Thyroid Hormone Economy in Pregnant Rats Near Term: A "Physiological" Animal Model of Nonthyroidal Illness?*

ROSA CALVO, MARIA JESÚS OBREGON, CARMEN RUIZ DE OÑA,
BEATRIZ FERREIRO, FRANCISCO ESCOBAR DEL REY, AND
GABRIELLA MORREALE DE ESCOBAR

Unidad de Endocrinología Experimental, Instituto de Investigaciones Biomédicas, Consejo Superior de Investigaciones Científicas, and Facultad Autónoma de Medicina, Madrid, Spain

ABSTRACT. We have studied the changes in thyroid hormone economy that occur in normal pregnant rats between 17–22 days of gestation. T_4 and T_3 decreased in all extrathyroidal tissues studied, namely plasma, liver, kidney, lung, heart, and skeletal muscle. The exception is the concentration of T_3 in cerebral cortex, which remains unchanged, possibly as a consequence of an increase in type II 5'-iodothyronine deiodinase activity. The marked decrease observed in most T_4 and T_3 pools was not accompanied by a commensurate increase in circulating TSH levels, which at 21 days gestation were either unchanged or actually decreased. The TSH response to TRH appeared to be prolonged. α -Glycerophosphate dehydrogenase activity was decreased in the liver, in accordance with its thyroid hormone

deficiency. Hepatic type I 5'-iodothyronine deiodinase activity, however, did not decrease, but was slightly increased. Thus, thyroid hormone economy in the pregnant rat near term shows striking similarities with several (but not all) of the changes described in patients with nonthyroidal illness and in several animal models used to study this condition. It is suggested that attenuation of the negative feedback response to the decrease in thyroid hormone pools, leading to low levels of thyroid hormones in most tissues, is the normal physiological response to situations where preservation of energy (and protein) represents a distinct adaptive advantage, as is the case of the pregnant rat and her conceptus. (*Endocrinology* 126: 10–16, 1990)

WE HAVE observed that plasma levels of L- T_4 (T_4) and L- T_3 (T_3) decrease markedly during gestation in rats (1), confirming previous observations by others (2–4). We did not, however, find the expected elevation in circulating plasma TSH (5), although increased levels have been reported by Kojima *et al.* (3) and Fukuda *et al.* (4). The absolute concentration of circulating free T_4 (as assessed indirectly from T_4 -binding activities of the plasma) was unchanged (2) or decreased (4). This last finding suggested that T_4 levels might be decreased in extrathyroidal tissues. This was confirmed not only for T_4 , but also for T_3 , in the liver of pregnant rats near term; T_3 concentrations in the brain, however, did not change, although T_4 levels decreased progressively (5, 6).

Considering the discrepancy between the changes in

liver and brain, it appeared of interest to study other maternal tissues late in pregnancy and to determine whether the changes in T_4 and T_3 concentrations were accompanied by alterations in biological end points of thyroid hormone action, such as basal TSH levels and the TSH response to TRH, and the activities of hepatic intramitochondrial α -glycerophosphate dehydrogenase (α GPD) and hepatic and cerebral 5'-iodothyronine deiodinases (5'D).

As will be seen, both T_4 and T_3 concentrations decreased in all tissues studied, cerebral T_3 levels being the exception. The biological responses tested in liver and brain were indicative of primary hypothyroidism (7, 8). Basal TSH levels did not increase, however, as expected in primary hypothyroidism, and the TSH response to TRH was suggestive of central hypothyroidism (9). Thus, thyroid hormone economy in the pregnant rat near term presents several (but not all) of the features described in some animal models of nonthyroidal illness (10–12). This includes a variety of conditions in which serum T_4 and/or T_3 levels decrease markedly, usually with a commensurate decrease in both their concentration and biological effects in tissues, but without the increase in TSH levels

Received November 22, 1989.

Address all correspondence and requests for reprints to: Dr. G. Morreale de Escobar, Unidad de Endocrinología Experimental, Facultad Autónoma de Medicina, Arzobispo Morcillo 4, 28029 Madrid, Spain.

