0013-7227/00/$03.00/0
Endocrinology

Vol. 141, No. 7
Printed in U.S.A.

Effects of Selenium Deficiency on Tissue Selenium Content, Deiodinase Activity, and Thyroid Hormone Economy in the Rat during Development*

JOANNE M. BATES, VICKIE. L. SPATE, J. STEVEN MORRIS, DONALD L. ST. GERMAIN, AND VALERIE ANNE GALTON

Departments of Physiology and Medicine, Dartmouth Medical School, Lebanon, New Hampshire 03756-0001; and Reactor Research Laboratories, University of Missouri, Columbia, Missouri 65211

ABSTRACT

The iodothyronine deiodinases, D1, D2, and D3, all contain selenium (Se) in the form of selenocysteine at their active sites, and they play crucial roles in determining the circulating and intracellular levels of the active thyroid hormone (TH), T_3. However, not only are serum T_3 levels normal in Se-deficient rats but phenotypic and reproductive abnormalities are minimal, and it has been suggested that regulatory mechanisms exist to conserve Se in critical tissues.

The present study was designed to determine, in rats: 1) whether the effects of Se-deficiency are greater in the fetus and neonate than in the adult; 2) whether there are tissues other than brain and thyroid in which deiodinase activities are maintained; 3) whether the maintenance of deiodinase activity in a specific tissue is associated with a concomitant preservation of Se level in that tissue; and 4) whether TH economy and general health is maintained over several generations. The tissues studied included liver, cerebrum, thyroid, pituitary, skin, brown adipose tissue, uterus, ovary, testis, placenta, and the implantation site (uterus plus contents) at E9.

The results have revealed that, with the exception of liver, skin, and nonpregnant uterus, all of the tissues studied maintained substantial deiodinase activity (>50%) during prolonged Se-deficiency. Second, although the ability of a tissue to maintain deiodinase activity in the face of dietary Se deprivation was associated in some tissues with a concomitant local preservation of Se concentration, this was not the case for all tissues. Only when Se levels were decreased by more than 80% was deiodinase activity markedly decreased. Third, the effects of Se-deficiency were no greater in the fetus than in the adult; and fourth, at the level of Se-deficiency employed in this study, TH economy and general health were successfully maintained over six generations of Se-deficient rats. How Se levels are maintained in specific tissues, whether Se is sequestered in specific cells of a tissue or organ during dietary Se deprivation, and the precise mechanisms by which plasma T_3 levels are maintained in Se-deficient animals remain unanswered. Further insights may be gained by using diets that are even lower in Se than those that were used herein and/or by conducting studies using radioactive forms of Se and thyroid hormones. (*Endocrinology* **141:** 2490–2500, 2000)

THYROXINE (T_4) IS the major thyroid hormone (TH) secreted by the thyroid gland. It can be deiodinated in tissues either to T_3, which is responsible for most of the physiological activity of the thyroid hormones or to the relatively inactive iodothyronine, rT_3 (1, 2). Three iodothyronine deiodinase isoforms, type 1 (D1), type 2 (D2), and type 3 (D3), have been identified. D1 and D2 catalyze primarily outer-ring or 5′-deiodination (5′D) and thus are responsible for the conversion of T_4 to T_3, whereas D3 catalyzes inner-ring or 5-deiodination (5D) of both T_4 and T_3, thereby generating inactive products from the hormones (1, 2).

The two 5′-deiodinases are differentially expressed in tissues; D1 is found primarily in liver, kidney, and thyroid; whereas D2 is expressed in brain, pituitary, and brown adipose tissue (BAT) (1, 2). D3 is found in cerebral cortex and skin (3, 4) and is expressed at very high levels in placenta (5–7) and pregnant uterus (7), and at much lower levels in several fetal rat tissues (7–9).

All three deiodinases contain selenium (Se), which is present in the form of the amino acid selenocysteine located at their active sites (10–16). Thus, one would predict that a nutritional Se deficiency would result in significant changes in deiodinase activities and, hence, in TH economy. However, in the adult rat, the effects of nutritional Se deprivation on the thyroid axis are relatively modest and seem to be limited to a few select tissues (17, 18). The most notable effects included a marked decrease (>90%) in hepatic and renal D1 activity (19) and protein (20) and a 40–50% increase in serum T_4 concentration. Serum T_3 and TSH levels and thyroidal D1 and brain D3 activities were largely unchanged (18, 21, 22). Brain D2 activity was decreased in these animals, but this was attributed to the down-regulating effects of the elevated circulating T_4 level (23, 24), rather than to a direct effect of Se deficiency (22, 25). These findings have led to the suggestion that brain and thyroid contain mechanisms for local conservation of Se (22, 26, 27).

The impact of Se status on various clinical parameters is currently being investigated. For example, the plasma T_3/T_4 ratio is low in individuals prone to Se deficiency, such as the elderly (28), patients with phenylkenouria (29), and cystic fibrosis (30), and it normalizes upon Se supplementation (31). This may reflect Se-induced alterations in D1 activity. Other studies have suggested that Se deficiency is related to adverse outcomes of pregnancy. Thus, maternal blood Se levels are low in women who experience a first trimester miscar-

Received January 5, 2000.
Address all correspondence and requests for reprints to: Valerie Anne Galton, Department of Physiology, Dartmouth Medical School, 1 Medical Center Drive, Borwell Building, Lebanon, New Hampshire 03756-0001. E-mail: val.galton@dartmouth.edu.
* This work was supported by USPHS Grants T32-DK-07508 (to J.M.B), DK-42271 (to D.L.S.), and HD-09020 (to V.A.G.)

riage, when compared with women at the same stage of pregnancy who carry to term (32). Maternal Se levels were also found to be decreased in women with preterm deliveries; and the activity in cord blood of glutathione peroxidase, another selenocysteine containing enzyme, was diminished in the premature infants (33). The authors suggested that this could be a factor in the higher rate of retinopathy and respiratory distress syndrome observed in preterm infants.

Information regarding the impact of Se deficiency on TH economy during development is limited. Because the fetus develops *de novo*, one would expect it to be more prone than the adult to the adverse effects of Se deficiency. However, based on the limited information available, it is uncertain whether or not this is the case. Although it has been shown that severe Se deficiency can effect fertility in both male and female rodents (34), rats can be carried through three generations on a diet low in Se (22). In some reports, however, significant abnormalities involving sparse hair, delayed growth, retarded motor skills, and ocular abnormalities have been observed in the Se-deficient offspring (35, 36). One group has reported significant decreases in glutathione peroxidase and D1 activity in liver of fetuses from Se-deficient rats, but no changes in the levels of T_4, T_3, rT_3, or TSH in fetal serum were observed, and fetal brain D2 and placental D3 activities were unaffected (37). However, this study involved a relatively modest, short-term Se-deficiency (4 weeks) in the dams before pregnancy, and the investigators examined only selected tissues in near-term (gestation day 21) fetuses.

The present study was designed to answer several questions. First, are the effects of Se-deficiency greater in the fetus and neonate than in the adult? Second, are there tissues other than brain and thyroid in which deiodinase activities are maintained in Se-deficient rats? Third, can the maintenance of deiodinase activity in a specific tissue of Se-deficient rats be explained by a concomitant preservation of Se level in that tissue? Fourth, are TH economy and general health maintained over several generations in Se-deficient animals?

Materials and Methods

Animals

Weanling female Sprague Dawley rats and adult male rats (12 weeks old) were purchased from Charles River Laboratories, Inc. (North Wilmington, MA). The female rats were randomly divided into two groups. Rats and subsequent offspring in one group were fed *ad libitum* a Torula yeast-based, semisynthetic Se-deficient diet (TD 86298, Teklad Premier, Madison, WI), which contained 5 μg Se/kg as Na_2Se0_3. The other group received an Se-sufficient diet (TD 87177) which contained 200 μg Se/kg. The two diets were identical except for their Se content. These rats also received distilled water *ad libitum*. The water was analyzed and found to contain minimal levels of Se. The male rats, which were used for breeding, were fed Purina rat chow and tap water *ad libitum*. Animals were weighed regularly. Females were bred starting at 10–12 weeks of age. The presence of sperm in the morning vaginal smear was taken as day 1 of pregnancy, birth normally occurred on embryonic day 22 (E22). The majority of the studies were carried out in second-generation Se-sufficient and Se-deficient rats and their offspring. This study included fetuses at E21, neonates at postpartum day 12 (P12), their respective dams, and age-matched nonpregnant controls. Female rats from the third and subsequent generations were raised for breeding through to the sixth generation, and some studies used fourth-generation P30 weanlings and sixth-generation pregnant rats at E9. All groups contained a minimum of four rats. When possible, male and female pups were separated to determine whether or not gender differences existed in the parameters measured.

