# INCREASE IN ATP CONTENT IN WORKING SKELETAL MUSCLE

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In studies of changes in the biochemical composition of working isolated skeletal muscle of the frog we repeatedly observed that the ATP content of the muscle appeared higher than the resting level in all experiments in which an increase in amplitude of muscular contraction is accompanied by a rapid relaxation with preservation of elasticity, which is expressed by depression of the recording pen beneath the resting base line. This phenomenon was observed both in the gastrocnemius and in the sartorius muscles under various experimental conditions (single contraction, short tetanic contraction, sympathetic phenomena).

Below we briefly describe experiments in which an increase in contraction amplitude was observed in combination with retention of the elasticity and relaxation capacity of the muscle.

## METHODS

The method for quantitative measurement of ATP was briefly described previously [4]. The ATP content in the resting, not-working, contralateral muscle was usually used as control.

In experiments using the grass frog observations were made on single contractions of the gastrocnemius muscle, moistened in Ringer's solution, which contracted in air. Stimulation of 60 impulses/min was introduced via the nerve with a GRAKh-1 apparatus. An optimal load (7-10 g) was fixed to the recording-lever at the same distance from the axis of rotation as the ligature connecting the lever with the muscle. At the necessary moment the muscle was frozen in liquid nitrogen in the working chamber during prolonged nerve stimulation.

To obtain brief tetanic contractions the muscle was stimulated via the nerve with a GRAKh-1 apparatus. The duration of tetanus was one second, the interval between tetanic contractions four seconds, the stimulation frequency 30 hertz, the duration of the impulse 0.3-0.4 msec. An optimal load of seven g was hung at a distance from the muscle equal to that from muscle to the axis of the recording-lever. The sartorius muscle of the large lake frog contracted in air after moistening with drops of Ringer's solution.

## RESULTS AND DISCUSSION

At the end of amplitude increases for single contractions (at the end of the "staircase") an increased muscle ATP content was invariably observed. In summertime experiments the initial concentration and rise in ATP (see table), as well as the duration and height of the "staircase" effect, were significantly higher than in wintertime. At the end of the "staircase" in winter experiments, the ATP content rose by 10% over the resting level. In another series of experiments (see table) the muscle operated to the end of the "plateau" stage which follows the "staircase" (the period of relatively constant amplitude of contraction). Despite the increased ATP content of 14% at the end of this period, this change appeared statistically not valid.

Thus, the period of increase in muscular work-capacity is characterized by increase in the ATP content. To verify this statement, we conducted experiments in which the effect of work during the "staircase" period and during subsequent rest on the muscle ATP content was studied. If at the end of the "staircase" stage the increase in ATP in summer experiments was 45%, then after similar work and $1^1/_2$ -2 min of rest the ATP content exceeded the resting level only by 13% (see table), i.e. it had practically returned to the resting level. If the muscle were not frozen at the end of the rest period, but was again stimulated, the first contractions after the rest appeared to occupy a middle

Changes in ATP Content (in Mg%) in Frog Muscle During Work

| Time of year | Type of expt. | | Number of expts | State of muscle | Mean ATP content and limits of variability (in mg%) | Rise | | P |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Mg | % | |
| Winter experiments | Single contraction, 60 imp/min rhythm | Work to end of "staircase" | 4 | Rest | 203 ± 30.3 | 20 | 10 | < 0.05 |
| | | | 4 | Experiment | 223 ± 33.1 | | | |
| | | Work to end of "plateau" | 16 | Rest | 195 ± 7.9 | 27 | 14 | > 0.05 |
| | | | 6 | Experiment | 222 ± 21.1 | | | |
| Summer experiments | | Work to end of "staircase" | 6 | Rest | 245 ± 10.4 | 109 | 45 | < 0.05 |
| | | | 6 | Experiment | 354 ± 13.1 | | | |
| | | Same + rest of $1^1/_2$-2 min | 4 | Rest | 270 ± 27 | 34 | 13 | > 0.05 |
| | | | 4 | Experiment | 304 ± 31 | | | |
| | | Same + rest of 1 min | 4 | Rest | 243 ± 15.2 | 99 | 41 | < 0.05 |
| | | | 4 | End of "staircase" | 342 ± 16.5 | | | |
| | | | 4 | End of "staircase" + rest | 314 ± 19.6 | 71 | 30 | < 0.05 |

position between the amplitudes of the beginning and end contractions of the preceding "staircase." The new "staircase" began as if in the middle.

