NUCLEIC ACID METABOLISM IN MYOCARDIUM OF RABBITS WITH EXPERIMENTAL THYROTOXICOSIS

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Thyrotoxicosis in rabbits is shown to be accompanied by a marked increase in inorganic phosphorus concentration in the myocardium; the RNA content in the myocardium of animals with experimental thyrotoxicosis is increased although the rate of its renewal is considerably lowered. Thyrotoxicosis causes no appreciable changes in synthesis and content of DNA in the heart.

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It has previously been shown [3], that prolonged saturation of the body with thyroid hormones leads to a decrease in the rate of incorporation of labeled amino acids into myocardial proteins. It was, therefore, decided to investigate the state of the nucleic acid metabolism in this organ in experimental thyrotoxicosis.

EXPERIMENTAL METHOD

Experimental thyrotoxicosis was produced by feeding rabbits with thyroid in progressively increasing doses for 28 days [11]. During this period the experimental animals lost up to 40% of their initial weight; their heart rate increased by 45%. The animals received a subcutaneous injection of $Na_2HP^{32}O_4$ in a dose of 50,000 pulses/min/g body weight 4 h before sacrifice. Myocardial nucleic acids were fractionated by Davidson's modification [12] of the Schmidt-Thannhauser method, and inorganic phosphorus was extracted by the method of Weil-Malherbe [18]. The phosphorus content was determined colorimetrically [16]. The total activity (pulses/min/g tissue), the specific activity (SA; pulses/min/mg phosphorus of test fraction), and the relative specific activity (RSA; ratio between specific activity of test fraction and specific activity of inorganic phosphorus – P_{inorg}) were determined in each fraction. The activity was measured with a type "AS-2" cylindrical counter tube.

EXPERIMENTAL RESULTS AND DISCUSSION

The results given in Table 1 show that thyrotoxicosis is unaccompanied by an increase in DNA concentration in the myocardium. The RNA concentration rose on the average by 36%. Since it has previously been shown [5] that the increase in weight of the heart (by 15%) in experimental animals is unconnected with

TABLE 1. DNA and RNA Concentration (mg P/g tissue) in Myocardium of Rabbits (M±m)

Group of animals	DNA	RNA
Control (n=8)	0,07±0,009	0,154±0,007
Experiment (n=10)	0,08±0,004	0,210±0,008
P	0,3	0,001

TABLE 2. Concentration and Specific Activity of P_{inorg} in Myocardium of Rabbits (M±m)

Group of animals	Conc. of P_{inorg} (in mg/g tissue	Specific activity P_{inorg} (pulses/min/mg phosphorus)
Control (n=8)	0,28±0,016	364 125±54 324
Expt. (n=10)	0,46±0,020	240 330±35 000
P	0,0001	0,08

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TABLE 3. Indices of DNA and RNA Activity in Myocardium of Rabbits

Index	Control (n = 7)	Experiment (n = 7)	P
Relative specific activity of DNA	0.012±0.001	0.014±0.002	0.5
Activity of RNA	4295±600	2788±327	0.0001
Specific activity of RNA	31,837±3440	13,732±5270	0.0001
Relative specific activity of RNA	0.095±0.01	0.065±0.006	0.02

changes in dry weight of the myocardium, these results evidently indicate maintenance of the total DNA content in the thyrotoxic heart.

The content of inorganic phosphorus in the heart tissue is given in Table 2.

These results demonstrate a considerable increase in organic phosphorus concentration in the myocardium of the experimental animals. Remembering that the activity of phosphorus per gram tissue in the experiment was only slightly greater than in the control, it may be considered that this increase took place not so much because of increased permeability of the heart tissue in the experimental series as because of increased breakdown of organic phosphates in this tissue. Further evidence of this is given by the decrease in ATP and CP contents in the thyrotoxic heart [2], and an increase in ATPase activity [6].

The results obtained by calculating the relative specific activity of DNA and RNA are given in Table 3.

The rate of renewal of DNA in the myocardial cells remained practically unchanged, whereas the relative specific activity of RNA fell by 31.5%. However, some doubts were felt regarding the determination of the intensity of RNA synthesis from its relative specific activity. Most of the RNA determined consists of its cytoplasmic fraction [10], while incorporation of the label takes place mainly into rapidly labeled nuclear fractions [1]. To be on the safe side, therefore, the results obtained in the control and experimental series should be compared not in units of relative specific activity, but in other units in which no allowance was made for changes in the total RNA content which could reflect an increase in its unlabeled fractions:

$$\frac{\text{Activity of RNA (in pulses/min/g tissue)}}{\text{Specific activity of } P_{\text{inorg}} \text{ (in pulses/min/mg)}}.$$

Corresponding calculations showed that even by making this assumption, the rate of incorporation of label into myocardial RNA of rabbits with experimental thyrotoxicosis was lowered.

In our experiments, the RNA/DNA ratio remained considerably elevated in thyrotoxicosis, despite the long duration of cardiac hyperfunction [4, 11]. This evidently shows that the myocardium, under these pathological conditions, does not reach the stage of stabilized hyperfunction [7] characterizing achievement of hypertrophy of the heart and restoration of the normal "quantity of function" per unit mass of organ (IFS). Other evidence of this is given by our previous discovery [3] of inhibition of protein synthesis in the heart of animals with thyrotoxicosis.

However, it is also known [13, 19], that RNA and protein synthesis in the tissues is activated under the influence of small doses of thyroid hormones or in the early stages of thyrotoxicosis. It may, therefore, be considered that the increased content of ribosomal RNA, which is also present in the late stages of this pathological process, reflects previous activation of its synthesis.

The biphasic nature of the action of thyroid hormones on biosynthetic processes may be explained by the character of the effect of different doses of these hormones on oxidative phosphorylation in the mitochondria. Whereas small doses, which weaken respiratory control, increase the output of high-energy compounds in unit time and thereby facilitate increased protein synthesis [15, 17], during prolonged saturation of the body with large doses of thyroid hormones, the energy-producing efficiency of tissue respiration is sharply diminished [9, 14]. This is reflected particularly severely in the myocardium, for under free oxidation conditions it must increase its ATP utilization sharply for its specific function. As a result, the flow of energy directed into plastic processes in this organ is inevitably reduced, affecting first and foremost the most energy-demanding processes in the chain of protein biosynthesis: the synthesis of RNA nucleotides and the supply of amino acids to the ribosomes. The disagreement between our findings and the results of D. K. Parsadanyan's experiments [8], in which an increase in the content of both RNA and DNA

was observed in the myocardium of hyperthyroidized rabbits, may be explained from this point of view. In Parsadanyan's experiments, the degree of thyrotoxicosis was evidently not severe enough to limit the flow of energy intended for synthesis of new protein compounds completely. In fact, in these experiments slight hypertrophy of the heart was observed. The lower degree of thyrotoxicosis could have been due to administration of constant doses of thyroid, and not of increasing doses (as in our experiments) to the animals. As our observations showed, under such conditions "insensitivity" to thyroid often develops, probably because of mobilization of mechanisms for inactivation of thyroid and its elimination from the rabbit's body.

Absence of hypertrophy of the heart in the late stages of thyrotoxicosis makes it impossible to maintain the raised level of functioning of this organ for a long time, a result in agreement with clinical observations indicating the frequent and rapid onset of cardiac failure in thyrotoxicosis.

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