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Thyroid, Pituitary, and Hypothalamic Hormone Levels in Neonatal Rat Pups following Maternal Protein Deficiency (40535)[1]

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The effects of malnutrition, calorie restriction, and protein deficiency on thyroid structure and function in adult experimental animals have been well documented (1–5). Srebnik *et al.* (5) reported radioiodine uptake to be the same for protein-deficient and control female rats. However, iodine uptake was higher in the protein-deprived animals when it was expressed per milligram of thyroid weight. In contrast, Meites and Wolternick (1) and Guerin and Aschkenasy (6) reported decreased uptake of radioiodine in protein-deficient rats.

Radioiodine uptake and incorporation have also been used to study functional development and physiological maturity of the thyroid gland (7, 8). In the fetuses of protein-deficient dams, a marked delay in the formation of follicles, reduction in size of the follicular lumen, reduced staining of the colloid with PAS, and a marked decrease in the number of follicles containing labeled iodine were found between 17 days of gestation and birth (9).

Despite the evidence of retarded development and reduced incorporation of radioactive iodine in the thyroid glands of fetal and newborn young of protein-deprived dams the extent of reduced thyroidal function was unclear, as was the involvement of other portions of the thyroid axis. The present study sought answers to these questions by determining plasma levels of total thyroxine (T_4), total plasma triiodothyronine (T_3), free T_4 and free T_3, plasma and pituitary thyrotropin (TSH), the metabolic clearance rate of exogenous TSH, and the thyrotropin-releasing hormone (TRH) content of the hypothalamus.

Materials and methods. Virgin, female, Sprague–Dawley rats were mated as previously described (9). Each mated female was assigned at random to one of two dietary groups. The control animals received a diet containing 24% casein as the sole source of protein; the protein-deprived animals received a diet containing 4% casein and an additional 20% dextrose. Diets were fed *ad libitum* throughout gestation, and water was freely available. Temperature and light cycles were carefully controlled. Details of the diet composition have been published previously (10).

Newborn young of control and of protein-deprived dams, with birth weights of 5.9–7.0 and 2.7–5.6 g, respectively, were the experimental subjects. All blood samples were drawn from the jugular vein and centrifuged, and the plasma from the littermate donors was pooled and frozen at −20° until assayed. Pituitary and hypothalamic tissues from littermate young were also pooled and stored in the frozen state.

Total thyroxine (TT_4) and total triiodothyronine (TT_3) contents of the plasma were determined using the radioimmunoassay method of Black *et al.* (11) modified to increase the volume of the unknown from 50 to 100 μl, reduce the dilution of the antisera so that each tube received ⅛ of a unit, increase the incubation time to 16 hr at 4°, increase the centrifugation speed to 2500*g*, and increase the time to 35 min. These modifications increased the sensitivity and reproducibility of the technic. For TT_3 assays, radioactive iodinated T_3 (sp act 1200 μCi/μg, Amersham Searle, Chicago, Ill.) was used to increase the sensitivity of this assay. Samples consisted of pooled plasma from four young per litter. Ten such samples were assayed for TT_4 and TT_3 for each dietary treatment group. Assays were done in duplicate, standard curves in triplicate.

Free thyroxine (FT_4) and free triiodothyronine (FT_3) were determined using the dou-

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ble-labeling Sephadex-binding technique of Finucane and Griffiths (12). The samples were assayed in duplicate and counted for both ^{131}I and ^{125}I content simultaneously using two channels on a Packard gammacounter (Packard Instruments Co., Downers Grove, Ill.). The calculated efficiency of the machine for both isotopes was approximately 85%. Each sample represented the plasma from at least six littermates and the determinations were done on 10 samples of plasma from control newborn and 10 from prenatally protein-deprived (PPD) progeny.

Plasma and pituitary TSH was determined by homologous radioimmunoassay, using the NIAMDD materials generously supplied by Dr. Albert F. Parlow from the Rat Pituitary Hormone Distribution Program. Newborn animals in each dietary treatment group were divided into three subgroups: uninjected, TRH injected, and saline injected. Five young were needed in each of these groups to provide sufficient plasma and tissue for one assay sample. The injected animals all received intraperitoneally 0.01 ml of fluid/g body wt. Synthetic TRH was given at a dose level of 1.25 ng/g body wt, as indicated by Kieffer *et al.* (13) to elicit a maximal response in newborn rats. Samples were taken 30 min following injection. Pituitaries were weighed on a Cahn Microbalance, and homogenized in 0.5 ml EDTA–phosphobuffered saline before freezing. Ten samples of plasma and pituitary tissue from control and PPD animals receiving no injections, the same number from animals injected with TRH, and five samples from each dietary group injected with saline were assayed immediately after thawing to prevent damage to the TSH molecule (14). Results are reported in terms of the highly purified preparation, NIAMDD-Rat-TSH-I-1, which has a biological potency of about 35 IU/mg in the McKenzie assay.

