreased under such conditions, indicating that the decreased hormonal secretion by the thyroid is considered as a protective mechanism against energy loss and excessive catabolism (5–7).

Diabetes mellitus is considered as a nonthyroidal disease leading to alterations of thyroid hormone status typical of the so-called "low T3 syndrome" (5–8). The major alterations in thyroid hormone economy are a reduction in the TSH stimulation of the thyroid gland, probably caused by "central" hypothyroidism, and in the peripheral generation of T3 from T4 (8). The injection of streptozotocin (STZ) in rats is frequently used to obtain an experimental model for the study of nonthyroidal illness, by inducing diabetes mellitus.

In the STZ-diabetic adult rat, as in other situations of nonthyroidal illness, both the thyroid secretion of T4 and T3 and the extrathyroidal monodeiodination of T4 to T3 are clearly impaired, as a consequence of which circulating T4 and T3 are very low, as well as the concentrations of both iodothyronines in most tissues (5–10). To our knowledge little is known about the possible influence of maternal diabetes on the thyroid hormone status of the fetus, except for a preliminary study from our group (11) showing that STZ-induced maternal diabetes mellitus also affects fetal thyroid hormone economy, causing a decrease of T4 and T3 in plasma and most fetal tissues, brain included, with possible impairment of the normal response of 5'D-II to low T4 concentrations. These alterations were mitigated or avoided by appropriate treatment of the dams with insulin.

The present study has been undertaken to assess the potential benefits for the fetus of the correction of maternal hypothyroxinemia when caused by nonthyroidal illness. The underlying cause of the maternal hypothyroxinemia might not be readily ascertained or treated, and treatment with T4 might be attempted to normalize maternal T4 levels, in view of the beneficial effects of this treatment for fetal brain T3 in case of primary thyroid failure (1). Diabetes mellitus was used as a model of nonthyroidal illness. As will be seen, T4 treatment, without correction of the diabetic state, is of no benefit for fetal tissues, brain included, and actually appears to be harmful both for the mother and for the outcome of pregnancy.

**MATERIALS AND METHODS**

**Experimental design**

Female Wistar rats were used for this study. The guidelines for humane treatment of animals were followed in compliance with the principles outlined in "The care and use of animals" and the study was approved by the committee of our Institute. They were maintained at 22°C with 12-h periods of light and darkness and fed ad libitum with a standard diet (18 g protein, 39 g carbohydrate, 2.5 g lipid, and 4.5 g cellulose/100 g plus salt and vitamin mixtures, with an estimated caloric content of 2.54 kcal/g). They were mated with normal males and the morning of appearance of the vaginal plug was considered as day 0 of gestation. Twenty-four pregnant rats were divided into four groups. One group served as normal pregnant controls (C). At day 7 of gestation, the other three groups of rats (D groups) were injected into the femoral vein with 4.5 mg/100 g body weight (BW) of streptozotocin (STZ) dissolved in 50 mM citrate buffer, pH 4.5 (12). From the three groups of D rats, one was left without further treatment (D group), a second group was treated with insulin (D+Ins), and a third group with T4 (D + T4). The group of D+Ins dams was injected sc once daily with 0.5 U bovine insulin/100 g BW/day from 9 to 20 days of gestation (dg). The group of D+T4 dams was implanted under the dorsal skin with Alzet 2ML2 osmotic minipumps (Alza Co., Palo Alto, CA) delivering at a constant rate 2.4 μg T4/100 g BW/day from 9 to 21 dg. The infusion of T4 was carried out as already described (1) with modifications: T4 (free acid form, Sigma Chemicals Co., St. Louis, MO) was dissolved in the minimum volume of 0.05 N NaOH and then taken to the required volume in 50% propylene glycol.

At 21 dg all dams were anesthetized with ether, bled, and perfused with 40–50 mL of 0.05 M phosphosaline buffer, pH 7.4, as described (1). Maternal plasma, liver, brain, heart, lung, and mamma were obtained and frozen. The uterus was dissected out and carefully rinsed and blotted free of maternal blood. The fetuses were then dissected out, bled, separated from the placenta, weighed, and immediately placed on ice. The fetal brain, liver, and lung were dissected out and quickly frozen on dry ice; the thyroid, adhering to the trachea, was withdrawn and frozen. The placentas were separated, weighed, and divided into the basal (maternal) and labyrinthine (fetoplacental) sides with blunt forceps and frozen rapidly, as described (1,13).

