THE BIOCHEMICAL CHARACTERISTICS OF THE SKELETAL MUSCLE OF THE FROG IN VARIOUS STAGES OF FATIGUE AND RECEIVING SINGLE STIMULI FROM THE NERVE

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Studies of the process of fatigue in the isolated skeletal muscle of the frog during application of a rhythmic sequence of single maximal stimuli from its nerve, carried out by M. V. Kirzon and collaborators [4, 5, 7], have shown that the diminution in the height of the muscular contractions taking place in these circumstances passes through several stages. After an initial increase in height (staircase) and a short period of maintenance of the contractions at the same level (plateau), there follows a stage of a relatively rapid decrease in the height of the contractions (steep drop), and only then does a uniform and slow decrease in their height ensue, ending in the complete cessation of the responses of the muscle to stimulation (Fig. 1). Depending on the frequency of repetition of the single contractions of the muscle, the load placed upon it, and other conditions the duration of these stages of fatigue and the degree to which they occur may vary. If considerable residual shortenings (contractures) develop, the course of fatigue may vary significantly from that described above.

We have, therefore, every reason to suppose that the process of loss of working capacity of a skeletal muscle deprived of its blood supply does not obey a linear relationship, and that certain qualitative (including biochemical) differences must exist between the processes characteristically occurring in the muscle in these stages of fatigue we have described.

The physiological signs of these stages can be found not only in the changes in the height and rate of the contractions, but also in the different forms of temporary recovery of working capacity by the muscle (including during the action of sympathetic influences) in the various stages of fatigue [4, 5]. It has been concluded from these differences that the stage of the sharp drop in the heights of the contractions is a critical period, before and after which the action of all manner of influences on the nerve-muscle preparation may have different or even opposite after-effects.

Investigations of fatigue in the same conditions but conducted on a single innervated muscle fiber [7] have led to the conclusion that a decrease in the working capacity of the muscle develops as the result of a decrease in the contractile power of the muscle fibers and also of a deepening block to the transmission of impulses in the neuromuscular synapse, affecting an increasing number of fibers. It is remarkable that it is during this period of the sharp drop in the heights of the contractions that the blocking of the transmission of impulses from the nerve fiber to the muscle fiber first appears and increases sharply in intensity.

Since in all the investigations of fatigue we have described the frequency of stimulation from the nerve was very low (20-120 per minute), i.e., the shortest interval between stimuli was 500 millisec, it is obvious that in this case the blocking of the neuromuscular synapse cannot be interpreted as indicating a pessimum of frequency, in which the principal role would belong to what N. E. Vvedenskii [3] calls "fatigue from an inadequate interval". In such a case the principal sign of fatigue associated with a low frequency of stimulation from the nerve must be the biochemical changes taking place in the contractile structure of the muscle fiber itself.

The aim of the present investigation was to study the biochemical changes taking place in the isolated muscle at different stages of fatigue and during the application of single stimuli from the nerve.

EXPERIMENTAL METHOD

The test object was a nerve-muscle preparation of the gastrocnemius muscle of the frog. Single isotonic contractions of the muscle were recorded during application of a 10 g load; the frequency of stimulation of the sciatic nerve was 60/min, the stimuli being of maximal strength and of short duration (GRAKh-1 apparatus). The preparation was moistened in Ringer's solution only during dissection, and for 4-10 min the muscle worked in the air. At

