MITOGENETIC RADIATION OF THE NEUROMUSCULAR SYSTEM AS A METHOD OF ANALYSING ITS MOLECULAR SUBSTRATE

REPORT III. THE REGULATING EFFECT OF THE SPINAL CORD CENTERS ON THE MOLECULAR SUBSTRATE OF THE MUSCLES AT DIFFERENT AGE PERIODS OF THE ANIMALS AND THE SIGNIFICANCE OF THIS REGULATION FOR THE MUSCLE METABOLSIM

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 51, No. 4, pp. 57-61, April, 1961
Original article submitted April 25, 1960

In previous publications[2,3,6], data were presented on the antibathic processes (processes of a reverse nature) arising in spinal cord centers (presumably areas rich in cells—the motor cells of the anterior horns, the interneurons) and in muscle fibers (neuromuscular junctions).

The action of the muscles imparts a prepolymer character to the substrate, while the effect of the centers, on the other hand, results in a dispersed arrangement. The resultant state of the neural and muscular substrates is extremely labile, and may be characterized as a state of unequilibrated molecular regularity.* The unequilibrated nature of the substrate in the muscle fibers has been demonstrated particularly clearly during the resting state of the muscles in experiments in situ.

Along with this, a previous report [3] introduced the concept of the constant regulation of the three-dimensional parameters in the molecular substrate of the muscles as a specific physiological phenomenon.

Further study of this regulation, serving as the goal of the present work, was carried out in the following directions.

1. We studied the evolution of the molecular substrate character in the muscles throughout the development of the animal, i.e., in the period when the full functional relationships are formed.

2. We studied the action of the centers on the muscle substrate as a factor limiting and, in this way, regulating the character of the metabolism. In this case we were thinking of mediators, specifically acetylcholine. Reducing the influence of the centers, we investigated the possibility of acetycholine arising under this condition in a resting muscle.

METHOD

The first part of the work was performed on rabbits, the second, on frogs. We investigated the mitogenetic radiation of the gastrocnemius muscle in the resting state within the living animals. The adult rabbits were secured to a horizontally placed stand. The muscle was exposed, and the radiating area, 10-12 mm^2, was delineated in the upper portion. The detector was placed at a distance 10 mm from the muscle. The same conditions were observed in the work with the young rabbits, except that because they were so active it was necessary, in addition to the regular fastenings, to hold them in place manually.

*The unequilibrated state of the molecular substrate in living systems is one of the basic concepts in the problem of mitogenesis [4]. This state, of course, does not exclude the presence of myosine protein chains, etc.

The adult rabbits were also left in the horizontal positon for the spectral analysis of the radiation, directed into the objective aperture of the spectrograph* by reflection from a concave mirror. The young rabbits were placed in a the vertical position, directly in front of the spectrograph aperture.

The frogs were also centered in the vertical position, fastened to a cork disk.

Degradation radiation [3, 4, 6] was caused by irrigation of the muscle with chilled physiological saline (6-8° for the rabbits, and 1-2° for the frogs).

Separation of the narrow spectral bands was accomplished with the aid of a monochromatic aperture. However, the majority of the data was obtained by coarser separation of the spectrum into portions 100 A in width.

The biodetection method was used for the observation of the radiation. This method has been described in detail in a number of publications [4, 5, 6].

TABLE 1

Radiation Spectra of the Rabbits' Gastrocnemius Muscle

Wave length (in A)	Resting radiation	Degradation radiation
	15-18 second exposure	6-8 second exposure
	effects (in %)	
1 900—2 000	—3	3
2 000—2 100	4	—7
2 100—2 200	—1	5
2 200—2 300	52	45
2 300—2 400	4	0
2 400—2 500	8	—3
2 500—2 600	6	5
2 600—2 700	—2	—
2 700—2 800	—8	13
2 800—2 900	10	—3
2 900—3 000	4	—3
3 000—3 100	—1	5
3 100—3 200	—3	—

Note: Averages of 5 trials are presented in column 2; averages of 2 trials are presented in column 3.

