Chemical Energetics of Force Development, Force Maintenance, and Relaxation in Mammalian Smooth Muscle

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ABSTRACT   High-energy phosphate utilization ($\Delta\sim$P) associated with force development, force maintenance, and relaxation has been determined during single isometric tetani in the rabbit taenia coli. ATP resynthesis from glycolysis and respiration was stopped without deleterious effects on the muscle. At 18°C and a muscle length of 95% l_0, the resting rate of energy utilization is 1.8 ± 0.2 nmol/g·s^{-1}, or 0.85 ± 0.2 mmol $\sim$P/mol of total creatine (C_t)·s^{-1}, where $C_t = 2.7$ μmol/g wet wt. During the initial 25 s of stimulation when force is developed, the average rate of $\Delta\sim$P was -8.2 ± 0.8 mmol/mol C_t·s^{-1}, some four times greater than during the subsequent 35 s of force maintenance, when the rate was -2.0 ± 0.6 mmol $\sim$P/mol C_t·s^{-1}. The energy cost of force redevelopment (0 to 95% P_0) after a quick release from the peak of a tetanus is very low compared with the initial force development. Therefore, the high rate of energy utilization during force development is not due only to internal work done against the series elasticity nor to any high rate of cross-bridge cycling inherently associated with force development. The high economy of force maintenance compared with other muscle types is undoubtedly due to a slower cross-bridge cycle time. The energy utilization during 45 s of relaxation was not statistically significant, and $\int Pdt/\Delta\sim$P was higher during relaxation than during force maintenance in the stimulated muscle.

INTRODUCTION

We have recently described a mammalian smooth muscle preparation that is suitable for direct measurement of high-energy phosphate utilization during contraction (Butler et al., 1978). The treatment used stops ATP synthesis from both oxidative phosphorylation and glycolysis but does not change the high-energy phosphate contents or the mechanical responses of the muscle. Thus, we were able to measure the total high-energy phosphate utilization by the stimulated muscle from net changes in the contents of phosphorylcreatine (PCr) and adenine nucleotides.

We use this preparation to determine the chemical energy usage during

various phases of an isometric tetanic contraction. The energy requirements were determined for the periods of force development, maximum force maintenance, and relaxation, as well as during force redevelopment after a quick release from the plateau of an isometric tetanus. This information could not be satisfactorily obtained through the exclusive use of indirect measures of ATP utilization, such as total oxygen consumption and lactate production. These methods fail to provide a good time-resolved record of ATP utilization, for they depend on either steady-state measurements, which demand that force and/or the rate of energy utilization be constant during the period of observation, or measurements of total change during a complete contraction-relaxation cycle. From the knowledge of the energy inputs required for various mechanical situations, insight may be gained into the mechanism through which chemical energy utilization leads to the appropriate mechanical output by the muscle. Similarities in the relationships between energy utilization and resulting mechanical responses among functionally different smooth and striated muscles would suggest the operation of certain common basic mechanisms, whereas drastic differences between these relationships would be expected to reflect biochemical and/or structural specializations.

We have found from direct measurement of high-energy phosphate utilization that the chemical energy requirement for initial development of isometric force (25 s) is some four times greater than that for subsequent force maintenance (35 s). The energy cost of force redevelopment after a quick release from the peak of a tetanus is significantly lower than that required during initial force development. The energy utilization during relaxation was not statistically significant, although $\int Pdt/\Delta\sim P$ was higher during relaxation than during maximum isometric force maintenance in a stimulated muscle.

METHODS

1. Preparation of Tissues

Strips of taenia coli were excised from immature female rabbits (New Zealand strain, white) weighing 1.5–2.5 kg, which had been sacrificed by cervical fracture. The method of isolation of the taenia coli from adhering connective tissue and circular muscle of the caecum has been described (Gordon and Siegman, 1971). Each muscle was divided transversely into three segments, each 1.5 cm long and weighing ~15 mg.

The muscles were mounted in Plexiglas chambers containing flowing, oxygenated Krebs-bicarbonate solution and were allowed to equilibrate for at least 2 h under 2 × 10^{-2} newton (N) at 21°C. When cooled to 18°–22°C, the muscles are atonic (Gordon and Siegman, 1971). One end of the preparation was connected to an isometric force transducer (Kistler-Morse Corp., Bellevue, Wash., DSK-3), and the other end was attached to a micrometer drive or to a servomotor (Gould, Inc. Instruments Div., Cleveland Ohio, model 440) to impose changes in preparation length. Recordings were made on a Grass model 7 (Grass Instrument Co., Quincy, Mass.) or Brush model 440 (Gould, Inc.) polygraph.The muscles were supramaximally stimulated in a transverse field with platinum-plantinum chloride electrodes and 10 V rms, 60-Hz AC pulses.

2. Solutions

a) normal Krebs' solution (mM) NaCl, 118; KCl, 4.7; MgSO₄·7H₂O, 1.18; KH₂PO₄,1.2; CaCl₂, 1.9; NaHCO₃, 25.01; and glucose, 11.0. The solution was bubbled with a mixture of 95% O₂–5% CO₂ and the pH was 7.4.

b) FAA Krebs' solution Similar to normal Krebs' solution but containing 5.0 mM sodium fluoroacetate (FAA, ICN Pharmaceuticals Inc., Plainview, N. Y.). The solution was bubbled with a mixture of 95% N₂–5% CO₂ and the pH was 7.4.

c) IAA-FAA Krebs' solution Similar to normal Krebs' solution but without glucose and containing 0.5 mM sodium iodoacetate (IAA, Eastman Organic Chemicals Div., Eastman Kodak Co., Rochester, N. Y.) and 5.0 mM sodium fluoroacetate. The solution was bubbled with a mixture of 95% N₂–5% CO₂ and the pH was 7.4.

3. Freezing and Extraction

To freeze the muscles, the bath was rapidly drained (1 s) and the muscle was clamped between two stainless steel plates precooled to −190°C with liquid nitrogen. Extraction with 0.5 N HClO₄ was performed as described previously (Kushmerick et al., 1969). The extracts were neutralized with 0.57 M KOH buffered to pH 7.4 with 0.2 M triethanolamine and stored at −76°C until analysis. Extracts used for analyses of lactate were not neutralized or buffered.

4. Chemical Analyses

Free creatine (C_f) was assayed by the method of Eggleton et al. (1943), and total creatine (C_t) was similarly assayed after hydrolysis with 0.1 N HCl for 9 min at 65°C. Phosphorylcreatine (PCr) was estimated as the difference between total and free creatine corrected for 10.8% creatinine formation during acid hydrolysis. ATP, ADP, and AMP were assayed by liquid chromatography (Butler et al., 1978) or fluorometric analyses (Butler, 1974). Lactate was assayed fluorometrically acording to the method of Hohorst (1963). Values for chemical contents are expressed on the basis of total creatine, thereby taking into account any differences in muscle mass. The total creatine content of the taenia coli is 2.7 μmol/g wet tissue (Butler et al., 1978). ~P refers to high-energy phosphate and is calculated as indicated in Results.

