Effects of marginal iodine deficiency during pregnancy: iodide uptake by the maternal and fetal thyroid

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Versloot, P. M., J. P. Schröder-Van der Elst, D. van der Heide, and L. Boogerd. Effects of marginal iodine deficiency during pregnancy: iodide uptake by the maternal and fetal thyroid. Am. J. Physiol. 273 (Endocrinol. Metab. 36): E1121–E1126, 1997.—Iodide uptake by the thyroid is an active process. Iodine deficiency and pregnancy are known to influence thyroid hormone metabolism. The aim of this study was to clarify the effects of iodine deficiency and pregnancy on iodide uptake by the thyroid. Radiiodide was injected intravenously into nonpregnant and 19-day pregnant rats receiving a normal or marginally iodine-deficient diet. The uptake of radiiodide by the thyroid was measured continuously for 4 h. The absolute iodide uptake by the maternal and fetal thyroid glands at 24 h was calculated by means of the urinary specific activity. Pregnancy resulted in a decrease in the absolute thyroidal iodide uptake. Marginal iodine deficiency had no effect on the absolute iodide uptake by the maternal thyroid. The decreased plasma inorganic iodide was compensated by an increase in thyroidal clearance. A similar compensation was not found for the fetus; the uptake of iodide by the fetal thyroid decreased by 50% during marginal iodine deficiency. This can lead to diminished thyroid hormone production, which will have a negative effect on fetal development, especially of the brain.

it is known that thyroid function is affected by various physiological conditions, for instance, food deprivation (24), pregnancy (10, 11, 12), and iodine deficiency (19, 23). Iodine is an essential element for the production of thyroid hormones. The uptake of iodide by the thyroid gland is an active process that is regulated by thyroid-stimulating hormone or thyrotropin (TSH) (26) and the thyroidal blood flow (3). Alterations in the thyroidal uptake of iodide can cause changes in the production of thyroid hormones.

Iodine deficiency affects the physical and mental development of humans in large areas of the world (13). In rats it has been shown that during iodine deficiency the plasma thyroxine (T4) level decreased while the 3,5,3′-triiodothyronine (T3) level remained unchanged (23). The weight of the thyroid and the T3-to-T4 ratio in the thyroid of rats on a low-iodine diet increased (19, 23). Studies of 10-day-old rats have shown that the thyroidal iodide uptake was considerably higher in iodine-deficient rats than in controls (31).

Physiological changes in thyroid function also occur during pregnancy. Normal pregnancy in humans is accompanied by a rise in T4-binding globulin and total T4 and T3 (12). However, the free T4 level is decreased at the end of gestation (29). The availability of iodide for the maternal thyroid is decreased because of increased renal clearance (1) and transport to the fetoplacental complex during the late phase of gestation (10). The maternal thyroid is enlarged and the radiiodide uptake, expressed as a percentage of the dose, is increased during pregnancy (1, 10, 13).

In the rat normal pregnancy results in a decrease in plasma total T4 and T3 concentrations (4). This is comparable to the decrease in free T4 in the third trimester of human pregnancy (29). The thyroid uptake of 131I is decreased in the pregnant rat (9, 11, 15). No changes were found in the urinary excretion of iodide during the last days of gestation (9). Iodine deficiency induces a further decrease in plasma T4 in the near-term pregnant rat (8). Also the weight of the thyroid is increased by iodine deficiency in the pregnant rat (7).

Most studies of the iodide uptake in rats are performed by tracer injection of a radioactive isotope of iodide (131I, 125I, or 123I). After a certain period the thyroid is removed and the percentage of the injected radioactivity is calculated (9, 11, 15, 31). In our laboratory we developed a method for measuring continuously the in vivo uptake of 125I by the thyroid. By means of this method it is possible to study not only the amount of radiiodide taken up by the thyroid but also the kinetics of the thyroidal uptake of iodide. We were also able to calculate the absolute iodide uptake by the thyroid.

