INCORPORATION OF METHIONINE-H^3 INTO NUCLEI OF SKELETAL MUSCLE TISSUE IN RATS RECEIVING CORTISONE

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UDC 612.744-06:612.453.018-087.45

Experiments in which methionine-H^3 was administered showed that prolonged injection of cortisone into rats induced a marked decrease in the content of radioactive label in the nuclei of skeletal muscle tissue. Analysis of the distribution of the nuclei by size and shape shows that the number of small round nuclei was significantly increased in the experimental animals. This may have resulted from inhibition of their differentiation, as results showing a decrease in the content of labeled proteins confirm.

Investigation of the hormonal regulation of protein synthesis is of great importance in connection with the discovery of the role of hormones as regulators of the activity of the genetic apparatus of the cell [3, 4, 6, 7]. Glucocorticoid hormones have an antianabolic effect [2, 5] on skeletal muscle tissue, and as the authors' autoradiographic investigations have shown, this effect is manifested primarily in the sarcoplasm and the myofibrillary system of the muscle fibers [1].

The object of the present investigation was to study the intensity of incorporation of methionine-H^3 into proteins of skeletal muscle tissue nuclei during administration of cortisone.

EXPERIMENTAL METHOD

Sixteen male Wistar rats weighing 95-105 g were used. Eight of these rats received daily intramuscular injections (triceps brachii) of cortisone acetate (N. V. Organon Oss, Holland) in a dose strictly corresponding to the animal's weight.*

The remaining eight rats acted as the control and instead of hormone they were given injections of physiological saline at the same times of day as the experimental animals. Half of the experimental rats were sacrificed 10 days (series I), the other half 15 days (series II) after the beginning of injection of cortisone. Methionine-H^3 was injected subcutaneously into all the control and experimental rats 16 h before the decapitation in a dose of 3.5 μCi/g (specific activity 90 mCi/g). As the result of a preliminary histoautoradiographic investigation using methionine-H^3 in a dose of 3.5 μCi/g showed, the intensity of incorporation of the labeled amino acid into the nuclei of the muscle tissue after 16 h was comparatively high.

The muscles (tibialis anterior) were fixed with Carnoy's fluid, embedding in paraffin was by the usual method, and sections were cut to a thickness of 6 μ; the sections through muscles of the control and experimental animals were mounted on the same slide. To obtain histoautographs type M emulsion was used. The duration of exposure of the autographs was 40 days. The sections were stained with hematoxylin-eosin.

*The dose of cortisone was calculated by the equation $a = a_0(P/P_0)^{2/3}$, based on the daily therapeutic dose (0.1 g) used in clinical practice (a_0 is the dose for man, P_0 the weight of a man, P the weight of the animal).

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 75, No. 6, pp. 89-92, June, 1973. Original article submitted September 14, 1972.

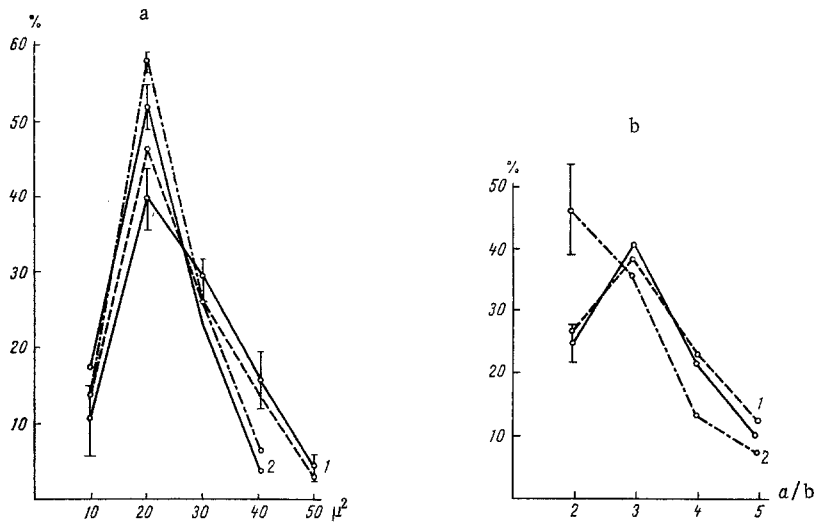

Fig. 1. Distribution of nuclei by size (a) and shape (b): 1) 10 days; 2) 15 days after daily administration of cortisone. Continuous lines, control; broken lines, experiment (in graph b, the control for the two series of experiments virtually coincides and is shown as a single curve). Abscissa, classes of nuclei (in μ^2) (graph a) and classes of nuclei by ratio between axes (graph b); ordinate, percentage of nuclei of each class. In this and subsequent figures, vertical lines show error of mean.

The intensity of incorporation of methionine-H^3 was determined by counting the number of grains of reduced silver above the nuclei. The nuclei were simultaneously drawn by means of a projection drawing apparatus (objective 90×, ocular 10). Nuclei were studied in 100 fields of vision in each muscle. A paper model of each nucleus was weighed, milligrams were converted into square microns, and the mean number of grains of silver per nucleus was determined for nuclei of the particular class. Classes of nuclei were formed by reference to their size and shape. The shape of the nuclei was determined from the ratio a/b between their axes (a the long axis, b the short axis).

