BREAKDOWN OF ADENOSINE TRIPHOSPHATE DURING A SINGLE CONTRACTION OF WORKING MUSCLE*

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Experiments with isolated enzymes, muscle protein solutions, reconstituted actomyosin fibrils and glycerinated muscle models have suggested that adenosine triphosphate (ATP) is the immediate energy source for muscle contraction. However, despite enormous efforts, numerous attempts to demonstrate changes in the concentration of ATP during a single contraction cycle have repeatedly shown that no quantitatively sufficient changes occur. This has been interpreted as being due to a very rapid regeneration of ATP from phosphorylcreatine (PCr) catalyzed by the enzyme creatine phosphoryltransferase. Although single contractions of muscle without measurable changes in PCr have been reported (Fleckenstein, Janke, Davies and Krebs 1954; Mommaerts 1954), recent results with improved techniques have shown clearly that approximately 0.6 μmole/gm muscle of this compound does in fact break down to form creatine (Cr) and inorganic phosphate (Pi) when work is done during a single contraction (Davies, Cain and Delluva 1959; Cain and Davies 1960; Cain 1960; Davies and Cain 1961; Davies, 1961; Davies, Cain, Infante, Klaupiks and Eaton 1962; Cain, Infante, Klaupiks, Eaton

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and Davies 1962). This result has been confirmed independently by Mommaerts (1960; 1961; 1962).

It is of interest that a muscle performing 125 gm-cm work/gm muscle should require about 0.5 μmole of high-energy phosphate/gm muscle assuming that 10 mcal of free energy/μmole become available and are utilized at about 50% thermodynamic efficiency. If creatine phosphoryltransferase could be inhibited *in situ* a direct demonstration of the role of ATP in muscular contraction might be obtained. In this case, an inhibited muscle should be able to do only a few contractions before the ATP stores become exhausted, and a quantitative relationship between the change in the ATP content in the muscle and the amount of work done could be found. Kuby and Mahowald (1959) showed that crystalline creatine phosphoryltransferase is completely inhibited by 1-fluoro-2,4-dinitrobenzene (FDNB) and, using this inhibitor, we have now found the long-sought-for changes in ATP.

After pretreatment of the muscle with FDNB, the number of full, normal contractions which could be performed by an isolated muscle was reduced from more than 30 to about 3, yet these muscles still contained the same amount of phosphorylcreatine as resting, untreated muscle. This communication shows that the energy for these contractions came from the breakdown of ATP.

Methods

The paired rectus abdominus muscles of female frogs (Rana pipiens) were dissected and allowed to rest at room temperature for at least one hour in a physiological bicarbonate saline solution. They were then transferred to a similar solution containing 3.8×10^{-4} M FDNB at 0°C for 40 min. and mounted on an isotonic lever at rest length. They were then frozen rapidly in a 1:1 mixture of Freon 12 and 13 (CF_2Cl_2 and CF_3Cl, -170°C) either at once (control) or when they had nearly reached the peak of a single smooth contraction following stimulation for 1 - 1.5 sec. at 12 supramaximal shocks/sec. The

frozen muscles were cut down, rapidly weighed, and transferred whilst frozen to stainless steel centrifuge tubes at -196°C. Each muscle was pulverized at this temperature with a cold steel ball on a handle and ground into 0.3 ml of 0.5 M $HClO_4$ to form a smooth powder. The powdered muscle plus perchloric acid was stored in liquid nitrogen.

Extracts were thawed in pairs. 1.0 ml of 0.5 M $HClO_4$ was added to each extract and the tube transferred to a water bath at 35°C. The contents were stirred for 4 min., spun in the high-speed head (no. 296) of an International Centrifuge, then 1.0 ml of the supernate was rapidly removed and added to 0.45 ml of a solution containing 0.5 m-mole KOH plus 0.15 m-mole triethanolamine buffer, pH 7.5. The solution was refrozen and stored at -78°C until ready for analysis. After rethawing, the precipitate of $KClO_4$ was allowed to settle or was centrifuged out at 0°C.

Analytical Procedure

ATP, ADP and AMP were measured fluorimetrically with the enzymatic assay kits from Boehringer and Soehne (Mannheim, Germany) according to a modification of Estabrook and Maitra (1962), on a Turner Model 111 Fluorometer and a recorder. All analyses of ATP and ADP were carried out in duplicate. In some experiments the ATP content of control and contracted muscle was also measured by the use of the specific firefly luminescence technique and a photomultiplier coupled to a recorder.

