Reçu le 15 janvier 1964.

COMMUNICATION BRÈVE

Phosphorylcreatine and ATP changes during shortening and lengthening of stimulated muscle, by G. Maréchal [1] (*Laboratoire de Physiologie Générale, Université de Louvain, Belgium*).

The energy which is given out by a stimulated muscle increases when the muscle shortens and decreases when the muscle is forcibly lengthened. Several authors have shown that the increased energy output of the shortening muscle (heat + work) is simultaneous with an increase in phosphorylcreatine (PC) hydrolysis (Mommaerts, Seraydarian and Maréchal, 1962; Carlson, Hardy and Wilkie, 1963). Abbott, Aubert and Hill (1951) have demonstrated that 40 % of the work done by stretching a muscle during a short tetanus does not appear as heat but is absorbed, and, presumably, turned into some kind of potential energy. Abbott and Aubert (1951) showed that the whole of the negative work might disappear in very slow isotonic stretches. Aubert (1956) reached the same conclusions by careful heat and work measurements, but Hill and Howarth (1959) found a complete absorption of work even during fairly rapid stretches. This report demonstrates that the work absorbed by the stretch must be accounted for by an economy in PC.

Methods

Sartorius muscles of male *R. temporaria* were left overnight in oxygenated Ringer, with 5 mM lactate then poisoned with 0.4 mM iodoacetate for 1 hour. The paired muscles were mounted in a quick freezing apparatus similar to that of Mommaerts and Schilling (1955), and connected to a pneumatic ergometer which could shorten or stretch them with a

[1] Chargé de recherches du *F. N. R. S.* (Belgium).

constant speed of 0.23 cm/sec. The muscles kept in a nitrogen atmosphere at 2°C could be stimulated one at a time with alternating condenser discharges (24/sec) given by a digital transistorized stimulator. The stimulator controlled the movements of the ergometer which began after a preset number of stimuli, usually 12. The tensions of the two muscles were measured with two strain gauges and recorded with a dual channel Sanborn electrocardiograph. Each muscle of the 156 used received one of the four following treatments : complete rest ; 6 tetanus of 3 sec given alternatively at length $l_0 - 3$ mm and $l_0 + 2$ mm at 1 min interval ; 6 tetanus of 3 sec at 1 min interval with a shortening from $l_0 + 2$ mm to $l_0 - 3$ mm beginning 0.5 sec after the first stimulus ; 6 tetanus of 3 sec at 1 min interval with a lengthening from $l_0 - 3$ mm to $l_0 + 2$ mm beginning 0.5 sec after the first stimulus. The four treatments are given at random to the muscles, according to an experimental design known as « balanced incomplete blocs ». 30 sec after the last tetanus the muscles are frozen in isopentane, cooled with liquid nitrogen, ground in stainless steel cartridges, and extracted with perchloric acid. Free creatine and total creatine are measured by the diacetyl-alpha naphtol reaction (Ennor and Stocken, 1948), and ATP by the firefly method (Strehler and Totter, 1952).

Results

Table I summarizes the results. The muscle stimulated isometrically have less PC than resting muscles and the stretched muscles, and more PC than the shortened muscles ; they have also less ATP than the resting muscles, more ATP than the shortened muscles, and as much ATP as the stretched muscles.

The excess hydrolysis of PC associated with a positive work of 33.7 ± 1.6 mcal/g is 2.17 ± 0.59 micromoles/g. The mechanochemical coefficient estimated from these figures is 15.5 kcal/mole. But the muscles have also used 0.88 ± 0.21 micromole ATP/g. If we suppose that this ATP should be added to the PC hydrolyzed, the mechanochemical coefficient for positive work becomes 11.1 kcal/mole. The regression

TABLE I

	Resting muscles	Isometric muscles	Shortened muscles	Stretched muscles	Standard error of means
Free creatine micromoles/g	7.340	21.625	23.869	19.374	0.388
PC micromoles/g	18.638	6.224	4.054	7.903	0.422
ATP micromoles/g	4.907	4.192	3.308	4.199	0.149
Tension kg/cm^2		1.866			0.059
Work positive mcal/g			33.794		1.546
Work negative mcal/g				65.222	3.138
Number of muscles	39	39	39	39	

analysis of the PC content of the shortened muscles on the work yields a slope of -0.142 ± 0.035 micromole PC/mcal, from which a mechanochemical coefficient of 7.04 kcal/mole is calculated. The change in ATP is probably connected with the low content of the PC in the shortened muscle.

The stretched muscles have more PC than the isometrically stimulated muscles : 1.68 ± 0.59 micromole/g ($t = 2.84$; $P < 0.01$) and less free creatine : -2.25 ± 0.55 micromoles/g ($t = 4.1$; $P < 0.001$). The ATP level has not changed. The saving effect of the stretch does not depend on the amount of negative work received by the muscle, as the correlation coefficient between the PC level of the stretched muscle and the negative work is —0.050, obviously not significant. The muscle cannot probably absorb negative work at the rate which is actually fed (5.4 mcal/g/ sec). Indeed, in the experiments of AUBERT and MARÉCHAL (1963), the muscles were stretched at 1.8 cm/sec, and the negative work rate was 60 mcal/g/sec : there occurs an increase in the PC split and

not a decrease as in the present experiments. This high rate of stretching was choosen in those experiments because HILL and HOWARTH (1959) had shown that the absorption of work could be complete in muscle of *Bufo bufo* stretched at a rather high speed. All our results fall in line with those of AUBERT (1956) who found that the efficiency of the work absorption increase as the rate of stretch decreases. In order to calculate the efficiency of the stretching process in our experiments, an assumption as to the size of the mechanochemical coefficient for negative work must be made. The most reasonable hypothesis is to assume that it is equal to the mechanochemical coefficient for positive work, which is taken as 11.1 /kcal/mole. Then the spared 1.7 micromole PC/g are a reserve of 19 mcal/g. This is about 30 % of the mechanical energy which has been fed into the muscle. AUBERT (1956) measures an efficiency of 20 to 40 % when comparing heat output and negative work in sartorius of *R. temporaria* stretched at a velocity equal to that used in our experiments. We conclude then that the work absorbed during slow stretches is recovered as an economy in PC.

REFERENCES

ABBOTT, B. C., AUBERT, X. and HILL, A. V. (1951). — *Proc. Roy. Soc.* (*B.*), **139**, 86-104.

ABBOTT, B. C. and AUBERT, X. (1951). — *Proc. Roy. Soc.* (*B.*), **139**, 104-117.

AUBERT, X. (1956). — *Le couplage énergétique de la contraction musculaire.* Ed. Arscia, Brussel.

AUBERT, X. and MARÉCHAL, G. (1963). — *J. de Physiol.*, **55**, 186-187.

CARLSON, F. D., HARDY, D. J. and WILKIE, D. R. (1963). — *J. gen. Physiol.*, **46**, 851-882.

ENNOR, A. H. and STOCKEN, L. A. (1948). — *Biochem. J.*, **42**, 557-563.

HILL, A. V. and HOWARTH, J. V. (1959). — *Proc. Roy. Soc.* (*B.*), **151**, 169-193.

MOMMAERTS, W. F. H. M. and SCHILLING, M. O. (1955). — *Am. J. Physiol.*, **182**, 579-584.

MOMMAERTS, W. F. H. M., SERAYDARIAN, K. and MARÉCHAL, G. (1962). — *Biochim. biophys. Acta*, **57**, 1-13.

STREHLER, B. L. and TOTTER, J. R. (1952). — *Arch. Biochem. Biophys.*, **40**, 28-41.