5. Histology

Taenia coli muscles were divided transversely into four segments, prepared and treated as described in section 1, and then stretched to 1.45 times their slack length (l_s).

After equilibration at that length for 15 min, one segment from each taenia was placed in IAA-FAA Krebs' solution gassed with 95% N₂–5% CO₂ at 5°C for 30 min. The muscle was then rapidly rewarmed to 18°C in the same solution for 3.5 min, then fixed in a glutaraldehyde solution for 2 h as described below. Another segment from each taenia remained in normal Krebs' solution, but at 18°C, for the corresponding period of 33.5 min, and then was fixed in glutaraldehyde for 2 h. Remaining muscle segments were incubated in normal Krebs' solution at 18°C and then placed in the glutaraldehyde fixative for 5, 10, 20, 60, or 120 min, after which they were quickly frozen in liquid nitrogen, extracted, and analyzed for ATP.

The tissues were fixed with 2% glutaraldehyde in 0.1 M sodium cacodylate containing 6% sucrose at pH 7.4. They were postfixed in 2% osmium tetroxide in cacodylate buffer, rinsed with buffer, and stained with uranyl acetate. The tissues

were then dehydrated in graded alcohols and embedded in Spurr's resin (Ashton et al., 1975). Thin sections were stained with lead acetate and examined for ultrastructural detail with a Hitachi HU 11E (Hitachi Ltd., Tokyo, Japan) or Zeiss EM9 (Carl Zeiss, Inc., New York) electron microscope.

6. Myosin Heavy Chain Determinations

Sartorius muscles were isolated from northern frogs (*Rana pipiens*) and equilibrated for 1 h or more in a normal Ringer's solution gassed with 95% O_2–5% CO_2. The muscles were weighed and quick frozen between stainless steel blocks precooled with liquid nitrogen. The rabbit taenia coli muscles were dissected as usual, equilibrated for at least 1 h, and frozen.

Relative myosin heavy chain contents were determined by a modification of a method described by Cohen and Murphy (1978). The tissues were pulverized while frozen and extracted with a solution containing 1% sodium dodecyl sulfate, 1% mercaptoethanol, and 25 mM sodium phosphate, pH 7.0. The sartorius muscles were extracted using a concentration of 7.5 mg wet wt/ml, and the taenia coli muscles were adjusted to 15 mg wet wt/ml. The samples were incubated for 1 h at 60°C and subsequently cooled and diluted from 2 to 20 times with the same extraction solution. 0.2 ml of glycerol and 0.1 ml of 0.05% bromophenol blue was then added to 1 ml of diluted sample.

The gels were prepared according to Weber and Osborn (1969) with a 4.5% acrylamide concentration. A 10-μl sample containing from 4 to 60 μg of tissue (wet wt) was added to each gel. The electrophoresis was carried out at 11 mA/gel for ~2.75 h. The gels were stained with Coomassie Blue (0.25% wt/vol) in 45% methanol, 9.2% acetic acid for 1 h at 60°C, and then destained by diffusion at 60°C with 7.5% acetic acid, 5% methanol. The gels were scanned at 570 nm on a Beckman model 25 spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.), and areas of the 200,000-dalton peaks were determined. In every run, the relationship between the peak area and tissue wet weights added to the gels was determined using at least four different samples for both rabbit taenia coli and frog sartorius. The ratio of the slopes relating peak area and wet weight was taken as the measure of the relative myosin heavy chain content in the two tissues. Myosin was purified from rabbit taenia coli and frog sartorius according to the methods of Chacko et al. (1977) and Barany (1967), respectively. Equal quantities of myosin from each muscle were added to separate gels, and the areas of the myosin heavy chain peaks determined as described above. The ratio of the areas of the peaks for taenia coli:frog sartorius was 0.99 ± 0.05 ($n = 8$). Therefore, any differences in the slopes relating peak area and wet weight for the two tissues would not reflect differences in staining but, rather, differences in myosin heavy chain content.

7. Statistics

All values are means ± standard error, and n = number of observations. Comparisons were made using Student's t test.

RESULTS

1. Relative Myosin Heavy Chain Contents in the Frog Sartorius and Rabbit Taenia Coli

The energetics of contraction in the frog sartorius has been studied in detail and it was of interest to determine to what extent the differences in the

energetics of the frog sartorius and rabbit taenia coli could be attributed to differences in myosin content.

Fig. 1 shows the relationship between area of the myosin heavy chain peak and equivalent tissue wet weight added to each gel for one electrophoresis run. The ratio of the slopes in six such paired analyses showed that the frog sartorius muscle contained 3.3 ± 0.2 times more myosin heavy chain per gram wet weight than the rabbit taenia coli.
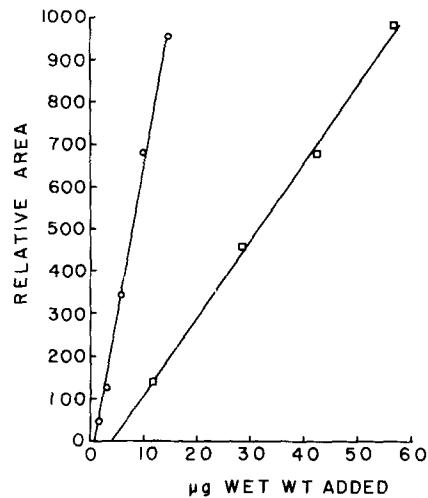

Figure 1. Relationship between the area under the 200,000-dalton peak of densitometer tracing and tissue wet wt applied per gel from a typical SDS gel electrophoresis run. Aliquots of whole-muscle extracts of frog sartorius (O) or rabbit taenia coli (□) were applied to each gel. The ratio of the slopes of the two curves is used to calculate the relative myosin content of the muscles. Slope sartorius/slope taenia coli = 3.3 ± 0.2 ($n = 6$).

2. Histology

Although previous mechanical and chemical determinations indicated that treatment with metabolic inhibitors and lowered temperatures had no adverse effect on the muscles (Butler et al., 1978), it was of importance to learn whether there were any changes in ultrastructure, particularly filament organization. For this reason, tissues subjected to the treatment with metabolic inhibitors described above were prepared for electronmicroscopy, along with paired control segments that were bathed in normal Krebs' solution. The results are shown in Figs. 2 and 3.