The metabolic clearance rate (MCR) of TSH was examined using NIAMDD-Rat-TSH-I-3 labeled with ^{125}I using the method of Greenwood *et al.* (15). The ^{125}I rat TSH had a specific activity of 200 $\mu Ci/\mu g$, was repurified prior to each assay using Sephadex G-75, and diluted with phosphobuffered saline to give 30,000 dpm/ml. For each determination of MCR, approximately 20–30 newborn young representing five to six litters for each diet group were given 1.5 μl ^{125}I-Rat-TSH/g body wt by intracardiac injections. The TSH content of 50 μl of plasma from blood samples taken 5, 10, 15, or 20 min later were determined in the TCA precipitate by counting in a well-type scintillation counter. The specific activity of the labeled TSH given was determined on 10-μl aliquots of the injection solution.

Determination of hypothalamic TRH content was done on pooled hypothalamic samples from at least six newborn pups representing each dietary treatment. The hypothalamic-containing fragment of brain tissue was bordered anteriorly by the optic chiasm, posteriorly by the brain stem, laterally by the cerebral peduncles and pyriform lobes, and extended 2 to 3 mm rostrally. After excision, this tissue was frozen on dry ice.

Hypothalamic TRH was determined in duplicate by radioimmunoassay using methods outlined by Bassiri and Utiger (16). Antibody was kindly provided by R. D. Utiger of the University of Pennsylvania.

The statistical methods used to assess the data include analysis of variance and Student's t test (17). MCR calculations followed the previously described method of Sterling and Chodos (18).

Results and discussion. Body weights of newborn young are shown in Table I. The mean pituitary weight of the PPD pups is significantly lower ($P < 0.001$) than those of the control animals. There were, however, significantly higher pituitary wt/body wt ratios in PPD pups than in controls, suggesting a sparing action on gland growth. This hypothesis is further supported by the observations of Ferlatte and Zeman (22) that pituitary volume/body wt ratios were significantly higher in 21-day fetal PPD young than in controls of corresponding age. Srebnick *et al.* (5) have reported similar findings in postnatally malnourished young rats.

Values for TT_4 and TT_3, as determined by RIA are shown in Table II where it can be seen that TT_3 concentration is significantly reduced in PPD young as compared with controls. The values for TT_4 in the plasma of control and PPD rat pups did not differ. Interassay variation was 5%. The coefficient of variation for TT_4 was 3% and that for TT_3 was 4%. The relatively high values for TT_4

TABLE I. EFFECT OF PRENATAL PROTEIN DEFICIENCY ON BODY AND PITUITARY WEIGHTS OF NEWBORN RAT PUPS

		Control	Prenatally protein deprived
Mean body weight (g)		6.36 ± 0.03 (231)[a]	3.29 ± 0.02[b] (257)
Mean pituitary weight (mg)		1.22 ± 0.06 (137)	0.78 ± 0.03[b] (146)
$\frac{\text{Pituitary weight} \times 10^{-3}}{\text{Body weight}}$	=	0.18 ± 0.01	0.23 ± 0.01[c]

[a] Mean ± SEM; numbers in parentheses indicate number of determinations.
[b] Significantly different from controls, $P < 0.001$.
[c] Significantly different from controls, $P < 0.01$.

TABLE II. EFFECT OF PRENATAL PROTEIN DEFICIENCY ON THYROID HORMONE LEVELS IN PLASMA OF NEWBORN RAT PUPS

Hormone	Control	Prenatally protein deprived
TT_4 (μg/dl)	2.4 ± 0.1 (10)[a]	2.2 ± 0.1 (10)
TT_3 (ng/dl)	57.3 ± 7.4 (10)	15.7 ± 3.3 (10)[b]
Percentage free T_4	$7.6 \times 10^{-3} \pm 8 \times 10^{-4}$ (10)	$7.5 \times 10^{-3} \pm 5 \times 10^{-4}$ (10)
Percentage free T_3	$4.1 \times 10^{-2} \pm 3.4 \times 10^{-3}$ (10)	$4.9 \times 10^{-2} \pm 3.2 \times 10^{-3}$ (10)

[a] Mean ± SEM; numbers in parentheses indicate number of pooled samples assayed.
[b] Significantly different from controls, $P < 0.001$.

and TT_3 (23–26) found in the present study could be the result of modifications in methods, i.e., our use of homologous antisera or, alternatively, the comparatively high iodine content of our diet (15 mg KI/kg) may have produced elevated TT_4 and TT_3 levels (27). There were no significant differences between the control and deficient young in the free T_4 and free T_3 when these were expressed as a percentage of total circulating hormone (Table II).

Since the RIAs for TT_4 and TT_3 were done on blood collected from one set of animals and the FT_4 and FT_3 determines on blood samples obtained from a corresponding, but separate, group of animals, no statistical evaluation of FT_4 or FT_3 concentrations could be made.

