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NEURAL AND ENDOCRINE DEVELOPMENT AFTER CHRONIC TRYPTOPHAN DEFICIENCY IN RATS: II. PITUITARY–THYROID AXIS

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SUMMARY

Long-Evans female rats, 21 days of age, were weaned and placed on a control (Purina Rat Chow) diet, on a tryptophan deficient diet (T–) and on a diet complete in quality (Purina Rat Chow) but restricted to the daily amount consumed by the rats on the T– diet (pair-feeding). All animals were maintained on these diets for 1 and 2 months and then one group of T– rats was returned to the complete (Purina) diet and kept on this diet for several periods of time (up to 2 months). Growth was interrupted during the period of tryptophan deficiency and pair-feeding, but was restored to normal when the animals were returned to the Purina diet. In control rats, blood levels of TSH, T_4 and T_3 as determined by radio-immunoassay showed characteristic developmental patterns. In T– rats, hormonal developmental patterns were similar to those of controls after 1 month on the T– diet but levels fell significantly below control values after 2 months. On the other hand, hormonal levels of pair-fed rats were already significantly low 1 month after treatment and continued to remain low after 2 months. The decrease in thyroid function reported in these experiments as a result of severe dietary restrictions not only may explain the retardation of growth and development characteristic of nutritionally deficient animals, but also suggest some long-term interaction of nutrition and thyroid function on the aging process.

INTRODUCTION

Previous studies have shown that restriction of total food intake as well as deficiency in a specific dietary component such as tryptophan, initiated at an early age during development, not only induce retardation or arrest of growth and maturation, but also retard the rate of aging and may extend the lifespan of rats [1–3]. With regard to the

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mechanisms by which dietary deficiencies alter development, a number of investigations have reported that pituitary cells are reduced in size and in staining capacity by these conditions and that immaturity or impairment of the pituitary function may lead to endocrine deficits manifested by alterations in growth and sexual function [4, 5]. Furthermore, parallel studies have shown a relationship between brain maturation, especially in terms of monoamine systems and endocrine development. Thus, nutritional deficiencies, such as caloric or tryptophan restriction would lead to impairment of both brain and endocrine maturational patterns. We know that tryptophan is not only an essential amino acid but also the precursor of brain serotonin, and that tryptophan deficiency as well as caloric restriction induce alterations of brain monoamine levels capable of affecting the pituitary function [5–7]. The present study was designed to examine the function of the pituitary–thyroid axis in the course of long-term tryptophan deprivation and food restriction and to investigate the possible role of thyroid hormones in the delay of growth, maturation and aging induced by the dietary restriction.

MATERIALS AND METHODS

Diets

Two different types of diets were used: Purina Rat Chow and tryptophan deficient (T–) diets. The T– diet was the same as that used in previous experiments and its tryptophan level was approximately 15% that of the Purina diet [3, 5].

Experimental protocol

A total of 82 Long-Evans female rats of 21 days of age were divided into three major groups: group I consisted of control animals fed Purina Rat Chow *ad libitum*; group II consisted of animals fed the T– diet *ad libitum* (T– animals); group III consisted of pair-fed (PF) animals fed the same amount of Purina Rat Chow as consumed daily by the rats on the T– diet.

Weights of the animals and of the food consumed were measured daily. The rats were sacrificed between 9 a.m. and 10 a.m. at the following ages: 21 days, age of weaning; 51 days, 1 month after the beginning of the administration of the special diets, and 81 days, 2 months after the beginning of the administration of the special diets. The animals were always sacrificed under ether anesthesia (lasting less than 3 minutes) and blood was collected from the heart into an heparinized syringe. Blood samples were centrifuged, the collected plasma was frozen in acetone–dry ice and stored at −20 ℃.

An additional experimental group was added at the end of this first phase of our study. This group consisted of some animals from group II (T– diet) not sacrificed at 81 days and kept to test the effects of refeeding them with normal (Purina Rat Chow) diet (refeeding experiment). Thus, at 81 days of age, the T– diet was replaced by Purina Rat Chow given *ad libitum* and the animals were kept on this diet for 2, 5 and 10 days and 2 months. They were sacrificed at these intervals and samples were processed as described above.

Radio-immunoassays

TSH was assayed by double-antibody radio-immunoassay according to the directions supplied with the assay reagents obtained from the NIAMDD Rat Pituitary Hormone Program. The values were expressed in terms of NIAMDD-Rat-RP-1. Triiodothyronine (T_3) and thyroxine (T_4) were assayed by means of commercial radioimmunoassay kits (Immuno-T_3 and Immuno-T_4, Pantex). Most of the measurements were performed in duplicate. Student's *t* test was used to measure significance of differences between control and experimental animals.

RESULTS

Changes in body weight and food consumption during the experimental period are shown in Fig. 1 and are consistent with previous reports [2, 3]. None died between 21 and 81 days in controls; in the T– animals, mortality was 5% in the first 30 days and 40% in the second month; in the PF animals mortality was 20% in the first month and 17% in the second month. Growth was almost completely blocked in T– and PF animals as indicated by failure to gain body weight from 21 to 81 days of age (Fig. 1). T– rats resumed very rapid growth immediately after feeding Purina Rat Chow *ad libitum*.