*This work was supported by Grant M85-0005 from Comisión Interministerial de Desarrollo Científico y Técnico (Spain) and Grant PM-88-0005 from DGICYT (Spain). A preliminary report was presented at the 18th Annual Meeting of the European Thyroid Association, Copenhagen, Denmark, and has appeared in *Ann Endocrinol* (Paris) [50:74A, 1989 (Abstract)].

expected in primary hypothyroidism. In many of these conditions there is evidence that extrathyroidal generation of T_3 from T_4 is decreased, with a relative increase in T_4 deiodinating pathways leading to generation of rT_3 . A recent study by Valverde and Acevesi (13) has reported similarities between models of the so-called euthyroid sick syndrome and the lactating rat.

Materials and Methods

Experimental design

Female rats of a Wistar strain, bred in our colony, were fed a stock diet containing 1–2 μg iodine/g. They were housed in temperature-controlled quarters, with alternating light-dark cycles of 14-h light, 10-h dark, respectively. They were mated at 2.5–3 months of age with normal males; the morning of appearance of a vaginal plug was taken as day 0 of pregnancy. With this dating, birth takes place at 22.7 ± 0.2 days (6).

Exp A. Two or three pregnant (P) rats were killed daily between 17–22 days gestation, following the procedure previously described (1), except that propylthiouracil (PTU) was omitted from the perfusion buffer to avoid interferences in the 5'D assays. There were six P rats in the group studied at 21 days gestation. Results from P dams were compared to those of six age- and sex-paired nonpregnant (NP) rats.

Exp B. Seven NP rats and five P rats were anesthetized lightly with ether at 21 days gestation. A 0.5- to 1-ml aliquot of blood was withdrawn from one jugular vein. TRH (1 ng/1 g BW $\cdot$ 0.5 μl) was injected into the femoral vein. A blood sample was obtained from the contralateral jugular vein 5 min later and from the abdominal aorta 10 min after the injection of TRH, when the animals were killed. The dose of TRH was injected in proportion to the total body weight, not in proportion to the calculated conceptus-free weight, because TRH is readily transported across the rat placenta in both directions (14). All animals were submitted to the test on the same morning, alternating rats from each of the two groups, at 5-min intervals.

Preparation of samples

The plasma was kept at -20°C for the determination of T_4 , T_3 , rT_3 , and TSH. The liver, lung, and cerebral cortex were dissected out, weighed, and kept at -20°C . At 21 days gestation, the kidney, heart, and an aliquot (1–2 g) of skeletal muscle (musculus quadriceps femoris) were obtained for the determination of T_4 and T_3 . Separate liver aliquots were used for the determination of intramitochondrial αGPD and type I 5'D activities. An aliquot of the cerebral cortex (Cx) was used for the determination of type II 5'D activity.

Determinations of T_4 , T_3 , and rT_3

The iodothyronines were determined by specific RIAs in purified extracts from plasma or tissues. Direct determinations in whole plasma were avoided, because of the presence in plasma of pregnant rats near term of substances interfering with the RIAs (1, 5, 6). The procedure used for the T_4 and T_3

assays has been described in detail previously (1). In brief, homogenization in methanol is followed by extraction in chloroform-methanol, back-extraction into an aqueous phase, and purification of this phase through Bio-Rad AG 1 $\times$ 2 resin columns (Bio-Rad Laboratories, Richmond, CA). The iodothyronines are eluted with 70% acetic acid, which is then evaporated to dryness. RIA buffer is added, and the samples are submitted to highly sensitive RIAs. Each sample is processed in duplicate or triplicate at two or more dilutions. In the case of the anterior pituitary, only T_3 was assayed and only in duplicate at a single dilution because of the small amount of tissue. Results are calculated using individual recovery data obtained by the addition of tracer amounts of [^{131}I] T_4 and [^{125}I] T_3 to each homogenate. The amounts of tracer are such that the radioactivity carried over into the RIA tube is very low compared to that of the labeled antigen added for the RIA. For the determination of plasma rT_3 we used the same purification procedure as for T_4 and T_3 , but [^{125}I] rT_3 was added as recovery tracer. The antibody used was a highly specific one, kindly supplied by Dr. D. van der Heide (University Hospital, Leiden, The Netherlands) and was used at a final dilution of 1:20,000.