Careful examination of multiple random sections from eight muscle segments revealed that there were no conspicuous differences between the treated and untreated preparations. Low magnification electron micrographs of representative populations of cells show that the tissues did not swell as a result of the treatment (Figs. 2 *A* and 3 *A*). Filament organization, best seen in high magnification, was the same in both groups, with thick (myosin) and thin
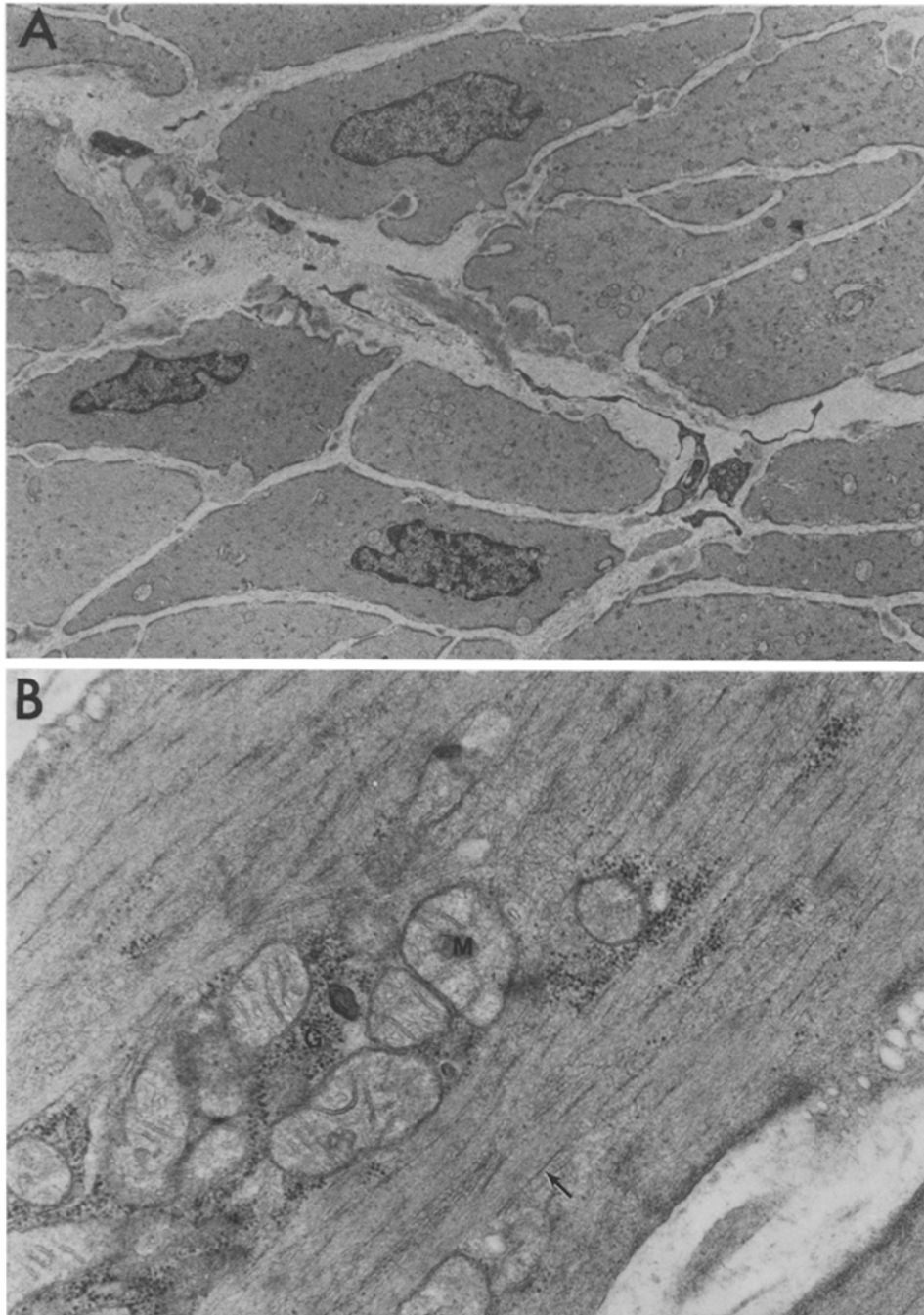
FIGURE 2. Rabbit taenia coli, bathed in Krebs' solution at 18°C under aerobic, isometric conditions at the length of 0.95 l_o (1.45 × slack length). (A) Transverse section, × 7,600. (B) Longitudinal section, × 38,000. Glycogen granules (G), intact mitochondria (M), and surface vesicles are evident. Longitudinally oriented thick (myosin, arrow) and thin filaments can be seen within the cytoplasmic matrix.
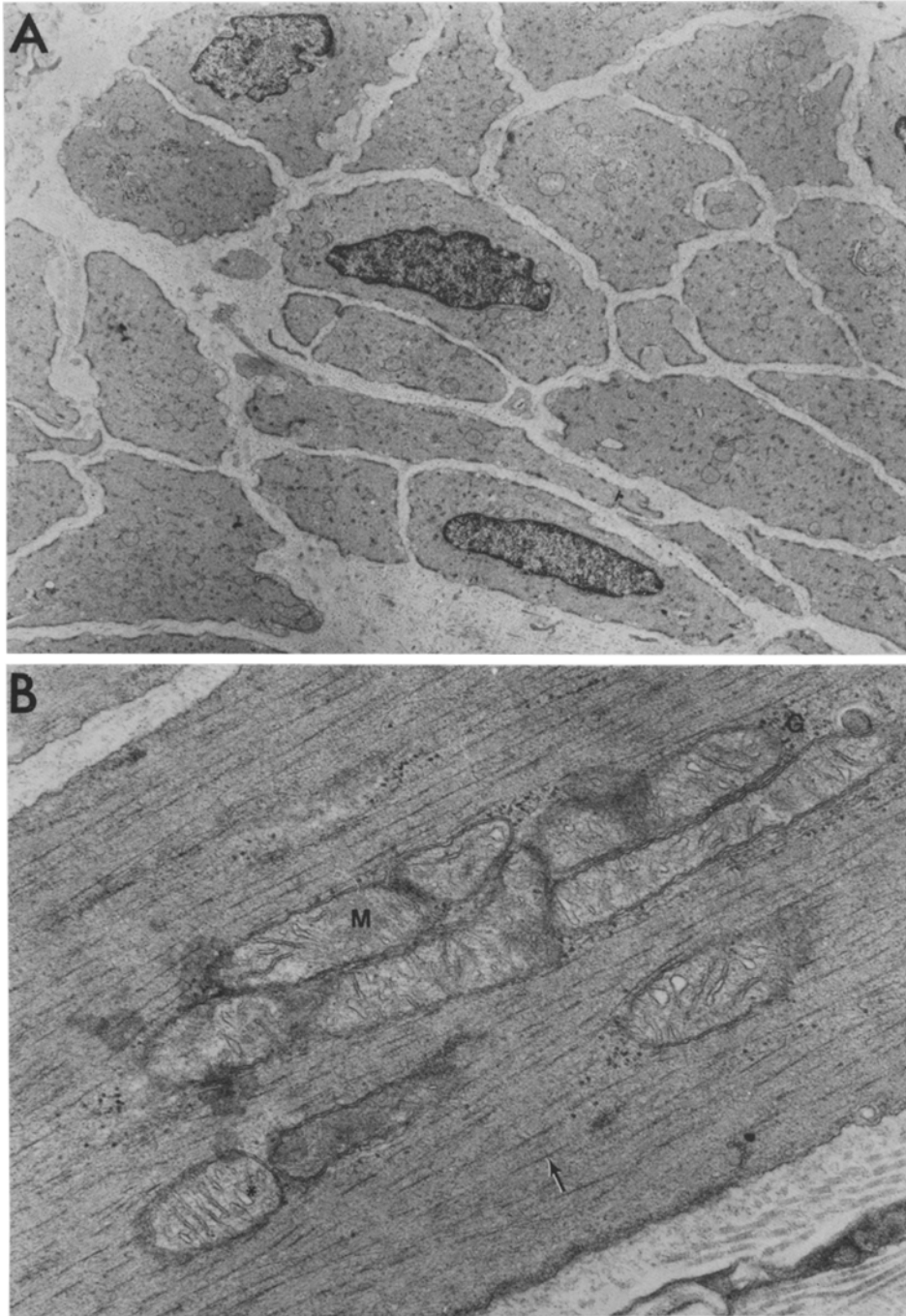
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FIGURE 3. Rabbit taenia coli, bathed in 0.5 mM IAA–5.0 mM FAA, glucose-free Krebs' solution at 5°C under anaerobic conditions for 30 min and then rewarmed to 18°C for 3.5 min. Muscle held under isometric conditions at 0.95 l_0 (1.45 slack length). (A) Transverse section, × 7,600. (B) Longitudinal section, × 38,000. Longitudinally oriented thick (myosin, arrow) and thin myofilaments can be seen within the cytoplasmic matrix. Glycogen granules (G) are evident. Mitochondria (M) are intact, but some evidence of swelling of cristae is noted (*).