**Determination of thyroid hormone concentrations**

Thyroid hormones were determined by RIAs after extraction and purification of plasma and tissues (14–17). In brief, methanol is added to the still frozen tissue sample and homogenized with tracer amounts of [131I]T4 and [125I]T3 being added to each homogenate. This is followed by extraction of more than 90% of the endogenous and added iodothyronines using chloroform–methanol (2:1). The iodothyronines are then backextracted into an aqueous phase, and purified by passing this aqueous phase through Bio-Rad AG 1 × 2 resin columns. After a pH gradient, the iodothyronines are eluted with 70% acetic acid, which is then evaporated to dryness and dissolved in RIA buffer. Each extract is extensively counted to determine the recovery of the [131I]T4 and [125I]T3 added to each sample during the initial homogenization process. Average recovery is 50–60% for [131I]T4 and 60–70% for [125I]T3. The samples are submitted to highly sensitive RIAs for the determination of T4 and T3, the limits of sensitivity being 2.5 pg T4 and 1.5 pg T3/tube. Each sample is processed in duplicate or triplicate at two or more dilutions. Concentrations are then calculated using the amounts of T4 and T3 found in the respective RIAs, the individual recovery of the
[131I]T₄ and [125I]T₃ added to each sample during the initial homogenization process, and the weight of the tissue sample submitted to extraction. Maternal samples were processed individually. Plasma from different fetuses were pooled to obtain 300–400 µL aliquots. Fetal tissues were pooled (2 to 3 organs per pool) for the determination of T4 and T₃. Pools were always obtained from fetuses of the same litter.

**Percentage of circulating “free” T₄ and T₃**

The method described by Mendel et al. (18) was used, with modifications. High specific activity [125I]T₄ or [125I]T₃ (approximately 300,000 cpm) was added in a 5 µL volume to 300 µL of plasma, and incubated at room temperature for 1 h. A 280-µL aliquot of each was submitted to ultrafiltration using Microcon 10 microconcentrators (Amicon Division, W.R. Grace and Co, Beverly, MA) and a 20 min centrifugation at 14,000 rpm. A measured volume of each ultrafiltrate was added to 0.5 mL bovine serum and submitted to precipitation with 10% trichloroacetic acid (TCA) and centrifuged, the pellet being washed twice with the same solution of TCA. The washed pellet was counted and its radioactivity calculated as percentage of the initial added tracer, submitted to the same TCA precipitation and washing procedure. This percentage of “free” T₄ (% FT₄) or “free” T₃ (% FT₃) and the T₄ and T₃ concentrations determined by RIA were used to calculate the concentrations of free T₄ (FT₄) or free T₃ (FT₃), respectively.

**Iodothyronine S’- and S-deiodinase activities**

Before each assay [125I]rT₃ (3,3’,5’-triodothyronine) or [125I]T₄ was purified by paper electrophoresis to separate the iodide. Iodothyronine S’-deiodinase (S’D) activity was assayed as described (17,19), using 2 mM DTT and 400 or 200 nM rT₃ for maternal and fetal liver, respectively, and 2 nM rT₃ and 20 mM DTT for maternal and fetal lung. Maternal and fetal brain S’D-II activity was assayed (2,19) using 2 nM T₄ + 1 µM T₃ and 20 mM DTT in the presence of 1 mM 2-N-propyl-6-thiouracil (PTU). The [125I] released was separated by ion exchange chromatography on Dowex-50W-X2 columns equilibrated in 10% acetic acid. The production of equal amounts of iodide and 3’,3-T₂ was checked in some assays. The protein content was determined by the method of Lowry et al. (20), after precipitation of the homogenates with 10% TCA to avoid interferences from DTT in the colorimetric reaction.

