Deiodination of ^{131}I-Labeled Thyroxine in the Fetal Rat (37457)

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(Introduced by M. E. Conrad)

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The initial description of triiodothyronine (T_3) in 1952 (1) was soon followed by evidence for the extrathyroidal conversion of thyroxine (T_4) to T_3 (2, 3). Recent technological advances in the measurement of T_3 have confirmed the physiologic importance of T_4 to T_3 conversion (4–7). Hotelling and Sherwood (8) reported that T_3 concentrations in cord sera were significantly lower than in their paired maternal specimens. These findings have been confirmed in other laboratories (9, 10) and recently reviewed (11). Larsen (9, 11) has found a 3–4-fold increase in serum T_3 concentrations in humans within the first 24 hr of life, which he believes parallels the increased TSH secretion that has been observed at this time (12, 13). The explanation for the low cord T_3 levels is obscure, but might be due to either: (a) a decreased conversion rate of T_4 to T_3 in the fetus; (b) an enhanced rate of T_3 disposal unique to the fetoplacental unit; or (c) to a decreased T_3 secretion rate relative to the rate of T_4 secretion from the fetal thyroid. The present study was performed to examine whether T_4 or T_3 conversion might be decreased in tissue of the intrauterine fetal rat as determined by *in vitro* assessment of the deiodination of ^{131}I-labeled T_4.

Materials and Methods. The *in vitro* deiodination of thyroxine was assessed by methods previously described (14, 15). In brief, pregnant female Wistar rats (200–250 g) estimated to be between 19–21 days of gestation, were sacrificed and the liver and kidneys immediately removed from the fetal rats. Control liver and kidney tissues were obtained from adult male Wistar rats weighing approximately 200–250 g. The liver and kidney tissues were dissected free, washed in iced saline and blotted dry. The tissues were homogenized in chilled Teflon grinders using 1 g tissue per 25 ml of ice-cold Krebs–Ringer–Tris–glucose (KRTG) buffer[1]. Homogenates were centrifuged at 4° for 15 min at 1000 rpm to remove erythrocytes, connective tissue, and intact cells. Protein was determined in the supernatant homogenate by the biuret technique (16). Following the addition of ^{131}I-T_4,[2] 2 ml aliquots of this supernatant were incubated in duplicate for 90 min at 37° in a covered Dubnoff metabolic shaker continuously flushed with 95% oxygen:5% CO_2. Pairs of duplicate flasks for each age group contained homogenate from approximately 3–4 fetal livers or 6–8 kidneys, or 2–3 livers or 4–6 kidneys from 1 or 3 day old animals. ^{131}I-T_4 was diluted in 1% human serum albumin and added to give approximately 2–4 μCi/flask and a final hormone concentration of 2–5 $\times$ 10^{-6} *M*. Following incubation, 1 ml human plasma was added to each flask to inhibit further deiodination.

Chromatographic analysis was carried out in butanol–dioxane–2 *N* ammonium hydroxide (4:1:5) by an ascending technique. Aliquots (20 μl) of the incubation mixtures were spotted on strips of Whatman No. 1 paper. The position of T_4, T_3, and iodide was determined by the use of appropriate markers after exposure to X-ray film in standard

[1] KRTG buffer: NaCl (0.154 *M*) 100 parts; Tris buffer, (pH 7.4) (0.05 *M*) 10.6 parts; $CaCl_2$ (0.11 *M*) 4 parts; KCl (0.154 *M*) 4 parts; $MgSO_4$ (0.154 *M*) 1 part; Glucose 2 mg/ml.

[2] ^{131}I-Labeled thyroxine was obtained from Abbott Laboratories, North Chicago, IL.

TABLE I. Degradation of ^{131}I-Thyroxine by Homogenates of Rat Liver and Kidney.