(actin) filaments running parallel to the longitudinal axes of the cells; there was no evidence of filament aggregation (Figs. 2 *B* and 3 *B*). The only remarkable, but not unexpected, change was slight swelling of mitochondrial cristae with some dissolution in the metabolically inhibited tissues (Fig. 3 *B*). The average ATP content of tissues fixed in glutaraldehyde for 2 h was 0.95 ± 0.09 μmol/g wet wt ($n = 5$), whereas unfixed tissues contained 1.52 ± 0.05 μmol ATP/g (Butler et al., 1976). Although this represents a significant loss of ATP (which occurred within the first 5 min of fixation), both the ATP contents and the magnitude of the change in ATP are similar to those observed in contracting unfixed muscles (to be described; Table I). Importantly, the ATP content of fixed tissues is far greater than that of muscles in the rigor state ($0.07 ± 0.02$ μmol/g, $n = 7$, Butler et al., 1976). Therefore, the structural similarity of the control and metabolically inhibited tissues cannot be attributed to a loss of ATP before or during fixation. Rather, their normal appearance is consistent with evidence from mechanical and biochemical studies (Butler et al. [1978] and this study), showing that the specific treatment with the metabolic inhibitors employed had no untoward effects on the tissues.

3. Energetics Studies

a) EXPERIMENTAL PROCEDURES   After the initial equilibration period at 2×10^{-2} N, the muscle length was reduced for the determination of l_s, the length at which passive tension was just zero. Unless otherwise stated, the muscle was then stretched to the length $1.45 \times l_s$, which is 95% l_0 (l_0 is the length at which maximum active force, P_0 is developed), where active force is 92% P_0 (Butler et al., 1978). A period of 15 min was allowed for equilibration at the new length, during which time stress relaxation occurred and tension stabilized.

The bathing medium (normal Krebs' solution) was then replaced by IAA-FAA Krebs' solution at 5°C, and treatment with the metabolic inhibitors was continued, under anaerobic conditions, for 30 min. The cold medium was then rapidly replaced by the same solution at 18°C, and treatment continued for an additional 3.5 min before further experimental maneuvers. The rationale for this regimen, which blocks oxidative metabolism and glycolysis with no diminution of contractility, has been discussed in detail (Butler et al., 1978).

Our purpose in this study was to distinguish the energy utilization association with force development, maximum force maintenance, and relaxation during an isometric tetanus, taking into account resting metabolism. Therefore, in a given experiment, the three muscle segments derived from each taenia coli were distributed and used according to the procedures listed in the legend to Fig. 4, as specifically required and as described in subsequent sections.

At the end of each of the procedures, the muscles were quickly frozen. The constancy of the adenine nucleotide, phosphorylcreatine contents, and mechanical behavior along the length of the taenia coli made it possible to subject the segments to different experimental procedures and to compare the results obtained (Butler et al., 1978).

b) RESTING ENERGY UTILIZATION   The resting rate of oxygen consumption of smooth muscle is as much as one-quarter to one-half that of the steady-state rate during maximum isometric force maintenance (Paul and Peterson, 1975; Glück and Paul, 1977; Hellstrand, 1977). For this reason, estimates of the energy utilization associated with the various phases of an isometric tetanus required correction for energy utilization by the resting, unstimulated muscle. In the determination of the energy utilization during force development, such a correction was automatically made by comparison of the PCr and ATP contents of the stimulated muscle with that of a paired muscle that
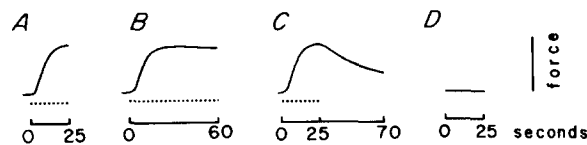
FIGURE 4.   Experimental procedures applied to segments of rabbit taenia coli under isometric conditions at 18°C after treatment with metabolic inhibitors, as described in text. (*A*) Stimulation for 25 s. (*B*) Stimulation for 60 s. (*C*) Stimulation for 25 s followed by 45 s of relaxation. (*D*) Unstimulated control. The duration of suprathreshold 60-Hz AC stimulus is shown by the dotted lines. Muscles were quickly frozen upon termination of each procedure. From differences in muscle PCr and ATP contents and correction for resting metabolism, the chemical energy utilization during force development (*A—D*), maximum force maintenance (*B—A*) and relaxation (*C—A*) can be calculated, as described in text.

was unstimulated but otherwise identically treated (procedure *A*—procedure *D*). But a comparable correction was not practical for determining the net energy utilization during maximum force maintenance and relaxation. This would have required the use of four muscle segments from each taenia coli, providing an unstimulated control appropriate for each of the different stimulus durations. The availability of only three segments of adequate size from each taenia coli required that the necessary comparisons be made among segments derived from different muscles or, as an alternative, an independent determination of the rate of resting energy utilization.