Synthesis of labeled T_4 , T_3 , and rT_3

The high specific activity ^{125}I - or ^{131}I -labeled T_4 and T_3 used as labeled RIA antigens and/or recovery tracers were obtained as previously described (1). [^{125}I] rT_3 was obtained with the same methods using 3,3'-diiodothyronine (Henning Berlin GMBH, Berlin, West Germany) as substrate.

Other determinations

Plasma TSH levels were measured using immunoreactants kindly supplied by the Rat Pituitary Agency of the NIDDK, NIH (Bethesda, MD), as previously described (15). Results are expressed as weight equivalents of the NIDDK rat TSH RP-2 reference preparation.

PTU-insensitive (type II) 5'D activities were measured in homogenates of cerebral Cx, as outlined by Leonard *et al.* (16) and detailed by Ruiz de Oña *et al.* (17), using [^{125}I] T_4 (2 nM) as substrate and 20 mM dithiothreitol in the presence of 1 mM PTU. PTU-sensitive (type I) 5'D activities were measured in liver homogenates as indicated above for cerebral Cx, but using [^{125}I] rT_3 (200 nM) as substrate and 2 mM dithiothreitol for 15 min. Type I 5'D represents 90% of the total activity.

Hepatic intramitochondrial αGPD (EC 1.1.99.5) activities were measured by the method of Lee and Lardy (7).

Statistical analysis

Mean values ($\pm\text{SEM}$) are given. Comparisons between two groups were performed using Student's *t* test. When more than two groups were involved, one- or two-way analysis of variance was applied, after ensuring homogeneity of variances by Bartlett's test. Square root or logarithmic transformation ensured homogeneity when this was not found with the raw data. Statistically significant differences were then identified by the least significant difference method. All calculations were performed as described by Snedecor and Cochran (18).

TABLE 1. Mean ($\pm$ SEM) weights (in grams) of the body, liver, and cerebral Cx of P rats at 21 days gestation and NP control animals

Group	BW ^a	Liver	Cerebral Cx
P	360 $\pm$ 4	10.9 $\pm$ 0.9	0.98 $\pm$ 0.04
NP	241 $\pm$ 4	7.2 $\pm$ 0.5	0.86 $\pm$ 0.06
P	<0.001	<0.001	<0.01

^a Including the concepta.

Results

Changes in weights and in plasma and tissue iodothyronine concentrations

The dams near term weighed 120 g more than age- and sex-paired NP rats (Table 1). Growth and development of the conceptus accounts for approximately two thirds of this increase (19). There is a net increase in maternal conceptus-free weight of about 40 g, of which only 25% is due to mammary tissue.¹ The total protein content of the maternal (conceptus-free) body is increased during pregnancy (20) as well as the total carcass fat (21). The weight of many organs increases as well, as shown in Table 1 for the liver and cerebral Cx at 21 days gestation. At least for the liver, this increase is not merely due to water retention, as there is a net increase in total hepatic DNA and protein (22).

The changes in plasma T₄, T₃, and TSH are shown in Fig. 1, compared to data from the NP rats. T₄ was already markedly decreased (to 38% of NP values) at 17 days gestation and remained low up to delivery. T₃ in P dams was lower than in NP rats at 17 days gestation and continued to decrease to about 36% of NP values by term. The changes in plasma T₄ preceded those in T₃. Although mean circulating TSH levels were slightly higher at 18–20 days gestation than in NP rats, the differences were not statistically significant.

