INCORPORATION OF GLYCINE-1-C^{14} INTO HEMATOPOIETIC ORGANS AND BLOOD OF RABBITS WITH THYROTOXICOSIS

V. V. Ivleva

UDC 616.441-008.61-07:
[616.41+616.15]-073.916

In the late stages of thyrotoxicosis in rabbits, incorporation of glycine-1-C^{14} into proteins of the hematopoietic organs and blood is depressed.

* * *

Data on protein synthesis in individual organs in hyperthyroidism are highly contradictory, although according to some reports the way in which thyroid hormones act depends on the dose of the hormone. If large doses of hormone are given, or in animals with thyrotoxicosis, incorporation of amino acids into proteins is depressed [3, 4]. Investigations in the author's laboratory have shown that by the 14th day of thyroid feeding to rabbits the intensity of incorporation of methionine-S^{35} and glycine-1-C^{14} into total protein of a myocardial homogenate from the left ventricle is increased, but by the 28th day this index has fallen [1, 2].

The object of the present investigation was to study the intensity of synthesis of proteins of the hematopoietic organs and blood as reflected in the incorporation of glycine-1-C^{14} into total protein in vivo during the development of thyrotoxicosis produced by thyroid administration.

EXPERIMENTAL METHOD

Experiments were carried out on male chinchilla rabbits with a mean body weight of 3080 ± 68.97 g. Experimental thyrotoxicosis was produced by feeding the animals with tablets containing thyroid extract in progressively increasing doses, resulting in loss of weight of 15-18% of the initial body weight by the 14th day and 25-30% by the 28th day [2]. The behavior and clinical state of the animal (increase in heart rate and respiration rate, diarrhea, tremor of the limbs) and the degree of loss of weight were used as criteria of the severity of thyrotoxicosis. The mean loss of weight in these experiments by the 7th day of thyrotoxicosis was 7.8 ± 1.03%, on the 14th day 16.68 ± 0.69%, and on the 28th day 29.53 ± 1.22%.

The incorporation of a labeled amino acid (glycine-1-C^{14}) was used as the index of intensity of protein synthesis.

Experiments were carried out on the 7th, 14th, and 28th days of thyrotoxicosis. Intact rabbits served as controls. Labeled amino acid was injected intravenously 6 h before sacrifice in a dose of 10,000 pulses/min/g. Blood was taken from the marginal vein of the rabbit's ear into heparinized centrifuge tubes and placed on ice. Next, under ether anesthesia, the spleen was removed and the femora amputated. The spleen was carefully washed with 0.14 M NaCl solution and dried with filter paper. The femur was sawn along its length and the bone marrow extracted. The spleen and bone marrow were then frozen. The blood was centrifuged at 3000 rpm for 20 min, after which the plasma was carefully aspirated. Targets were loaded with 0.1 ml plasma and 0.1 ml blood cells. Weighed samples of spleen and bone marrow tissue, each of 1 g, were obtained and homogenized with 10 ml 0.14 M NaCl solution; 0.1 ml of the homogenate was placed on the target. Protein from the plasma, blood cells, and spleen and marrow homogenates was precipitated with equal volumes of 20% TCA solution and then washed with 10 and 5% TCA solutions and treated with alcohol and ether. The precipitated protein was dried, ground to powder, and samples of 10 mg were placed on targets. Radioactivity on the targets was determined by means of the BFL-25 end-window counter on a "Volna" apparatus.

Laboratory of Pathological Physiology, Institute of Experimental Endocrinology and Hormone Chemistry, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Yudaev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 68, No. 9, pp. 35-38, September, 1969. Original article submitted December 20, 1968.