Tissue preparation

Rats were killed by decapitation and exsanguination. Blood, cerebrum, liver, skin, BAT, and placenta were taken from E21 fetuses. Blood, cerebrum, liver, BAT, thyroid, and pituitary were obtained from the P12 neonates, the dams of the fetuses and neonates, and the age-matched nonpregnant controls. Ovaries, uteri, and testes were obtained from fourth-generation weanlings at P30; and individual implantation sites, which consisted of uterus and its contents (decidual tissue and embryo), were obtained at E9 from sixth-generation pregnant rats. From preliminary studies, it was determined that the activities of the deiodinases in muscle, heart, and adrenals were too low to allow significant comparisons to be made.

Representative samples of tissue and serum were snap-frozen in 2-ml microfuge tubes on dry ice and then stored at −80 C until they were shipped on dry ice to the University of Missouri for analysis of their Se content. Aliquots of serum were also stored at −20 C for subsequent analysis of TH levels.

For determination of 5′D and 5D activities, tissues were homogenized in a deiodinase buffer (0.25 mM sucrose and 20 mM Tris-Hcl, pH 7.6) using a Tissumizer (Tekmar Co., Cincinnati OH). The homogenates were centrifuged at 1000 × *g* for 15 min, and the supernates were stored at −20 C for subsequent assay.

5D and 5′D assays in rat tissue homogenates

Tissue samples were assayed for 5D and 5′D, according to published methods (38, 39). The reaction mixture (total vol, 50 μl) contained between 25 and 250 μg tissue protein for the 5D assay, and 1 μg (adult liver) to 500 μg (most fetal and neonatal tissues) for the 5′D assay. Protein concentrations for both assays were adjusted to ensure that deiodination was less than 20%. The incubation time for both assays was 1 h. For the 5D assays, 1 nM [^{125}I]T_3 was used as substrate and 50 mM dithiothreitol as cofactor; activity is expressed as femtomoles or picomoles of 3,3′-diiodothyronine (T_2) generated per hour per milligram of protein. For the 5′D assays, EDTA (1.2 mM) was included in the incubation mixture, the substrate was 1.0 nM [^{125}I]rT_3, and the cofactor was 20 mM dithiothreitol; activity is expressed as femtomoles or picomoles of iodide generated per hour per milligram of protein. In determining 5′D activity, the percent of iodide generated was multiplied by 2 because the specific activities of the labeled products were only half that of the substrate. D1 and D2 5′D activities were distinguished, respectively, by the inclusion of 1 mM 6-n-propyl-2-thiouracil and/or 100 nM nonradioactive T_4 in the incubation medium. [^{125}I]iodothyronines ([^{125}I]rT_3: specific activity ~959 μCi/μg; [^{125}I]T_3: ~3390 μCi/μg) were obtained from Dupont de Nemours (Boston, MA) and were purified by chromatography using Sephadex LH-20 (Sigma, St Louis, MO) before use. Protein concentrations of all samples were determined, according to the method of Comings and Tack, using BSA as the standard (40). Because initial studies revealed that D1, D2, and D3 activity in P12 Se-deficient and Se-sufficient rats was not influenced by gender, most assays in P12 rats used tissues from both sexes.

Determination of Se content of serum and tissues

Tissue samples were transferred to clean polyethylene vials (0.25 ml) that had been previously tared. The samples were dried and then weighed using an analytical balance (Mettler AT261, Fisher Scientific, Pittsburgh, PA), which had a sensitivity of 0.00001 g. Finally, spacers were used to fix the samples in the bottom of the vials during all subsequent analysis steps. Samples prepared in this way, along with Se standards and quality-control samples (NIST Standard Reference Material 1577, Bovine Liver) were placed in shuttle capsules and irradiated for 7 sec at a thermal neutron flux of 8E13 n/cm**2/sec using the pneumatic-tube irradiation facility at the Missouri University Research Reactor. During the irradiation, naturally occurring, nonradioactive Se-76 atoms capture neutrons to produce radioactive Se-77m (half life, 17.4 sec). After the irradiation, samples and standards were removed from the shuttle capsule and placed in a fixed counting position approximately 5 mm from a high-purity germanium detector (EG&G OR-

TEC, Oakridge, TN) coupled to a state-of-the-art high-resolution γ-ray spectrometer (Canberra-Nuclear Data, Meridian, CT). At precisely 15 sec, measured from the end of the irradiation, each sample and standard was real-time counted for a period of 25 sec to quantify the γ-ray emissions (161.9 keV) from the photon decay of Se-77m. Pulse pile-up corrections were made by the Westphal virtual-pulse method using a loss-free counting module (Canberra-Nuclear Data). Data reduction to produce the Se concentration for each tissue and quality control sample was carried out by standard comparison. Se concentrations are expressed as ppm. For liver, cerebrum, BAT, skin, pituitary, and thyroid, ppm = μg Se/g dry tissue). For all other tissues ppm = μg Se/g wet tissue. Each sample was analyzed at least twice by the above method, and the small samples were analyzed three times. Results are reported as means for the two or three determinations for each sample. The absolute sensitivity for an Se measurement made by this method is approximately 0.1 ng, and this permitted Se levels to be quantified in tissue samples of less than 50 mg from Se-deficient animals.

Analysis of plasma thyroid hormone concentration by RIA

Serum total T_3 and T_4 concentrations were determined, in duplicate, by species-adapted specific RIAs, using commercial kits according to the manufacturer's directions (Diagnostic Products, Los Angeles, CA). A T_3 resin uptake assay was also performed using a kit from the same company.

Statistical analysis

Student's *t* test was used to compare differences between the mean values obtained in Se-deficient and Se-sufficient in each tissue analyzed at each developmental period (41). Data are expressed as mean ± SE. Statistical significance was defined as $P < 0.05$. To test the significance in multigroup comparisons, one-way ANOVA was used; differences at the 5% level ($P < 0.05$) were assessed by Tukey's *post hoc* probability test (42).

Results

General effects of a Se-deficient diet on rat growth and development

Rats maintained on the Se-deficient diet gave birth to pups that grew at a slower rate during the first few months than did pups born to dams on the Se-sufficient diet. However, this growth retardation was no longer observed once the rats reached maturity. Many Se-deficient pups exhibited sparse hair growth in the early weeks, but this also was not evident after the first 3 months. Unilateral cataract development was observed in 12 of the Se-deficient rats; none was noted in the Se-sufficient group. Apart from these problems, the Se-deficient rats exhibited no obvious effects, and normal reproductive capacity was retained through six generations.

Effects of a Se-deficient diet on tissue Se content and deiodinase activity

Serum Se levels were much lower in Se-sufficient fetal and neonatal rats than in their dams ($P < 0.004$), and Se levels in these dams were lower than in the age-matched nonpregnant rats ($P < 0.004$) (Fig. 1). However, in all groups, Se-deficiency resulted in a substantial decrease in serum Se levels, ranging from an 82% decrease in fetal serum to a greater-than-92% decrease in serum from the adult rats.