The changes in the concentrations of T₄ and T₃ in liver and cerebral Cx are shown in Fig. 2. T₄ concentrations decreased markedly in both tissues, to 45% and 27%, respectively, of the NP value. Although not shown here, similar changes were observed for T₄ concentrations in the lung, which decreased to 60% of the NP level. The decrease in plasma T₄ preceded the changes observed in the liver, cerebral Cx, and lung. Hepatic concentrations of T₃ also decreased during this phase of pregnancy, to 57% of the NP value (Fig. 2). Lung T₃ levels (not shown) also decreased. In contrast, and despite the marked reduction in T₄, T₃ concentrations in the cerebral Cx of the P dams were the same as in NP rats (Fig. 2). Changes in tissue T₄ concentrations preceded those in tissue T₃ levels.

Other maternal tissues (lung, heart, skeletal muscle,

¹ Prof. E. Herrera (Centro Ramón y Cajal, Madrid, Spain), manuscript in preparation, personal communication.

FIG. 1. Changes in plasma T₄, T₃, and TSH levels in P rats between 17 and 22 days gestation. Data are the mean $\pm$ SEM. The shaded areas represent the mean ($\pm$ SEM) values for NP age- and sex-paired controls. The asterisks indicate differences between P and NP data that were statistically significant.

FIG. 2. Changes in T₄ and T₃ concentrations in liver and cerebral Cx during the last days of pregnancy. See Fig. 1 for meaning of symbols.

FIG. 3. Comparison of the concentrations of T_4 and T_3 in different tissues from P dams at 21 days gestation with levels in NP controls. Bars represent the mean $\pm$ SEM. See Fig. 1 for meaning of symbols.

TABLE 2. Concentrations of rT_3 and molar ratios of rT_3 to T_4 in the plasma of P rats at 21 days gestation and NP controls

Group	rT_3 (nmol/liter)	$rT_3:T_4$
P	0.080 ± 0.014	0.0074 ± 0.0016
NP	0.122 ± 0.007	0.0037 ± 0.0003
P	<0.05	<0.05

Data are the mean $\pm$ SEM.

FIG. 4. Plasma TSH levels of P rats at 21 days gestation and of NP controls at different times after the iv injection of TRH. n.s., not significant; *, statistically significant difference.

and kidney) were studied only at 21 days gestation (Fig. 3). T_4 and T_3 concentrations were lower than in NP rats. The changes in liver and cerebral Cx T_4 and T_3 , described

above, were confirmed (see Fig. 5). The concentration of T_3 in anterior pituitary was 47 ± 12 pg/gland in NP rats and 19 ± 7 in P animals; the difference neared statistical significance ($0.10 < P < 0.05$).

Plasma rT_3 levels at 21 days of pregnancy were lower in P than in NP rats (Table 2), but the molar ratio of rT_3 to T_4 increased 2-fold.

Biological responses to changes in thyroid hormone economy

Figure 4 shows the results of the TRH stimulation test, performed for Exp B. The basal TSH levels of P dams were lower than those of the NP rats.² TSH levels attained 5 min after iv TRH were the same in both groups; at 10 min they were higher in P than in NP rats. Although later intervals were not studied, the response to TRH in P dams appears more prolonged than that in NP rats.

Liver intramitochondrial α GPD activity of P dams decreased to a similar degree, as did iodothyronine levels (Fig. 5). Type II 5'D activity increased in the cerebral Cx of P rats, the degree of change being commensurate with the decrease in cerebral Cx T_4 level.

Practically all of the 5'D activity measured in liver homogenates was type I (PTU sensitive). It increased slightly in the liver of P dams (305 ± 10 pmol/h·mg protein) compared to that in NP rats (258 ± 13 pmol/h·mg protein; $P < 0.05$).

Discussion

The present results confirm and extend our previous ones (1, 5, 6) and those of others (2-4), which show that the rat undergoes important changes in thyroid hormone economy during the last stages of pregnancy. Plasma T_4 decreases near term to about 30% of levels found in nonpregnant females, with a similar decrease in plasma T_3 . In other studies (4) the change in plasma T_3 was less marked. We should like to point out that in studies from our laboratory we have used purified plasma extracts to avoid the presence of substances interfering with the T_4 and, especially, the T_3 RIAs (1), whereas in other studies unextracted serum or plasma was used.

