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VERY HIGH TENSION WITH VERY LITTLE ATP BREAKDOWN BY ACTIVE SKELETAL MUSCLE

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The tension developed and the chemical change occurring during stretching at velocities up to 2 l_0 sec^{-1} and during isometric contraction of dinitrofluorobenzene-treated and untreated frog sartorius muscle have been measured. At all velocities of stretching the tension was greater than during shortening at the same velocity and the mean rate of chemical change was less than or equal to the rates found in similar studies of shortening. For velocities 0.18, 0.33 and 0.66 l_0 sec^{-1}, the mean rate of chemical change was significantly less ($P < 0.05$) than during shortening at the same velocity. At the highest velocity of stretching the tension at l_0 was about 0.8 P_0 and the mean rate of chemical change was about the same as that for isometric contraction. At low velocity (0.1 l_0 sec^{-1}) the tension was as high as 1.4 P_0 and the mean rate of chemical change during the stretches at the three slowest velocities was significantly less ($P < 0.05$) than during isometric contraction; the lowest mean rate of chemical change amounted to only about 25% of that for isometric contraction. The fact that the chemical change was so low suggests that during stretching crossbridges can form links and develop tension without breaking down ATP.

As most people realize, the arm muscles can steadily lower a much heavier load than can be lifted or held in a constant position. It is also well known that activities which depend on the stretching of muscles are less tiring than the corresponding ones which depend on shortening. It may seem that the actions which involve stretching are easier because the muscles are working with gravity. However, the same amount of force is required for with-gravity and against-gravity tasks, if both are done at the same, constant velocity. Our experiments show that a large part of the difference between tasks requiring shortening of muscle and those requiring lengthening is due to the fact that the necessary force can be developed and maintained with less ATP breakdown during lengthening.

In some of the first and most provocative experiments on stretching of active isolated muscle, Fenn (1923, 1924) found that the net energy output, that is, the heat output minus the work done on the muscles, was lower during stretching than during contraction under isometric conditions or isotonic shortening. Fenn's results have been confirmed by more recent studies of heat production (Abbott, Aubert, and Hill, 1951; Abbott and Aubert, 1951; Hill and Howarth, 1959). These results showed that the processes responsible for the energy changes are different in some way during stretching and during contraction under other conditions. However, the heat measurements alone do not show whether these processes involve ATP or some other compound, nor do they show the nature of the change in the process. Thus several possibilities for the actual chemical basis for the low energy output during stretching have been given (Abbott *et al.*, 1951; Abbott and Aubert, 1951; Hill and Howarth, 1959; Wilkie, 1960, 1966, 1968). Perhaps the most interesting suggestion was that the mechanochemical event was reversed during stretching (Abbott *et al.*, 1951; Abbott and Aubert, 1951; Hill and Howarth, 1959); this could cause a resynthesis of ATP. However none of the measure-

Dedicated to the memory of Professor A. Katchalsky.

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ments of ATP or phosphorylcreatine, the compounds which are known to be closely involved in the contractile process, give any evidence that these compounds are formed at the expense of the work done on the muscle during stretching (Aubert and Maréchal, 1963; Infante *et al.*, 1964a; Maréchal, 1964a, b; Maréchal and Beckers-Bleukx, 1965; Wilkie, 1968; Curtin, Drobnis, Larson and Davies, 1969; Curtin, Svensson and Davies, 1970; Butler, Curtin and Davies, 1972). Although isotope experiments on both intact muscle and glycerol-extracted muscle without diffusion barriers have shown that inorganic phosphate-ATP exchange reactions do occur during stretching (Gillis and Maréchal, 1969, 1971a, b; Mannherz, 1970; Maréchal, Mommaerts and Seraydarian, 1971; Ulbrich and Ruëgg, 1971) these processes also occur during shortening and isometric contraction (Gillis and Maréchal, 1971a, b). Furthermore the amount of labeled ATP that appears during stretching is very small; it is insignificant compared to the amount of ATP breakdown that we have found under similar conditions of stretching. We therefore, conclude, as does Wilkie (1960, 1966) in reviewing the literature on heat production during stretching, that there is no compelling evidence for the actual reversal of the mechanochemical event.