TABLE 2

Intensity of the "Resting" and Degradation on Radiation of the Gastrocnemius Muscle in Rabbits at Various Age Periods

Age (in days)	Resting radiation		Degradation radiation	
	exposure, sec**	effect (in %)	exposure, sec	effect (in %)
1—2	2—3	40	4—5	5
			8	36
4—5	2—3	—3	2—3	—2
	4—5	28	4—5	27
9	2—3	10	2—3	—10
	4—5	36	4—5	20
16	4—5	2	2—3	48
	8	24		
Adults	4—5	—3	2—3	39
	8	45		

Note: Averages of the figures from 2 trials are presented.

** The threshold exposures are indicated in boldface type.

RESULTS

Just as in the case of the spectrum described for the corresponding muscle in the frog [3], the radiation spectrum of the gastrocnemius muscle in the adult rabbit is characterized by a single radiation maximum in the range of 2200-2300 A (Table 1). Only with finer separation was a small difference seen in the spectra: the greatest radiation intensity for the frog muscle lay in the range 2000-2260 A, while for the rabbit it was in the range 2210-2270 A.

The same type (as in the frog) and spectrum of degradation radiation was seen to arise in the rabbit muscle in association with its cooling.

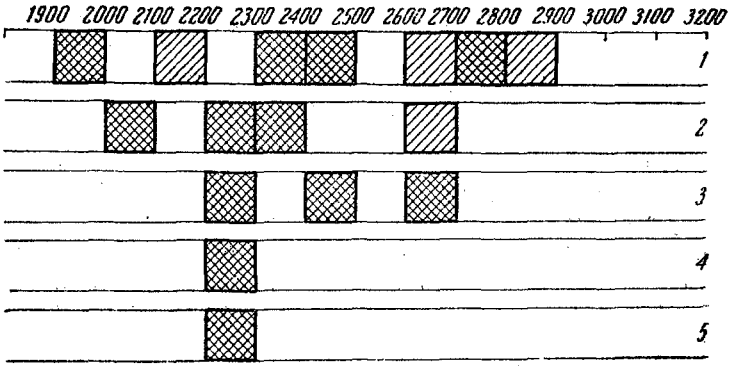
Spectral analysis of the radiation encountered in the resting gastrocnemius muscle of the rabbits in the different age periods yielded results which are presented in the figure.

The clear evolution of the spectra toward reduction in the number of active bands makes these data completely conclusive, despite the small number of animals. The radiation spectrum of the muscle in the 16-day old rabbit is already comparable to the spectrum observed in the adult animals. It must be noted that at this age, i.e., after maturation of the animal, degradation radiation of the muscle also acquires the same spectral composition.

* Fuess Quartz Spectrograph, possessing adequate dispersion.

These data make it possible to postulate that the molecular substrate of the muscle fibers gradually acquires the character of an unequilibratedly regular system. The data were supported by the results obtained from comparing the intensity of "resting" and degradation muscle radiation in the young and adult rabbits (Table 2).

Intensity and threshold duration were inversely related: $I.t = \text{const}$. [5, 6] (I – intensity, t – threshold exposure). In these experiments the constant radiation area (10 mm^2) was strictly observed, as was the constant distance of the detector from the muscle, 10 mm.



Spectra of radiation encountered in the resting gastrocnemius muscle of the rabbits at various age periods: 1) 1-2 days; 2) 4-5 days; 3) 9 days; 4) 16 days; 5) adults.

TABLE 3

Radiation Spectrum of the Frog Gastrocnemius during Cooling of the Spinal Cord Centers (the acetylcholine line is shown in boldface type)

Wavelength (in A)	Exposure (in seconds)	Effect (in %)	Wavelength (in A)	Exposure (in seconds)	Effect (in %)
1 900—1 930	8—10	**43**	1 940—45	20—30	0
1 930—1 960	8—10	0	**1 945—50**	20—30	**40**
1 960—2 000	8—10	12	1 950—55	20—30	—10
2 000—2 100	8—10	3			
2 100—2 200	8—10	—3	2 330—35	20—30	6
2 200—2 300	8—10	—6	2 335—40	20—30	39
2 300—2 400	8—10	2	2 340—45	20—30	0
2 400—2 500	8—10	—8			
2 500—2 600	8—10	3			
2 600—2 700	8—10	12	2 370—75	20—30	3
2 700—2 800	8—10	6	2 375—80	20—30	**45**
2 800—2 900	8—10	—1	2 380—85	20—30	6
2 900—3 000	8—10	**33**			
3 000—3 100	8—10	**20**	2 610—20	20—30	0
3 100—3 200	8—10	**16**	**2 620—30**	20—30	**60**
			2 630—40	20—30	10

Note: The averages of two trials are presented in column 3, and the averages of 3 trials in column 6.