**Other determinations**

rT₃ concentrations in maternal and fetal plasma, and in placental extracts, were determined by RIA, as previously described (13,21). Maternal and fetal plasma glucose levels were determined by the glucose oxidase method (22) using 10–25 µL of plasma. Insulin levels in maternal and fetal plasma were measured using the specific RIA adapted for rat insulin with reagents supplied by Novo BioLabs (Denmark). We used rat insulin as standard, anti-porcine antiserum, and human [125I]-labeled insulin as antigen.

TSH was determined in 200-µL aliquots of maternal plasma using the immunoreactants for RIA kindly supplied by the National Institutes of Health (Bethesda, MD), and made available through the Rat Pituitary Agency of the National Institutes of Diabetes, Digestive and Kidney Diseases. Concentrations are expressed in weight equivalents of the rat TSH RP-3 reference preparation (23).

**Drugs and reagents**

T₄, T₃, 3,5-diiodothyronine (3,5-T₂), PTU, and DTT were obtained from Sigma Chemical Co. (St. Louis, MO). rT₃ and 3’,3-T₂ were obtained from Henning Berlin GMBH (Berlin, Germany).

High specific activity [131I]T₄, [125I]T₃, [125I]T₄, and [125I]rT₃ (3000 µCi/µg) were synthesized in our laboratory (14,24) and used for highly sensitive T₄, T₃, and rT₃ RIAs, as recovery tracers for extraction, and as substrates for S’-deiodinases.

**Statistical analysis**

After testing for homogeneity of variance using Bartlett’s procedure, data were submitted to one-way analysis of variance. Square root or logarithmic transformations usually ensured homogeneity of variance when this was not achieved with the raw data. Significant differences among groups were assessed using the protected least significant difference (LSD) test. All statistical calculations were performed as described by Snedecor and Cochran (25). The SE appearing in the figures is the mean standard error calculated by ANOVA, and used for the identification of statistically significant differences between groups by the LSD test. For the sake of clarity, the ± SE is shown in figures only on the C value bar.

**RESULTS**

**Degree of maternal illness**

Figure 1 shows the insulin and glucose concentrations in the maternal plasma (M-plasma) from the different groups,

**FIG. 1.** Mean circulating insulin and glucose concentrations are shown for the dams from the different groups, as well as the calculated mean changes in the BW of the mother herself (M–BW) between 7 and 21 days of gestation, calculated as described in the Results section, by subtracting the weight of the conceptus from the total body weight. In this and the following figures, the SE shown only on the C value bar is the mean standard error calculated by ANOVA. The shaded area corresponds to the mean C value ± SE. An asterisk (*) identifies statistically significant differences versus C dams; number sign (#) identifies statistically significant differences versus the D group. Other statistically significant differences are not identified for the sake of clarity.
at 21 dg. Circulating glucose levels were very high in the STZ-injected dams, which did not receive insulin, whether or not they were infused with T4. Insulin decreased significantly in the D dams; the infusion of T4 resulted in insulin levels that were even lower than those of D dams. The injection of insulin affected both the insulin and glucose levels in the maternal circulation: Normal levels of insulin, as measured 24 h after the last injection, were found in the D+Ins group, with circulating glucose being somewhat higher than C values, although markedly decreased as compared to D animals.

Figure 1 also shows the calculated change in the body weight of the pregnant rats, free of the conceptus (M-BW), between 7 and 21 dg. The actual change of the dam plus conceptus appears in Table 1, as well as the number of fetuses per litter and the body weights of the fetuses (F-BW). The weights of the total placenta, as well as those of the maternal side (M-Placenta) and fetal side (F-placenta) are also shown in Table 1. The change in M-BW was calculated by subtracting the weight of the conceptus from the measured change in total weight. The weight of the conceptus was calculated for each animal from the sum of the weights of all the fetuses and placentas in each dam. Although extraembryonic fluids and membranes had not been collected, the sum of the fetal and placental weights appears to be a reasonable approximation to the total weight of the conceptus. As may be seen (Fig. 1), the mean net increase in M-BW of C dams was 27.8 ± 4.3 g. In contrast, in the D dams there was a net loss of M-BW, which was corrected by insulin treatment. The most severe loss in M-BW was found for the D+T4 group, being more than twice that of D dams.