A direct measurement of the high-energy phosphate utilization during rest was obtained from paired muscles that had been treated with metabolic inhibitors as described in Methods. One muscle of each pair was frozen 3.5 min after rewarming to 18°C, whereas the other was incubated an additional 70 s and frozen. The change in high-energy phosphate content (ΔATP/C_t + ΔPCr/C_t) was -0.022 ± 0.028 mol/mol C_t ($n = 19$), which is a rate of -0.3 ± 0.4 mmol/mol $C_t \cdot s^{-1}$. This rate of resting energy utilization is small and not significantly different from zero. A more accurate direct determination of the resting rate of energy utilization might be obtained by longer incubation times, but prolonged anaerobic incubation in the IAA-FAA Krebs' solution after rewarming to 18°C could not be tolerated by the tissue (Butler et al., 1978). Therefore, resting energy usage was also determined in separate experiments in which lactate production and changes in ATP and PCr content

were measured over a 10-min period in muscles in which respiration was blocked.

Muscle segments were allowed to equilibrate under 2×10^{-2} N tension in normal Krebs' solution at 18°C for 2 h. The muscles were then transferred, without disturbing their mechanical arrangement, to individual test tubes containing 4 ml Krebs' solution with 5 mM sodium fluoroacetate, and incubation was continued under anaerobic conditions at 18°C. The tubes were replaced by others containing fresh medium every 10 min for a total of 30 or 40 min. In this way, ATP synthesis could proceed through glycolysis, and the steady-state flux through glycolysis could be measured from the formation of lactate and its appearance in the medium as incubation progressed. In addition, changes in ATP, PCr, and lactate could be measured from differences in the contents of the tissues at the end of specific periods of incubation.

A steady-state rate of lactate production was reached within the first 10-min period, but all calculations are based on samples taken after 30 and 40 min of incubation. There was no significant difference in the rate of lactate production during these two time periods, nor in the tissue lactate concentration at the end of these time periods. The mean steady-state rate of lactate production was 0.98 ± 0.06 μmol/g wet tissue·10 $\min^{-1}$ ($n = 10$). Separate experiments showed the same average rate of lactate production when glucose was present or absent from the bathing medium, suggesting that glycogen is the source of the lactate produced. The measured rate of lactate production was equivalent to a ΔATP of 1.40 ± 0.09 μmol/g wet tissue·10 $\min^{-1}$, assuming 1.5 ATP per lactate produced from glycogen (White et al., 1968). To take into account changes in ATP and PCr that might have occurred, we froze some muscles immediately after the third 10-min incubation and still others after the fourth 10-min incubation period and determined and compared their ATP and PCr contents. The decrease in ATP and PCr that occurred during the final 10 min of incubation was 0.22 ± 0.32 μmol/g·10 $\min^{-1}$, which is not statistically significant, and the concomitant ATP from lactate was 1.19 ± 0.09 μmol/g·10 $\min^{-1}$ ($n = 4$). This represents a resting energy utilization of 2.3 ± 0.5 nmol/g·s^{-1}. On the basis of total creatine (2.7 μmol/g wet wt; Butler et al., 1978), this value is 0.85 ± 0.19 mmol ~P/mol C$_t$· s^{-1}. This value is significantly different from zero but not significantly different from that observed by direct measurement of high-energy phosphate change (-0.3 ± 0.4, $n = 19$). Because of the lower standard error, we used the value derived from lactate production for correction of results concerning maximum isometric force maintenance and relaxation. Use of the value from direct high-energy phosphate utilization would give very slightly different results, but would not change any of the conclusions.

c) ENERGY UTILIZATION DURING FORCE DEVELOPMENT AND FORCE MAINTE-NANCE   The energy used during the development of maximum isometric force was taken as the difference in the PCr and ATP contents of muscles stimulated for 25 s (procedure *A*) and that of unstimulated controls (procedure *D*, Fig. 4, and Table I). Previous findings have shown that under these conditions there is no significant change in AMP and/or IMP (Butler et al., 1978). During

TABLE I

CHANGES IN PHOSPHORYLCREATINE (PCr) AND ATP CONTENT OF TAENIA COLI DURING AN ISOMETRIC TETANUS AT 18°C

Expt.	n	Chemical Change	A—D Force development	B—A* Force maintenance	C—A* Relaxation	B—C* Force maintenance vs. relaxation
		$mol/molC_t$				
IV-38	5	ΔPCr		−0.028 ±0.039	0.007 ±0.037	−0.035 ±0.048
	5	ΔATP		−0.014 ±0.079	0.002 ±0.073	−0.016 −0.034
	5	ΔPCr+ΔATP		−0.042 ±0.076	0.010 ±0.069	−0.052 ±0.070
IV-53	5	ΔPCr		−0.166 ±0.034‡	−0.135 ±0.056	−0.032 ±0.035
	5	ΔATP		0.043 ±0.041	0.047 ±0.077 (n=4)	−0.008 ±0.032 (n=4)
	5	ΔPCr+ΔATP		−0.124 ±0.051	−0.110 ±0.091 (n=4)	−0.016 ±0.028 (n=4)
V-60	8	ΔPCr	−0.097 ±0.028‡		−0.150 ±0.021§	
	8	ΔATP	−0.063 ±0.027‖		0.083 ±0.021‡	
	8	ΔPCr+ΔATP	−0.161 ±0.025§		−0.067 ±0.024‖	
VI-1	7	ΔPCr	−0.135 ±0.029‡	−0.114 ±0.020§ (n=8)		
	7	ΔATP	−0.124 ±0.031‡	−0.006 ±0.045 (n=8)		
	7	ΔPCr+ΔATP	−0.259 ±0.020§	−0.118 ±0.040‖ (n=8)		
V-1	9	ΔPCr				−0.068 ±0.036
	9	ΔATP				−0.003 ±0.031
	9	ΔPCr+ΔATP				−0.071 ±0.036

	AVERAGE OF ALL EXPERIMENTS			
Chemical change	A—D Force development	B—A Force maintenance	C—A Relaxation	B—C Force maintenance vs. relaxation
ΔPCr	−0.115 ±0.019§	−0.104 ±0.020§	−0.102 ±0.025§	−0.050 ±0.022‖
n	15	18	18	19
ΔATP	−0.092 ±0.021§	0.005 ±0.029	0.050 ±0.028	−0.008 ±0.018
n	15	18	17	18
ΔPCr+ΔATP	−0.206 ±0.020§	−0.098 ±0.029‡	−0.054 ±0.030	−0.053 ±0.024‖
n	15	18	17	18
Average rate, $mmol \sim P/mol\ C_t \cdot s^{-1}$	8.2 ±0.8 (25 s)	2.8 ±0.8 (35 s)	1.2 ±0.7 (45 s)	

* Values are not corrected for resting energy utilization. See text for description.

‡ $P < 0.01$, § $P < 0.001$, ‖ $P < 0.05$, values significantly different from zero. All others, $P > 0.05$.

force development, there is a significant breakdown of PCr (−0.115 ± 0.019 mol/mol C_t) and ATP (−0.092 ± 0.021 mol/mol C_t). From the total chemical energy change, $\Delta PCr/C_t + \Delta ATP/C_t$, the average rate of ~P change during the 25 s of force development was −8.2 ± 0.8 mmol/mol $C_t \cdot s^{-1}$.

