ACTA ENDOCRINOLOGICA
52 (1966) 383–390

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THYROID UTILIZATION IN NON-PREGNANT,
STEROID-INDUCED PSEUDOPREGNANT,
AND PREGNANT MONKEYS

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ABSTRACT

The utilization of thyroxine was studied in pregnant, non-pregnant and steroid-induced pseudopregnant rhesus monkeys. The PBI concentration in the blood serum was found to be higher during pregnancy and pseudopregnancy than in the non-pregnant state. The turn-over rate of radio-thyroxine, following its intravenous injection, was found to be higher during and outside of pregnancy than during pseudopregnancy. The extra-thyroidal thyroxine pool was calculated to be more expanded during pregnancy and (probably) pseudopregnancy than in the non-pregnant state. The utilization of thyroxine per unit time proved to be smaller in the non-pregnant monkey than in the pregnant and (probably) greater than in the pseudopregnant animal. It is concluded that thyroxine utilization during pregnancy is higher than during pseudopregnancy. The increased activity of the thyroid of the pregnant monkey, as judged from the increase in thyroxine utilization, is not only caused by the high level of placental steroids that characterize this state.

With the notable exception of the increase in thyroxine binding capacity of the serum (Dowling et al. 1956; Robbins & Nelson 1958), the results of the usual methods of investigation of the thyroid gland (¹³¹I-tracing, PBI, basal metabolic rate) point to a higher activity of this gland during pregnancy. A conclusive answer to the important question whether such an increased activity does in fact exist seems to be still lacking. Even more uncertain is the mechanism by which, if one accepts such a hyperfunction, this is achieved.

Starting from the assumption that the synthesis and the secretion of thyroid hormone is equal to its utilization, the determination of thyroxine utilization
can be used as an effective measure of thyroid function. *Ingbar & Freinkel* (1960) proved that in euthyroid man this assumption holds true.

*Dowling et al.* (1961) estimated thyroxine utilization in seven non-pregnant and in eight pregnant monkeys by means of intravenous injections of radio-thyroxine. The utilization was calculated from the per cent decline in serum radioactivity per day, the extra-thyroid thyroxine space and the butanol extractable iodine of the serum. During monkey pregnancy the thyroxine utilization, expressed in microgram/kg/unit time, proved to be not higher than in the non-pregnant state.

The present authors measured thyroxine utilization in *macacus rhesus* and *macaca mulatta* monkeys during pregnancy, in non-pregnants, and in non-pregnants after treatment with a combination of oestradiol and progesterone. The latter treatment was intended to create a state of pseudopregnancy. In view of the large inter-individual differences in the values of thyroxine utilization, care was taken to compare thyroxine utilization in the same animal under different conditions so that each animal could be used as its own control. Since the amount of maternal thyroxine that crosses the placenta is, at its best, extremely small (*Kock 1965*), the thyroxine utilization was not expressed as micrograms/kg/time unit but as micrograms PBI/animal/day.

**M A T E R I A L  A N D  M E T H O D S**

The terminology of *Ingbar & Freinkel* (1960) will be used. Thyroxine distribution space (TDS) is the volume which a tracer dose of 131I-labeled thyroxine is supposed to occupy after intravenous injection, assuming that the concentration of thyroxine in the whole body is taken to be equal to the concentration found in the blood. Since the thyroxine concentration is known to be different in different parts of the body, this space has no anatomical equivalent. The extra-thyroidal thyroxine pool (ETTP) is the amount of thyroxine present outside the thyroid gland. The PBI concentration of the serum is considered to adequately represent thyroxine concentration.

Intravenous injection of 131I-labeled thyroxine is initially followed by a sharp rise, after which a rapid fall in the radio-thyroxine content of the serum occurs. This fall is caused by the filling of the TDS (*Sterling & Chodos 1956; Lennon et al. 1961*). After this rapid fall, and as a consequence of utilization by the tissues, the serum radio-thyroxine concentration declines exponentially. Since the volume of the TDS can be expected constant, the change in the total amount of radio-thyroxine is a function of its change in concentration. Therefore, the slope of the line that connects the logarithm of the activity with time represents the disappearance rate of thyroxine from the ETTP. From this slope the percentual decline can be calculated. The activity at \( t = 0 \) is found by extrapolation. The following calculations are made: 1) the dose injected and the activity at \( t = 0 \) give TDS; 2) the serum PBI and TDS give ETTP; 3) the per cent decline of the radio-activity in the serum after equilibration and the ETTP give the thyroxine utilization in micrograms PBI/unit time. Changes in the thyroxine utilization can be the result of, or become apparent by, changes in
serum PBI, in the ETTP, or in the disappearance rate of $^{131}$I thyroxine from the ETTP.