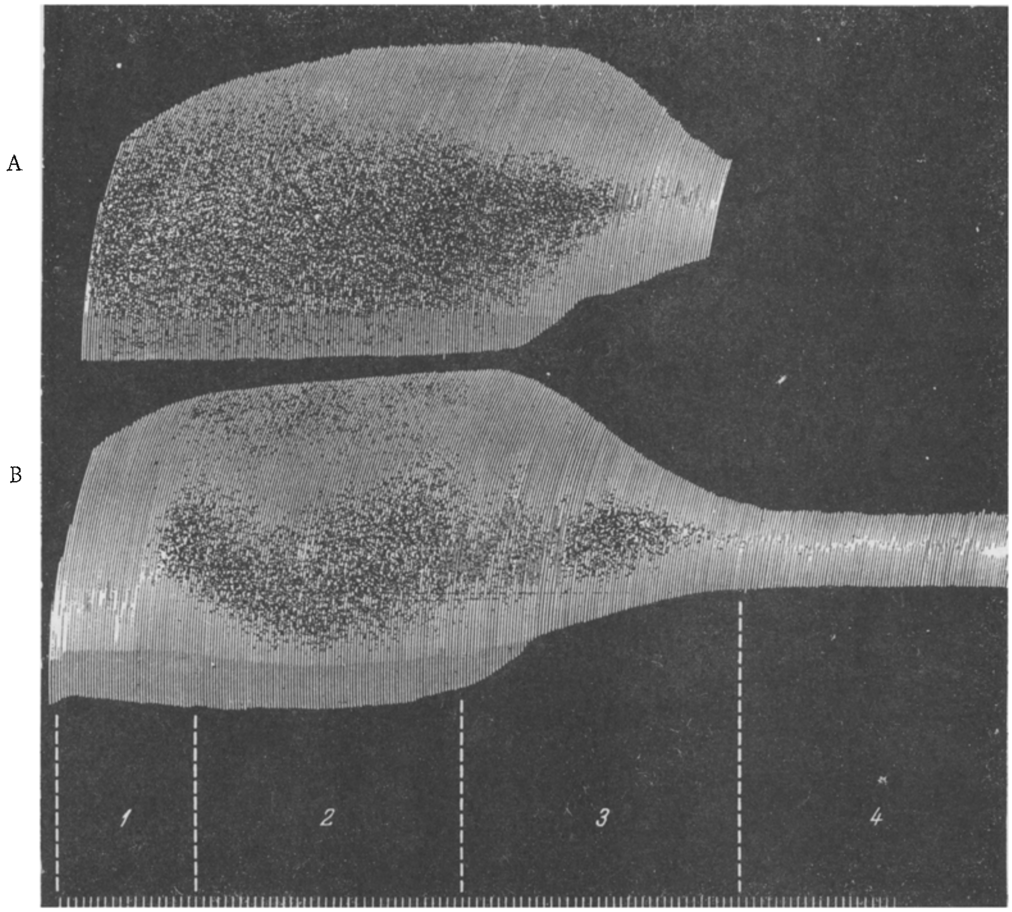

Fig. 1. Kymograms of parallel work of the gastrocnemius muscles of the same frog. Stages of fatigue: 1–staircase; 2–plateau; 3–sharp drop; 4–profound fatigue. The work of both muscles was stopped at different moments of fatigue: A–at the end of the stage of the sharp drop; B–in the stage of profound fatigue. Time marker 5 sec.

definite stages of fatigue the muscle was frozen in liquid nitrogen and subjected to biochemical analysis. A trichloroacetic centrifugate was obtained and the concentration of inorganic phosphate (IP) determined by the method of Lowry and Lopez [15] as modified by Bruemmer and O'Dell [14], creatine phosphate (CP) by Alekseeva's method [1], and lactic acid (LA) by the method of Barker and Summerson [12]. The adenylic system was separated by paper chromatography in an isoamyl alcohol–sodium citrate system [8]. ATP and ADP were determined quantitatively by a spectrophotometric method in accordance with their adenine content [13], or colorimetrically by means of Meibaum's pentose method [6]. As a control we used a nerve-muscle preparation of the symmetrically opposite muscle of the same frog, left attached to the recording lever while the experimental muscle was working, carrying the same load, but not subjected to stimulation.

EXPERIMENTAL RESULTS

The first series of experiments was carried out during the autumn-winter period of 1956-57, on grass frogs. In these experiments we determined the CP, ATP, and IP at different stages of fatigue (Table 1 and Fig. 2).

From the very earliest stages of fatigue, a considerable decrease in CP was observed, so that in the plateau stage approximately half the initial concentration was observed, and at the beginning of the stage of the steep drop the concentration had fallen to about one third of the initial value. In the stage of the steep drop the decrease took place much more slowly, and at the end of this stage its concentration was one-quarter the initial value. No further decrease in CP was observed in the deeper stages of fatigue.

TABLE 1. Concentration of High-Energy Phosphorus Compounds and Inorganic Phosphorus (in mg%) in the Gastrocnemius Muscle of the Frog at Rest and in Different Stages of Fatigue

State of Muscle	Substance											
	IP				CP				ATP			
	No. of experiments	Limits of variation	Mean	Change	No.of experiments	Limits of variation	Mean	Change*	No. of experiments	Limits of variation	Mean	Change*
Rest	21	23-69	46	–	13	36-51	42	–	11	28-45	35	–
End of staircase	3	24-58	44	0	3	13-22	19	−54	3	32-36	34	0
End of plateau	5	49-90	60	+30	3	13-15	14	−68	2	45-47	46**	–
Steep drop	9	43-71	62	+30	4	9-11	10	−78	3	27-38	30	−14
Profound fatigue	4	52-100	68	+50	3	7-11	10	−78	3	19-23	20	−45

* Given as a percentage of the value of the index at rest, taken as 100%.

** The ATP concentration at rest in these two experiments was 40 mg%.

Despite the rapid loss of CP, in the initial stages of fatigue (staircase) no increase in the IP concentration was observed, and not until the beginning of the steep drop did its concentration show an increase amounting to 30% of its initial value. Although during the steep drop the utilization of CP continued, not only did the IP concentration not continue to increase, but it actually fell slightly. After the stage of the steep drop, the IP concentration again began to increase.