Tissue T_4 concentrations also decrease markedly during the last days before term, although with some delay compared to the changes in plasma T_4 . All of the extra-thyroidal tissues we studied were clearly T_4 deficient. The same occurred with the T_3 concentrations in all

² The TSH levels of P rats at 21 days gestation were not different from those of NP rats in Exp A (Fig. 1). It is possible that the different results of Exp A compared to those of Exp B are related to the fact that for the latter the basal blood samples of all rats were obtained within a 1-h interval. The variations of values around the group means were one third or less of those in Exp A.

FIG. 5. The upper panel compares cerebral Cx T_4 and T_3 concentrations with type II 5'D activities. The lower panel compares hepatic T_4 and T_3 levels with α GPD activities. Data were obtained at 21 days gestation and in NP controls. Symbols are defined in Fig. 4.

tissues, with the exclusion of the cerebral Cx [and brain (5, 6)]. Preservation of the concentration of T_3 in the brain may be explained by the increase in type II 5'D activity, although an increase in uptake of T_4 and/or T_3 cannot be excluded. Activity of type II 5'D is, however, not increased in all tissues containing this type of deiodinase. Type II 5'D activity in brown adipose tissue, for example, is markedly decreased in pregnant rats near term, and concentrations of both T_4 and T_3 are low (23).

Two of the thyroid hormone-dependent responses that we have measured, namely hepatic α GPD and cerebral cortex type II 5'D, showed the changes expected in hypothyroidism (16, 17). On the contrary, we did not find the decrease in hepatic type I 5'D activity, which has also been described in hypothyroidism (8) and which accounts for increased levels of rT_3 in nonthyroidal illness and in experimental models of this condition (8–12). The opposite was actually observed, namely a slight increase in hepatic type I 5'D activity at 21 days gestation, confirming the report by Valverde and Acevesi (13) for pregnant rats near term. In our study circulating rT_3 levels were slightly decreased, but the molar ratio of rT_3

to T_4 was increased 2-fold. As the effect of pregnancy on the MCR of rT_3 is not known, it is difficult to interpret these results in connection with possible changes in the relative proportions of T_4 deiodinated by different pathways.

Several studies have reported an increased rate of T_4 deiodination in the rat in late pregnancy and increased T_4 MCR and volume of distribution (2, 24). Such changes were not found for T_3 (25). We have recently measured the net contribution of maternal T_4 to the extrathyroidal T_4 of the total fetal compartment at 21 days gestation (25a). This contribution represents 12% of the total maternal extrathyroidal T_4 pool in the average case of a dam bearing 12 fetuses. A preliminary rough estimation (25a) suggests that the amount of maternal T_4 actually transferred plus the T_4 actively deiodinated [mostly in the placenta (26)] may account for approximately 20% of the maternal extrathyroidal T_4 pool. This increased demand should be easily compensated for by activation of thyroidal T_4 secretion. Despite the fact that the negative thyroid-pituitary feedback mechanism is operative in pregnant rats, as shown by the observation that thyroidectomy, goitrogen treatment, or iodine deficiency result in increased plasma TSH (5, 19, 27) plasma TSH was unchanged or actually decreased at 21 days gestation (see Footnote 2), when extrathyroidal T_4 and T_3 pools were markedly decreased. Although the decrease in mean anterior pituitary T_3 was only bordering statistical significance, and concentrations in the thyrotrophs might not be adequately represented by those in the total gland, the results suggest that it is unlikely that the unchanged (or decreased) basal TSH levels and the lack of TSH response to the low T_4 and T_3 pools are due to an increased local production of T_3 from T_4 . The decreased basal TSH level and the somewhat prolonged response to TRH, as found in Exp B, are suggestive of central hypothyroidism (9). Other researchers have described an increase in basal (3, 4) and TRH-stimulated plasma TSH levels in pregnant rats near term (4). The 4-h radioiodide uptake was measured (28) at different stages of pregnancy, with inconclusive results. Although mean values were lower in pregnant rats, differences were only significant after 20 days gestation, when a marked decrease was observed, which was due in part to competing uptake by the fetal thyroids and the mammary gland. However, whether thyroid secretion increases or not, the response is clearly insufficient to avoid marked T_4 and T_3 deficiency in maternal tissues.