During maximum isometric force maintenance (B—A), there was a significant breakdown of PCr (-0.104 ± 0.020 mol/mol C_t) but not of ATP (0.005 ± 0.029 mol/mol C_t). Taken together for each muscle pair, the total chemical energy change during the 35 s of maximum force maintenance was -0.098 ± 0.029 mol/mol C_t, and the average rate of $\Delta{\sim}P$, therefore, was -2.8 ± 0.8 mmol/mol $C_t \cdot s^{-1}$. When corrected for the resting chemical energy change, the average rate of chemical energy change during maximum force maintenance is -2.0 ± 0.8 mmol ${\sim}P$/mol $C_t \cdot s^{-1}$, or only one-fourth that seen during force development.

Because C_t is proportional to muscle mass (see Methods), C_t divided by muscle length (L) for comparable muscles is a measure of cross-sectional area. Force per cross-sectional area is taken from maximum PL/C_t. The value PL/C_t for all muscles used to determine the energy utilization during maximum force maintenance was 1.4 ± 0.1 N·cm/μmol C_t, $n = 36$; this is equivalent to a force of 3.7 N/cm^2.

d) ENERGY UTILIZATION DURING FORCE REDEVELOPMENT   The striking difference in the average rates of energy utilization during force development and force maintenance led us to consider the possible causes. Experiments were designed to directly determine whether the high rate of energy utilization is associated with work done against a series elasticity (SE) during force development or, perhaps associated with the initial activation of the muscle. Accordingly, the energy cost of force development was compared with that of force redevelopment following a quick-release imposed after maximum isometric force had been attained. By the use of a length perturbation sufficient to reduce active force to zero, an amount of internal work equal to that done during force development would be done later in the contraction. The method is shown in Fig. 5.

For these experiments, after the initial equilibration period, muscle length was set at 1.55 l_s, or 102% l_o. Experimental muscles were stimulated tetanically and allowed to develop force for 20 s, then quickly released (0.2 l_o/s, 7 × V_{max}) to 93% l_o. This length change was sufficient to fully discharge the SE.[1] As a result, active force fell to the passive tension at the new length and then redeveloped from $0 \pm 4\%$ to $95 \pm 6\%$ of the initial maximum active force in the 25 s after the quick-release. From a comparison of the energy utilization of muscles treated in this way and control muscles that were stimulated for 20 s, the chemical energy change during force redevelopment was -0.078 ± 0.050 mol ${\sim}P$/mol C_t ($n = 7$). When corrected for resting energy metabolism, this becomes -0.057 ± 0.050 mol ${\sim}P$/mol C_t and is significantly less ($0.05 > P > 0.02$) than that used for initial force development (-0.206 ± 0.020 mol ${\sim}P$/mol C_t, $n = 15$). Although the amount of internal work done under the two conditions is the same, the energy utilized during the initial force development is more than three times greater than that utilized during force redevelopment. Furthermore, the total rate of energy change during force redevelopment (-3.0 ± 2.0 mmol ${\sim}P$/mol $C_t \cdot s^{-1}$) is not significantly different

[1] Siegman, M. J. Unpublished observation.

from that for maximum force maintenance (-2.8 ± 0.8 mmol $\sim$P/mol $C_t \cdot$ s^{-1}). The data shows that the high rate of energy utilization during force development in an isometric tetanus is not due only to the internal work done against the SE, nor is it due to any high rate of cross-bridge cycling inherently associated with force development. Rather, there seems to be an energetically expensive process associated with the initial activation of the muscle under these isometric conditions.

e) ENERGY UTILIZATION DURING RELAXATION The energy utilization during relaxation was measured by taking the difference in the $\sim$P of muscles
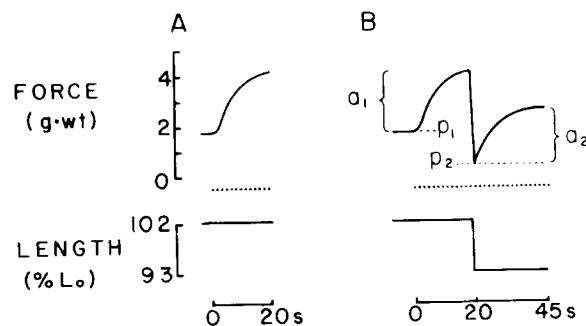

FIGURE 5. Experimental design for determining the biochemical energetics of force redevelopment. (*A*) Control muscle tetanically stimulated for 20 s at length of 102% l_0. (*B*) Experimental muscle tetanically stimulated for 20 s at length of 102% l_0 and then quickly released to length 93% l_0 with continued stimulation for an additional 25 s. a_1 = active force at 102% l_0, taken as the difference between maximum force on stimulation and steady-state force p_1 of resting muscle. a_2 = active redeveloped force at 93% l_0, taken as the difference between maximum force on stimulation and steady-state force p_2 of the resting muscle at the shorter length. Duration of suprathreshold 60-Hz AC stimulus is shown by the dotted lines. Muscles were quickly frozen upon termination of each procedure.

subjected to procedures C (25 s stimulation + 45 s relaxation) and A (25 s stimulation; see Fig. 4 and Table I). The change in $\sim$P during 45 s of relaxation was -0.054 ± 0.030 mol $\sim$P/mol C_t. After correction for a 45-s component of resting metabolism, this value reduced to -0.016 ± 0.031 mol $\sim$P/mol C_t, which is very small and not statistically different from zero.

It was of interest to determine the relative energy utilization associated with maximum force maintenance during stimulation and relaxation. This was accomplished by comparing paired muscles that developed and maintained maximum force (procedure B) with those that also developed maximum force but were then allowed to relax (procedure C; see Fig. 4 and Table I). Preliminary trials showed that the average force·time ($\int P dt$, impulse) was the same in muscles that were stimulated for 60 s (procedure B) and in those stimulated for 25 s followed by 45 s of relaxation (procedure C). The total chemical energy change was greater for muscles maintaining maximum

isometric force than for those allowed to relax, -0.053 ± 0.024 mol $\sim$P/mol C_t. For the 10 s difference in observation time for groups B and C, the correction for basal metabolism is -0.0085 ± 0.0019 mol $\sim$P/mol C_t, making the difference in total chemical energy change during the two mechanical events even greater, -0.0615 ± 0.0241 mol $\sim$P/mol C_t ($n = 18$, $P < 0.02$). For group B, $\int Pdt$ was 144 ± 11, and, for group C, $\int Pdt$ was 141 ± 13, $n = 18$, and not significantly different. These results show that force can be exerted with a lower expenditure of energy during relaxation than when maximum force is maintained during stimulation.