The aim of this study was to clarify the effects of pregnancy and iodine deficiency on iodide uptake by the thyroid. We used four groups of rats: nonpregnant and near-term (day 19) pregnant rats receiving a normal iodine diet or a marginally iodine-deficient diet. This marginally iodine-deficient situation closely reflects the iodine status of large populations of humans in the world. As previously described, pregnancy as well as iodine deficiency affects plasma thyroid hormone levels. However, the effects on the absolute iodide uptake by the thyroid are unknown. In particular iodine-deficient, near-term pregnant rats represent an important group. What are the effects of iodine deficiency on iodide metabolism in the near-term pregnant rat? Are the low amounts of iodide available taken up totally by the maternal thyroid, or is there still iodide available for the fetuses?

MATERIALS AND METHODS

Animals. Three-month-old female Wistar rats (CPB/MU, IFCA CREDO, Brussels) were used. The rats were individually housed in metabolic cages at 22°C, with an alternating 14-h light and 10-h dark periods. The animals were fed a semisynthetic American Institute of Nutrition (AIN) diet (2) mixed with distilled water (60% dry weight-40% water) and potassium iodide: 55 ng [normal iodine dose (NID)] or 2.9 ng [marginally iodine dose (MID)] per gram of feed. The margin-
ally iodine-deficient groups received 1% KClO₄ in the drinking water during the first 2 days of the MID ≥4 wk before measurement was started. KClO₄ was given to accelerate thyroidal depletion of total iodine stores.

After two regular estrus cycles the rats were mated. Mating was confirmed by the presence of sperm in a vaginal lavage the following morning, called day 0 of pregnancy.

Design of the study. In this study four groups of animals were studied: 1) nonpregnant rats on a normal diet (NID, C); 2) pregnant rats on a normal diet (NID, P); 3) nonpregnant rats on a marginally iodine-deficient diet (MID, C); 4) pregnant rats on a marginally iodine-deficient diet (MID, P). The pregnant rats were assessed at the end of gestation, i.e., day 19. Fetuses were delivered on day 21. The more frequently used day 20 was not suitable in this experiment, because the animals were killed and bled 24 h after the injection of radiiodide.

Daily feed intake and urinary iodide excretion were determined for all rats. The mean feed intake was 30 g, resulting in a daily iodide intake of 1.3 µg for the NID groups and 66 ng for the MID groups.

Urinary iodide was determined as described by Sandell and Kolthoff (22).

The experiments were approved by the University Committee on Animal Care and Use of the Agricultural University of Wageningen.

Thyroidal iodide uptake. The rat was anesthetized with xylazine (25 µl of 2% Rompun/100 g body wt sc), atropine (5 µg/100 g body wt sc), and ketamine (50 µl of 10% ketamine/100 g body wt ip). The anesthesized rat was fixed in a bed of plaster. The body temperature was monitored via the rectum and maintained at 38°C by a warming jacket.

The scintillation probe (type 42A NaI crystal 23 mm long × 1 mm thick, connected to mini-Assay type 6–20 (Mini Instruments, Essex, UK)) was placed as close as possible to the thyroid region.

To measure the thyroidal iodide uptake in the rat, a cannula was inserted into the right vena jugularis (20) and a 400-µl bolus of saline containing 10 µCi carrier free Na¹²⁵I (Amersham, Aylesbury, UK) was injected. The radioactivity taken up by the thyroid was measured automatically every 30 s for 4 h and calculated as a percentage of the injected dose. The percentage dose of iodide taken up by the thyroid was fitted to the equation %dose = A [1 - exp(-γt)] by nonlinear regression analysis using the program NLfit/FIT4EXP, which is based on the Levenberger-Marquart method. A represents the maximum percentage dose taken up by the thyroid, and γ is the time constant (min).

Perchlorate discharge test. To investigate the effects of perchlorate on radioactivity in the thyroid, three animals from each group underwent a perchlorate-discharge test (17). Four hours after the administration of Na¹²⁵I, potassium perchlorate (10 mg/kg body wt ip) was administered. Thyroidal radioactivity was measured for another 30 min.

Iodide kinetics in plasma. To study the disappearance of the injected¹²⁵I, plasma samples (50–100 µl) blood were taken via the cannula in the vena jugularis 1, 3, 5, 10, 16, 25, 40, 80, 150 and 240 min after injection of the¹²⁵I. Radioactivity in the plasma samples was expressed as percentage dose per milliliter.