The total number of nuclei in the experimental and control series was counted per section of a myon 0.2 mm in length, in 100 muscle fibers of each muscle. The numerical results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The karyometric investigations showed that classes of nuclei could be distinguished in the muscle tissue both of the control and of the experimental rats in accordance with their size (Fig. 1a) and shape (Fig. 1b), as follows: by area – 10, 20, 30, 40, and 50 μ^2; by shape – with ratios between axes a/b of 2, 3, 4, and 5. Nuclei with a mean area of 20 μ^2 were found most frequently; the distribution of the nuclei was unimodal and approximately normal in character. The modal class in the distribution of the nuclei by ratio between their axes was the class with the ratio of 3, while nuclei with ratios of 2 and 4 between their axes were found with almost equal frequencies.

As the graph in Fig. 1 shows, no difference was found in the distribution of nuclei by classes in the control and experimental series 10 days after administration of cortisone.

In the control animals, as a first approximation a linear relationship was observed between the dimensions of the muscle nuclei and the intensity of methionine-H^3 incorporation (Fig. 2a: 2). The results indicate that the content of radioactive label in the large nuclei was higher than in the smaller nuclei. This is in agreement with the results of autoradiographic investigations by Schultze et al. [9], who studied the intensity of incorporation of labeled amino acids into the nuclear proteins of other tissues. These workers concluded from their results that, if calculated per unit volume, the intensity of protein synthesis in large nuclei is the same as in small nuclei. However, our own calculations of the specific density of the tracks calculated per 100 μ^2 area of nuclei showed that large nuclei have a lower content of labeled proteins and, consequently, a lower intensity of metabolism than small nuclei (Fig. 2a: 4).

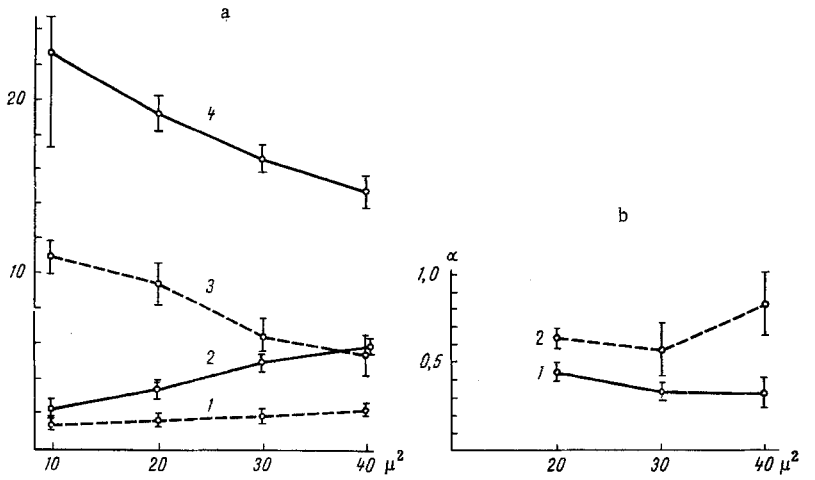

Fig. 2. Distribution of radioactive label in nuclei of intact rats and during cortisone administration: a) mean number of grains above nuclei (1, 2) and density of distribution of grains per 100 μ^2 area of nuclei (3, 4) 10 days after daily injections of cortisone (continuous lines – control, broken lines – experiment); b) ratio between specific density of grains in experiment control (control taken as 1) after administration of cortisone for 10 (1) and 15 (2) days. Abscissa, classes of nuclei (in μ^2); ordinate, number of grains of silver (graph a) and ratio $\alpha = M_2^*/M_1$ (graph b). M_1 and M_2 represent densities of grains per 100 μ^2 in control and experiment, respectively.

Administration of cortisone for 10 days led to a marked decrease in the incorporation of methionine-H^3 into nuclei of all classes (Fig. 2a: 1, 3).

In rats receiving injections of cortisone for 15 days the total number of nuclei in the muscle fibers was increased (458 ± 29 in the control and 716 ± 25 in the experiment). The increase in number of nuclei took place chiefly on account of nuclei with a ratio between their axes of 2 (Fig. 1b). The number of nuclei with an area of 20 μ^2 increased significantly, and the relative distribution of the nuclei showed no significant change (Fig. 1a).

On the 15th day of cortisone administration the quantity of radioactive label in the muscle tissue nuclei also was reduced, but by a rather lesser degree than after its administration for 10 days (Fig. 2b).

The shape of the muscle nuclei is evidently not determined by mechanical pressure from the contractile elements of the muscle fiber [8]. During differentiation of muscle cells the nuclei, which originally were round, became increasingly elongated. The appearance of many small (20 μ^2 in area) round nuclei in the experimental animals could evidently be attributed, first, to inhibition of differentiation of the nuclei, as is confirmed by the fact of a decrease in the content of labeled proteins in the nuclei of the specimens examined, and second, to the appearance of new generations of nuclei.

The experimental results show that administration of cortisone for 10 days leads to a statistically signficant inhibition of incorporation of methionine-H^3 into the nuclear proteins. By the 15th day of cortisone administration the inhibitory effect on incorporation of labeled amino acid was weaker.

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