Expt	Actual deiodination (% ± SD)				Deiodination/mg protein (% ± SD)			
	Fetal rats	1 Day old	3 Days old	Adult	Fetal rats	1 Day old	3 Days old	Adult
				Liver				
1	41.7 ± 4.5	6.7 ± 4.1	12.3 ± 1.2	24.5 ± 11.2	10.3 ± 3.6	1.5 ± 0.8	3.8 ± 0.8	3.5 ± 1.4
2	53.6 ± 5.6	23.4 ± 3.0	7.3 ± 3.2	2.8 ± 1.3	13.1 ± 2.9	10.4 ± 1.5	2.4 ± 1.1	0.3 ± 0.0
3	57.6 ± 2.1	24.7 ± 15	4.0 ± 0.0	9.3 ± 1.4	16.3 ± 0.7	7.9 ± 4.7	1.0 ± 0.0	1.2 ± 1.8
4	45.5 ± 1.3	3.5 ± 0.2	3.3 ± 0.0	13.5 ± 1.4	8.6 ± 1.0	1.0 ± 0.0	0.4 ± 0.1	1.5 ± 1.3
5	54.3 ± 1.5	3.0 ± 0.1	3.7 ± 0.1	13.2 ± 3.8	7.4 ± 0.3	1.4 ± 0.0	1.4 ± 0.1	1.3 ± 0.3
6	53.7 ± 5.7	—	—	9.7 ± 1.0	11.5 ± 3.0	—	—	1.2 ± 1.2
7	51.9 ± 6.1	—	—	15.4 ± 1.3	13.9 ± 1.2	—	—	1.4 ± 2.0
Mean ± SE	51.1 ± 2.0	12.2 ± 4.8	6.1 ± 1.7	12.6 ± 2.5	11.6 ± 1.1	4.4 ± 1.9	1.8 ± 0.5	1.5 ± 0.3
				Kidney				
1	44.6 ± 9.0	14.6 ± 1.7	13.5 ± 1.0	42.8 ± 6.0	16.6 ± 2.3	4.1 ± 1.3	6.0 ± 0.5	8.6 ± 1.0
2	34.6 ± 3.4	22.8 ± 5.0	11.3 ± 3.0	22.2 ± 4.0	23.7 ± 4.7	14.0 ± 4.5	3.9 ± 0.3	3.7 ± 0.5
3	13.1 ± 3.6	24.8 ± 8.7	9.7 ± 1.7	37.5 ± 3.0	6.9 ± 3.0	10.5 ± 1.0	4.0 ± 0.1	6.2 ± 1.0
4	20.9 ± 3.5	15.9 ± 0.1	6.6 ± 0.9	21.8 ± 3.0	8.6 ± 3.8	14.4 ± 3.8	5.6 ± 2.8	3.7 ± 0.5
5	30.8 ± 1.1	4.3 ± 0.0	3.7 ± 1.2	34.9 ± 1.0	11.6 ± 4.3	1.1 ± 0.2	0.1 ± 0.3	5.0 ± 0.5
6	25.8 ± 3.5	—	—	41.8 ± 3.0	9.6 ± 0.6	—	—	7.2 ± 1.0
7	47.1 ± 7.0	—	—	42.2 ± 3.0	21.2 ± 8.2	—	—	7.6 ± 0.4
Mean ± SE	30.9 ± 4.6	16.4 ± 3.6	8.9 ± 1.4	34.7 ± 3.0	13.9 ± 2.4	8.8 ± 2.6	3.9 ± 1.0	6.0 ± 0.7

cassettes. The only other ^{131}I-labeled material identified was the so-called "origin material." All spots were cut out and counted in a well-type scintillation counter. The percentage deiodination of T_4 was calculated as the sum of the percentile generation of its products, after correction for nonthyroxine ^{131}I contaminating the ^{131}I-T_4 as assessed by chromatography of zerotime control samples.

Results. Actual deiodination rates for liver and kidney tissue, and deiodination rates corrected for protein content of the homogenates for all ages of animals studied are shown in Table I. Uncorrected deiodination rates in homogenates of fetal liver were 400% of the deiodination rate observed in homogenates of liver from either 1 day old or adult rats ($p < 0.001$). After correction for protein content of the homogenates, the differences were still significant ($p < 0.001$). Within the narrow limits of this experiment, there appears to be a trend toward decreasing deiodinative activity with age (Fig. 1).

Deiodinative rates in homogenates of kidneys from fetal rats did not differ from those in adult animals unless corrected for protein content in the homogenate ($p < 0.025$). Although there was some variation, the rate of deiodination per milligram of protein in kidney also tended to decrease with age of the animals (Fig. 1).

Discussion. Larsen (11) reported that the concentration of T_3 in sera from 8 pregnant women at delivery averaged 164 ± 47 ng%, while T_3 levels in the matched cord sera from their infants was 53 ± 15 ng%, or in the range usually seen in adult hypothyroidism. These latter values were thought to reflect the circulating T_3 concentration in the infant, since other studies by Larsen (9) had indicated that T_3 levels in paired umbilical arterial and venous samples were similar. Other workers have also observed higher T_3 values in maternal than cord serum (8, 10). The resultant elevation in the serum T_4:T_3 ratio in the fetus is unexplained, and presumably indicates differences between the prenatal fetus and the newborn, in either rates of secretion, production, or disposal of T_3.