The disappearance of iodide from plasma can be described by an exponential function. The data were fitted, together with the plasma volume at t(0), individually to sums of n = 1–3 exponentials (6)

\[ Y(t) = A_1 \exp(\lambda_1 \cdot t) + A_2 \exp(\lambda_2 \cdot t) + A_3 \exp(\lambda_3 \cdot t) \]

by use of the program DIMSUM, whereby Aᵢ coefficients are expressed in percentage dose per milliliter and exponents λᵢ are expressed per minute (16).

Absolute iodide uptake after 24 h. After measurement the rats were placed in metabolic cages for collection of urine.

Twenty-four hours after injection of the¹²⁵I the rats were killed by bleeding and perfusion with saline under ether anesthesia. The maternal thyroid, mammary gland, placenta, and fetuses were removed. The fetal thyroid was collected by excising that part of the trachea containing the thyroid.

All radioactivities measured (i.e., maternal and fetal thyroxin, plasma, urine, mammary gland, placenta, and fetuses minus thyroid at 24 h) were expressed as percentages of the injected dose.

The assumption can be made that, during a steady-state situation, the specific activity of iodine (ratio of¹²⁵I to¹²³I) in urine is the same as that in the plasma from which it originated (28). The absolute iodide uptake (AIU) by the maternal and fetal thyroids, mammary gland, placenta, and fetuses minus thyroid, as well as the plasma inorganic iodide (PII), can be calculated after determination of the specific activity of iodine in urine (1)

\[ \text{AIU} = \frac{\% \text{ dose after } 24 \text{ h}}{\% \text{ dose urine} / \text{ng iodide urine}} \times (\text{ng} / 24 \text{ h}) \]

\[ \text{PII} = \frac{\% \text{ dose ml plasma}}{\% \text{ dose ml plasma} / \text{ng iodide urine}} \times (\text{ng} / \text{ml}) \]

Radioiodide clearance from the plasma by the thyroid (C-Th) and by the kidneys (C-R) is calculated from the increment between 120 and 240 min divided by the radioactivity in a plasma sample collected simultaneously (1)

\[ C_{Th} = \frac{\% \text{ dose min in thyroid}}{\% \text{ dose ml plasma}} \times (\text{ml/min}) \]

\[ C_{R} = \frac{\% \text{ dose ml plasma} - \text{1.440 min}}{\% \text{ dose ml plasma}} \times (\text{ml/min}) \]

Statistical analysis. All data are expressed as means ± SE. Data were analyzed using the Statistical Package for Social Sciences (25). All data were subjected to one-way analysis of variance, and statistical differences between groups were determined using the least significant difference method.

RESULTS

Marginal iodine deficiency had no effect on either the body weight of the rats or the number of fetuses. No significant alterations were found for plasma TSH. Plasma T₄ and T₃ did not change significantly during marginal iodine deficiency. Pregnancy resulted in a

<table>
<thead>
<tr>
<th>Table 1. Body weight and plasma thyroid hormone concentrations</th>
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<tr>
<td><strong>Contrast</strong></td>
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<tr>
<td>Body weight, g</td>
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<tr>
<td>No. of fetuses</td>
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<td>T₄, nM</td>
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<td>T₃, nM</td>
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<td>TSH, ng/ml</td>
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Values are means ± SE. NID and MID, normal and marginal iodine deficiency, respectively; C, control rats; P, near-term pregnant rats; T₄, thyroxin; T₃, 3,5,3'-triiodothyronine; TSH, thyrotropin. *P < 0.05, P vs. C.
decrease in plasma T_4 and T_3 in NID as well as MID rats (Table 1).

Feed intake and urinary iodide excretion. At the start of the experiments, the marginally iodine-deficient groups received potassium perchlorate for 2 days. The effect of this treatment on urinary iodide excretion is shown in Fig. 1. Potassium perchlorate treatment from day 0 to day 2 resulted in an increase in iodide excretion. Within 1 wk after perchlorate treatment, urinary iodide excretion had decreased to 0.4 µg/day; it remained constant during the rest of the experimental period.