Results and Discussion

Table 1 shows the results obtained with 9 pairs of muscles in which one of each pair had done a single contraction and performed about 125 gm-cm work/gm (i.e. 2.9 mcal/gm).

TABLE 1

Changes in the adenine nucleotides during contraction of frog rectus abdominus muscles after treatment with fluorodinitrobenzene

(External work was approx. 125 gm-cm/gm muscle/contraction)

	ATP	ADP	AMP
	(all quantities in μmoles/gm wet wt. of muscle)		
SINGLE CONTRACTION			
Rest	1.25	0.64	0.10
1 Contraction	0.81	0.90	0.24
Change ± standard error of the mean for 9 pairs	-0.44 ± 0.046	+0.26 ± 0.023	+0.14 ± 0.027
DOUBLE CONTRACTION			
Rest	1.24	0.61	0.07
2 Contractions	0.59	0.88	0.41
Change ± standard error of the mean for 3 pairs	-0.65 ± 0.045	+0.27 ± 0.051	+0.34 ± 0.037

In all cases there was a decrease in the ATP content and an increase in ADP and AMP. The decrease in ATP was not quite as large as would have been expected from the results of earlier experiments which showed an increase of 0.64 ± 0.14 μmole/gm of Pi and 0.83 ± 0.20 μmole/gm of free Cr during a single contraction under similar conditions except for the pretreatment with FDNB. In these earlier experiments, the Lohmann reaction was apparently the energy-supplying mechanism, i.e.

$$\text{ATP} \xrightarrow{\text{actomyosin ATPase}} \text{ADP} + \text{Pi}$$

$$\text{PCr} + \text{ADP} \xrightarrow{\text{creatine phosphoryltransferase}} \text{ATP} + \text{Cr},$$

so one would expect, as was found, that the changes in Pi, Cr and PCr were identical in magnitude. Table 1 shows further that the ADP increases are less than the decreases in ATP and that there is a real increase in AMP. This shows that myokinase is active in these muscles under these conditions, so the following reactions occur:

$$\text{ATP} \xrightarrow{\text{actomyosin ATPase}} \text{ADP} + \text{Pi}$$

$$2\ \text{ADP} \xrightarrow{\text{myokinase}} \text{ATP} + \text{AMP}.$$

This is advantageous for several reasons. A low level of ADP is maintained which is helpful since ADP inhibits myosin ATPase (Nanninga 1962). The ratio of ATP to ADP is kept higher, and free energy is effectively released from the breakdown of both labile phosphates of ATP, despite the fact that the actomyosin system seems to require only ATP. The combined action of actomyosin ATPase and myokinase leads to the generalized equation:

$$n\text{ATP} \longrightarrow (n-m)\text{ADP} + m\text{AMP} + (n+m)\text{Pi}.$$

The averages obtained from the experiments shown in Table 1 together with our previous work show that the actual quantities are - in μmole/gm muscle:

$$0.44 \pm 0.046\ \text{ATP} \longrightarrow 0.26 \pm 0.023\ \text{ADP} + 0.14 \pm 0.027\ \text{AMP} + 0.64 \pm 0.14\ \text{Pi}.$$

On the basis of the figures for ATP and ADP, the calculated values are 0.18 AMP and 0.62 Pi.

A few experiments with muscles which had performed two consecutive contractions were also done and the results are shown in Table 1. It is clear that these results also fit the generalized equation as follows:

$$0.65 \pm 0.045\ \text{ATP} \longrightarrow 0.27 \pm 0.051\ \text{ADP} + 0.34 \pm 0.037\ \text{AMP} + 1.20 \pm 0.17\ \text{Pi}.$$

The calculated values are 0.38 AMP and 1.03 Pi.

It is of interest that in these experiments the ADP changes remained low and that the apparent discrepancy between the relatively large change in Pi compared with the changes in the nucleotides is completely accounted for by the action of myokinase.

These results, together with those obtained previously, make it clear that in working muscle creatine phosphoryltransferase can make available the energy stored in PCr, and that myokinase also operates and can make available twice as much energy from ATP whilst preventing large increases in ADP. They also demonstrate directly and clearly that the energy comes directly from the breakdown of ATP. Much previous work (reviewed by Davies and Cain 1961) has shown that no sufficient net changes occur in other phosphorus compounds and that the concentration of Pi does not change during relaxation (Cain and Davies 1960).

Thus ATP is the primary energy source and PCr is the secondary energy source for contraction of working muscle.

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