$^{131}$I thyroxine, manufactured by Philips-Duphar in a 50% solution in propylene-glycol, was used in the experiments. The specific activity was 30 mc/mg. To avoid disintegration the batches were used within five days after delivery. Electrophoresis of the thyroxine dissolved in monkey serum showed that the activity due to free $^{131}$I was less than 5%. Prior to injection the thyroxine was always dissolved in monkey serum.

The PBI in the monkey serum was determined with the method of Barker (1948). Macacus rhesus and macaca mulatta monkeys were used because their placentation is to a large extent similar to that in man and because thyroxine binding in serum of monkeys, as in man, is to the albumin, prae-albumin and inter-alpha-globulin fraction (Blumberg & Robbins 1960).

Thyroxine utilization was determined (Table 1): 1) in four monkeys during pregnancy, six weeks to six months after delivery, and also during treatment with oestradiol combined with progesterone (no. 11, 12, 15, 19); 2) in three monkeys during pregnancy and from six weeks to six months after delivery (no. 44, 46, 47). Monkey no. 1 was investigated during treatment with oestradiol combined with progesterone, and prior to it.

As a result of this arrangement, we were able to study the influence on thyroxine utilization exerted in the same animal by pregnancy in seven monkeys, and by oestradiol combined with progesterone in five monkeys. In four monkeys (no. 11, 12, 15, 19) a comparison could be made in the same animal between the influence of pregnancy and a combination of oestradiol and progesterone (Table 2).

The treatment with steroid was started six months after the determination of the thyroxine utilization in the non-pregnant state. Ten micrograms of oestradiol benzoate and 10 mg micro-crystals of progesterone were given daily for 13 days. On the sixth day after the beginning of the treatment, five $\mu$c of $^{131}$I-labeled thyroxine was injected intravenously, after which blood samples were taken every other day for a period of eight days.

The animals were in a healthy state during the experiments. The weight of the m. rhesus monkeys (no. 11, 12, 15, 19) was 6–7 kg. that of the m. mulatta (no. 1, 44, 46, 47) 3–4 kg. The results were statistically evaluated with parameter free tests: the Dixon-Mood sign test, the Wilcoxon rank-sign test or the Wilcoxon rank correlation test. Student’s $t$-test on paired observations also was applied when the number of observations was too small to go beyond the $P = 0.05$ level. In that case, only the results in the rhesus monkeys were considered. Mixing these experiments with those in the mulatta monkeys would certainly have created a state of non-normalcy in the distribution. Although not strictly proven, it is assumed that the data procured from experiments in which the same rhesus monkeys were exposed to a different condition or treatment are normally distributed.

**RESULTS**

1) PBI: The finding of Kerr (1962) that the PBI concentration of monkey serum is elevated during pregnancy was confirmed (Dixon-Mood sign test $P = 0.05$). During treatment with the combination of oestradiol and progesterone the PBI levels were higher in all five animals treated than in the
Table 1.
Serum PBI, turn-over rate of radioactive thyroxine, ETTP and thyroxine utilization in non-pregnant, steroid-induced pseudopregnant and pregnant monkeys no. 11, 12, 15, 19 *m. rhesus*; no. 44, 46, 47, 1 *m. mulatta*.