This experiment was also performed in somewhat altered conditions. Both gastrocnemius muscles from one frog were worked to the end of the "staircase" stage, then one of them was frozen rapidly, the other after a minute's rest. The ATP content in the shank of the same frog served as control. At the end of the "staircase" the ATP content of the muscle rose to 41% over the resting level and after one minute of rest only to 30% (see table).

We observed similar biochemical changes with the so-called sympathetic effect [2]. Against a background of sharply decreasing single contractions and steady non-relaxation of the muscle, stimulation of the sympathetic nerve led to rapid and repeated increase in the amplitude of each contraction, accompanied by an improvement in the relaxability and partial return of elasticity. With this an increase in the ATP content to 37% was observed in comparison with the level in the control, contralateral muscle, which worked in parallel under the same conditions but with the sympathetic chain removed (see table). It is known that the effect of cooling [3] is distinguished from sympathetic effect by the lack of improvement in relaxability and elasticity and is accompanied not by a rise but by a fall in ATP [1].

With brief tetanus the amplitude and elasticity of the muscle reach a maximum by the seventh to tenth contraction, and then begin gradually to fall. No contracture occurs. When the ATP content is measured under these conditions at the twelfth, eighteenth and 58th contraction, its rise is noted to be 30, 25 and 15%, respectively. The greatest contraction amplitude corresponded to the greatest rise in ATP (twelfth contraction).

The data obtained from isolated skeletal muscle of the frog, are confirmed by similar results from experiments on rats. These experiments were performedon ether-anasthetized rats fastened to a special operating table supplied with warming apparatus. Both gastrocnemius muscles were prepared together. The sciatic nerve on the side of the experimental muscle was cut and the distal end was stimulated with the GRAKh-1 apparatus with short tetanic impulses. The impulse frequency was 40 hertz, the duration of the tetanus one-half second, the interval between tetani five seconds, the duration of impulse 0.3 millisec, the load seven g. Both muscles were moistened throughout the experiment with Ringer's solution heated to 38° (for homoiotherms). The experimental muscle and its contralateral control were frozen simultaneously after 20-40 contractions of the experimental muscle. The ATP content of the experimental muscles exceeded that of the control by 12, 6.7, 1.2 and 11% (mean of 7.35%) with no signs of contracture on the kymogram.

An increase in rat muscle ATP content after certain types of indirect electrical stimulation of the muscle has been noted previously [5]. A similar effect in frog gastrocnemius muscle was obtained by S. V. Shestakov in 1957 (unpublished data).

In our experiments a rise in the ATP content of working muscle has been repeatedly noted under various conditions. These data are not related to the stimulation regime, since an increase in ATP concentration was observed both in single, and in tetanic contractions; in the sartorius muscle of the large lake frog contracting in air, in the gastrocnemius muscle of the grass frog functioning in Ringer's solution, where the access to oxygen is considerably more difficult for the muscle, and also in rat muscle studied under conditions in which the blood flow is preserved. Special experiments have established that whereas work under certain laboratory conditions increases the muscle ATP content, subsequent rest again lowers it to a resting level. Together with the loss of ATP the amplitude of contraction decreases, as observed when impulses are resumed after rest.

The experiments which lead to a rise in muscle ATP content possess the following common feature: during the observation period the muscles contract without losing their relaxability and elasticity. Under these circumstances, when for some reason muscle relaxability is lost, a decrease in ATP relative to the resting level is invariably observed.

Since the normal work of muscles in the healthy organism is not accompanied by contracture, we suggest that the rise in ATP content (relative to the resting level) accompanies any natural work done by the muscle and is a reflection of its active functional status.

## LITERATURE CITED


1. T. A. Allik, Energy metabolism in isolated skeletal muscle during work, exhaustion and pathological decrease in work capacity [in Russian], Avtoref. diss. kand., Moscow (1963).
2. A. Ginetzinskii, Russk. fiziol. zh. 6, No. 1-3, (1923), p. 139.
3. M. V. Kirzon, Study of the mechanism of sympathetic effect of skeletal muscle and the similar phenomenon of temporary removal of exhaustion [in Russian], Diss. dokt. Moscow, (1948).
4. M. V. Kirzon, T. A. Allik, and S. V. Shestakov, Byull. eksper. biol. No. 9, (1962), p. 29.
5. M. N. Kondrashova, Biochemical and physiological characteristics of certain functional states [in Russian], Diss. kand., Moscow (1957).


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