This decrease in serum Se concentration was associated with a decrease in Se concentration in all tissues studied at almost all stages of development. However, the extents to which tissue Se content was depleted, and deiodinase activity affected, varied greatly among tissues. In liver, skin, and

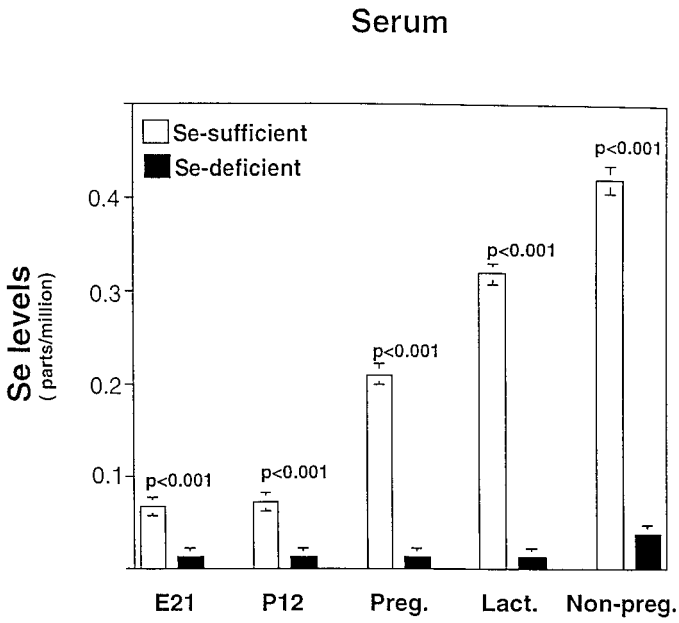

FIG. 1. Se levels in serum of Se-sufficient and Se-deficient fetal (E21), neonatal (P12), pregnant (Preg.), lactating (Lact.), and nonpregnant (Non-preg.) rats. Data are expressed as mean ± SE, n = 4 (minimum). The *P* value indicates significant differences between animals in the two diet groups at each developmental stage.

uterus, Se-deficiency resulted in very marked decreases in the levels of both Se and deiodinase activity (Fig. 2). In liver, the Se level was decreased by 90% or more and D1 activity was greatly diminished (Fig. 2, A and B); D2 and D3 were not expressed to a measurable extent in this tissue at the stages of development studied. It was also noted that, as in serum, Se concentration in liver was significantly lower in the fetal and neonatal rats than in adult rats ($P < 0.004$). In neonatal skin, the Se level was decreased by 85% (Fig. 2C) This tissue expresses all three deiodinases and, with the exception of D2 in the P12 rats (which was minimal, even in the Se-sufficient rats), deiodinase activities were greatly decreased at both E21 and P12 (Fig. 2D). In uterus from P30 rats, the Se level was decreased by 90%, and this was associated with a marked decrease in tissue D3 activity (Fig. 2, E and F). Although substantial 5′D activity, which proved to be entirely attributable to expression of D2, is present in adult uterus (Galton, unpublished observations); in the present study, this deiodinase was only minimally expressed at P30.

In contrast, cerebrum, thyroid, and pituitary from Se-deficient rats exhibited a much less marked decrease in Se levels, and deiodinase activity in these tissues was only minimally effected (Fig. 3). In cerebrum from Se-deficient rats, the decrease in the Se level was less than 45%; and, with the exception of D1 activity in nonpregnant rats, the activities of all three deiodinases were unchanged (Fig. 3, A and B). It was also noted that, in contrast to serum and liver, Se levels were significantly higher in fetal than in adult brain ($P < 0.003$). In thyroid, the decrease in Se level was less than 43% in all but the P12 rats, and no decrease in deiodinase activity was observed (Fig. 3, C and D). Similarly, the decrease in Se level in pituitary was less than 41%, and deiodinase activity was minimally affected (Fig. 3, E and F). Under the conditions of

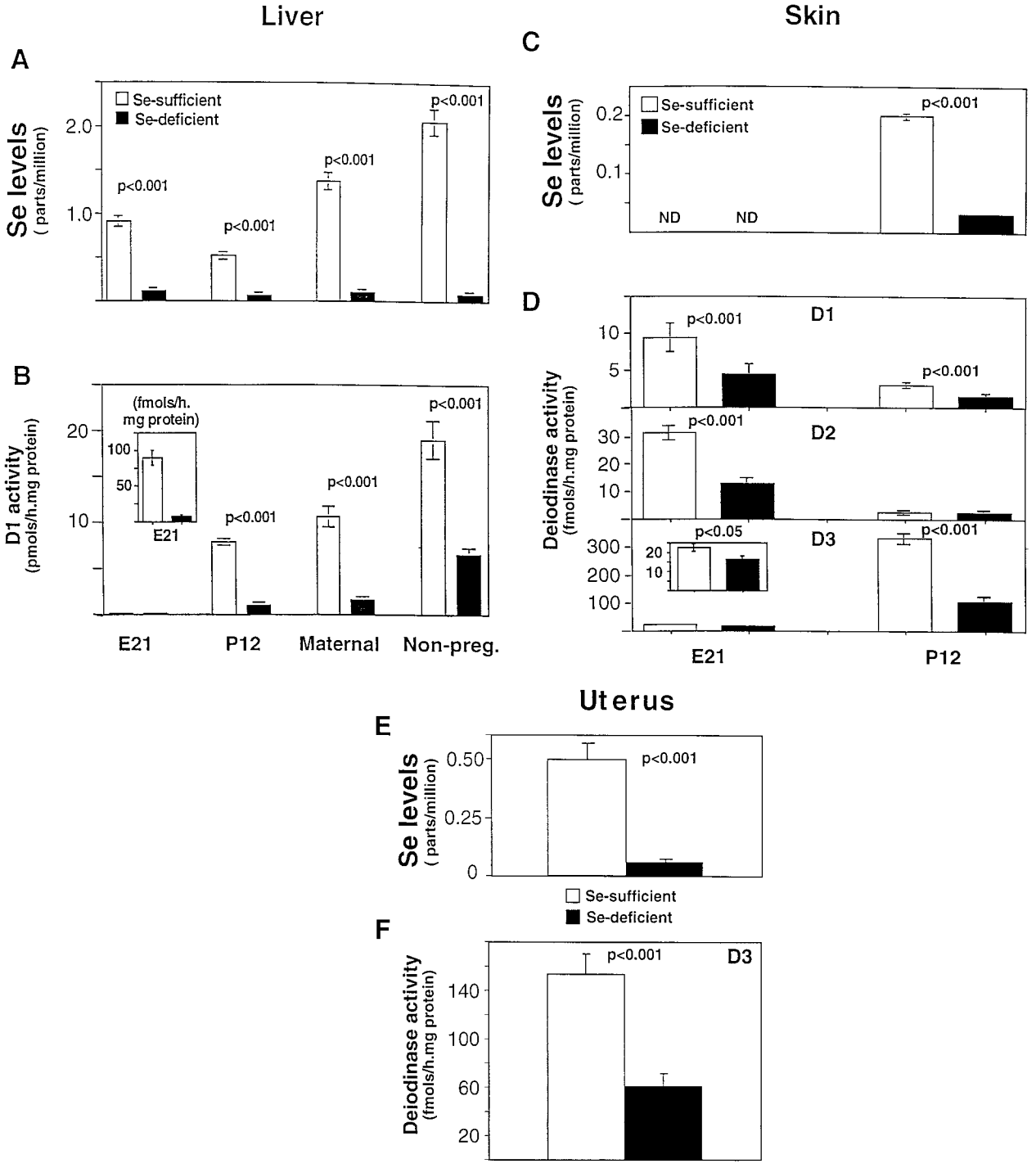

FIG. 2. Se levels in liver (A), skin (C), and uterus (E), and deiodinase activities in liver (B), Skin (D), and uterus (F) of Se-sufficient and Se-deficient rats at different stages of development. The type of deiodinase activity is specified. ND, Not determined. Data are expressed as mean ± SE, n = 4 (minimum). The *P* value indicates significant differences between animals in the two diet groups at each developmental stage.

these studies, no D3 activity was expressed in either thyroid or pituitary, and D1 constituted all the 5′D activity in thyroid and more than 95% of that in pituitary.

