

# CHANGES IN THE TRI-iodothyronine AND THYROXINE CONCENTRATIONS IN RABBIT FETUSES AFTER ENCEPHALECTOMY

E. V. Proshlyakova, M. S. Mitskevich,  
and O. N. Rumyantseva

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In previous investigations on species of mammals differing in their degree of development at birth (rats, rabbits, and guinea pigs) the presence of hypothalamic control of thyroid function in the fetuses could be demonstrated only in guinea pigs [1, 4]. In these experiments a change in the degree of development of the thyroid response to methylthiouracil was used as the test. After removal of the hypothalamus (encephalectomy) signs of the thyroid response were significantly weaker than in intact fetuses only in the case of guinea pigs, which are animals born with sight. The absence of any marked hypothalamic control in the prenatal period in rats was later confirmed by the method of quantitative determination of thyroxine ( $T_4$ ) and tri-iodothyronine ( $T_3$ ) in the thyroid gland and blood serum [3]. Since the rabbit occupies an intermediate position between the rat and guinea pig as regards the degree of its development at birth, it might be expected that with the use of a more sensitive method it would be possible to demonstrate hypothalamic control of thyroid function in rabbits, at least at the end of intrauterine life.

## EXPERIMENTAL METHOD

Experiments were carried out on 63 fetuses from 10 pregnant rabbits. The hypothalamic region of the brain was removed (encephalectomy) by the method described previously [2] from some fetuses at the age of 22-23 days; intact fetuses from the same litter served as the control. Under pentobarbital anesthesia, at the stages of 29 and 30 days of development fetuses were removed from the mothers and blood was taken immediately from the region of the heart. The isolated thyroid glands were weighed, placed in closed glass capsules, and frozen. The blood was allowed to stand for 1 h at 37°C and the clot was separated by centrifugation. All material taken separately from each fetus was stored at -70°C. For quantitative determination of  $T_3$  and  $T_4$  in the thyroid gland a homogenate of the gland tissue was prepared in borate buffer, pH 8.6, incubated for 16 h at 37°C with trypsin, and centrifuged; the supernatant was used for assay of the hormones [5]. The assay was carried out by means of radioimmunologic kits: TETRAK-1 (CEA-Sorin, France), for  $T_4$  and  $T_3$  RIA kit (Radiochemical Centre, Amersham, England) for  $T_3$ . The results were subjected to statistical analysis on the Mir computer. Significance of differences was estimated by Fisher's and Student's criteria.

Thyroid glands for histological study were fixed in Bouin's fluid and sections were stained with iron-hematoxylin and with azan by Heidenhain's method.

## EXPERIMENTAL RESULTS

As the results (Table 1) show, the weight of the thyroid glands in fetuses aged 29 and 30 days after encephalectomy remained at the control level. Determination of  $T_3$  and  $T_4$  in thyroid homogenates of 29-day fetuses of the two groups revealed no significant differences, although in the encephalectomized fetuses some decrease was observed in the concentrations of the two hormones compared with intact fetuses. Meanwhile attention was drawn to considerable individual fluctuations in all the quantitative parameters, a feature observed previously also in similar experiments on rat fetuses [4].

A marked increase in the content of both thyroid hormones was found in the glands of the 30-day fetuses compared with 29-day fetuses: In the control at this time the mean  $T_3$  concentration was 2.87 ng/mg and  $T_4$

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Laboratory of Hormonal Regulations, N. K. Kol'tsov Institute of Developmental Biology, Academy of Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 91, No. 1, pp. 71-73, January, 1981. Original article submitted December 29, 1979.

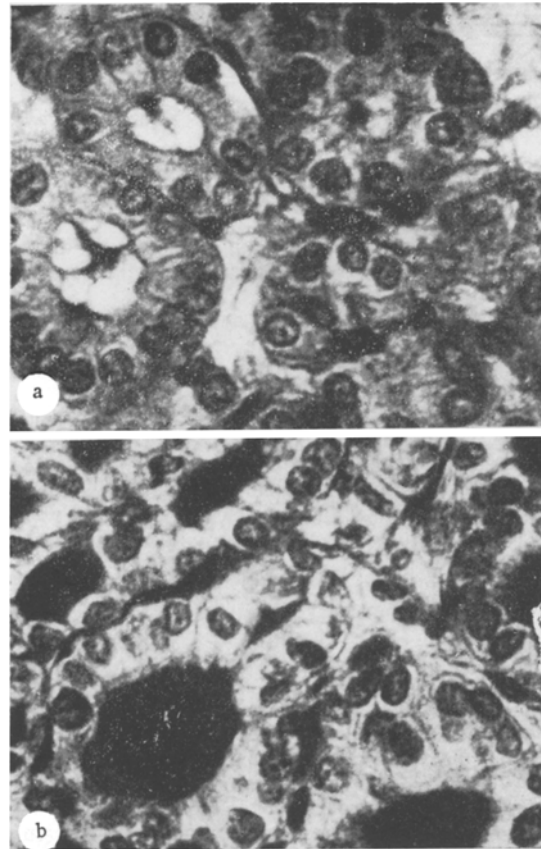
**TABLE 1. Concentrations of T<sub>3</sub> and T<sub>4</sub> in Thyroid Gland and Blood Plasma of Rabbit Fetuses after Encephalectomy**