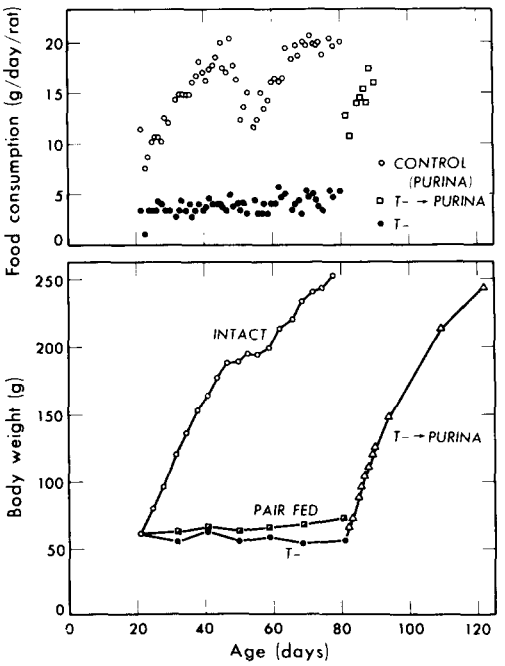

Fig. 1. Growth retarding effects of tryptophan deficiency and food restriction.

Changes in blood levels of TSH, T_4 and T_3 during the experimental period are shown in Fig. 2(a), (b), (c). In control animals, TSH, T_4 and T_3 levels show characteristic patterns of development: TSH increases significantly between 51 and 81 days (Fig. 2a); T_4 remains constant between 21 and 81 days (Fig. 2b) and T_3 decreases significantly between 21 and 51 days but remains unchanged thereafter (Fig. 2c). These results suggest that the reactivity of the thyroid gland to TSH declines between 51 and 81 days of age inasmuch as no response to increased TSH levels are detected in T_4 and T_3 levels at this time. Alternatively, the metabolism of increased thyroid hormones may increase with age. The decrease in T_3 concentration in the presence of constant levels of T_4 may be interpreted as a reduction in the effectiveness of peripheral tissue to deiodinize T_4 into T_3 between 21 and 51 days of age.

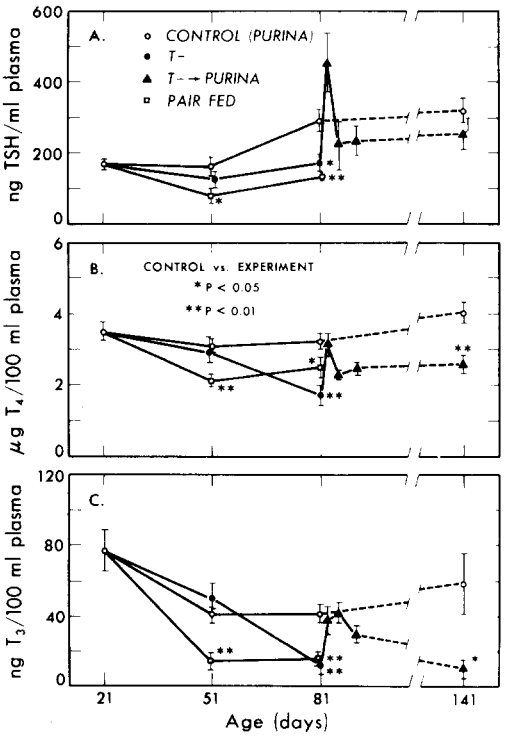

Fig. 2. Plasma levels of TSH, T_4 and T_3 ± S.E.M. in normal, tryptophan deprived and pair-fed rats.

No significant difference in TSH, T_3 and T_4 levels was detected between control and T– animals at 51 days [Fig. 2(a)–(c)]. However, all three hormones in T– rats were significantly lower than in controls after the treatment lasted for 2 months (81 days old). On the other hand, hormonal levels of PF rats were already decreased after a one-month treatment and remained low at 81 days. In the animals which were refed a normal diet following the tryptophan deficiency for 2 months, the contents of TSH, T_4 and T_3 returned to control levels in the first 2 days, but decreased again afterwards. The hormone levels of refed animals were still much lower than those of control even 2

months after the beginning of refeeding, although the difference in TSH was not statistically significant. These results suggest that the pituitary–thyroid system suffers some long-term alterations when animals are subjected to a period of tryptophan deficiency for 2 months.

DISCUSSION

It has been reported that acute fasting results in diminished circulating levels of TSH [8, 9] and alterations of peripheral thyroxine metabolism [10–13]. The results of the present study indicate that long-term calorie or tryptophan deprivation, either of which conditions appear to retard aging and may prolong the lifespan of the rat, bring about a diminution in the function of the pituitary–thyroid axis. The most profound consequences of the dietary manipulations were represented by a significant reduction in the levels of T_3, which, according to current evidence, is the most active form of thyroid hormone [14]. Even though our experiments covered only a short portion of the lifespan, from weaning to adulthood, it is possible to hypothesize that thyroid deficiency during this life period might have long-term effects on the aging processes. Our results support the view that the effect of nutritional restrictions, either of total dietary calories or of special dietary components, may be related to the reduction in thyroid function which occurs under these circumstances. That thyroid hormones may influence the aging process has been suggested by several investigators. Hyperthyroidism has been associated with a shortening of the lifespan [15, 16]; long-term treatment of rats with thyroxine increases the aging of collagen fibers as measured by their breaking time in urea [17]. Moreover, the age-related decrease in minimal O_2 consumption can be prevented by thyroidectomy or hypophysectomy [18]. That thyroid hormones have a profound influence on several developmental changes in vertebrates is well known; the present findings suggest that these hormones may play an equally important role in regulating the timetable of aging as a programmed biological process.

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