DISCUSSION

Relative Myosin Contents in Frog Sartorius and Rabbit Taenia Coli

Our measurements have shown that the frog sartorius muscle contains 3.3 ± 0.2 ($n = 6$) times more myosin heavy chain per gram wet weight than the rabbit taenia coli. Assuming a value of $\sim$0.13 μmol of myosin/g of skeletal muscle (Ebashi et al., 1969), this corresponds to a value of 0.04 μmol myosin/g of rabbit taenia coli. This is similar to the values of 0.03–0.04 μmol/g found by Cohen and Murphy (1978) for myosin contents in a variety of nonarterial smooth muscles from the pig.

Resting Energy Utilization

Ideally, the rate of resting energy utilization should be determined by direct measurement of high-energy phosphate utilization under the same conditions of metabolic inhibition used for the studies of contraction. From such determinations, a value of -0.3 ± 0.4 mmol $\sim$P/mol $C_t \cdot s^{-1}$ ($n = 19$) was obtained; this is not significantly different from zero. Among the factors that might have contributed to the large variance are the low metabolic rate at rest and the mandatory limitation of the measurement to a 70-s period. We prefer to use the value 0.85 ± 0.19 nmol $\sim$P/mol $C_t \cdot s^{-1}$ ($n = 10$) obtained from the measurement of steady-state lactate production under anaerobic conditions, which is significantly different from zero, but not different ($P > 0.05$) from the directly measured value. In our view, because of its lower standard error, the rate of resting energy utilization measured from lactate production is more reliable, although it requires certain assumptions about the stoichiometry of glycolysis. Corrections of data in Table I to account for resting metabolism may be made using either value. Although this would give very slightly different numerical values, none of the conclusions would be altered.

Our values for the rate of resting energy utilization (from $\sim$P utilization or steady-state lactate production) are similar to that reported from the same muscle from the guinea pig (Mulvany and Woledge, 1972), most vascular smooth muscles (Lundholm and Mohme-Lundholm, 1965; Paul and Peterson, 1975; Glück and Paul, 1977), and the frog sartorius muscle (Kushmerick and Paul, 1976 a). This suggests that similar processes, occurring to about the same extent, may account for the resting energy utilization in all of the muscles studied. Separate experiments have shown that the resting energy

utilization of the rabbit taenia coli is unchanged by removal of calcium.[2] The postulation that the basal rate of ATP utilization in the frog sartorius is due primarily to the actomyosin ATPase (Mahler, 1978), if true, is not general, since one would expect a substantially lower basal rate in smooth muscle, reflecting its much lower rate of specific myosin ATPase activity and lower myosin content.

Energetics of Force Development

The development of isometric force by the rabbit taenia coli at 18°C and 95% l_0 is associated with a chemical energy utilization of 0.21 ± 0.02 mol/mol C_t, or 0.56 μmol/g. This agrees reasonably well with previous measurements on bovine carotid (0.35 μmol/g at 37°C, Daemers-Lambert and Roland [1967]), frog sartorius at 0°C (~0.4 μmol ~P/g, Butler [1974]; Homsher et al. [1975]; Kushmerick and Paul [1976 b]), frog sartorius at 20°C (0.7 μmol ~P/ g, Mahler [1979]), and estimates from myothermal studies in tortoise rectus femoris (Woledge, 1968). Despite the fact that the rates of energy utilization during force development and subsequent force maintenance vary by more than 300-fold, the total energy requirements for force development are very similar. This suggests that there is an inverse relationship between the rate of energy utilization and the time required to develop maximum isometric force. It is unlikely that the work done against the SE is the main determinant of energy utilization during force development, because of the results reported here on the energetics of force redevelopment.

Energy Utilization During Force Maintenance

The average rate of total high-energy phosphate utilization during mainte- nance of nearly constant isometric force in the taenia coli at 18°C is 2.8 ± 0.8 mmol ~P/mol $C_t \cdot s^{-1}$. This is equivalent to 7.6 nmol/g$\cdot$s^{-1}, and after correc- tion for the basal rate of energy utilization, the value is 5.3 nmol/g$\cdot$s^{-1}.

Previous investigators, measuring steady-state oxygen consumption and lactate production during constant force maintenace, have reported values which, when corrected to 18°C with a Q_{10} of 2.5, are 1.8 nmol/g$\cdot$s^{-1} for the hog carotid artery (Glück and Paul, 1977) and 14 nmol/g$\cdot$s^{-1} for the rat portal vein (Hellstrand, 1977). It is interesting that, despite the variety of tissues, techniques, and methods of stimulation used in these studies, there is close agreement on the rate of energy utilization during force maintenance.

Other workers have reported values that are difficult to reconcile with those reported here. On the basis of myothermal measurements in the rabbit rectococcygeus muscle, the rate of high-energy phosphate utilization was 48 nmol/g$\cdot$s^{-1} (Davey et al., 1975), but this might have included a substantial quantity of recovery metabolism. For the bovine carotid artery, very much lower rates were obtained than those reported here, and this may be traced to the conditions under which metabolic inhibitors were used, which did not exclude oxidative recovery reactions and may have limited the high-energy

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[2] Mooers, S. U., T. M. Butler, and M. J. Siegman. Unpublished observations.

phosphate supply (Glück and Paul, 1977) and caused drastic changes in the mechanical properties of the muscles (Daemers-Lambert, 1969).

The rate of energy utilization during force maintenance in the rabbit taenia coli is 400-fold lower than that in the frog sartorius muscle at the same temperature (DeFuria and Kushmerick, 1977). The V_{max} for shortening, time to maximum force development, and the rates of energy usage during force development, are approximately 300-fold different in these two muscles (Gordon and Siegman, 1971; Kean and Homsher, 1974; DeFuria and Kushmerick, 1977; Mahler, 1979), suggesting that there is an invariant relationship among all of the above parameters (see Rüegg [1971]). The fraction of the energy usage for force maintenance that is due to activation process is not known, but we may assume this to be 20–40%, based on the work of others on a variety of muscles (Homsher et al., 1972; Smith, 1972; Rall and Schottelius, 1973; Paul and Peterson, 1975). After appropriate correction, a 300- to 400-fold difference in the rate of ATP utilization by the actomyosin systems of the two muscle types would also obtain.

If it is assumed that each myosin molecule is one cross-bridge, that during a cross-bridge cycle one ATP is split by each myosin molecule, and that all cross-bridges are cycling, then it is possible to calculate an average cross-bridge cycle duration by dividing the molar myosin content by the rate of ATP utilization (Curtin et al., 1974; Paul et al., 1974; Ferenczi et al., 1978). The taenia coli contains 0.04 μmol myosin/g and the rate of energy utilization during force maintenance is 5.3 nmol/g·s^{-1}, so that the average cross-bridge cycle time is 7.5 s. A similar calculation for frog sartorius using a value of 0.13 μmol myosin/g and an ATP splitting rate of 2.7 μmol/g·s^{-1} at 20°C (DeFuria and Kushmerick, 1977) gives a mean cross-bridge cycle time of 0.05 s, or 150-fold shorter than the taenia coli. This is consistent with the observed differences in myosin ATPase activities determined on actin-activated subfragment-1 from frog skeletal muscle (Ferenczi et al., 1978) and the isolated contractile proteins from chicken gizzard (Marston and Taylor, 1978) and hog carotid artery (Mrwa and Rüegg, 1977).