The mean feed intake and urinary iodide excretion for the four groups of rats are shown in Fig. 2. No effect of MID on feed intake was found. During pregnancy, feed intake and urinary iodide excretion increased.

Thyroidal ¹²⁵I uptake. Table 2 shows the data on thyroidal ¹²⁵I uptake. The weight of the thyroid increased significantly in both marginally iodine-deficient groups; pregnancy had no effect on the weight of the thyroid.

The 4-h ¹²⁵I uptake by the thyroid increased in C and P MIDs rats. Pregnancy induced a decrease in ¹²⁵I uptake by the thyroid in both NID and MID rats. The 4-h ¹²⁵I uptake is a direct measurement of the thyroid; A and γ are the results of fitting the data to the one-exponential function %dose = A [1 − exp(−t/γ)]. Two- and three-exponential functions did not yield satisfactory results. The time constant (γ) of the ¹²⁵I uptake was unchanged in all groups. The mean fitted thyroidal ¹²⁵I uptake is given in Fig. 3, where we also see that the thyroidal ¹²⁵I uptake was lower in P rats and increased in the MID groups.

Perchlorate discharge test. No discharge of radioiodide from the thyroid was found for any of the four groups. The radioactivity of the thyroid remained unchanged (results not shown).

Iodide kinetics in plasma. Table 3 shows the parameters of iodide kinetics in plasma. No significant changes were found except for A_1 in pregnant rats.

AIU after 24 h. Table 4 shows the specific activity of urine and the results of calculations of the AIU by the maternal thyroid, PII, C,Th, and C_R. The urinary specific activity was unchanged during pregnancy, but marginal iodine deficiency caused an increase in specific...
activity. The AIU by the thyroid was decreased by pregnancy, whereas marginal iodine deficiency had no effect on the AIU. PII was decreased in the MID groups, whereas pregnancy had no effect. Unidirectional iodide CTh was increased in the MID groups. Cn was increased by pregnancy, whereas MID resulted in a decrease in Cn in C and near-term P rats (Table 4).

The AIU values at 24 h by the maternal thyroid, placentas, fetal thyroids, remaining part of the fetuses, and mammary gland are shown in Fig. 4. Whereas MID had no effect on the AIU by the maternal thyroid, a pronounced decrease was found for the fetal thyroids and the remaining part of the fetuses. The amount of iodide taken up by the mammary gland was also decreased by MID, whereas no significant effect was found for the placentas.

**DISCUSSION**

The process by which the thyroid gland adapts to an insufficient iodine supply is to increase the trapping of iodide. When iodine intake is low, adequate secretion of thyroid hormones may still be achieved by marked modifications of thyroid activity. The thyroid is stimulated by an increased thyroid blood flow (3) and TSH (19, 23). The T1-to-T4 ratio of iodothyronines secreted by the thyroid is increased during iodine deficiency, especially because of a decrease in T4 production (23).

Marginal iodine deficiency was induced in our rats by feeding them a MID diet. Within 2 wk of the start of the MID diet, urinary iodide excretion remained at the same level. So, at the moment of measurement, ≈4 wk after start of the MID diet, the rats were in a steady state. The fact that only a marginal iodine-deficient state was achieved, and not a severe iodine-deficient state, is demonstrated by the unchanged plasma T4 and T3 levels. Despite a slight increase in plasma TSH, the weight of the thyroid had already increased by 50%. The unchanged number of fetuses during pregnancy also demonstrates that the induced iodine deficiency was not severe (8).

In this study we were able to measure thyroidal radioiodide uptake continuously. For the kinetic analysis of radioiodide uptake in the human thyroid, a three-compartmental model is used (3, 14, 30). In this model, a plasma iodide pool and an inorganic and an organic thyroidal iodide pool can be distinguished. We tried to fit our data to this model; however, our data could only be fitted to a one-exponential function. Therefore, we assume that, even in euthyroid rats, iodide transport from plasma into the thyroid is unidirectional because of an extremely avid iodine organification. This idea is confirmed by the total absence of a perchlorate discharge in all rats, meaning that there is no efflux of inorganic iodide from the thyroid (17).