Fisher *et al.* (17) in more recent studies,

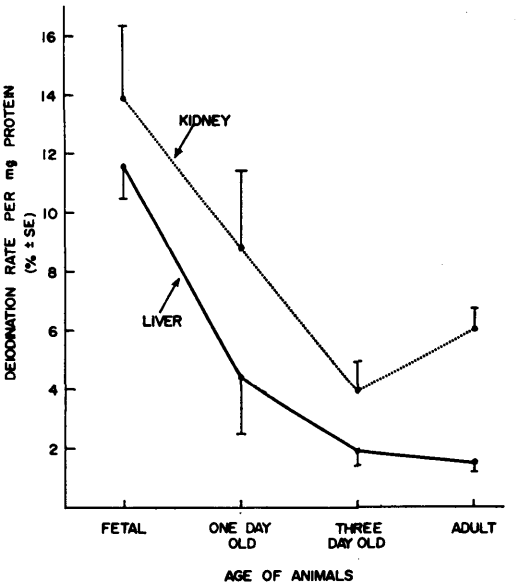

FIG. 1. Values for deiodination rates in liver and kidney homogenates (mean ± SE). Individual values and the number of experiments represented are listed in Table I. The number of animals per experiment is indicated in the text.

measured T_3 concentration in blood of 8 fetuses 13–24 wk and in 16 infants delivered between 25–40 wk, as well as thyroidal T_3 content in 14 fetuses between 13–31 wk. They found that T_3 was undetectable prior to 24 wk, averaged 34 ± 5.9 ng% at 25–34 wk, and was 77 ± 8.7 ng% at 35–40 wk. (The maternal sera averaged 193 ± 7.7 at 13–40 wk). The T_4/T_3 content in fetal thyroid was 14.3, which these workers deemed similar to the value (19.5) seen in adult glands, and concluded therefore that decreased T_3 secretion did not account for the low T_3 values in blood, and that decreased T_4 to T_3 conversion might be the mechanism.

It has been estimated that 20% of daily T_3 production in the rat (7), and 41% in the human (6), may derive from the extrathyroidal monodeiodination of T_4. Consequently, we examined rates of deiodination of ^{131}I-labeled T_4 by liver and kidney tissues *in vitro* as a means of assessing whether conversion of T_4 to T_3 might be decreased in these tissues in the fetus. The results (Table I; Fig. 1) suggest that the rate of T_4 de-

iodination in the fetus is not decreased in comparison to the neonatal or adult rat, and in fact may be increased when corrected for protein content of the tissue homogenates. These observations would not support the concept of a difference in peripheral T_3 production in the fetus insofar as the present method reflects *in vivo* deiodination, and/or the monodeiodination of T_4 to T_3. Certainly, more sensitive techniques or a rigorous experimental design with kinetic analyses such as that employed by Oppenheimer and associates (7, 18) might resolve any remaining doubt.

If the rate of conversion of T_4 to T_3 is not increased after delivery, then what accounts for the rise in serum T_3 in the first 24 hr of life (11)? The obvious alternative explanation for an increased serum T_4/T_3 ratio in the fetus would be a relatively lower secretion rate of T_3 than T_4 from the thyroid gland itself. The observed increases in TSH secretion following delivery (12, 13) alone could account for a rise in T_3:T_4 secretion rates from the thyroid, since TSH will acutely induce a relatively greater secretion of T_3 than T_4 (9, 19). And the data of Fisher *et al.* (17) suggesting that the T_3 content of the fetal thyroid is not significantly different from that in the adult gland should be confirmed, since a higher T_3/T_4 ratio in the fetus would permit an even greater T_3 secretion than usual to an exaggerated TSH stimulus. These hypothetical explanations for the observed rise in serum T_3 with delivery do not satisfactorily explain why T_3 levels are in the hypothyroid range in cord blood, however, since TSH concentration in serum is generally believed to be comparable in cord and maternal sera at term (20).

The enigma is made greater by the observation that T_3 is not detected prior to 24 wk (17) while T_4 and TSH are measurable at 12 wk and approach term levels by 16 wk of gestation (20). The rising fetal serum T_3 with length of gestation (17) would be compatible with increases in either T_4 to T_3 conversion or thyroid T_3 secretion, but the rapidity of the rise within the first 24 hr of life is more suggestive of an increased rate of secretion. Some additional factor, perhaps related to T_3 disposal by the fetoplacental unit, might also account for the remarkable correlation between delivery and the rising T_3 concentration. Conceivably, the fetus might exist in a milieu of high environmental iodide, which is more favorable to synthesis and release of T_4 rather than T_3, as has been recently reviewed by Greer (21). Finally, the low fetal T_3 concentrations may represent a combination of factors unique to intrauterine existence, such as both an altered T_3 metabolism by the fetoplacental unit, and a limited fetal pituitary–fetal thyroid axis in regard to TSH stimulation of T_3 secretion, which then becomes "normal" with delivery.

Summary. In vitro deiodination of ^{131}I-T_4 as an index of T_4 to T_3 conversion, was assessed in homogenates of fetal and adult rat liver and kidney, to explore whether differences existed which might account for the low fetal concentrations of serum T_3 observed by others. The deiodination rate per milligram of protein was greater for both kidney and liver homogenates in the fetus than in adult rats. These results suggest that fetal T_4 deiodination is not decreased in comparison to the neonatal or adult rat. Thus, the mechanism for the low serum T_3 concentration in the fetus does not appear to be a decreased production of T_3 from T_4, insofar as *in vitro* T_4 deiodination may reflect *in vivo* T_4 to T_3 conversion.

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