Both the increment in total weight (M-BW plus weight of the conceptus) and the change in M-BW were significantly related to decreasing glucose and increasing insulin concentrations in the maternal circulation, and appeared to be good indices of the degree of maternal illness. The closest fit was found for the change in M-BW versus the logarithm of the M-plasma insulin levels (n = 24; r = 0.76, p < 0.001).

Effects of maternal illness on the outcome of pregnancy

No reproductive abnormalities were observed in the control (C) group, whereas reabsorbed fetuses were found in many D dams. Treatment with insulin prevented these abnormalities, whereas they were most frequent in the D+T4 dams. Either the number of fetuses per litter or the F-BW (or both) were decreased in the diabetic animals, the lowest number and F+BW being found for the D+T4 group. Treatment with insulin increased F-BW, but normal weights were not achieved. Observed changes in placental weights caused by the maternal diabetic state were greatest in the D+T4 dams.

In summary, the outcome of pregnancy was affected by the maternal diabetic state and illness. This was prevented, or at least ameliorated, with the administration of 0.5 U of insulin/100 g BW/day, and was actually worsened by the infusion of T4 (2.4 µg/100 g BW per day) into the D dams.

Effects on thyroid hormone status of the mothers

The concentrations of T4, T3, rT3, and TSH in the maternal plasma are shown in Figure 2, as well as the circulating % FT4, FT3, % FT3, and FT3. Figure 3 shows the concentrations of T4 and T3 in the liver, lung, brain, heart, and mammary tissue.

Mean concentrations of T4, T3, and rT3 in the maternal circulation and of T4 and T3 in most tissues studied (Figs. 2 and 3) were lower in the D dams as compared to the C mothers, the differences being statistically significant except for M-brain T4 and M-mamma T3. Circulating TSH (Fig. 2) was also lower in the D animals.

**Table 1. Mean (± SEM) Increments in Total Body Weight (BW) between 7 and 21 Days of Gestation, Mean Number of Fetuses/Dam, BW of Fetuses (F-BW), and Weights of the Placenta (Total, Maternal and Fetal Sides), at 21 Days of Gestation, of Normal (C) and Streptozotocin-Injected Dams (D), and of D Dams Treated with Insulin (D + Ins), or Infused with T4 (D + T4)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Increment in total weight (g)</th>
<th>Number of fetuses/dam</th>
<th>F-body weight (mg)</th>
<th>Placental weight (mg)</th>
<th>M-placental weight (mg)</th>
<th>F-placental weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>100.0 ± 2.5</td>
<td>12.4 ± 1.0</td>
<td>4872 ± 32</td>
<td>510.6 ± 7.9</td>
<td>154 ± 9</td>
<td>339 ± 11</td>
</tr>
<tr>
<td>D</td>
<td>31.6 ± 8.8^a</td>
<td>10.0 ± 1.3</td>
<td>3196 ± 66</td>
<td>568.1 ± 12.2^a</td>
<td>116 ± 4^b</td>
<td>361 ± 11</td>
</tr>
<tr>
<td>D + Ins</td>
<td>71.3 ± 10.6^ab</td>
<td>10.8 ± 1.8</td>
<td>4097 ± 65</td>
<td>531.2 ± 14.6^b</td>
<td>136 ± 5</td>
<td>326 ± 11</td>
</tr>
<tr>
<td>D + T4</td>
<td>-11.3 ± 3.8^ab</td>
<td>6.7 ± 1.5^b</td>
<td>2746 ± 92</td>
<td>655.0 ± 40.4^ab</td>
<td>165 ± 28^b</td>
<td>432 ± 66^ab</td>
</tr>
</tbody>
</table>

^aStatistically significant differences (p < 0.05) versus C group.

^bStatistically significant differences (p < 0.05) versus D group.

**FIG. 2.** The mean concentrations of T4, % FT4, and F T4, T3, % FT3, and F T3, as well as rT3 and TSH in the maternal circulation are shown for the different groups of dams. See the legend to Figure 1 for the meaning of the shaded area and of the asterisk and #.
The circulating % FT₄ was increased in the diabetic dams (Fig. 2) to twice normal values of the normal pregnant dams. This increase was comparable to the decrease in total circulating T₄, as a result of which the mean total amount of circulating FT₄, although lower in D as compared to C dams, was not statistically different from that of the C mothers. The % FT₃ also increased in D as compared to C dams, but not to the extent that it could compensate for the decrease in circulating total T₃, and the FT₃ concentration was lower than that of C dams.