Several other tissues showed substantial decreases in Se levels, yet exhibited little (if any) decrease in deiodinase activities. Thus, in BAT, although the decrease in the Se level was at least 75%, mean values for D2 activity were only slightly reduced in the Se-deficient rats; the reduction was significant only in the nonpregnant group (Fig. 4, A and B). BAT was another tissue in which Se levels were much higher in the fetus than in the adult. In ovary, the Se level was decreased by 62%, but the activities of the two deiodinases expressed (D1 and D3) were unchanged (Fig. 4, C and D). In testis, the Se level was decreased 67%, but D1 activity was

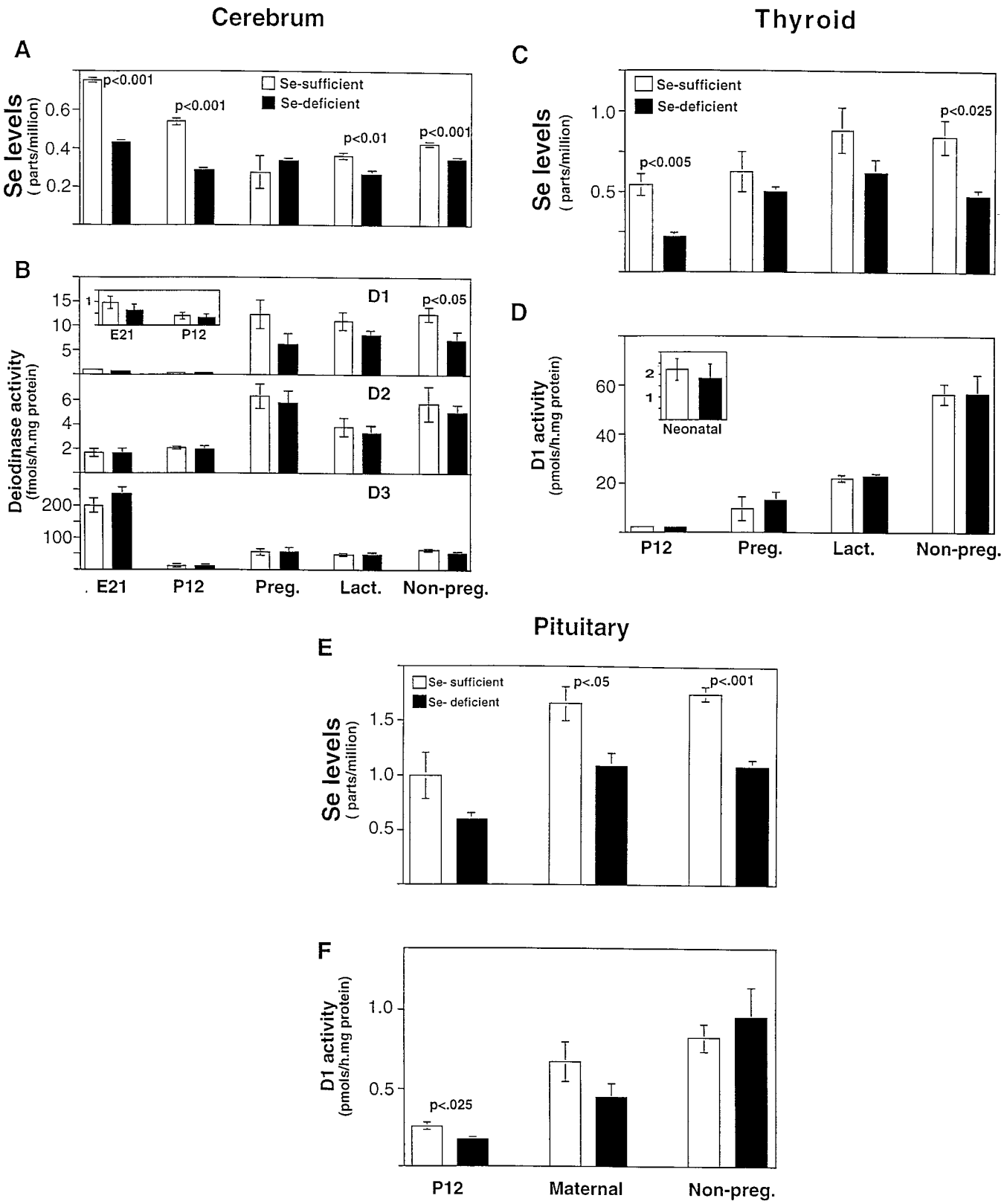

FIG. 3. Se levels in cerebrum (A), thyroid (C), and pituitary (E), and deiodinase activities in cerebrum (B), thyroid (D), and pituitary (F) of Se-sufficient and Se-deficient rats at different stages of development. The type of deiodinase activity is specified. Data are expressed as mean ± SE, n = 4 (minimum). The *P* value indicates significant differences between animals in the two diet groups at each developmental stage.

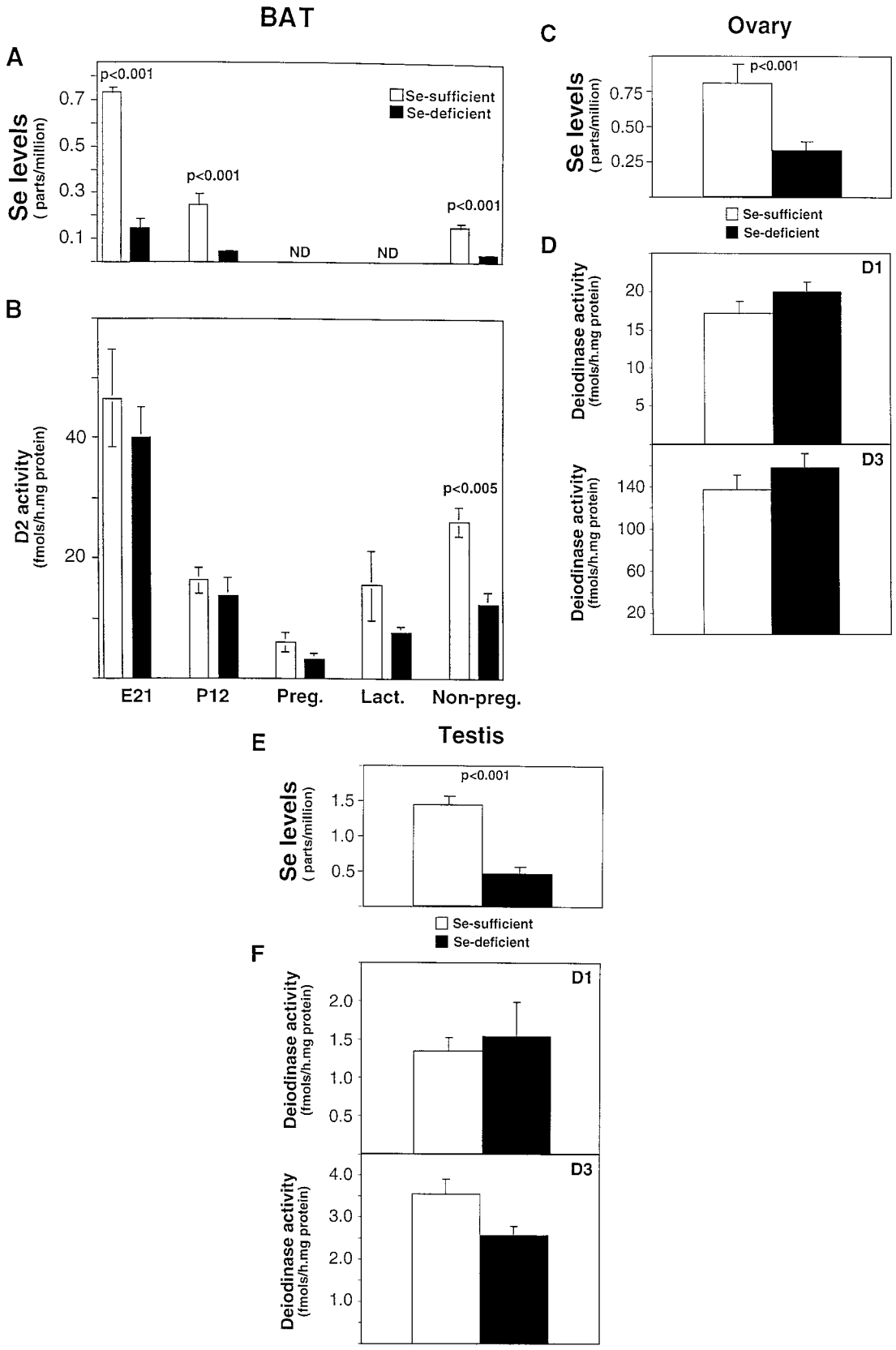

FIG. 4. Se levels in BAT (A), ovary (C), and testis (E), and deiodinase activities in BAT (B), ovary (D), and testis (F) of Se-sufficient and Se-deficient rats at different stages of development. The type of deiodinase activity is specified. ND, Not determined. Data are expressed as mean ± SE, n = 4 (minimum). The *P* value indicates significant differences between animals in the two diet groups at each developmental stage.

