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LOAD CLAMP ANALYSIS OF MAXIMAL FORCE POTENTIAL OF MAMMALIAN CARDIAC MUSCLE

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SUMMARY

1. Abrupt alterations in load (load clamps) have been imposed on cat papillary muscle during isotonic shortening and relaxation of afterloaded twitch and tetanic contractions, to assess the maximal force potential for a given contractile state.

2. These load clamps were accompanied by an initial fast lengthening reflecting an undamped series compliance. Even when exceeding isometric twitch and tetanic force, these loads could be borne for a considerable time, accompanied by a slower lengthening after the initial extension of the series compliance. At sufficiently high loads the muscle was pulled out very rapidly; a maximal supra-isometric force potential was defined as the load the muscle could bear momentarily and this was measured at times throughout contraction and relaxation.

3. This maximal force potential was determined at different initial muscle lengths. Depending on the instantaneous loading conditions, various length–force relations were obtained from: (*a*) peak force values of isometric twitches at different starting lengths, (*b*) the shortest length reached during afterloaded isotonic twitches, and (*c*) the forces obtained in overloaded isotonic twitch contractions.

4. These results are consistent with a crossbridge model in which the delayed lengthening during isotonic overloading is due to back rotation and detachment of attached crossbridges and in which the initial phase of spontaneous isotonic relaxation is governed by the same mechanism.

INTRODUCTION

From the study of length transients after load clamps in isotonically contracting cat papillary muscle, this muscle was shown to resist augmented clamped loads for a considerable time (Housmans & Brutsaert, 1976). The accompanying slow extension of the muscle proceeded in two phases that were proposed to reflect back rotation and eventual detachment of attached crossbridges in accordance with recent crossbridge models of contraction (Huxley & Simmons, 1971; Simmons & Jewell, 1974).

In the present study the analysis of the delayed lengthening after load clamps was extended to loads either at or beyond isometric force levels both in twitch and tetanic contractions. In twitch contractions, this technique was used to explore the ability of the muscle to resist supra-isometric loads both during the rising phase of the contraction and during relaxation, and at various initial muscle lengths below $l_{\text{max.}}$, the initial muscle length at which active force development is maximal.

TABLE 1. Muscle characteristics under control conditions at $l_{\text{max.}}$, i.e. the length at which active twitch force development was maximal

Muscle	$l_{\text{max.}}$ (mm)	Mean cross-sectional area (mm^2)	Resting force (R) (mN/mm^2)	Total force (T) of isometric twitch (mN/mm^2)	R/T
1	5·50	0·56	5·25	59·24	0·09
2	6·00	0·65	3·02	33·20	0·09
3	6·75	0·47	4·17	39·23	0·11
4	9·00	0·93	9·49	72·02	0·13
5	4·75	0·61	6·43	67·20	0·10
6	4·25	1·03	7·62	65·89	0·12
7	5·75	0·65	4·53	63·97	0·07
8	7·75	0·85	9·23	80·76	0·11
9	6·75	0·90	7·63	56·55	0·14
10	7·00	0·43	6·84	66·14	0·10
11	15·00	0·53	11·10	144·33	0·08
12	6·00	0·61	6·43	70·58	0·09
13	5·00	0·32	6·13	66·81	0·09
14	6·00	0·83	5·91	53·88	0·11
15	10·00	0·24	12·26	124·22	0·10
16	7·25	0·50	9·80	112·39	0·09
17	6·50	0·61	8·04	77·97	0·10
18	7·00	0·36	5·45	61·29	0·09
19	5·00	1·24	4·75	56·23	0·08
20	5·50	0·89	6·61	62·92	0·11
21	9·00	0·44	8·92	76·23	0·12
22	4·50	0·69	8·53	76·04	0·11
23	6·00	0·41	9·57	130·84	0·07
24	9·00	0·61	12·86	134·08	0·10
Mean	6·89	0·64	7·52	77·17	0·10
± S.E. of mean	0·47	0·05	0·52	6·07	0·01

METHODS

Twenty-four papillary muscles of the right ventricle of the cat were used in this study. Table 1 summarizes the basic characteristics of these muscles under control conditions at the initial muscle length $l_{\text{max.}}$, i.e. the length at which active force development was maximal. Only undamaged muscles with lengths at $l_{\max.}$ longer than 4 mm were considered in this study. Suitable muscles were selected on the basis of their ratio of resting to total force at $l_{\max.}$. Muscles with a ratio of above 0·15 were rejected, the mean ratio being 0·10 ± 0·01 (S.E. of mean, $n = 24$) at 29 °C and 12 beats/min.