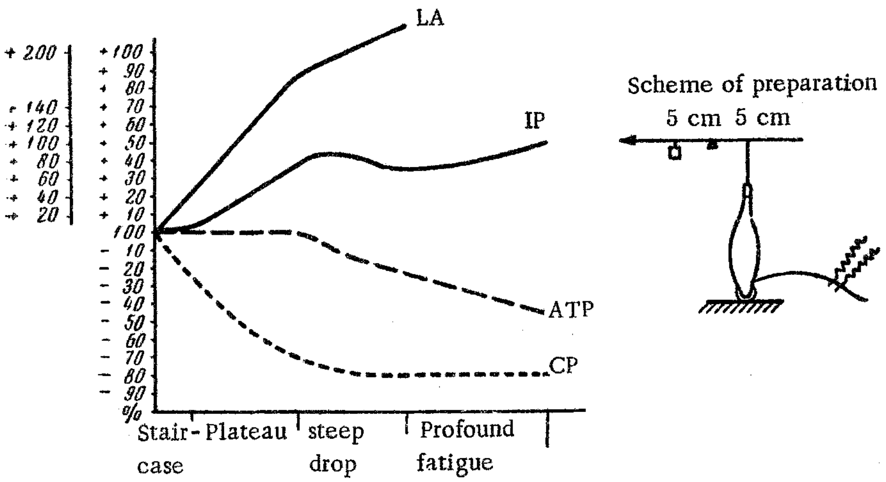

Fig. 2. Schematic graph showing the changes in the concentrations of ATP, creatine phosphate, and lactic acid in the gastrocnemius muscle of a frog at different stages of fatigue and during application of single indirect stimuli (from the figures in Tables 1 and 2). Scale on the left–increase in LA concentration in percent.

The ATP concentration underwent distinctive changes during the various stages of fatigue. Although the work done by the muscle in unit time was greater during the stages of the staircase and plateau than in the later stages, the ATP concentration in these stages of fatigue remained unchanged, and at the end of the plateau stage it actually showed a small increase (this is not reflected in Fig. 2).

In order to clarify the details of the metabolism of the high-energy phosphorus compounds in the stage of the steep drop, a second series of experiments was carried out in the spring of 1957 (Table 2). Besides CP, ATP, and IP, we also determined the LA, from which we could estimate the rate of glycolysis in the various stages of fatigue.

TABLE 2. Concentrations of ATP, ADP, CP, IP, and LA (in mg%) in the Muscle of a Frog at Rest and at the Beginning and End of the Steep Drop in the Heights of Single Contractions

Substance	State of Muscle	Experiment No.					Mean	% increase or decrease*
		1	2	3	4	5		
IP	Rest	50	30	47	44	48	44	–
	Beginning of steep drop	55	60	60	55	53	58	+32
	End of steep drop	58	70	68	53	60	62	+40
CP	Rest	36	42	51	31	58	44	–
	Beginning of steep drop	24	20	24	22	39	26	−40
	End of steep drop	8	10	11	17	24	14	−68
ATP	Rest	33	30	32	28	30	31	–
	Beginning of steep drop	34	29	31	26	32	30	0
	End of steep drop	31	18	28	21	24	24	−22
ADP	Rest	6	5	5	6	5	5	–
	Beginning of steep drop	5	4	5	6	4	5	–
	End of steep drop	3	5	3	5	3	4	–
LA	Rest	162	128	200	312	172	195	–
	Beginning of steep drop	562	575	595	595	362	538	+176
	End of steep drop	631	645	774	634	506	638	+127

*The concentration of the substance at rest is taken as 100%.

These experiments confirmed the basic facts. The ATP concentration fell quite clearly at the end of the stage of the steep drop, whereas at the beginning of this stage practically no decrease in ATP was present. As in the first series of experiments, however, this was not accompanied by an increasing concentration of IP: whereas at the beginning of the steep drop the increase in IP was 32% (close to the value characterizing the end of the plateau stage, see Table 1), at the end of this drop the further increase in IP was slowed, and amounted to about 40% of the initial value.

Lactic acid (see Table 2) accumulated more slowly during the stage of the sharp drop than in the plateau stage. This indicates that glycolysis was slowed during the stage of the steep drop. This may be why the rate of resynthesis of ATP fell and why its concentration fell sharply at this stage of the work.