Treatment with insulin resulted in normal circulating T₄, T₃, rT₃, and TSH concentrations, and normal % FT₄, FT₅, % FT₃, and FT₃. Thus, the maternal hypothyroxinemia caused by the diabetic state was avoided in the insulin-treated D dams. Treatment with insulin also reversed the effects of the diabetic state on the concentrations of T₄ and T₃ in maternal tissues, with the exception of cardiac T₄.

The amount of T₄ infused into the D rats effectively prevented maternal hypothyroxinemia near term (Fig. 2). As the % FT₄ was markedly increased by the uncorrected diabetic state, the FT₄ levels actually increased 2-fold as compared to normal C rats. When the marked decrease in available T₄ of the D dams was avoided by the infusion of T₄, circulating rT₃ concentrations also increased to normal values and the rT₃/T₃ ratio increased 3-fold both with respect to the C and D groups. On the contrary, total T₃ and FT₃ concentrations did not increase with the infusion of T₄, and were as low as in D dams. The infusion of T₄ into the D rats did not alter their very low circulating TSH. In the T₄-infused diabetic animals, the concentration of T₄ in the maternal tissues increased as compared to that of D dams.

Normal concentrations were reached in most tissues, with higher than normal levels being reached in liver and mammary tissue. As already observed for circulating T₃, the concentration of T₃ in most tissues did not improve with the infusion of T₄, with T₃ concentrations in the heart being actually lower than those of D dams.

5'D-I activity in the liver and lung was decreased in the diabetic dams (Fig. 4), a finding consistent with the decreased hepatic and pulmonary thyroid hormone concentrations. Despite the decreased plasma T₄ concentrations, no significant change was observed in the 5'D-II activity of the cortex, a finding consistent with the lack of decrease in cerebral T₄ concentration. Treatment with insulin normalized liver and lung 5'D-I activities. On the contrary, the mean 5'D-II activity in the maternal cortex decreased with insulin treatment, but the difference with respect to both D and C dams was not statistically significant. With

**FIG. 4.** The activity of the outer-ring 5'D-deiodinases are shown: type I for liver (pmol I⁻⁻/min/mg protein) and lung (pmol I⁻⁻/h/mg protein) and type II for mamma (pmol I⁻⁺/h/mg protein). The meaning of the shaded areas, asterisk, and # is the same as in the legend to Figure 1.

**T₄ treatment** the activities of liver and lung 5'D-I and of cortex 5'D-II were the same as those of D animals.

**Effects on thyroid hormone status of the placenta**

Figure 5 shows the concentrations of T₄, T₃, and rT₃ in the M- and F-placenta. As described for these iodothyronines in the M-plasma, their concentrations decreased in the D dams, and improved with insulin treatment, although C levels were not always reached.

Treatment of the D dams with T₄ increased the concentrations of T₄ and rT₃ to normal values in the F-placenta, whereas T₃ remained as low as in D dams. These changes were similar to those observed in the M-plasma. On the contrary in the M-placenta, T₄ and rT₃ levels did not reach normal values and were lower than expected from the changes in M-plasma T₄ and rT₃ values, whereas T₃ was normal and higher than expected from the changes in M-plasma T₃.

**Effects in the fetus**

Figure 6 shows the glucose and insulin concentrations in F-plasma, as well as the corresponding T₄ and T₃ concentrations. F-plasma glucose was markedly elevated, and insulin decreased, in the D progeny. Both were clearly ameliorated by treatment of the mothers with insulin, whereas the infusion of T₄ had no effect.

The concentrations of T₄ and T₃ in the fetal circulation were decreased in the D group, with T₄ returning to nor-

**FIG. 5.** The concentrations of T₄, T₃, and rT₃ in the maternal and fetal placenta are shown for the different groups of dams in ng/g. The meaning of the shaded areas, asterisk, and # is the same as indicated in the legend to Figure 1.
mal fetal values with insulin treatment of the mothers. Treatment of the D dams with T4 did not improve fetal T4 concentrations and T3 levels were actually lower than those of D fetuses.