The evidence, both thermal (Wilkie, 1960, 1966) and chemical (Aubert and Maréchal, 1963, Infante *et al.*, 1964a; Maréchal, 1964a, b; Maréchal and Beckers-Bleukx, 1965; Wilkie, 1968; Curtin *et al.*, 1969, 1970, Butler *et al.*, 1972) for the suppression of heat-releasing reactions by stretching is, on the other hand, good. However, in one investigation (Aubert and Maréchal, 1963) in which a rather high velocity of stretching was used, the chemical breakdown was greater than during isometric contraction. We have therefore made a systematic study of the influence of the velocity of stretching on chemical change. In view of the low ATP breakdown usually found during stretching the large forces developed (Gasser and Hill, 1924; Katz, 1939; Aubert, 1956; Abbott and Aubert, 1952; Joyce, Rack and Westbury, 1969; Joyce and Rack, 1969; Sugi, 1969, 1971, 1972; Chaplain, 1972) are striking. We have therefore designed our experiments so that a comparison of tension and ATP breakdown could be made.

MATERIAL AND METHODS

All of the experiments were done on the sartorius muscle of the frog *Rana pipiens*. Muscles were dissected on the day of the experiment and were allowed to recover at room temperature in bicarbonate buffered Ringers solution for at least an hour. Before each muscle was used it was cooled in Ringers solution at 0°C, then just before stimulation the muscle was in air at room temperature for about 15 seconds. Thermistor measurements have shown that the muscles were not above 2°C when they were stimulated (Larson, 1970). The muscles were stimulated at a frequency of 25 pulses sec^{-1} and for the experiments on stretching and shortening the velocity and extent of movement was controlled by a Levin-Wyman ergometer. The velocity of movement is expressed as l_0 sec^{-1}, where l_0 is the tendon-to-tendon length of the resting muscle *in situ* with the legs in the standard position. The tension was measured by a transducer (Grass Instruments, Model FT 0.03) firmly mounted at the end of the ergometer arm, and was recorded on a pen recorder (Beckman Instruments, Offner Type RS Dynograph). In the tension-velocity experiments each muscle was stretched and released at several different velocities. In the chemical experiments the experimental muscle received 8 stimuli before stretching began and stimulation continued until the muscle was frozen immediately after the stretching ended. Muscles that contracted under isometric conditions were frozen immediately after the last stimulation. The muscles were frozen by rapid immersion in a 1:2 mixture of dichlorodifluoromethane and chlorotrifluoromethane (Freon 12 and 13) at −160°C. The amounts of inorganic phosphate (P_i), phosphorylcreatine (PCr), total hexose monophosphate (HMP) and ATP in the muscles were measured by methods reported previously (Kushmerick and Davies, 1969). For each muscle pair, we determined the average rate of chemical change during stimulation by dividing the measured chemical change by the duration of stimulation. From the set of average rates for each type of experiment we then calculated a mean and standard error shown in the Tables and in Figure 2. The mean value of the average rate of chemical change during stimulation determined in this way will be referred to as the mean rate.

Occasionally the result from one muscle pair was very different from the others in the experiment. We excluded such a result only if it was more than two standard deviations away from the mean for the experiment (mean and standard deviation used for this test were calculated from all the results, including the one in question).

RESULTS

Tension-Velocity Relationship

The relationship shown in Figure 1 was determined by measuring the tension developed at 100% l_0 during isovelocity (constant velocity) stretches and releases of tetanized muscle. The tension is expressed as a proportion of the isometric tension of the same muscle at 100% l_0. Negligible tension was developed in control experiments on unstimulated muscles. The distance stretched or shortened was constant within a series of experiments on a particular muscle and was at least 15% l_0 or 200 nm per half sarcomere. This distance is much greater than the 10 nm distance believed to be traversed by a crossbridge in a single cycle of operation (Huxley, 1960; Davies, 1971).

The highest tension (about 1.4 P_0) was produced at a velocity of stretching of about 0.2 l_0 sec $^{-1}$, and then at higher velocities less tension was developed. This decrease in the tension was not due to fatigue or damage of the muscle because: (1) the $P_{0\,final}$, which was measured at the end of the experiment, was within 7% of the $P_{0\,initial}$, which was measured at the beginning of the experiment; and (2) muscles that had been stretched at high velocities (about 1.0 l_0 sec^{-1}) and had developed tensions of about 1.0 P_0 could subsequently develop higher tensions (1.4 P_0) when they were stretched at a lower velocity (about 0.2 l_0 sec^{-1}).