Although there is no obligatory energy requirement for constant force maintenance in physical systems, it has long been recognized that there is a variety of energy requirements for isometric force maintenance in different muscle types (for a review, see Rüegg [1971]). The energy requirements for force maintenance in different muscles may be compared on the basis of their "economy" (force per cross-sectional area divided by the rate of energy utilization per gram). The economy of force maintenance in the rabbit taenia coli at 18°C, obtained by direct measurements of high-energy phosphate utilization, is 700 (N/cm^2)/(μmol/g·s^{-1}). Similar values for other mammalian smooth muscles have been derived from measurement of oxygen consumption or heat production when corrected for temperature differences (Mulvany and Woledge, 1972; Paul and Peterson, 1975; Davey et al., 1975; Hellstrand, 1977; Glück and Paul, 1977). In contrast, the high economy of the anterior byssus retractor muscle (ABRM) in the catch state is an order of magnitude greater than that of the taenia coli (Baguet and Gillis, 1968). However, when myosin filament length is accounted for (30 μm for the ABRM, Lowy and Hanson

[1962]; 2.2 μm in mammalian smooth muscle, Ashton et al., [1975]), the economy of force maintenance by the individual cross-bridges in the ABRM would be approximately the same as in the taenia coli, assuming similarity in other structural and metabolic relationships. The unique specialization in smooth muscles showing catch may then not be the ability of the cross-bridges to maintain force economically but rather their ability to vary economy according to the stimulation parameters and to have a relatively large V_{max} for shortening compared to other smooth muscles (Rüegg, 1971).

The rabbit taenia coli has a 100-fold higher economy than the frog sartorius and this is undoubtedly due, in part, to a slower cross-bridge cycle in the smooth muscle (i.e., a greater impulse per cross-bridge force-time cycle) and a 40% longer myosin filament (Ashton et al., 1975). Although such differences alone could quantitatively account for the difference in economy, it is not yet known whether other structural and/or biochemical specializations also contribute.

The rate of chemical energy utilization during constant force maintenance is 350% of the basal rate in the taenia coli. This is similar to what others, using techniques involving oxygen consumption and lactate production (Paul and Peterson, 1975; Glück and Paul, 1977; Hellstrand, 1977), have found in smooth muscles and is in contrast to the 9,000% increase in the rate of chemical energy utilization above basal during force maintenance in the frog sartorius muscle (DeFuria and Kushmerick, 1977; Kushmerick and Paul, 1976 *b*).

Energetics of Force Development vs. Force Maintenance

The average rate of high-energy phosphate utilization during the first 25 s of an isometric tetanus is more than four times greater than the average rate (corrected for basal metabolism) during the next 35 s of force maintenance. This is shown both in a comparison of the means of all estimates of the energy utilizations and in experiment VI-I, where the high energy phosphate breakdown for force development and force maintenance were determined on segments from the same taenia coli muscle (Table I).

An initial high rate of high-energy phosphate utilization during an isometric tetanus has been reported for frog sartorius at 0°C (Butler, 1974; Homsher et al., 1975; and Kushmerick and Paul, 1976 *b*) and at 20°C (Mahler, 1979; DeFuria and Kushmerick, 1977). Although it is difficult to determine accurately from the published data, it appears that the rate of chemical energy utilization is three to eightfold higher during the period when force is increasing compared with that when force is at its maximum value.

Energetics of Force Redevelopment

Experiments have shown that the initially high rate of chemical energy usage during the development of isometric tetanic force is not associated with force development per se, nor can it be accounted for on the basis of work done against the SE. The transition from the resting to the activated state leads to an extra 0.45 μmol ~P breakdown per gram during initial force development compared with an equivalent period of force maintenance, and may be due

to the process of activation. The phosphorylation of the 20,000-dalton myosin light chain, implicated in the activation of smooth muscle (Aksoy et al., 1976; Chacko et al., 1977; Small and Sobieszek, 1977) can account for only a small part of this high-energy phosphate utilization, for there is only 0.08 μmol light chain per gram of muscle.

Energetics of Relaxation

The change in high-energy phosphate during 45 s of relaxation from an isometric tetanus was very low, only -0.054 ± 0.030 mol/mol C_t. When corrected for resting metabolism, this figure was reduced further to -0.016 ± 0.031 mol $\sim$P/mol C_t and was not significantly different from zero. More force is maintained per ATP split during relaxation than during maximum isometric force maintenance under stimulated conditions. The measured energy utilization correlates well with the extent to which force is maintained through ATP-driven cross-bridge cycling or "active state" during relaxation (Siegman et al., 1977). It was shown that the "active state" decays more rapidly than the force exerted by the muscle, leaving a component of "extra force" that is maintained without a measurable expenditure of energy. In the frog sartorius muscle at 0°C, the energy utilization during relaxation from an isometric tetanus is also very low, but, in contrast to smooth muscle, can fully explain the measured force (Curtin and Woledge, 1974). The inference is that isometric relaxation in skeletal muscle is a progressively decreasing continuation of contractile activity occurring during the tetanus, and that a more complex process may operate in smooth muscle.

The extra force exerted during relaxation in smooth muscle may be due to the occurrence of a state in which force is maintained by attached but noncycling (non-ATP-driven) cross-bridges (Siegman et al., 1976), which are strained as the SE shortens. Then, the time-course of relaxation would be determined by the time-course of calcium removal (which presumably governs the time course and magnitude of redeveloped force after quick release) and by the stress-relaxation of the attached but noncyling cross-bridges. The stress-relaxation may be viewed as a progressive process in which the recoil of the SE causes temporary detachment of the strained cross-bridges, followed by their reattachment under unstrained conditions (Siegman et al., 1976) until again strained by the SE. An alternative interpretation is that the "extra" force is derived from the strain of fully activated, cycling cross-bridges. For skeletal muscle, the stress that the cross-bridges can bear when undergoing slow isovelocity strain exceeds that which they can develop actively and is associated with little, if any, ATP breakdown (Curtin and Davies, 1973). The present experiments cannot distinguish the operation of these two possible mechanisms. Yet, such interpretations are generally consistent with the cross-bridge model of Brutsaert and Housmans (1977), which was derived from mechanical studies on cardiac muscle. In their model, the delayed lengthening during isotonic overloading and early phases of spontaneous isotonic relaxation are governed by the back rotation and eventual detachment of attached and strained cross-bridges, which are momentarily able to resist large forces.

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