The concentrations of T4 and T3 in different F-tissues were decreased in the D group (Fig. 7). Treatment of the mothers with insulin usually resulted in improved T4 and T3 concentrations in most F-tissues, as compared to D fetuses, whereas infusion of T4 into the D dams did not improve the low T4 and T3 concentrations of T-tissues, with the exception of a modest increase of F-brain T4.

The 5'D activity in F-liver and F-lung was slightly decreased as compared to C fetuses (Fig. 7), being restored to normal both with insulin and T4 treatment of the mothers. 5'D-II activity in the F-brain remained unchanged.

Comparison of the changes in thyroid hormone status observed in the dams and in their fetuses

Changes in thyroid hormone status of the fetuses from the D and insulin-treated dams were qualitatively similar to those observed for their mothers: the concentrations of T4 and T3 decreased in the circulation and in tissues, and were clearly improved when the mother was treated with insulin. On the contrary, in fetuses from the T4-treated D dams, the changes were different from those observed in the T4 and T3 concentrations of the mothers. Whereas in the mothers plasma and tissues T4 were normal, or higher than normal, the concentrations of T4 in plasma and most tissues of their fetuses are as low as in those of fetuses from D dams, or even lower (i.e., F-plasma T3, Fig. 6). In this respect, the changes of the concentrations of T4 in the fetuses from D+T4 dams were similar to those observed for T4 levels in the M-placenta.

There were very good correlations between indices of the fetal and the maternal diabetic state, as assessed both from the circulating glucose and the insulin concentrations. The F–BW (Table 1) was also affected by the diabetic condition of the mothers, an effect that was ameliorated, but not totally corrected, by the administration of insulin to the D mothers. On the contrary, treatment with T4 resulted in even smaller fetuses than those of D mothers. The F–BW appeared to be related to other indices of the outcome of pregnancy, such as the number of viable fetuses per litter \( (n = 24; r = 0.66; p < 0.001) \).

**DISCUSSION**

**Effects of STZ-induced diabetes mellitus and T4 treatment on the mother**

Alterations in thyroid hormone status have been extensively described for nonpregnant adult rats, treated with STZ to induce diabetes mellitus. These are considered typical of the alterations of thyroid function and thyroid hormone metabolism observed in "nonthyroidal illness" (5,6,10). At present they are considered as beneficial adaptive responses in situations of limited availability of intracellular energy, when a decrease in T3-dependent catabolic effects is desirable (5–7,26), although this idea is being reconsidered (26). The two best known mechanisms involved in these responses are a decreased thyroidal secretion of both T4 and T3, which would lower the pool of T4 available for extrathyroidal generation of T3, and a decrease in the activity of enzymes involved in the extrathyroidal generation of T3 from T4.

The sequence of events leading to the first of these mechanisms appears to involve decreased release of hypothalamic TRH (27–29), decreased secretion of TSH (27–28,30–33), and a decreased sensitivity of the thyroid to TSH (32). The normal feedback mechanism is superseded, and TSH levels actually decrease, despite the lower levels of circulating total and/or free T4 and T3. Indeed, a return to normal secretion of TSH, accompanied by normalization of the thyroidal release of hormones, is considered as an index that the illness is remitting, or that the metabolic alterations are under control. In the nonpregnant rat with STZ-induced diabetes mellitus these mechanisms are fully operative, and the decreased thyroidal production of the iodothyronines is quantitative very important (10).

In the present study diabetes mellitus was induced during pregnancy. The changes in circulating total and free T4 and T3, together with decreased TSH, and the low T4 and
T3 levels found in all the tissues studied in the present D dams are in agreement with the changes described for the nonpregnant diabetic rats (10), and for patients dying from different nonthyroidal illnesses (34), indicating that 2 weeks after the injection of STZ, the adaptive mechanisms involving decreased thyroid secretion of thyroid hormones are fully operative.