The thyroidal uptake of radioiodide was stimulated by marginal iodine deficiency. The kinetic analysis shows that the time constant for thyroidal radioiodide uptake was not affected by iodine deficiency, meaning that the time needed to achieve the maximum effect remained the same. However, the AIU by the thyroid remained normal. This was achieved by an increased CTh resulting from the increased thyroid volume and thyroidal blood flow (3).

The urinary specific activity was increased, whereas the radioiodide activity in plasma was not altered by marginal iodine deficiency. This resulted in a PII that was significantly lower, such as is found in humans residing in areas of iodine deficiency (5) and in 10-day-old iodine-deficient rats (31).

Our observations emphasize the need for caution in interpretation of results obtained by studies measuring the thyroidal radioiodide uptake only. We have demonstrated that a decrease or increase in radioiodide uptake does not automatically mean that the AIU has changed.

**Table 3. Parameters of iodide kinetics in plasma**

<table>
<thead>
<tr>
<th></th>
<th>NID, C (n = 13)</th>
<th>NID, P (n = 10)</th>
<th>MID, C (n = 8)</th>
<th>MID, P (n = 5)</th>
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<tr>
<td>A1, %dose/ml</td>
<td>7.05 ± 0.42</td>
<td>4.92 ± 0.13*</td>
<td>7.01 ± 0.16</td>
<td>5.34 ± 0.22*</td>
</tr>
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<td>λ1, 1/min</td>
<td>−1.78 ± 0.13</td>
<td>−2.32 ± 0.24</td>
<td>−2.47 ± 0.13</td>
<td>−2.28 ± 0.21</td>
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<tr>
<td>A2, %dose/ml</td>
<td>1.14 ± 0.15</td>
<td>1.19 ± 0.13</td>
<td>1.12 ± 0.07</td>
<td>1.22 ± 0.16</td>
</tr>
<tr>
<td>λ2, 1/min</td>
<td>−0.066 ± 0.014</td>
<td>−0.085 ± 0.013</td>
<td>−0.059 ± 0.004</td>
<td>−0.072 ± 0.009</td>
</tr>
<tr>
<td>A3, %dose/ml</td>
<td>0.806 ± 0.067</td>
<td>0.762 ± 0.049</td>
<td>0.837 ± 0.055</td>
<td>0.767 ± 0.069</td>
</tr>
<tr>
<td>λ3, 1/min</td>
<td>−0.0019 ± 0.0003</td>
<td>−0.0022 ± 0.0007</td>
<td>−0.0025 ± 0.0003</td>
<td>−0.0034 ± 0.0007</td>
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Values are means ± SE. P < 0.05, *P vs. C.

**Table 4. Urinary specific activity, absolute iodide uptake by thyroid at 24 h, plasma inorganic iodide, and clearance of iodide from plasma by thyroid and kidneys**

<table>
<thead>
<tr>
<th></th>
<th>NID, C (n = 7)</th>
<th>NID, P (n = 12)</th>
<th>MID, C (n = 5)</th>
<th>MID, P (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine, %dose/ng</td>
<td>0.022 ± 0.003</td>
<td>0.023 ± 0.003</td>
<td>0.034 ± 0.005</td>
<td>0.037 ± 0.005†</td>
</tr>
<tr>
<td>AIU, µg/24 h</td>
<td>1.29 ± 0.09</td>
<td>0.87 ± 0.16</td>
<td>1.57 ± 0.35</td>
<td>0.89 ± 0.14*</td>
</tr>
<tr>
<td>PII, ng/ml</td>
<td>24.5 ± 4.6</td>
<td>19.9 ± 2.4</td>
<td>17.7 ± 3.6†</td>
<td>9.11 ± 2.3†</td>
</tr>
<tr>
<td>CTh, ml/min</td>
<td>0.079 ± 0.014</td>
<td>0.079 ± 0.015</td>
<td>0.367 ± 0.172†</td>
<td>0.164 ± 0.044</td>
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<tr>
<td>Cr, ml/min</td>
<td>0.149 ± 0.010</td>
<td>0.228 ± 0.040</td>
<td>0.081 ± 0.019†</td>
<td>0.173 ± 0.032*</td>
</tr>
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</table>

Values are means ± SE. AIU, absolute iodide uptake; PII, plasma inorganic iodide; CTh and Cr, clearance of iodide from plasma by thyroid and kidneys. P < 0.05: *P vs. C; †MID vs. NID.
Also, during normal pregnancy, changes in iodide metabolism are found. The urinary iodide excretion was higher for P than C rats of the NID as well as MID groups. This can be explained by the increased feed intake and the increase in CR. Galton (11) also found an increased urinary iodide excretion for pregnant rats during the last days of gestation.