The tension-velocity experiments by Katz (1939) and Aubert (1956) showed that, although their results for shortening fit the curve calculated from the Hill (1938) equation, the equation did not correctly predict the results for stretching. At low velocities of stretching they found that more tension was developed than was predicted by the equation. Our results agree with these and similar experiments on isovelocity movement (Gasser and Hill, 1924; Abbott and Aubert, 1952; Aubert, 1956; Joyce, Rack and Westbury, 1969; Sugi, 1969, 1971, 1972; Chaplain, 1972) in showing that more tension is developed during stretching than during shortening at the same speed. Although the curve in Figure 1 is similar to the tension-velocity curves of Katz (1939), Aubert (1956) and Chaplain (1972), the exact shape of the curve is different from those given by Joyce and Rack (1969) for isotonic stretch and by Joyce *et al.* (1969) for isovelocity stretch. During a single isotonic stretch the velocity changes and the shape of the tension-velocity curve depends on which velocity is measured. Joyce and Rack (1969) measured the very early fast velocity; Katz

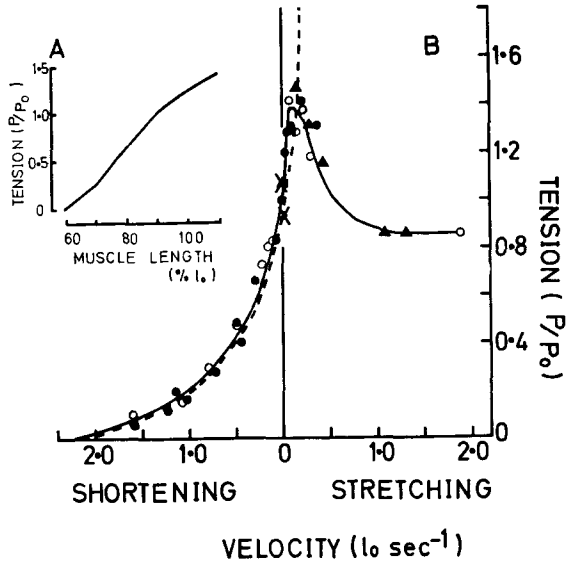

FIGURE 1 Tension—Velocity Relationship for Normal, Oxygenated Frog Sartorius Muscle.

The muscle was kept under isometric conditions, or was allowed to shorten, or was stretched at a constant velocity as it was being stimulated at 25 pulses sec^{-1}.

A. A typical record of the tension produced during stretching from 60% l_0 to 110% l_0 at a velocity of 0.3 l_0 sec^{-1}. Similar records were obtained during stretching at the other velocities.

B. Each symbol (●, ○, ▲) represents the results for a single muscle.

The tension produced at 100% l_0 during movement or during isometric contraction is expressed as $P/P_{0\,initial}$. $P_{0\,initial}$ is the tension produced during an isometric contraction at 100% l_0 at the beginning of the experiment. The $P_{0\,final}$, the tension produced during an isometric contraction at the end of the experiment, is shown as ⊠ ($P/P_{0\,initial}$ = 0.93) for muscle ○, and as ⧓ ($P/P_{0\,initial}$ = 1.07) for muscle ▲.

muscle	l_0 cm	wet weight g	$P_{0\,initial}$ l_0/wet weight N cm g^{-1}
●	2.70	—	—
○	2.50	0.070	20.3
▲	2.90	0.095	17.6

$P_{0\,initial}$ l_0/wet weight is roughly equivalent to the isometric tension developed per cm^2 of cross sectional area.

The solid line is the observed relationship of tension and the velocity. The broken line was calculated from the Hill (1938) equation

$$v/l_0 = b/l_0 \frac{(1 - P/P_0)}{(P/P_0 + a/P_0)}$$

Where v/l_0 is the velocity in units of l_0 sec^{-1}, P is the observed tension and P_0 is the maximum tension under isometric conditions. The values used for the ratios, a/P_0 and b/l_0 were 0.25 and 0.56 sec^{-1}, respectively; they were determined by the graphical method of Katz (1939).

(1939), in a similar study of isotonic stretch, measured the later slower velocity and his curve is quite similar to our Figure 1. In the isovelocity experiment by Joyce *et al.* (1969) the peak in the tension-velocity curve may have been missed because the velocities of stretching were not slow enough.

Chemical Changes

Some of the chemical experiments (Table I) were done on oxygenated muscles. Net breakdown of ATP (1) does not occur under these conditions because ATP is rapidly rephosphorylated by reacting with phosphorylcreatine (PCr) in the Lohmann reaction (2).