unchanged, and D3 activity only slightly decreased (Fig. 4, E and F). Finally, in placenta and E9 implantation site, although the Se levels were decreased 78% and 73%, respectively, the levels of D3 activity were not affected, and those of D2 activity were only minimally reduced (Fig. 5 A–D).

Effects of Se-deficiency on serum thyroid hormone levels

The effects of Se-deficiency on serum TH levels were minimal (Fig. 6). Mean values for serum T_4 levels were slightly increased in the Se-deficient rats, but this increase was not significant. The mean value for T_3 concentration was reduced in Se-deficient fetal rats, but again this difference was not statistically significant; and in all other groups, serum T_3 concentration was not changed in the Se-deficient group. T_3 resin uptake was unchanged in the serum of Se-deficient rats (data not shown), indicating that the TH binding activity of the serum was not affected by this condition.

Discussion

Previous studies in rats fed a Se-deficient diet have documented that levels of deiodinase activity are greatly decreased in liver and kidney but are maintained in brain, thyroid, and placenta (18, 19, 21, 22, 37). The present studies have shown that, in addition to these latter three tissues, deiodinase activities also remain relatively unaffected by Se deprivation in pituitary, ovary, testis, fetal and neonatal BAT, and E9 implantation site. In only three tissues were levels of activity decreased by more than 50%, and these were liver, skin, and nonpregnant uterus. It was also noted that when a tissue expressed more than one deiodinase activity, each one responded similarly to Se-deficiency.

It has been suggested that the ability of a tissue to maintain levels of deiodinase activity is a function of the extent to which it can maintain its local Se concentration (22, 26, 27). The present findings in liver, cerebrum, thyroid, and pituitary are consistent with that view. Thus, in liver, both Se levels and deiodinase activity were greatly diminished; whereas in cerebrum, thyroid, and pituitary, Se levels were decreased less than 50%, and deiodinase levels were well maintained. However, in many other tissues, deiodinase levels were also well maintained, in spite of greatly diminished local Se levels. To examine this relationship further, the percent decreases in the activities of D1, D2, and D3 in all tissues and at all stages of development were plotted against the corresponding percent decrease in Se concentration (Fig. 7A). Examination of the data in this way revealed that local Se levels can fall by almost 80%, and a tissue is still able to maintain substantial levels of deiodinase activity. Only when the decrease was more that 80%, such as occurred in liver, skin, and uterus, was it associated with a marked decrease

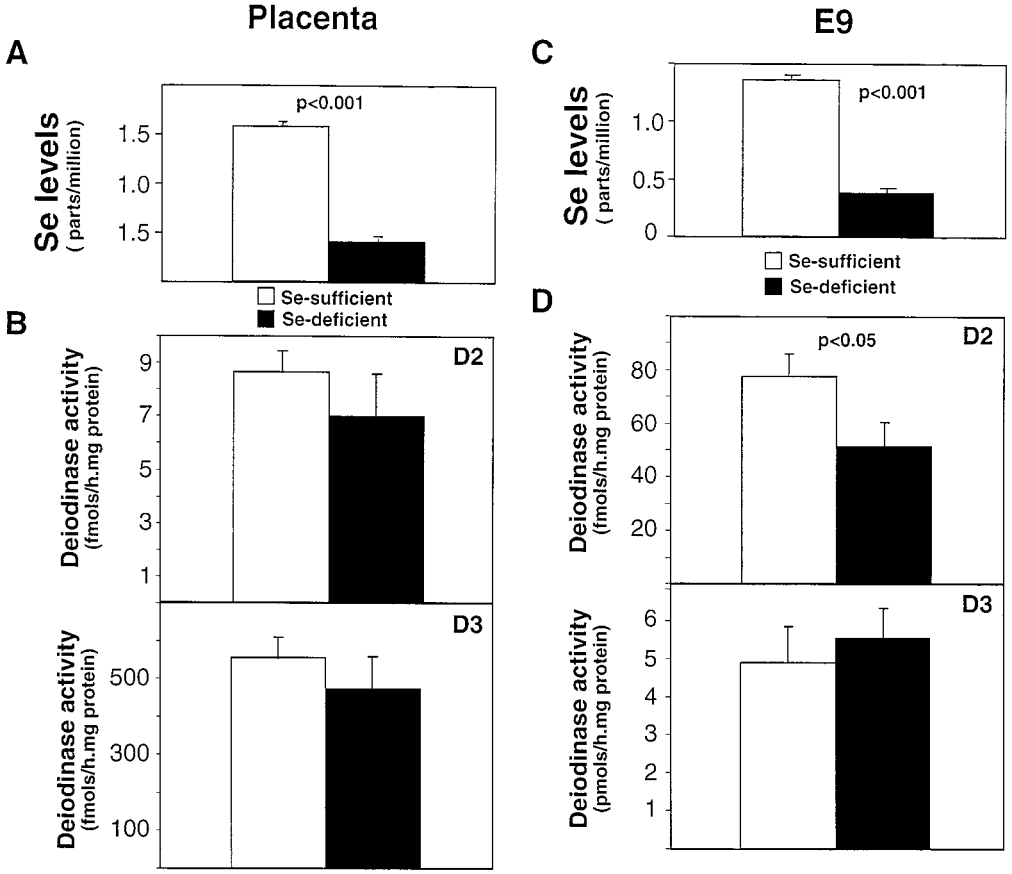

FIG. 5. Se levels in E21 placenta (A) and E9 implantation site (C), and deiodinase activities in E21 placenta (B) and E9 implantation site (D) of Se-sufficient and Se-deficient rats. The type of deiodinase activity is specified. Data are expressed as mean ± SE, n = 4 (minimum). The *P* value indicates significant differences between animals in the two diet groups at each developmental stage.

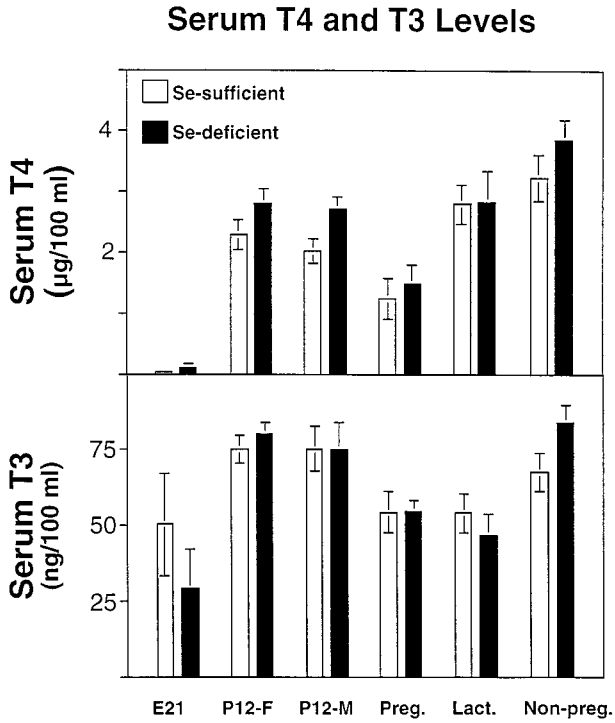

FIG. 6. Concentration of T_4 (μg/100 ml) and T_4 (ng/100 ml) in serum of Se-sufficient and Se-deficient rats at different stages of development. Data are expressed as mean ± SE, n = 4 (minimum). Differences between the mean values obtained in rats from the two diet groups at each developmental stage were not significant.