Many of the changes in thyroid hormone economy summarized here for the pregnant rat resemble those described in nonthyroidal illness (8, 11, 12) or in animal models used to understand this condition, such as the fasting, tumor-bearing, or diabetic rat (8, 10). Arem *et al.* (29) found decreased concentrations of T_3 in all tissues

obtained from patients who died of nonthyroidal illness; the best preserved T_3 levels were in pituitary and Cx. In such patients a compensatory increase in TSH secretion is not usually observed (9, 11, 12), although in most cases TSH levels increase when primary thyroid failure is also present. In several models of the so-called euthyroid sick syndrome (10), including the diabetic rat (30, 31), T_4 and T_3 are low in plasma and extrathyroidal tissues, although brain T_3 is often preserved (31). An increase in type II 5'D has been described in the brains of tumor-bearing rats (32). Decreased hepatic α GPD activities have been reported (10, 33). In most animal models studied so far, a compensatory response of pituitary TSH secretion does not occur, and in some this has been shown to be due at least in part to central hypothyroidism. *In vitro* and *in vivo* studies indicate that deiodination of T_4 to T_3 is impaired, probably because of decreased hepatic type I 5'D activity. Thus, one or more mechanisms become operative that decrease the availability of thyroid hormone (especially T_3) in most tissues. In the normal pregnant rats near term there appears to be evidence suggestive of central hypothyroidism and less evidence, so far, that generation of T_3 from T_4 is affected before lactation (13).

Why do such changes in thyroid hormone economy (usually associated with stressful conditions) occur in the pregnant rat nearing term? Pregnancy is a normal event occurring many times during the lifespan of the female rat. It is tempting to conclude that the changes reported here in thyroid hormone economy represent the physiological response that enables the dam to meet the many metabolic demands imposed by the rapid growth (and of protein) through a decrease in catabolic processes would provide a distinct adaptative advantage for the pregnant animal. There is a consensus (11) that the low T_3 state is metabolically protective in patients with severe illness and in animals used as models of the euthyroid sick syndrome. The present results strongly suggest that a readjustment of the hypothalamic regulation of the pituitary-thyroid axis, leading to low T_3 concentrations in most tissues, represents the normal response of the organism when faced with situations in which energy and protein need to be spared. The severely ill patient (or fasted, tumor-bearing and diabetic rats) would not be restoring to emergency measures to achieve this, but to physiological regulatory mechanism(s).

As summarized many years ago, "Pregnancy poses unusual metabolic problems. Theoretically, the mother should be geared to conserve more exogenous nutrients whenever she eats in anticipation of the more severe demands which the presence of the conceptus confers upon her endogenous reserves whenever food is withheld" (22). The pregnant rat is hyperphagic, and there is

a net increase in maternal weight and net nitrogen retention. Plasma glucose levels are low, and immunoreactive insulin levels are increased. There are important changes in lipid metabolism (34), leading to high plasma levels of triglycerides and FFA. Some of these metabolic changes might trigger responses that result in central hypothyroidism (9). As in the case of euthyroid sick syndrome, however, the primarily associated factors remain to be identified.

Present findings in rats might be pertinent to women (35). Weeke *et al.* (36) found an initial increase in circulating free T_4 and T_3 (measured by RIA in dialyzates) during the first trimester, followed by a persistent decline with progression of pregnancy. By term, both were lower than postpartum values, although the decrease was not as marked as described here for the rats. They also found a slight increase in plasma TSH, which was clearly insufficient to maintain normal free T_4 and T_3 concentrations. As a possible explanation they suggest that "the hormonal and metabolic alterations occurring during normal pregnancy somehow dampen the normal feedback regulation of thyroid function either at the hypothalamic/pituitary or at the thyroidal level."