(1) $ATP \longrightarrow ADP + P_i$
(2) $ADP + PCr \longrightarrow ATP + Cr$

Under these conditions there is a net breakdown of PCr and net increase in inorganic phosphate (P_i) (Table I B). It is possible for P_i to be used to form HMP's (Kushmerick and Davies, 1969), but this did not occur in our experiment (Table I B). We have, therefore, taken the measured increase in P_i to be equivalent to the amount of ATP used in the untreated muscles. However if the muscles are pretreated with 2,4-dinitrofluorobenzene (DNFB) (Cain and Davies, 1962) as in the experiments in Table II, the Lohmann reaction is prevented. Under these conditions there is no change in the PCr level in the muscle, but there is a net change in ATP and an equivalent amount of P_i is formed (Infante and Davies, 1965). Therefore, in all of these experiments we have measured the amount of P_i formed during contraction, since it gives a valid measure of the chemical change in both untreated and treated muscles.

For the experiments shown in Table I the experimental muscles were tetanized under the specified conditions and the control muscles were not stimulated. The chemical change measured by comparing each experimental muscle with its "resting" control is referred to as the "total chemical change" because it represents the ATP breakdown by the contractile elements, the ATP-ase of the sarcoplasmic reticulum which pumps Ca^{+2} and other reactions. In set A each experimental muscle was stimulated as it was held under isometric conditions at 100% l_0. In set B each experimental muscle was stimulated and was stretched at a constant velocity over a distance of 1.2 cm from 60% l_0 to 100% l_0, which is equivalent to a movement of 500 nm per half sarcomere.

For the experiments shown in Table II the experimental muscle of each pair was stimulated as

TABLE I

Mean rates of chemical change during contraction of untreated muscles

A. Isometric contraction Design Experimental—control	Duration of tetanus sec	n	Compound measured	Mean rate of total chemical change μmole g^{-1} sec^{-1}
Isometric—resting control	1.0	8	Pi	$+1.05 \pm 0.08$

B. Stretching Design Experimental—control	Velocity of stretching l_0 sec^{-1}	Duration of tetanus sec	n	Compound measured	Mean rate of total chemical change μmole $g^{-1}sec^{-1}$
Stretching—resting control	0.08	4.8	16	Pi	$+0.37 \pm 0.05$
			15	PCr	-0.35 ± 0.19
			10	HMP	$+0.001 \pm 0.017$
Stretching—resting control	0.66	0.6	10	Pi	$+0.56 \pm 0.16$

Normal oxygenated frog sartorius muscles at 2°C or less (see text) were used. The control muscles remained unstimulated (resting) and the experimental muscles were stimulated at a frequency of 25 pulses sec^{-1}. In A the experimental muscles performed an isometric contraction for 1 sec and in B they were stimulated as they were stretched from 60% l_0 to 100% l_0 at the velocities listed in the Table. The experimental muscles were frozen before relaxation. The mean rates of chemical change (experimental—control) are listed and followed by $\pm$ one standard error. P_i is inorganic phosphate, PCr is phosphorylcreatine and HMP is total hexose monophosphates. The number of pairs of muscles is listed under n. The mean l_0 was 3.06 ± 0.12 cm, range 2.60 to 3.80 cm; the mean wet weight was 0.81 ± 0.008 g, range 0.027 to 0.143 g.

TABLE II

Mean rates of chemical change during contraction of DNFB-treated muscles

A. "Stimulated controls" Design	Duration of tetanus sec	n		Mean rate of chemical change μmole g^{-1} sec^{-1}
Stimulated—resting	2.0	19		$+0.10 \pm 0.03$
B. Isometric contraction				
Design Experimental—control	Duration of tetanus sec	n	Mean rate of total chemical change μmole $g^{-1}sec^{-1}$	Mean rate of net chemical change μmole g^{-1} sec^{-1}
Isometric—resting control	1.0	7	$+0.72 \pm 0.09$	—
Isometric—stimulated control	1.0	10	—	$+0.64 \pm 0.13$
C. Stretching				
Design Experimental—control	Velocity of stretching l_0 sec^{-1}	Duration of tetanus sec	n	Mean rate of net chemical change μmole g^{-1} sec^{-1}
Stretching—stimulated control	0.13	3.0	11	$+0.08 \pm 0.03$
,, ,,	0.18	2.4	10	$+0.08 \pm 0.03$
,, ,,	0.33	1.2	7	$+0.12 \pm 0.14$
,, ,,	0.66	0.6	18	$+0.39 \pm 0.16$
,, ,,	1.33	0.3	10	$+0.35 \pm 0.17$
,, ,,	2.00	0.2	18	$+0.74 \pm 0.35$