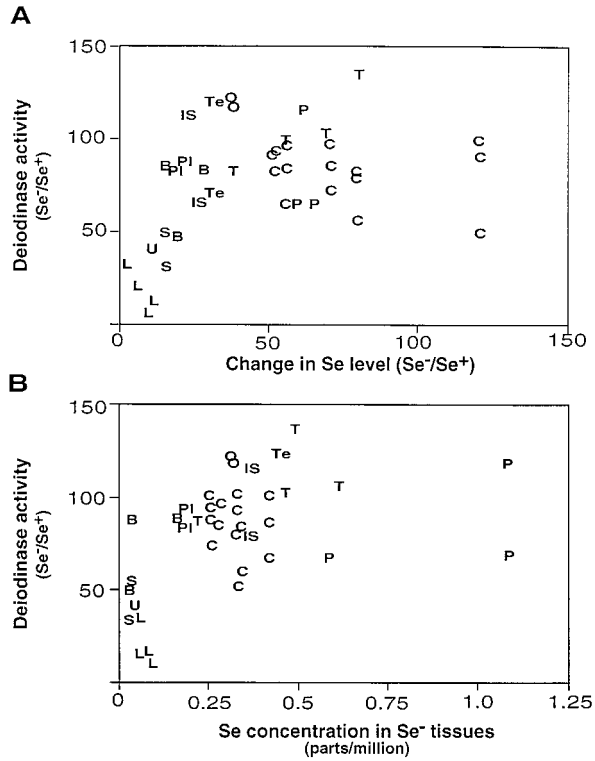

FIG. 7. Scattergrams depicting in Se-deficient rats the percent change in deiodinase activity plotted against the percent change in Se level (A) and the absolute Se concentration in Se-deficient tissues (B). The type of deiodinase activity and the stage of development are not distinguished. Tissues are indicated as follows: L, liver; C, cerebrum; B, BAT; S, skin; P, pituitary; Pl, placenta; T, thyroid; Te, testis; O, ovary; U, uterus; and IS, E9 implantation site.

in deiodinase activity. The decreases in deiodinase activity were also plotted against the absolute Se concentrations achieved during Se deprivation (Fig. 7B). These concentrations ranged from 0.01–1.1 ppm. In all tissues in which Se concentrations remained above 0.2 ppm, deiodinase activities were decreased less than 50%. However, several tissues exhibited Se levels below 0.1 ppm. At these low levels of Se, there seems to be no correlation with the change in deiodinase activity. Thus, ovary, testis, E9 implantation site, and BAT exhibited little or no decrease in deiodinase activity, whereas a marked decrease was observed in liver, skin, and nonpregnant uterus. These observations indicate that in Se-deprived rats, the decrease in levels of deiodinase expression correlates more closely with the fractional decrease in Se concentration than with the absolute Se concentration. However, many of these tissues are heterogeneous; and in tissues such as ovary, testis, and E9 implantation site, the possibility cannot be excluded that Se is sequestered in the cells that express the deiodinase activity.

The relationship between the decrease in Se content and tissue deiodinase activity seems to be independent of age; there was no evidence that the effects of Se-deficiency were greater in the fetus and neonate than in the adult. However, there were some striking differences in tissue Se content in the Se-sufficient animals, between developing and adult rats (Fig. 8). In serum, the Se concentration in adults was more than four times that in the fetus and neonate; and in liver; it was at least double. In contrast, the Se concentration in fetal and neonatal cerebrum was greater than that in adult cerebrum, and the concentration in fetal BAT was four times that in adult BAT. It was also noted that the highest levels of Se were found in adult liver and pituitary. The significance of these observations remains to be determined.

In contrast to the findings of previous investigators (18, 19, 21, 22), Se deficiency did not result in a significant increase in plasma T_4 at any stage of development. The reason for this difference is not clear, but it was noted that, in all but the lactating rats, the mean plasma T_4 concentration was slightly higher in the Se-deficient than in the Se-sufficient rats. Because there were only four rats per group and the standard error was relatively large, it is possible that the difference would have reached significance if more rats had been employed. As reported by others (18, 21, 22), plasma T_3 levels in the present study were not affected by Se deprivation. Although levels of free T_4 and T_3 were not determined, the results of a T_3-uptake assay indicated that the T_3 binding activity of the serum was comparable in rats on the two diets; and thus, the ratio of free-to-bound TH in the two groups was likely also to be comparable.

In view of the findings that plasma TH concentrations are essentially unaffected at the level of Se-deficiency attained in the present study, the lack of any distinct phenotype, including signs of altered thyroid status or reproductive capability, is not really surprising. The only problems observed were a slight retardation in growth, poor hair development in the neonatal phase, and an occasional cataract. The first two problems were temporary; adult Se-deficient rats were

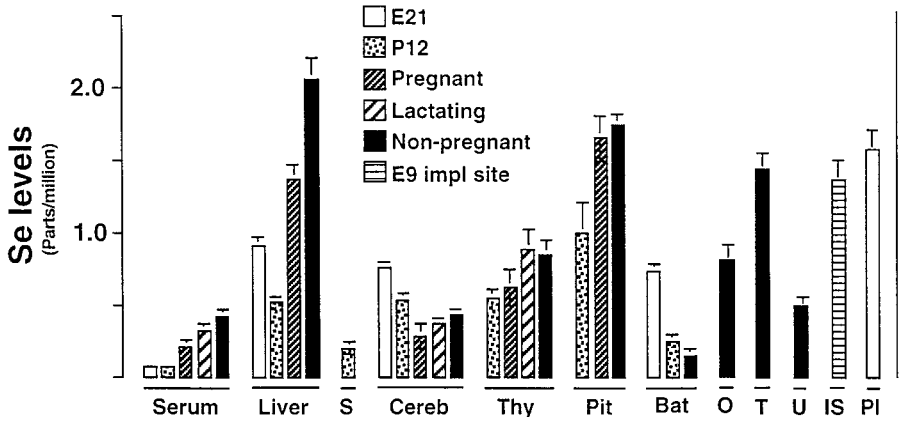

FIG. 8. Se levels in tissues of rats, at different stages of development, fed a Se-sufficient diet. Data are expressed as mean ± SE, n = 4 (minimum). Tissue abbreviations are as follows: S, skin; Cereb, cerebrum; thy, thyroid; pit, pituitary; Bat, brown adipose tissue; O, ovary; T, testis; U, uterus; IS, implantation site; and Pl, placenta.

comparable in weight and coat thickness with age-matched Se-sufficient rats. Furthermore, cataracts were a relatively rare occurrence. The thyroid is known to play a major role in mammalian growth (43–45); and in rats, this is achieved, in part, through a direct stimulation of the GH gene expression (46). In the present study, the pituitary was a tissue in which Se levels were not greatly depressed. However, whereas levels of D1 activity were unaffected in the adult nonpregnant rat, activity was reduced by 50% in the neonatal P12 rat. Because this is the period when the growth retardation was noted, it is tempting to speculate that, as a result of the temporary decrease in pituitary D1 activity, intrapituitary T_3 levels were reduced, leading to a decrease in transcription of the GH gene. However, given the known complex role of the thyroid in growth, this explanation is unlikely to be the whole answer. Furthermore, the possibility that these morphological abnormalities are the result of Se-deprivation *per se*, or secondary to an effect of this deficiency on a process unrelated to the thyroid-pituitary axis, cannot be excluded.