Thus, we can speak of significant intensity of the resting radiation from the rabbit muscle at early ages of the animals, and of approximately the same elevation in the intensity of the degradation radiation, beginning with the rabbit's second weak of age.

The entire body of results permits drawing the following conclusions.

In the first days following the birth of the animal the radiation of the muscle in the resting state is directly related to metabolic processes, and must be very varied in that period. Later the radiation arises as a result of

natural disturbances in the unequilibrated molecular substrate, i.e., appears as degradation radiation of a physiological type. Energy for the support of this unequilibrated state is supplied by the muscle metabolism. The three-dimensional regularities in the substrate's molecular order reproducible in general outline, are, as we already stated earlier, the product of an uninterrupted interaction of processes whose sources are the centers and the muscles.

Judging form the evolution of the spectra, the general steric regularities of the substrate gradually transform into more well defined structural parameters. Nevertheless the state of the substrate completely retains its unequilibrated character, i.e., requires a considerable expenditure of energy for its support.*

This latter conclusion leads to the concept, already advanced earlier, that the resting state of the neuromuscular system must be regarded as an active setting, and emphasizes that a high energy potential is specific for the muscle resting state.**

Judging from the low intensity of the "resting" radiation of the muscle, it is consumed very expeditiously and economically. The unequilibrated structural state of the substrate doubtlessly aids this.

We considered it of interest to compare these data with the results of detailed investigations by I. A. Arshavskii and V. D. Rozanova [1, 8], demonstrating a low lability and excitability, and also low resistence of the neuromuscular apparatus in the early age periods of the animals, and elevation of these properties with development of the animal. The gradual formation of the unequilibrated structural arrangement corresponds to the rise in lability and excitability. The constant reproducibility of the structural arrangement formed may serve as one of the factors elevating the resistence of the muscle tissue.

But along with this the question may be raised as to whether such dynamic constancy of the structural arrangement does not serve as the kind of regulating factor which limits the accomplishment of various chemical activities.

In this case, reduction in the interaction between the spinal cord centers and the muscles could lead to "enrichment" of the spectrum for the resting radiation of the muscle and to inclusion of bands in it which are characteristic of metabolites not found under normal conditions. We settled on acetylcholine, whose spectrum is not observed during analysis of the resting radiation from the frog gastrocnemius muscle, and which arises during its excitation [7]. The experiments presented below were also carried out on frogs.

Reduction of the action of the centers was accomplished by cooling them. This, along with direct stimulating action, yields an increasing inhibitory effect on the enzymatic activity of the tissue, i.e., indirectly also on the functional activity of the centers.

A flat piece of ice from frozen phsiological saline was affixed to the corresponding portion of the vertebrate, which had first been freed of the muscles covering it. After 5-8 min we began the spectral analysis of the radiation from the gastronemius muscle (Table 3).

We see, in this manner, that not only the general character of the spectrum changed, but weak acetylcholine lines also arose. The parallel occurrence of these two phenomena are of great interest from out point of view.

These data thus confirm the concept that regulation of the structuro-energy state in the molecular substrate of the muscle in association with the overall state of all systems involves the principle of limiting the various chemical processes, i.e., it determines, to a definite degree, the character of the metabolsim.

In line with this one must refer to the additional orientating data, showing that with adequate excitation of the muscles there occurs an "enrichment" of the total radiation spectrum in addition to the simultaneous genesis of acetylcholine.

SUMMARY

The authors present data which broadens the concept of the regulatory effect exerted by the spinal cord centers on the molecular substrate of muscles. Evolution of mitogenetic spectra of the resting rabbit gastrocnemius (connected with age) and the prevalence of mitogenetic radiation of degradational type in adult animals point to the gradual formation of the nonbalanced structural state of the substrate.

* The concept that a more limited spectrum reflects greater uniformity of the substrate's structural organization has been completely substantiated.

** Especially manifest, apparently, for heavy muscles.

It was shown that the nature of metabolism is also connected with the substrate regulation. This follows from the altering of the mitogenetic spectrum lines of the resting frog gastrocnemius caused by reduced influence of the centers. In such conditions, the spectrum contains acetylcholine lines as well.

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