Acknowledgments

We are grateful to Dr. D. van der Heide (University Hospital, Leiden, The Netherlands) for the generous gift of antiserum for the determination of T_3 by RIA. We are grateful to Socorro Durán, María Jesús Presas, and Arturo Hernández for skilled technical assistance, and to the animal care-taker, Pablo Sñor, for dating pregnancies. We thank Prof. E. Herrera (Centro Ramón y Cajal, Madrid, Spain) for permitting us to use unpublished information.

References

1. Morreale de Escobar G, Pastor R, Obregón MJ, Escobar del Rey F 1985 Effects of maternal hypothyroidism on the weight and thyroid hormone content of rat embryonic tissues, before and after onset of fetal thyroid function. *Endocrinology* 117:1890
2. Galton VA 1968 Thyroxine metabolism and thyroid function in the pregnant rat. *Endocrinology* 83:282
3. Kojima A, Hershman JA, Azukizawa M, DiStefano III JJ 1974 Quantification of the pituitary-thyroid axis in pregnant rats. *Endocrinology* 95:599
4. Fukuda H, Kohshima K, Mori M, Kobayashi I, Greer MA 1980 Sequential changes in the thyroid-pituitary axis during pregnancy and lactation in the rat. *Endocrinology* 107:1711
5. Escobar F, Mallol J, Pastor R, Morreale de Escobar 1987 Effects of maternal iodine deficiency on thyroid hormone economy of lactating dams and pups: maintenance of normal cerebral 3,5,3'-triiodo-L-thyronine concentrations in pups during major phases of brain development. *Endocrinology* 121:803
6. Escobar del Rey F, Pastor R, Mallol J, Morreale de Escobar G 1986 Effects of maternal iodine deficiency on the L-thyroxine and 3,5,3'-triiodo-L-thyronine contents of rat embryonic tissues before and after onset of fetal thyroid function. *Endocrinology* 118:1259
7. Lee YP, Lardy A 1965 Influence of thyroid hormones on alpha-glycerophosphate dehydrogenases and other dehydrogenases in various organs of the rat. *J Biol Chem* 240:1427
8. Kaplan MM 1986 Regulatory influences on iodothyronine deiodination in animal tissues. In: Hennemann G (ed) *Thyroid Hormone Metabolism*. Marcel Dekker, New York, p 231