DNFB-treated frog sartorius muscles at 2°C or less (see text) were used. The number of pairs of muscles is listed under n. The mean rate of chemical change is followed by ± one standard error of the mean; Pi is inorganic phosphate. The mean l_0 for the muscles was 3.05 ± 0.14 cm, range 2.55 to 3.75 cm; the mean wet weight was 0.083 ± 0.011 g, range 0.032 to 0.149 g.

A. Each experimental muscle was held at 60% l_0 and was stimulated at 25 pulses sec^{-1} for 2 sec. Each control muscle remained unstimulated (resting control).

B. Each experimental muscle was stimulated for 1 sec at 25 pulses sec^{-1} under isometric conditions at 100% l_0. The control muscle was either unstimulated (resting control), or it was stimulated under conditions of zero external load (stimulated control) as described in the text.

C. Each experimental muscle was stimulated as it was being stretched from 60% l_0 to 100% l_0 at the velocity listed in the Table. All of the control muscles were stimulated under conditions of zero external load (stimulated control) as described in the text.

it was being held under isometric conditions, or was being stretched. In some of the experiments, there were "resting controls", that is, the control muscles were not stimulated. Thus the total chemical change was found simply by comparing the experimental muscle with the control muscle. In the other experiments, the control muscles, noted as "stimulated controls", were tetanized for the same period of time as the corresponding experimental muscles. They were allowed to shorten under zero external load and remained at their fully shortened length (about 60% l_0) until they were frozen. They did not develop any externally detectable tension. The "net chemical change" listed in Table II was found by comparing the experimental muscle with the "stimulated control".

The ATP breakdown under the conditions used for the "stimulated controls" is not significantly different from the amount used when the muscle has been extended to the length at which the thick and thin filaments do not overlap (Infante, Klaupiks and Davies, 1964b, c; Sandberg and Carlson, 1966; T. M. Butler, personal communication). We, therefore, intended to use the stimulated controls to give a measure of the ATP breakdown for processes, such as Ca^{+2} pumping, which are not directly involved in the interaction of myosin and actin, the contractile event. However, in light of the finding of Taylor and Rüdel (1970) that in single fibres the entire fibre is not active when it is fully shortened, it now seems better to calculate the ATP breakdown for noncontractile processes in another way which will be described in the discussion. Nevertheless our conclusions are the

same regardless of which method is used for estimating the ATP breakdown for noncontractile processes.

The absolute amount of chemical change in the "stimulated control" muscles was 0.10 ± 0.03 μmol $g^{-1}sec^{-1}$ (Table II A) and this mean rate is constant for brief periods of stimulation (Table II A and B). It was, therefore, used to calculate the mean rate of total ATP breakdown from the "net" values measured with the "stimulated controls". The resulting values for the mean rates of total ATP breakdown by DNFB-treated muscles during stretching are listed in Table III B.

TABLE III

Rates of total ATP breakdown by DNFB-treated muscles during stretching and shortening

Velocity l_0 sec^{-1}	n	Mean rate of total chemical change μmole g^{-1} sec^{-1}
A. Isometric contraction		
0	7	$+0.72 \pm 0.09$
B. Stretching		
0.13	11	$+0.18 \pm 0.042$
0.18	10	$+0.18 \pm 0.042$
0.33	7	$+0.22 \pm 0.143$
0.66	18	$+0.49 \pm 0.163$
1.33	10	$+0.45 \pm 0.173$
2.00	18	$+0.84 \pm 0.351$
C. Shortening		
0.20	10	$+0.69 \pm 0.035$
0.33	13	$+0.75 \pm 0.072$
0.66	16	$+1.08 \pm 0.105$
0.91	9	$+1.41 \pm 0.099$
1.33	9	$+0.73 \pm 0.065$
2.10	10	$+0.94 \pm 0.149$

A. The mean rate of total ATP breakdown during isometric contraction at 100% l_0. See Table II B.

B. The mean rates of total ATP breakdown calculated from the results given in Table II C by adding the result from Table II A. See text.