<table>
<thead>
<tr>
<th>Monkey:</th>
<th>PBI μg/100 ml</th>
<th>% decline radioactivity in serum/day</th>
<th>ETTP μg PBI</th>
<th>Thyroxine utilization μg PBI/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>no. 11 non-pregnant</td>
<td>5.3</td>
<td>23.4</td>
<td>43.4</td>
<td>10.1</td>
</tr>
<tr>
<td>pseudopregnant</td>
<td>7.9</td>
<td>16.2</td>
<td>59.3</td>
<td>9.6</td>
</tr>
<tr>
<td>pregnant</td>
<td>5.3</td>
<td>24.7</td>
<td>51.0</td>
<td>12.6</td>
</tr>
<tr>
<td>no. 12 non-pregnant</td>
<td>4.5</td>
<td>25.4</td>
<td>38.1</td>
<td>9.7</td>
</tr>
<tr>
<td>pseudopregnant</td>
<td>5.4</td>
<td>16.2</td>
<td>39.2</td>
<td>6.3</td>
</tr>
<tr>
<td>pregnant</td>
<td>5.8</td>
<td>19.9</td>
<td>69.6</td>
<td>13.9</td>
</tr>
<tr>
<td>no. 15 non-pregnant</td>
<td>4.2</td>
<td>22.9</td>
<td>26.5</td>
<td>6.1</td>
</tr>
<tr>
<td>pseudopregnant</td>
<td>5.9</td>
<td>14.3</td>
<td>32.3</td>
<td>4.6</td>
</tr>
<tr>
<td>pregnant</td>
<td>5.9</td>
<td>22.7</td>
<td>45.0</td>
<td>10.2</td>
</tr>
<tr>
<td>no. 19 non-pregnant</td>
<td>4.5</td>
<td>25.9</td>
<td>35.0</td>
<td>9.1</td>
</tr>
<tr>
<td>pseudopregnant</td>
<td>5.0</td>
<td>16.2</td>
<td>36.3</td>
<td>5.9</td>
</tr>
<tr>
<td>pregnant</td>
<td>5.3</td>
<td>25.4</td>
<td>62.0</td>
<td>15.6</td>
</tr>
<tr>
<td>no. 44 non-pregnant</td>
<td>2.8</td>
<td>27.9</td>
<td>19.7</td>
<td>5.5</td>
</tr>
<tr>
<td>pregnant</td>
<td>3.1</td>
<td>29.3</td>
<td>23.7</td>
<td>6.9</td>
</tr>
<tr>
<td>no. 46 non-pregnant</td>
<td>2.3</td>
<td>41.4</td>
<td>12.7</td>
<td>5.2</td>
</tr>
<tr>
<td>pregnant</td>
<td>6.4</td>
<td>17.0</td>
<td>26.4</td>
<td>4.5</td>
</tr>
<tr>
<td>no. 47 non-pregnant</td>
<td>2.5</td>
<td>23.8</td>
<td>14.2</td>
<td>3.4</td>
</tr>
<tr>
<td>pregnant</td>
<td>5.9</td>
<td>24.1</td>
<td>19.1</td>
<td>4.6</td>
</tr>
<tr>
<td>no. 1 non-pregnant</td>
<td>3.2</td>
<td>34.6</td>
<td>13.9</td>
<td>4.8</td>
</tr>
<tr>
<td>pseudopregnant</td>
<td>4.6</td>
<td>24.6</td>
<td>15.4</td>
<td>3.8</td>
</tr>
</tbody>
</table>

Untreated ones. (Dixon-Mood sign test $P = 0.06$, the lowest value that can be reached with five animals.) Comparison of the PBI levels in five monkeys during pregnancy and during steroid-induced pseudopregnancy showed that in one monkey, the value during pregnancy was higher, in another monkey lower, and in two monkeys equal to the values found in pseudopregnancy.

2) Per cent decline in $^{131}$I thyroxine activity in the blood serum: no difference was observed between the decline in radioactivity in the blood of non-pregnant and pregnant animals.

During the treatment with the steroid combination, the decline was slower
Table 2.
Thyroxine utilization in pregnant and steroid-induced pseudopregnant rhesus monkeys.

<table>
<thead>
<tr>
<th></th>
<th>Thyroxine utilization in μg PBI/24hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>no. 11</td>
<td>pregnant: 12.6, pseudopregnant: 9.6</td>
</tr>
<tr>
<td>no. 12</td>
<td>pregnant: 13.9, pseudopregnant: 6.3</td>
</tr>
<tr>
<td>no. 15</td>
<td>pregnant: 10.2, pseudopregnant: 4.6</td>
</tr>
<tr>
<td>no. 19</td>
<td>pregnant: 15.6, pseudopregnant: 5.9</td>
</tr>
</tbody>
</table>

than in the untreated non-pregnant state (Wilcoxon rank correlation test \( P = 0.05 - 0.02 \)) and the pregnant state (\( t \)-test \( P = 0.02 \)).

3) ETTP: The extra-thyroidal thyroxine pool expanded during pregnancy (Dixon-Mood sign test \( P = 0.02 \)).

In all four animals treated with oestradiol and progesterone, this expansion also occurred (Dixon-Mood sign test \( P = 0.06 \)), but probably to a smaller degree.

4) Thyroxine utilization: Thyroxine utilization proved to be higher during pregnancy than in the non-pregnant state (Wilcoxon rank-sign test \( P = 0.05 - 0.02 \)), and higher than during steroid-induced pseudopregnancy (\( t \)-test \( P = 0.02 \)). In the non-pregnant animal the utilization is probably higher.

Table 3.
Schematic representation of differences in serum PBI, turn-over rate of radioactive thyroxine, ETTP and thyroxine utilization in non-pregnant, steroid-induced pseudopregnant and pregnant monkeys.

<table>
<thead>
<tr>
<th></th>
<th>pregnant (( P = 0.05 ))</th>
<th>? pseudopregnant (( P = 0.06 ))</th>
<th>&gt; non-pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turn-over</td>
<td>pregnant (( P = 0.05 ))</td>
<td>&gt; pseudopregnant</td>
<td></td>
</tr>
<tr>
<td>rate</td>
<td>non-pregnant (( P = 0.02 ))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ETTP</td>
<td>pregnant (( P = 0.02 ))</td>
<td>&gt; non-pregnant</td>
<td></td>
</tr>
<tr>
<td></td>
<td>? pseudopregnant (( P = 0.06 ))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyroxine</td>
<td>pregnant (( P = 0.05 ))</td>
<td>&gt; non-pregnant</td>
<td></td>
</tr>
<tr>
<td>utilization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>pregnant (( P = 0.02 ))</td>
<td>&gt; pseudopregnant</td>
<td>(( P = 0.06 )) pseudopregnant</td>
</tr>
</tbody>
</table>
than during treatment with oestradiol and progesterone (Dixon-Mood sign test
\[ P = 0.06 \]).