If the concentration of FT_3 (TT_3 × percentage free fraction) were calculated using the data given in Table II, the PPD young would appear to have far less free T_3 (0.77 nmole/liter) than controls (2.35 nmole/liter). Thus, the endocrine picture presented by the PPD newborn rat is one of depressed TT_3 and decreased free-T_3 concentrations when compared to control animals of the same age.

A low plasma FT_3 could occur if the peripheral kinetics of thyroid hormones were so altered that the metabolic clearance of T_4 remained the same and that of T_3 was increased in protein-calorie malnutrition. When disturbances in metabolic clearance of T_4 and T_3 occur, however, both hormones are usually found to be altered in the same direction (28). Portnay *et al.* (29) have reported that starvation does not affect the metabolic clearance rate of either T_4 or T_3 in adult rats. It appears unlikely that the decrease in FT_3 is related to alterations in circulating thyroid hormone-binding proteins since this presumably would also result in parallel changes in both total plasma T_4 and T_3. Abnormalities in thyroidal secretion could also account for the present findings relating to T_4 and T_3. Experimental protein deprivation has not been found to be associated with any appreciable change in the ratio of mono- and diiodotyrosine to that of iodothyronines and iodotyrosines in thyroglobulin (30). Studies of the relative proportions of T_3 and T_4 in thyroglobulin in protein-calorie malnutrition are still to be reported. Taking the above factors into consideration, it is suggested that a decrease in peripheral monodeiodination of T_4 to T_3 is the most likely explanation for the reduction of plasma T_3 observed in the PPD rats.

Plasma and pituitary levels of TSH were found to be similar in PPD and control newborn, both in response to and in the absence of exogenous TRH (Table III). These findings appear to rule out an effect of intrauterine protein deprivation on the TSH secretory

TABLE III. EFFECT OF PRENATAL PROTEIN DEFICIENCY ON PLASMA AND PITUITARY THYROTROPIN LEVELS IN NEWBORN RAT PUPS

	Control	Prenatally protein deprived
Plasma TSH (ng/ml)		
TRH injected	2.33 ± 0.09[a] (10)	2.76 ± 0.20 (10)
Uninjected	0.91 ± 0.05 (10)	0.85 ± 0.15 (10)
Saline injected	0.80 ± 0.17 (5)	0.93 ± 0.08 (5)
Pituitary TSH (ng TSH/pit)		
TRH injected	161.43 ± 10.41 (10)	102.93 ± 4.78[b] (10)
Uninjected	161.65 ± 16.21 (10)	114.87 ± 7.95[b] (10)
Saline injected	172.50 ± 21.72 (5)	119.75 ± 20.35 (4)
Pituitary TSH (ng TSH/mg pit)		
TRH injected	736.24 ± 59.21	730.53 ± 29.85 (10)
Uninjected	728.62 ± 87.86 (10)	811.03 ± 78.12 (10)
Saline injected	756.74 ± 121.51 (5)	904.02 ± 134.58 (4)

[a] Mean ± SEM; numbers in parentheses indicate number of pooled samples.
[b] Significantly different from controls, $P < 0.001$.

capability of pituitary thyrotroph cells. Earlier developmental studies of pituitaries of control and PPD fetuses and newborn young indicated that maternal protein deprivation had not adversely affected the differentiation of these TSH-secreting cells (22). Hypothalamic tissue concentration of TRH did not differ between control and PPD young, being 0.077 ± 0.017 ng/hypothalamus and 0.089 ± 0.017 ng/hypothalamus, respectively. These similarities in hypothalamic TRH content between control and PPD young suggest that function of this portion of the thyroid axis is also not affected by the maternal diet.

The metabolic clearance rate (MCR) of TSH was significantly lower in the PPD newborn ($P < 0.005$) when compared to control animals, being 0.014 ± 0.001 ml/min and 0.039 ± 0.006 ml/min, respectively. When expressed on a unit weight basis (MCR/100 g BW), this difference disappeared. Because of the difference in the MCR, the secretion rate (MCR × plasma TSH) was significantly lower ($P < 0.01$) in the deficient groups (6.0 ± 1.1 μg/day) as compared to that in control (19.0 ± 3.0 μg/day). No differences in the half-life or in plasma TSH concentration of PPD and control young were found. When these data were compared with those of Kojima *et al.* (31), it was apparent that, although actual values are low in the newborn as compared with adult female rats, both control and PPD newborn pups had slightly higher clearance rates than did adults when corrected for body size.

Summary. Thyroidal, pituitary, and hypothalamic hormones in the plasma and tissues of newborn young of rats fed diets containing 24 or 4% casein as the sole source of protein were quantitated by radioimmunoassay. Circulating levels of total plasma triiodothyronine (T_3) were significantly reduced and the free fraction of T_3 was lower in young of protein-deprived rats than in control pups. Total plasma T_4, free T_4, plasma TSH, pituitary TSH per milligram of pituitary, the metabolic clearance rate of exogenous TSH per 100 g of body weight, and the TRH content of the hypothalamus were not significantly affected by the maternal diet.

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