9. Lamberton P, Jackson I 1986 The hypothalamic-pituitary-thyroid axis. In: Van Middlesworth L (ed) *The Thyroid Gland. A Practical Clinical Treatise*. YearBook, Chicago, p 69
10. Tibaldi JM, Surks MI 1985 Animal models of nonthyroidal illness. *Endocr Rev* 6:87
11. Brent GA, Hershman JM 1986 Effects of nonthyroidal illness on thyroid function tests. In: Van Middlesworth L (ed) *The Thyroid Gland. A Practical Clinical Treatise*. YearBook, Chicago, p 83
12. Kaptein EM 1986 Thyroid hormone metabolism in illness. In: Hennemann G (ed) *Thyroid Hormone Metabolism*. Marcel Dekker, New York, p 297
13. Valverde C, Acevesi C 1989 Circulating thyronines and peripheral monodeiodination in lactating rats. *Endocrinology* 124:1340
14. Kojima A, Hershman JM 1974 Effects of thyrotropin-releasing hormone (TRH) in maternal, fetal and newborn rats. *Endocrinology* 94:1134
15. Santisteban P, Obregón MJ, Rodríguez-Peña A, Lamas L, Escobar del Rey F, Morreale de Escobar G 1982 Are iodine-deficient rats euthyroid? *Endocrinology* 110:1780
16. Leonard JL, Kaplan MM, Visser TJ, Silva JE, Larsen PR 1981 Cerebral cortex responds rapidly to thyroid hormones. *Science* 214:571
17. Ruiz de Oña C, Obregón MJ, Escobar del Rey F, Morreale de Escobar G 1988 Developmental changes in rat brain 5'-deiodinase and thyroid hormone during the fetal period: the effects of fetal hypothyroidism and maternal thyroid hormones. *Pediatr Res* 24:588
18. Snedecor GW, Cochran WG 1980 *Statistical Methods*, ed 7. Iowa State University Press, Ames
19. Bonet B, Herrera E 1988 Different response to maternal hypothyroidism during the first and second half of gestation in the rat. *Endocrinology* 122:450
20. Beaton GH, Beare J, Ryn MH, McHenry EW 1954 Protein metabolism in the pregnant rat. *J Nutr* 54:291
21. López-Luna P, Muñoz T, Herrera E 1986 Body fat in pregnant rats at mid and late gestation. *Life Sci* 39:1389
22. Herrera E, Knopp RH, Freinkel N 1969 Carbohydrate metabolism in pregnancy. VI. Plasma fuels, insulin, liver composition, gluconeogenesis, and nitrogen metabolism during late gestation in the fed and fasted rat. *J Clin Invest* 48:2260
23. Viñas O, Giralt M, Obregón MJ, Iglesias R, Villarroja F, Mampel T 1988 Iodothyronine 5'-deiodinase activity and thyroid hormone content in brown adipose tissue during the breeding cycle of the rat. *Biochem J* 255:457
24. Dussault JH, Coulombe P 1980 Minimal placental transfer of L-thyroxine in the rat. *Pediatr Res* 14:228
25. Dubois JD, Cloutier A, Walker P, Dussault JH 1977 Absence of placental transfer of L-triiodothyronine in the rat. *Pediatr Res* 11:116
- 25a. Morreale de Escobar G, Calvo R, Obregón MJ, Escobar del Rey F 1990 Contribution of maternal thyroxine to fetal thyroxine pools in normal rats near term. *Endocrinology* 126:2765
26. Roti E, Gnudi A, Braverman LE 1983 The placental transport, synthesis and metabolism of hormones and drugs which affect thyroid function. *Endocr Rev* 4:131
27. Morreale de Escobar G, MJ Obregón, Ruiz de Oña C, Escobar del Rey F 1988 Transfer of thyroxine from the mother to the rat fetus near term: effects on brain 3,5,3'-triiodothyronine deficiency. *Endocrinology* 122:1521
28. Iino S, Greer MA 1961 Thyroid function in the rat during pregnancy and lactation. *Endocrinology* 68:253
29. Arem R, Kaplan MM, Wiener G, Kaplan SG, Reichlin S, Tissue thyroxine and triiodothyronine concentrations in human nonthyroidal illness. 61st Meeting of the American Thyroid Association, Phoenix, Arizona, 1986, p T-4 (Abstract)
30. Jolin T, Ortiz-Caro J 1985 Secretion and metabolic clearance rates of thyroxine and triiodothyronine in streptozotocin-diabetic rats. *Acta Endocrinol (Copenh)* 110:395
31. Schröder-van der Elst JP, van der Heide D 1989 Drastic changes in intracellular T4 and T3 concentrations in diabetic rats. 18th Meeting of the European Thyroid Association, Copenhagen. *Ann Endocrinol (Paris)* 50:72 (Abstract)
32. Grüssendorf M, Baumgartner C, Horster FA 1988 Veränderung der T4-Dejodierung in vitro im Gehirn von Ratten mit nichtthyreoidaler Erkrankungen. *Acta Med Austr* 15:32
33. Ortiz-Caro J, Obregón MJ, Pascual A, Jolin T 1984 Decreased thyroxine to 3,5,3'-triiodothyronine conversion in tissues of Streptozotocin-diabetic rats. *Acta Endocrinol (Copenh)* 106:86
34. Herrera E, Gómez-Coronado D, Lasunción MA 1987 Lipid metabolism in pregnancy. *Biol Neonate* 51:70
35. Mestman JH 1986 Thyroid disease in pregnancy. In: Van Middlesworth L (ed) *The Thyroid Gland. A Practical Clinical Treatise*. YearBook, Chicago, p 149
36. Weeke J, Dybjaer L, Granlie K, Eskjaer Jensen L, Kjaerulf E, Laurberg P, Magnusson B 1982 A longitudinal study of serum TSH, and total and free iodothyronines during normal pregnancy. *Acta Endocrinol (Copenh)* 101:531