The results of our experiments can be summarized as given in Table 3.

**DISCUSSION**

The data evolving from our experiments seem to fit best with the conclusion
that thyroxine utilization is enhanced during monkey pregnancy. The results
appear to differ from those reached by Dowling et al. (1961) but these in-
vestigators expressed thyroxine utilization in micrograms/time unit/kg body
weight. In the body weight, the weight of the uterine contents was included.
It has been shown, however, that the amount of maternal thyroxine that
crosses the placenta is extremely small, perhaps not more than 0.2% of the
thyroxine utilized by the foetus during intra-uterine life (Kock 1965). More-
over, in view of the large inter-individual spread of the thyroxine utilization
in our experiments, each monkey was used as its own control, whereas Dowling
et al. (1961) compared a group of pregnant animals with a different group of
non-pregnant ones. Another possibility to explain the fact that Dowling et al.
(1961) got different results may be attributed to their treating the monkeys
with KI, the effect of which is not necessarily identical during and outside
of pregnancy. Since during human pregnancy \(^{131}\)I uptake is increased (Pochin
1952; Noble & Rowlands 1953; Halnan 1958; Aboul-Khair et al. 1964), it
seems reasonable that in our experiments the uptake of \(^{131}\)I derived from the
radiothyroxine was greater during pregnancy than outside of this state. The
radiothyroxine resynthesized from this \(^{131}\)I must have had a decreasing effect
on the turn-over rate of \(^{131}\)I thyroxine during pregnancy. So, the only effect
of inhibiting this recirculation of \(^{131}\)I by giving KI (which was omitted by
us) would have been a still greater enhancement of the calculated thyroxine
utilization during monkey pregnancy.

From our data it follows that the increase in thyroxine utilization during
pregnancy is primarily related to an expansion of the extra-thyroidal thyroxine
pool. The part played by the rise in serum PBI alone is distinctly smaller.
Robbins & Rall (1957) and Tanaka & Starr (1959) calculated »free«
thyroxine levels, basing themselves on the outcome of measurements of PBI
and inter-alpha-globulin thyroxine binding capacity of the serum during
human pregnancy. This concentration was found to be lower than in the non-
pregnant state. Osorio et al. (1962), who started from serum PBI and the \(T_3–
^{131}\)I erythrocyte uptake determination, came to the result that the concentra-
tions of »free« thyroid hormone in serum were equal during and outside of
pregnancy. Sterling & Hegedus (1962) determined the dialysable fraction of
the thyroid hormone in the serum of 29 healthy women in the first trimester

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of pregnancy and found $0.0044 \pm 0.0012 \, \mu g/100 \, ml$. The corresponding value in 29 non-pregnant women was significantly higher: $0.0065 \pm 0.0019 \, \mu g/100 \, ml$. Going from the assumption that these data, procured from investigations in the human, are valid for the monkey also, the rise in PBI concentration as such cannot be considered as proof of a higher activity of the thyroid gland during pregnancy.

The increase in thyroxine utilization during pregnancy found by us could possibly be explained by an increase in the excretion of thyroxine in the intestines. Among the objections to this possibility is the finding of Kock et al. (1963) that in pregnant Wistar rats, the thyroxine excretion in the faeces was not greater than in their non-pregnant litter mates.

Our data are incompatible with the concept that the enhancement of thyroxine utilization during pregnancy is only brought about by the high levels of oestrogens and progesterone that are characteristic for this state. As a matter of fact, thyroxine utilization during steroid-induced pseudopregnancy was found to be even lower than in non-pregnant animals. The main difference between pregnancy and pseudopregnancy is a difference in turn-over rate of radio-thyroxine in the serum, the slope in pseudopregnant animals being less steep than in pregnant and even non-pregnant monkeys. Since it can hardly be doubted that during monkey pregnancy a high level of oestrogens and progesterone exists, some other factor must be hypothesized that corrects this »anomaly«. The conclusion that steroids are not responsible for the increased activity of the thyroid gland during pregnancy as far as is evident from the increase in thyroxine utilization, is in accordance with the findings in a molar pregnancy (Kock 1965). The factor, although not identical with HCG, is most probably located in the chorionic tissue.

**REFERENCES**


Received on December 7th, 1965.