The muscles were mounted vertically, the lower non-tendinous end being held by a force transducer (compliance 0·3 μm/mN, resonant frequency in liquid 250 Hz) and the upper tendinous end being tied (7·0 braided thread, Deknatel, Surgical Tevdek, Code 103-T) to an electromagnetic lever system (compliance 0·2 μm/mN, equivalent moving mass 155 mg, step response 3 msec). The current through the coil of the electromagnet, or load on the muscle, was controlled by a current source, calibrated for step changes of 0·98 mN and 9·8 mN, and could be switched from one level to another by means of two reference voltage sources. A detailed description of force transducer, electromagnetic lever system and their response characteristics to abrupt load alterations has been published previously (Claes & Brutsaert, 1971; Brutsaert & Claes, 1974). The physiological and methodological aspects of the 'load clamp' technique have also been reported (Brutsaert, Claes & Sonnenblick, 1971; Brutsaert & Claes, 1974). For each contraction, shortening, shortening velocity and force were recorded simultaneously and displayed as functions of time on a Storage Display Unit (Tektronix 611) and photographed with a Hard Copy Unit (Tektronix 4601). In some experiments velocity–length (phase–plane) plots were simultaneously recorded on the Storage Display Unit.

All experiments were performed with the initial muscle length set to $l_{\max.}$. Before the actual experiments, the muscles were allowed to recover for 2–3 h at 29 °C. The bathing solution during recovery and experiment contained (mM): NaCl 118, KCl 4·7, $MgSO_4.7H_2O$ 1·2, KH_2PO_4 1·1, $NaHCO_3$ 24, $CaCl_2.6H_2O$ 2·5, and glucose 4·5, bubbled with a gas mixture of 95 % O_2–5 % CO_2. Homogeneous electrical stimulation (12 beats/min at 29 °C) was obtained by rectangular pulses of 5 msec duration about 10 % above threshold through two platinum electrodes arranged longitudinally along the entire muscle.

In order to avoid the long term effects on cardiac muscle of loading history in preceding contractions, all test contractions were separated by a series of at least eight equally loaded standardized contractions (Parmley, Brutsaert & Sonnenblick, 1969; Kaufmann, Lab, Hennekes & Krause, 1971; Jewell & Rovell, 1973; Brutsaert, 1974). The results obtained in this study were qualitatively similar irrespective of the loading conditions of the interpolated contractions as long as these conditions were the same throughout the experiment.

RESULTS

1. *Supra-isometric load clamps during isotonic twitch and tetanic contractions*

Fig. 1 *A* illustrates the effects of an abrupt increase of load (load clamp) during isotonic shortening of a heavily afterloaded twitch contraction at 29 °C. The fast step increase of load was accompanied by an initial fast lengthening of the muscle (phase 1), the amplitude of which varied with the magnitude of the load step. Subsequently, the muscle resisted the augmented clamped load. This was accompanied by a further slower extension of the muscle. This delayed isotonic lengthening was always followed by an abrupt fall in force during subsequent isometric relaxation. The distinction between a slow phase 2 and faster phase 3 delayed lengthening was much less apparent than previously illustrated for twitch contractions at lower temperatures and for tetanic contractions (Housmans & Brutsaert, 1976).

Fig. 1*B* shows that the muscle could resist clamped loads even when augmented to beyond peak isometric twitch force. Load clamps of increasing magnitude progressively accelerated this delayed lengthening (Fig. 1*C*). High enough load steps forced the muscle lever rapidly against the mechanical stop during phase 1. Because of the difficulty in the present study of obtaining a correct measurement of this limiting force response, i.e. of finding the load clamp where delayed lengthening became inseparable from phase 1, a 'maximal force potential' (P_0') was defined as the load for which delayed lengthening lasted about 5 msec. Although this measurement was close to the resolution of the apparatus, it allowed a reasonably accurate comparison to be made of the maximum load a muscle could bear under different conditions.