C. The mean rates of total ATP breakdown during isovelocity shortening from Kushmerick and Davies (1969), Table 6, p. 344 of their paper. The experimental muscles shortened from 130% l_0 to 60% l_0 as they were stimulated at 25 pulses sec^{-1}. The control muscles were not stimulated.

We have included in Table III C and in Figure 2 the results of similar chemical experiments by Kushmerick and Davies (1969) on isovelocity shortening of DNFB-treated muscles. Comparison between their results and our own is valid since measurements of isometric contraction are common to both and are in agreement ($\Delta P_i = +0.65 \pm 0.23$ μmol $g^{-1}sec^{-1}$, Kushmerick and Davies, Fig. 5; $\Delta P_i = +0.72 \pm 0.09$ μmol $g^{-1}sec^{-1}$, Table II B of this paper).

DISCUSSION

Figure 1 shows that tensions even greater than P_0 were developed during slow stretches. In terms of the crossbridge theories this means that if every crossbridge is linked during isometric contraction then more tension must be exhibited by each linked bridge during stretching than during isometric contraction.

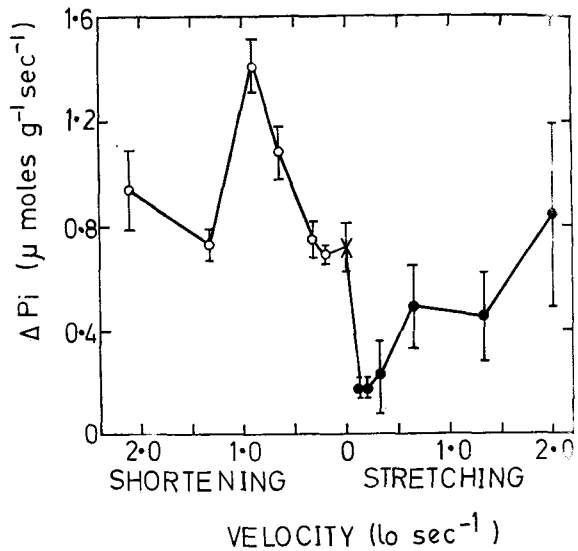

FIGURE 2 The mean rates of total ATP breakdown by DNFB-treated frog sartorius muscles during shortening (○) and stretching (●) at various velocities and during contraction under isometric conditions (×). The mean rates of ATP breakdown during isovelocity shortening are from Kushmerick and Davies (1969). The bars represent ± one standard error. See Table III.

Comparison of the results of the mechanical (Figure 1) and chemical (Table I B and Figure 2) experiments shows an important difference between stretching and shortening, which may be related to crossbridge operation. Even though the amount of tension developed was always greater during stretching than during shortening, the mean rate of breakdown of ATP during stretching was in every case lower than during shortening at that velocity. This difference was significant ($P < 0.05$) for velocities 0.18, 0.33 and 0.66 l_0 sec^{-1}.

An interesting feature of the results for slow stretches is that the rates of chemical breakdown are so low; this raises the question of whether the ATP breakdown might be due completely to the tension-independent processes. Smith (1972) and Homsher, Mommaerts, Ricchiuti and Wallner

(1972) have described the total heat output and the total chemical change during isometric contractions in terms of two components. One component is proportional to the tension developed by the contractile elements and the other component is independent of the tension and amounts to about 25% of the total change during contraction under isometric conditions at 100% l_0. This agrees with the chemical experiments of Infante *et al.* (1964b) and Sandberg and Carlson (1966); from the relationships they found the chemical change in a muscle in which there is no overlap of the filaments would be, respectively, 31% and 27% of the change at 100% l_0. We chose to use the value of 25% because it gives the smallest value for the tension-independent ATP breakdown and thus will provide the most extreme test of our hypothesis that all the ATP breakdown during slow stretches is due to tension-independent processes. Assuming that the figure of 25% also applies to DNFB-treated muscles used in these experiments, the tension-independent ATP breakdown would amount to 0.18 μmol $g^{-1}sec^{-1}$ (calculated from the first experiment in Table II B). Assuming that the rate of the tension-independent component is the same during stretching as during isometric contraction, and that the rate of the tension-dependent component is different, the results in Table III B show that for the three lowest velocities of stretching the rate of total ATP breakdown is not significantly greater than the tension-independent ATP breakdown. In other words, there does not seem to be any ATP breakdown associated with the very large amount of tension that is developed during these slow stretches. This raises strongly the possibility that during stretching the crossbridges can form links and develop tension without breaking down ATP.

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