At the end of gestation the percentage of radioiodide taken up by the thyroid was significantly decreased. This has also been reported for rats with a normal iodine intake (9, 11, 15). Because the urinary specific activity was not altered by pregnancy, this also resulted in a decrease in the absolute iodide uptake by the maternal thyroid at the end of gestation in NID as well as MID rats. It seems that the thyroid of the near-term P rat has less iodide available for the production of thyroid hormones. However, no change in thyroid hormone production was found at the end of gestation (27). This would seem to imply that the use of iodide in the near-term P rat is more efficient or that the thyroglobulin stores are depleted.

The difference in thyroid iodide uptake between C and near-term P rats cannot be attributed entirely to the increase in urinary iodide excretion. We suggest that the remaining part of the iodide is used by the mammary glands and the feto-placental compartment. For lactating rats it has been shown that, 4 h after injection, the mammary glands contain as much radioiodide as the thyroid, and it was suggested that during pregnancy radioiodide was already being transported to the mammary glands (15). The latter was contradicted by our measurements. One gram of the mammary gland had only taken up 6.5 ng iodide at 24 h; this is less than 1% of the thyroidal uptake.

The placenta possesses a mechanism for actively transporting iodine (21). Therefore, it is to be expected that a certain amount of radioiodide is transported to the feto-placental compartment. Our results, collected 24 h after injection, showed that the fetal thyroid is capable of concentrating iodide on day 20 of pregnancy. The thyroidal region of the fetus contained as much iodide as the rest of the fetus. However, the total amount of iodide in placentas and fetuses could not explain the decreased uptake of iodide by the maternal thyroid. Therefore, the feto-placental compartment and the mammary glands are not the only factors responsible for the difference in maternal thyroidal iodide uptake.

During marginal iodine deficiency the AIU of the maternal thyroid remained normal. However, there was already a tendency toward a shift in thyroid hormone synthesis; $T_4$ decreased and $T_3$ remained normal. In contrast to the maternal thyroid, no compensation by an increased thyroid clearance for the decreased PII concentration was found for the fetal thyroid. The AIU by the feto-placental compartment was decreased by 50%. This pattern can lead to lower $T_4$ availability for the fetus. This is supported by kinetic studies of marginally iodine-deficient, near-term pregnant rats showing that the maternal transfer of $T_4$ and $T_3$ to the fetuses is decreased during marginal iodine deficiency (unpublished data). As long as the increase in type II deiodinase is sufficient to maintain normal $T_3$ values in the fetal brain, problems need not be expected (18). If this is not achieved, defects in brain development will occur.

In conclusion, during pregnancy the AIU by the maternal thyroid gland is decreased. Marginal iodine deficiency does not affect the maternal thyroidal AIU. The low availability of iodide was compensated by increased activity of the maternal thyroid, whereas fetal thyroidal uptake of iodide decreased by 50%. The fetus is apparently not able to regulate its iodide metabolism in case of marginal iodine deficiency. Therefore, this level of marginal iodine deficiency, despite the near euthyroid status of the mother, already has an effect on the availability of iodide for the fetus. This can mean that fetal $T_4$ production is markedly diminished at a time when it is of eminent importance for the normal development of many organs, especially the brain.

We are grateful to J. S. Goense for setting up the measurements and G. van Niftrik (Gelderse Vallei Hospital, Bennekom, The Netherlands) for designing the measuring bed for the rats. We thank M. van Lieshout for contributing to the experiments, T. Viets for performing the iodide measurements, Dr. R. Bakker for developing the mathematical model, and G. P. Bieger-Smith for correcting the text.
References