The effect of Se deficiency on hair growth might reasonably be attributed to the substantial decreases in deiodinase activities resulting from the marked decrease in Se levels in skin. Skin and hair development are known to be influenced by TH (47), and thus would be expected to be influenced by changes in intradermal T_3 levels. The high level of D2 expression in skin at E21 coincides with a critical time of differentiation when there is formation of the skin permeability barrier in preparation for birth (48). T_3 and glucocorticoids accelerate this barrier development by stimulation of two important enzymes, steroid sulfatase and cholesterol sulfotransferase (49, 50). The situation in skin may be compared with that in tadpoles, where D2 expression in given tissues is highest at the time of that tissue's maximum T_3-dependent differentiation (51). In addition, as previously reported by us and others (4, 8, 9), D3 activity rises markedly after birth, reaching a peak around P12, the time that hair growth becomes significant. It has been postulated that in tissues undergoing T_3-dependent differentiation, the expression of both D3 and D2 serve to ensure that critical intracellular levels of T_3 are maintained (2, 51). If this is so, then the marked decreases in the levels of skin D2 and D3 activities in Se- deficient fetal and neonatal rats would be expected to have significant effects on intradermal T_3 levels, which would, in turn, influence T_3-dependent developmental processes.

Because of the high levels of D1 activity in liver and kidney, these two tissues are generally considered to be the major source of plasma T_3 (52). The present finding that plasma T_3 levels were unaffected by a 95% decrease in hepatic D1 activity seems inconsistent with this view. There are several possible explanations for this apparent paradox. First, it may be that, in spite of the marked decrease in hepatic D1 activity, the remaining activity was sufficient to maintain plasma T_3 levels. Second, because the D1 enzyme has both 5′D and 5D activities, it is possible that the decrease in hepatic T_3 generation was countered by a comparable decrease in hepatic T_3 degradation, thus leaving the amount released to the plasma unchanged. Third, a decrease in the amount of plasma T_3 generated in the liver or kidney may have been made up with T_3 generated in the thyroid and/or other tissues less affected by Se efficiency. Fourth, there may have been a decrease in the clearance of T_3 from plasma; and fifth, there may have been a shift in the enterohepatic circulation of TH, such that either T_3 secretion in the bile was reduced or T_3 resorption through intestinal wall into the circulation was increased in the Se-deficient rats.

The Se-deficient rat shares an important characteristic with the C3H/Hej inbred mouse. In this mouse strain, the hepatic D1 activity is only 10% of that in the C57BL/6j (C57) strain, which exhibits a D1 phenotype more typical of this species (53, 54). In spite of the low hepatic D1 activity, the C3H/Hej mouse exhibits a serum T_3 level in the normal range. However, these mice also exhibit a 2-fold increase in the serum free T_4 concentration (53) and a decrease in T_3 clearance (55), and the authors suggest that these are likely mechanisms through which serum T_3 concentration is maintained (53).

In summary, the results obtained in this study have provided the answers to the four questions posed regarding Se-deficient rats. First, there are tissues, in addition to brain and thyroid, in which substantial deiodinase activity is maintained during prolonged Se-deficiency; in fact, with the exception of liver, skin, and uterus, all of the tissues studied fell into this category. Second, the ability of a tissue to maintain deiodinase activity in the face of dietary Se deprivation could not be explained in all tissues by a concomitant local preservation of Se concentration. Third, the effects of Se-deficiency were no greater in the fetus than in the adult; and

fourth, at the level of Se-deficiency employed in this study, TH economy and general health were successfully maintained over six generations of Se-deficient rats. However, other questions, such as how Se levels are maintained in specific tissues, whether Se is sequestered in specific cells of a tissue or organ during dietary Se deprivation, and the precise mechanisms by which plasma T_3 levels are maintained in Se-deficient animals, remain unanswered. Further insights may be gained by using diets that are even lower in Se than those that were used herein, and/or by studies using radioactive forms of Se and thyroid hormones.

Acknowledgment

The authors gratefully acknowledge the excellent technical help of Mr. David Lori.