Experimental conditions	Age of fetuses, days		Weight of thyroid gland, mg	Thyroid gland tissue homogenate		Blood serum	
	at operation	at fixation		T <sub>3</sub> , ng/mg	T <sub>4</sub> , ng/mg	T <sub>3</sub> , ng/mg	T <sub>4</sub> , ng/mg
Control	23	29	10,87±1,13 (10)	1,114±0,13 (10)	22,85±5,79† (5)	—	—
Encephalectomy			10,78±1,47 (7)	1,062±0,09 (9)	18,69±4,40† (6)	—	—
Control	22	30	11,96±1,16 (28)	2,87±0,32 (27)	75,77±2,62 (7)	0,18±0,024 (25)	7,98±0,83 (7)
Encephalectomy			11,64±0,87 (28)	2,26±0,21* (26)	46,23±3,04* (6)	0,12±0,024 (17)	7,87±1,28 (6)

**Note:** Gland from one fetus taken for each determination. Number of fetuses given in parentheses.

\*P < 0.05 compared with control.

†Method of competitive binding was used (Res-O-Mat T<sub>4</sub> kit from Byk-Mallinckrodt, West Germany).



**Fig. 1.** Thyroid gland of 30-day intact (a) and encephalectomized (b) rabbit fetus. 200×

75.77 ng/mg. An important fact was that encephalectomy caused a significant decrease in the concentrations of T<sub>3</sub> and T<sub>4</sub> in the gland in fetuses of this age (Table 1).

By contrast with the higher level of thyroid hormones in the gland, their concentration in the blood serum of the 29-day fetuses was very low, and in some fetuses it was on the borderline of sensitivity of the method used. In 30-day fetuses the serum concentration of the hormones was 0.18 ng/ml for T<sub>3</sub> and 7.98 ng/ml for T<sub>4</sub> in the control, which did not differ statistically significantly from the level of these hormones in the anencephalics.

Investigation of the microstructure of the gland in 30-day fetuses revealed clear differences in the functional state of the thyroid in intact fetuses and in anencephalics (Fig. 1). In the former the gland had clear signs of increased activity: the follicles were lined with high epithelium, they were filled with foamy colloid, and were transparent or colored blue in the sections. In anencephalics at the same period of development the gland consisted chiefly of larger follicles, the lumen of which was filled with solid colloid, stained red and without its foamy appearance, characteristic of depressed function.

Analysis of the results shows that absence of the hypothalamic region of the brain in 29-day rabbit fetuses is not reflected in thyroid function. Similar results were obtained in preliminary experiments by Jost et al. [6]. A completely different picture was observed in 30-day fetuses, in which encephalectomy caused a significant decrease in the concentration of both thyroid hormones —  $T_3$  and  $T_4$  in the gland. Other evidence of the significant decrease in thyroid activity in the anencephalics at this stage of development is given by the characteristic changes in histological structure of the gland compared with that of intact fetuses.

It can be concluded from these results that hypothalamic control of pituitary thyrotrophic function is established in rabbits with effect from the 30th day of prenatal development, and it is manifested clearly in relation to  $T_3$  and  $T_4$  synthesis in the thyroid gland. In this investigation no significant differences could be detected in the serum concentrations of these hormones in the encephalectomized fetuses compared with the intact, and this effect is evidently exhibited at a later stage of development.

#### LITERATURE CITED

1. M. S. Mitskevich and O. N. Rumyantseva, *Ontogenez*, 3, 376 (1972).
2. M. S. Mitskevich, O. N. Rumyantseva, E. V. Proshlyakova, et al., *Ontogenez*, 1, 631 (1970).
3. E. V. Proshlyakova, K. Chandrasekhar, and O. N. Rumyantseva, *Byull. Éksp. Biol. Med.*, No. 12, 720 (1977).
4. O. N. Rumyantseva and M. S. Mitskevich, in: *Hormonal Factors of Individual Development* [in Russian], Moscow (1974), p. 303.
5. V. M. Samsonova, "The role of paraventricular nuclei in hypothalamic regulation of the pituitary-thyroid gland system," Author's Abstract of Candidate's Dissertation, Moscow (1968).
6. A. Jost, J. P. Dupouy, and A. Geloso-Meyer, in: *The Hypothalamus*, New York (1970), p. 1.