With respect to the second mechanism, the direct measure of 5'D-I activities in the liver and lung of the pregnant D rats of the present study has confirmed that generation of T3 from T4 is decreased. Such a decrease was also previously described for both nonpregnant (9,35,36) and pregnant (11) diabetic rats, as well as a decreased expression of 5'D-I mRNA (35). In contrast, in agreement with results in nonpregnant diabetic rats (37), 5'D-II activity in the cerebral cortex of the pregnant D rats was not changed. In other experimental situations not involving diabetes, however, a decrease in circulating T4 is accompanied by an increase in 5'D-II activity of the cortex. This response was not observed in the D dams, possibly because cerebral T4 was not decreased.

Although treatment with T3 has been recently found to be beneficial after coronary artery bypass surgery (38,39), it is suspected that treatment with T4 is of no benefit to patients with nonthyroidal illness (40,41). Even so, we had not foreseen that treatment of the D dams with T4 would actually markedly worsen their condition. Their circulating insulin levels were actually lower than those of D dams. They clearly lost more body weight, and the outcome of their pregnancy was very poor; their litters were the smallest, both as regards the number and weight of the fetuses, and the number of resorptions the greatest. This deterioration could be explained if the administration of T4, in a dose sufficient to compensate for the decreased thyroid secretion of hormone, increased the amount of substrate available to 5'D-I, and more T3 might be generated as compared to that of D dams, despite the decreased activity of the enzyme. The ensuing increase of extrathyroidal T3 pool would then be expected to aggravate T3-dependent catabolic events, further decreasing the availability of intracellular energy (41).

This possible explanation is not, however, supported by present findings, as there was no evidence whatsoever that the T3 pools increased: T3 concentrations in plasma and tissues were actually as low as in D dams, or even lower (i.e., heart T3 levels). This was an unexpected finding that suggests several possible explanations. More T3 might actually have been generated and its deiodination increased, the steady state levels of T3 remaining the same. T4 may be having a catabolic effect per se, either through nonnuclear mechanism(s) of action, or by binding to nuclear receptors, which are not saturated by T3 because of the decreased availability of T3. Further experiments are necessary to clarify this unexpected effect of the infusion of T4 into D dams.

Effects of STZ-induced maternal diabetes mellitus and T4 treatment on the fetus

Present results confirm and extend our preliminary observations on the effects of maternal STZ-induced diabetes mellitus on fetal thyroid hormone status, as studied at 20

CONCLUSIONS

Present results show that maternal diabetes mellitus, and possibly maternal nonthyroidal illnesses compromising intracellular energy availability, results in severe impairment
of the thyroid hormone status of the fetus. This includes low cerebral concentrations of T₃ during a very critical period of brain development. Although maternal diabetes as a cause of maternal hypothyroxinemia would be easily recognized, and controlled with insulin, other causes of maternal hypothyroxinemia might not always be evident, or might not be promptly controlled. Correction of the maternal hypothyroxinemia by treatment with T₃ might be attempted to protect the fetal brain (1). Unfortunately, the present results indicate that in such cases prevention of the maternal hypothyroxinemia might be of little benefit. If the present results are relevant for man and for causes of maternal hypothyroxinemia other than maternal diabetes, it would appear that correction of the illness is necessary to protect the brain, as compensatory mechanisms usually involved in maintaining cerebral T₃ homeostasis might not be operative, and correction of maternal hypothyroxinemia, without adequate control of the illness is of no benefit, and might actually be harmful. It would appear advisable to take the present tentative conclusions into consideration when faced with maternal hypothyroxinemia caused by nonthyroid illness, and not by primary thyroid failure.

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REFERENCES

24. Weeke J, Orskov H 1973 Synthesis of monolabeled 3,5,3'-
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T₄ treatment and fetal brain T₃ in diabetes.

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<td>34</td>
<td>Areš R, Wiener GJ, Kaplan SG, Kim HS, Reichlin S, Kaplan MM 1993 Reduced tissue thyroid hormone levels in fatal illness. Metabolism 42:1102-1108.</td>
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4. Rosa Maria Calvo, Rosa Forcen, Maria Jesus Obregon, Francisco Escobar Del Rey, Gabriella Morreale De Escobar, Javier Regadera. 1998. Immunohistochemical and morphometric studies of the fetal pancreas in diabetic pregnant rats. Effects of insulin administration. *The Anatomical Record* 251:2, 173-180. [CrossRef]