Our experiments thus confirmed the hypothesis that the process of fatigue of the isolated nerve-muscle preparation during application of single indirect stimuli cannot be regarded as a uniformly developing, linear process. It was shown perfectly clearly that the course of the biochemical processes before and after the steep drop in the heights of the contractions was different. Hence it follows that this period of the steep drop in the heights of the contractions is a critical period in the development of fatigue. This conclusion is reflected in several of the signs described above. The most convincing of these is the maintenance of the initial ATP concentration until the beginning of an appreciable decrease in the heights of the muscular contractions. Furthermore, in some experiments we observed a small increase in the ATP concentration, which appeared at the end of the initial staircase stage and was maintained until the end of the plateau period. Meanwhile, from the time that the muscle began to work CP was used up as LA accumulated rapidly. At the end of the stage of the steep drop in every case we observed utilization of ATP, accompanied by a slight decrease in ADP.

It was noteworthy that the commencing utilization of ATP during the period of the steep drop coincided in time with the increasingly slow contraction and relaxation of the muscle, with the development of some degree of contracture, and with a decrease in the height of the contraction of individual muscle fibers [9]. It was at this period that the muscular fibers began to give irregular responses to a regular rhythm of nervous impulses—a process terminating in the complete blocking of conduction through the neuromuscular synapses [7].

Whenever a muscle is provided with more suitable conditions for its biochemical processes to take place, it responds with a longer plateau period and a more gradual decline in the height of its contractions, so that in general the stage of the steep drop does not appear. This is observed, for example, when the blood flow through the muscle is intact, and the rhythm of contraction and the load are the same, and also in the isolated muscle at slower rhythms of contraction than in our experiments [4]. It may be concluded from these facts that the stage of the steep drop in our experimental conditions is the deadline beyond which the muscle undergoes certain irreversible changes, very intimately connected with the utilization of ATP by the muscle. On the other hand, earlier observations indicate that after the stage of the steep drop the muscle does not return to its initial level of working capacity, regardless of whether optimal conditions are provided for it or whether it is put at rest.

After the steep drop a stage of profound fatigue develops, characterized by low and slowly diminishing contractions, the continued accumulation of LA, the slower utilization of ATP and slow accumulation of IP, and the almost total using up of CP at the beginning of this stage, which is in agreement with reports in the literature [2, 10, 11].

SUMMARY

The content of ATP, creatine phosphate, inorganic phosphate and lactic acid was studied in the isolated frog skeletal muscle at various stages of fatique caused by single maximal stimulations of the nerve. There is no lineal alteration in the content of the substances studied during the muscle work. Before the stage of abrupt decline of contractions the ATP content remains on the initial level, whereas the quantity of creatine phosphate rapidly decreases and the content of lactic acid manifests a sharp rise. The stage of abrupt decline, characterized by deceleration of the contraction and relaxation of the muscle, is the turning point in the development of fatigue. In this stage, along with further decrease of creatine phosphate and accumulation of lactic acid, there occurs a drop in the content of ATP in comparison with the level of rest.

LITERATURE CITED

1. A. M. Alekseeva. Biokhimiya, 2, 243 (1956).
2. V. A. Belitser. Chemical Conversions in Muscle [in Russian]. Moscow-Leningrad, 1940.
3. N. E. Vvedenskii. Selected Works [in Russian], p. 47. Moscow, 1952.
4. M. V. Kirzon and Z. E. Khurges. In the book: Proceedings of the First Meeting of the Moscow Society of Physiologists, Biochemists, and Pharmacologists [in Russian], p. 110. Moscow-Leningrad, 1941.
5. M. V. Kirzon. Investigation of the Mechanism of Sympathetic Action on Skeletal Muscle and Similar Phenomena of Temporary Removal of Fatigue. Doctorate dissertation, 1948.
6. V. V. Meibaum. Biokhimiya, 4, 353 (1945).
7. N. R. Mirza. General Principles of the Process of Fatigue and Temporary Removal of Fatigue of a Single Muscle Fiber with Its Nerve Supply Intact. Author's abstract of candidate dissertation, Moscow, 1949.
8. E. A. Mishukova and L. N. Lebedeva. Vopr. Med. Khimii, 5, 369 (1956).
9. F. N. Serkov. Fiziol. Zh. SSSR, 1, 53 (1949).
10. D. L. Ferdman. The Biochemistry of Diseases of Muscles [in Russian]. Kiev, 1953.
11. N. N. Yakovlev. Essays on the Biochemistry of Sport [in Russian]. Moscow, 1955.
12. S. Barker and W. Summerson, J. biol. Chem., 1941, vol. 138, p. 535.
13. R. Block, R. Lestrange, and G. Zweig. Chromatography on Paper [Russian translation]. Moscow, 1954.
14. J. Bruemmer and B. O'Dell. J. biol. Chem., 1956, vol. 219, p. 283.
15. O. H. Lowry and J. A. Lopez. J. Biol. Chem., 1946, vol. 162, p. 421.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. ***Some or all of this periodical literature may well be available in English translation.*** **A complete list of the cover-to-cover English translations appears at the back of this issue.**