TABLE 1. Incorporation of Glycine-1-C^{14} (in %) into Fresh Tissues of Hematopoietic Organs and Blood of Rabbits with Thyrotoxicosis 6 h after Administration of Isotope in a Dose of 10,000 pulses/min/g

Tissues investigated	Statistical index	Control	Time after thyroid feeding		
			7 days	7 days	28 days
Spleen	$M+m$ n P	104,13±5,69 11	99,05±3,19 10 >0,2	119±5,02 14 >0,05	103,08±6,02 12 >0,5
Bone marrow	$M±m$ n P	104,5±4,89 10	142±6,42 10 <0,001	142,39±9,94 10 <0,01	113,27±7,86 12 >0,5
Plasma	$M±m$ n P	28,65±4,01 8	22,53±1,643 7 <0,2	55,01±2,569 10 <0,001	44,98±2,35 10 <0,01
Blood cells	$M±m$ n P	3,84±0,272 6	2,177±0,115 10 <0,001	5,579±0,460 10 <0,01	4,725±0,75 11 >0,2

TABLE 2. Incorporation of Glycine-1-C^{14} (in %) into Protein of Hematopoietic Organs and Blood of Rabbits with Thyrotoxicosis 6 h after Administration of Isotope in Dose of 10,000 pulses/min/g

Tissues investigated	Statistical index	Control	Time after thyroid feeding		
			7 days	14 days	28 days
Spleen	$M±m$ n P	355±16,62 11	310,6±18,02 10 >0,05	313±15,08 15 >0,05	274±13,2 14 <0,001
Bone marrow	$M±m$ n P	870±65,7 9	724±33,3 8 >0,05	789±37,3 12 <0,5	556±29,9 14 <0,001
Plasma	$M±m$ n P	484,5±25,3 10	492±45,1 10 >0,5	447±17,5 11 <0,5	406±23,6 13 <0,05
Blood cells	$M±m$ n P	30,4±3,84 7	25,61±3,76 10 <0,5	24,99±3,6 10 <0,5	21,6±1,99 11 >0,05

The percentage incorporation of labeled amino acids into the fresh tissues was calculated as the ratio between the number of pulses/min/g weight of the particular tissue and the number of pulses/min/g body weight administered.

The percentage incorporation of glycine-1-C^{14} into proteins of the test organs was also calculated as the ratio between the number of pulses/min/g protein and the number of pulses/min/g body weight administered.

EXPERIMENTAL RESULTS

The results given in Table 1 show that in animals fed with thyroid extract the incorporation of glycine-1-C^{14} into fresh spleen tissue was virtually unchanged.

Incorporation of glycine into bone marrow homogenate was increased by the 7th day of the experiment (from 104.5 ± 4.89 to 142 ± 6.42%; $P < 0.001$). On the 28th day a decrease in incorporation was observed and was significant relative to the values on the 7th and 14th days of the experiment ($P < 0.02$), but not relative to the control.

Incorporation of glycine into plasma was unchanged on the 7th day of thyroid feeding, almost doubled on the 14th day ($P < 0.001$), and began to fall on the 28th day (compared with the level on the 14th day of thyrotoxicosis) but did not reach the values in the control animals.

Incorporation of glycine-1-C^{14} into the blood cells was less on the 7th day of thyroid feeding than in the control animals ($P < 0.001$), greater on the 14th day ($P < 0.01$), and on the 28th day it had started to fall again, but did not reach the level of its incorporation in the intact rabbits.

It can be concluded from these results that incorporation of glycine-1-C^{14} into fresh bone marrow tissue, plasma, and blood cells is increased by the 14th day of thyrotoxicosis. On the 28th day the incorporation of glycine-1-C^{14} into these tissues starts to fall.

Results showing incorporation of label into protein are given in Table 2. It is clear that neither on the 7th, nor on the 14th day of thyroid administration was an increase observed in the incorporation of glycine-1-C^{14} into proteins of the hematopoietic organs and blood. On the 28th day of thyroid feeding a sharp decrease was observed in the incorporation of amino acid into protein of the spleen ($P < 0.001$), bone marrow ($P < 0.001$), and plasma ($P < 0.05$) compared with the control. The late stages of thyrotoxicosis resulting from thyroid administration are thus accompanied by a decrease in the rate of synthesis of protein of the hematopoietic organs and plasma protein.

LITERATURE CITED

1. V. I. Kandror, Problm. Endokrinol., No. 4, 88 (1965).
2. O. B. Svyatkina, Dynamics of Protein and RNA Renewal in the Myocardium in Experimental Thyrotoxicosis, Candidate's Dissertation [in Russian], Moscow (1968).
3. E. U. Nikkila and R. Pitkanen, Acta Endocrinol. (Copenhagen), 31, 573 (1959).
4. R. Sternheimer, Endocrinology, 25, 899 (1939).