References

1. **Leonard JL, Visser TJ** 1986 Biochemistry of deiodination. In: Hennemann G (ed) Thyroid Hormone Metabolism. Marcel Dekker, Inc., New York, pp 189–229
2. **Kaplan MM** 1986 Regulatory influences on iodothyronine deiodination in animal tissues. In: Hennemann G (ed) Thyroid Hormone Metabolism. Marcel Dekker, Inc., New York, pp 231–253
3. **Kaplan MM, Visser TJ, Yaskoski KA, Leonard JL** 1983 Characteristics of iodothyronine tyrosyl ring deiodination by rat cerebral cortical microsomes. Endocrinology 112:35–42
4. **Schroder-van der Elst JP, van der Heide D, Morreale de Escobar G, Obregon MJ** 1998 Iodothyronine deiodinase activities in fetal rat tissues at several levels of iodine deficiency: a role for the skin in 3,5,3′-triiodothyronine. Endocrinology 139:2229–2234
5. **Roti E, Fang S, Braverman LE, Emerson CH** 1982 Rat placenta is an active site of inner ring deiodination of thyroxine and 3,3′,5-triiodothyronine. Endocrinology 110:34–37
6. **Roti E, Fang SL, Green K, Emerson CH, Braverman LE** 1981 Human placenta is an active site of thyroxine and 3,3′,5-triiodothyronine tyrosyl ring deiodination. J Clin Endocrinol Metab 53:498–501
7. **Galton VA, Martinez E, Hernandez A, St. Germain EA, Bates JM, St. Germain DL** 1999 Pregnant rat uterus expresses high levels of the type 3 iodothyronine deiodinase. J Clin Invest 103:979–987
8. **Huang T, Chopra IJ, Boado R, Solomon DH, Chua Teco GN** 1988 Thyroxine inner ring monodeiodinating activity in fetal tissues of the rat. Pediatr Res 23:196–199
9. **Bates JM, St. Germain DL, Galton VA** 1999 Expression profiles of the three iodothyronine deiodinases, D1, D2 and D3, in the developing rat. Endocrinology 140:844–851
10. **Berry MJ, Banu L, Larsen PR** 1991 Type I iodothyronine deiodinase is a selenocysteine-containing enzyme. Nature 349:438–440
11. **St. Germain DL, Schwartzman R, Croteau W, Kanamori A, Wang Z, Brown DD, Galton VA** 1994 A thyroid hormone regulated gene in *Xenopus laevis* encodes a type III iodothyronine 5-deiodinase. Proc Natl Acad Sci USA 91: 7767–7771. Correction. Proc Natl Acad Sci USA 7791:11282–11994
12. **Croteau W, Whittemore SL, Schneider MJ, St. Germain DL** 1995 Cloning and expression of a cDNA for a mammalian type III iodothyronine deiodinase. J Biol Chem 270:16569–16575
13. **Davey JC, Becker KB, Schneider MJ, St. Germain DL, Galton VA** 1995 Cloning of a cDNA for the type II iodothyronine deiodinase. J Biol Chem 270:26786–26789
14. **Croteau W, Davey JC, Galton VA, St. Germain DL** 1996 Cloning of the mammalian type II iodothyronine deiodinase: a selenoprotein differentially expressed and regulated in the human brain and other tissues. J Clin Invest 98:405–417
15. **Buettner C, Harney JW, Larsen PR** 1998 The 3′-untranslated region of the human type 2 iodothyronine deiodinase mRNA contains a functional selenocysteine insertion sequence. J Biol Chem 273:33374–33378
16. **Davey JC, Schneider MJ, Becker KB, Galton VA** 1999 Cloning of a 5.8-kb cDNA for a mouse type 2 deiodinase. Endocrinology 140:1022–1025
17. **Arthur JR, Nicol F, Hutchinson AR, Beckett GJ** 1990 The effects of selenium depletion and repletion on the metabolism of thyroid hormones in the rat. J Inorg Biochem 9:101–108
18. **Chanoine J, Safran M, Farwell AP, Dubord S, Alex S, Stone S, Arthur JR, Braverman LE, Leonard JL** 1992 Effects of selenium deficiency on thyroid hormone economy in rats. Endocrinology 131:1787–1792
19. **Beckett GJ, MacDougal DA, Nicol F, Arthur JR** 1989 Inhibition of types I and II iodothyronine deiodinase activity in rat liver, kidney and brain produced by selenium deficiency. Biochem J 259:887–892
20. **DePalo D, Kinlaw WB, Zhao C, Engelberg-Kulka H, St. Germain DL** 1994 Effect of selenium deficiency on type I 5′-deiodinase. J Biol Chem 269:16223–16228
21. **Chanoine J, Braverman LE, Farwell AP, Safran M, Alex S, Dubord S, Leonard JL** 1993 The thyroid gland is a major source of circulating T_3 in the rat. J Clin Invest 91:2709–2713
22. **Meinhold H, Campos-Barros A, Walzog B, Köhler R, Müller F, Behne D** 1993 Effects of selenium and iodine deficiency on type I, type II, and type III iodothyronine deiodinases and circulating thyroid hormones in the rat. Exp Clin Endocrinol 101:87–93
23. **Leonard JL, Silva JE, Kaplan MM, Mellen SA, Visser TJ, Larsen PR** 1984 Acute posttranscriptional regulation of cerebrocortical and pituitary iodothyronine 5′-deiodinases by thyroid hormone. Endocrinology 114:998–1004
24. **St. Germain DL** 1988 The effects and interactions of substrates, inhibitors, and the cellular thiol-disulfide balance on the regulation of type II iodothyronine 5′-deiodinase. Endocrinology 122:1860–1868
25. **Chanoine JP, Safran M, Farwell AP, Tranter P, Ekenbarger DM, Dubord S, Alex S, Arthur JR, Becker GJ, Braverman LE, Leonard JL** 1992 Selenium deficiency and type II 5′deiodinase regulation in the euthyroid and hypothyroid rat: evidence of a direct effect of thyroxine. Endocrinology 130:479–484
26. **Buckman TD, Sutphin MS, Eckhert CD** 1993 A comparison of the effects of dietary selenium on selenoproteins expression in the rat brain and liver. Biochim Biophys Acta 1163:176–184
27. **Vadhanavikit S, Ganther HE** 1993 Selenium requirements of rats for normal hepatic and thyroidal 5′-deiodinase (type I) activities. J Nutr 123:1124–1128
28. **Olivieri O, Girelli D, Azzini M, Stanzial AM, Russo C, Ferroni M, Corrocher R** 1995 Low selenium status in the elderly influences thyroid hormones. Clin Sci 89:637–642
29. **Calomme MR, Vanderpas JB, Francois B, Van Caille-Bertrand M, Herchuelz A, Vanovervelt N, Van Hoorebeke C, Vanden Berghe DA** 1995 Thyroid function parameters during a selenium repletion/depletion study in phenylketonuric subjects. Experientia 51:1208–1215
30. **Kauf E, Dawczynski H, Jahreis G, Janitzky E, Winnefeld K** 1994 Sodium selenite therapy and thyroid-hormone status in cystic fibrosis and congenital hypothyroidism. Biol Trace Elem Res 40:247–253
31. **Calomme M, Vanderpas J, Francois B, Van Caille-Bertrand M, Vanovervelt N, Van Hoorebeke C, Vanden Berghe DA** 1995 Effects of selenium supplementation on thyroid hormone metabolism in phenylketonuria subjects on a phenylalanine restricted diet. Biol Trace Elem Res 47:349–353
32. **Barrington JW, Lindsay P, James D, Smith S, Roberts A** 1996 Selenium deficiency and miscarriage: a possible link? Br J Obstet Gynecol 103:130–132
33. **Dobrzynski W, Trafikowska U, Trafikowska A, Pilecki A, Szymanski W, Zachara BA** 1998 Decreased selenium concentration in maternal and cord blood in preterm compared to term deliveries. Analyst 123:93–97
34. **Bedwal RS, Bahuguna A** 1994 Zinc, copper and selenium in reproduction. Experientia 50:626–640
35. **McCoy KEM, Weswig PH** 1969 Some selenium responses in the rat not related to vitamin E. J Nutr 98:383–389
36. **Watanabe C, Satoh H** 1994 Brain selenium status and behavioral development in selenium-deficient preweanling mice. Physiol Behav 56:927–932
37. **Chanoine J, Alex S, Stone S, Fang SL, Veronikis I, Leonard JL, Braverman LE** 1993 Placental 5-deiodinase activity and fetal thyroid hormone economy are unaffected by selenium deficiency in the rat. Pediatr Res 34:288–292
38. **Sharifi J, St. Germain DL** 1992 The cDNA for the type I iodothyronine 5′-deiodinase encodes an enzyme manifesting both high K_m and low K_m activity. J Biol Chem 267:12539–12544
39. **St. Germain DL, Croteau W** 1989 Expression of phenolic and tyrosyl ring iodothyronine deiodinases in *Xenopus laevis* oocytes is dependent on the tissue source of injected poly(A)$^+$ RNA. Mol Endocrinol 3:2049–2053
40. **Comings DE, Tack LC** 1972 Similarities in the cytoplasmic proteins of different organs and species examined by SDS gel electrophoresis. Exp Cell Res 75:73–78
41. **Snedecor GW, Cochran WG** 1980 Statistical Methods, 7th ed. The Iowa University Press, Ames, Iowa, pp 64–82
42. **Godfrey K** 1985 Comparing the means of several groups. N Engl J Med 13:1450–1456
43. **Nataf B, Sfez M** 1961 Debut du fonctionnement de la thyroide foetale du rat. C R Soc Biol 156:1235–1238
44. **Schwartz HL** 1983 Effect of thyroid hormone on growth and development. In: Oppenheimer JH, Samuels HH (eds) Molecular Basis of Thyroid Hormone Action. Academic Press, New York, pp 413–444
45. **Legrand J** 1986 Thyroid hormone effects on growth and development. In: Hennemann G (ed) Thyroid Hormone Metabolism. Marcel Dekker, Inc., New York, pp 503–534
46. **Norman MF, Lavin TN, Baxter JD, West BL** 1989 The rat growth hormone gene contains multiple thyroid response elements. J Biol Chem 264:12063–12073
47. **Hausman GJ** 1992 The influence of thyroxine on the differentiation of adipose tissue and skin during development. Pediatr Res 32:204–211
48. **Aszterbaum M, Menon GK, Feingold KR, Williams ML** 1992 Ontogeny of the epidermal barrier to water loss in the rat: correlation of function with stratum corneum structure and lipid content. Pediatr Res 31:308–317
49. **Hanley K, Rassner U, Elias PM, Williams ML, Feingold KR** 1996 Epidermal

barrier ontogenesis: maturation in serum-free media and acceleration by glucocorticoids and thyroid hormone but not selected growth factors. J Invest Derm 106:404–411

50. **Hanley K, Jiang Y, Katagiri C, Feingold KR, Williams ML** 1997 Epidermal steroid sulfatase and cholesterol sulfotransferase are regulated during late gestation in the fetal rat. J Invest Dermatol 108:871–875
51. **Becker KB, Stephens KC, Davey JC, Schneider MJ, Galton VA** 1997 The type 2 and type 3 iodothyronine deiodinases play important roles in coordinating development in *Rana catesbeiana* tadpoles. Endocrinology 138:2989–2997
52. **Chopra I** 1977 A study of extrathyroidal conversion of thyroxine (T_4) to 3,3′,5-triiodothyronine (T_3) *in vitro*. Endocrinology 101:453–463
53. **Berry MJ, Grieco D, Taylor B, Maia AL, Kieffer JD, Beamer W, Glover E, Poland A, Larsen PR** 1993 Physiological and genetic analysis of inbred mouse strains with a type I iodothyronine 5′ deiodinase deficiency. J Clin Invest 92:1517–1528
54. **Maia AL, Berry MJ, Sabbag R, Harney J, Larsen PR** 1995 Structural and functional differences in the *dio1* gene in mice with inherited type I deiodinase deficiency. Mol Endocrinol 9:969–980
55. **Maia AL, Kieffer JD, Harney JW, Larsen PR** 1995 Effect of 3,5,3′-Triiodothyronine (T_3) administration on dio1 gene expression and T_3 metabolism in normal and type 1 deiodinase-deficient mice. Endocrinology 136:4842–4849