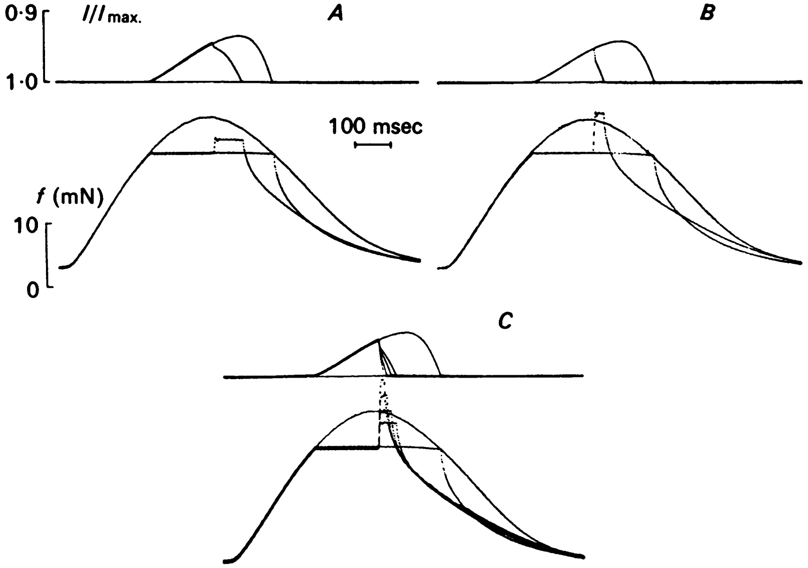

Fig. 1. Load clamps during afterloaded isotonic twitch contractions. Step increase in load (load clamp) during isotonic shortening of heavily afterloaded twitch contractions at 29 °C. Slow sweep tracings of shortening (upper) and total force (lower). Length and force scales shown in panel *A* were the same for panels *B* and *C*. Muscle 21. Panel *A*: load clamp of moderate amplitude during isotonic shortening of a heavily afterloaded twitch contraction, with superimposed control isotonic and isometric twitch contractions. Panel *B*: same as in panel *A* but for a higher clamped load which exceeded peak isometric twitch force. Panel *C*: series of contractions with load clamps of increasing magnitude.

(*a*) *Time course of* P'_0 *during twitch and tetanic contractions.* These force values (P'_0) were obtained by imposing load steps at different times during the contraction. For twitch contractions (Fig. 6*A*), the maximal ratio P'_0/P_0 averaged 106·1 ± 0·7, 115·2 ± 1·4 and 117·7 ± 3·1 % (mean ± S.E. of mean; n = eleven muscles; muscles 1–11) when both P'_0 and P_0 were meas-

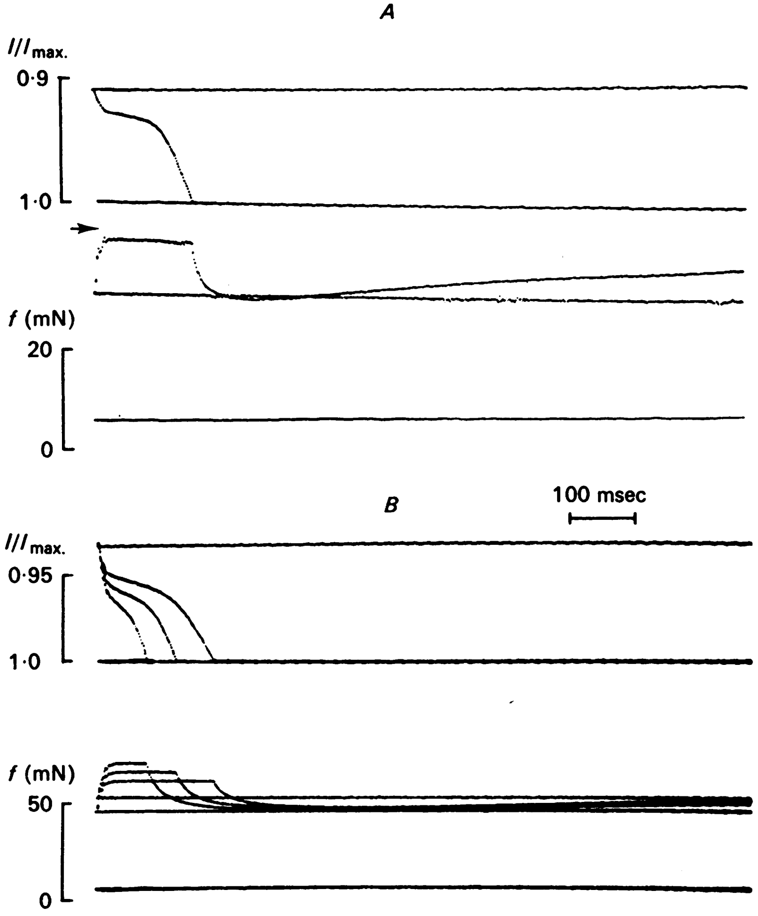

Fig. 2. Load clamps during afterloaded tetanic contractions. Fast sweep tracings of shortening (upper) and total force (lower). The oscilloscope sweep tracings were started just before the time of the clamps, so that only part of each contraction is shown. Panel *A* : a single load clamp which did not exceed the level of isometric force (indicated by the arrow at the left side of the panel) imposed during steady-state isotonic shortening of an afterloaded tetanic contraction. The control afterloaded tetanus was superimposed. Muscle 22. Panel *B*: three superimposed supra-isometric load clamps during steady isotonic shortening of an afterloaded tetanus. Both the control afterloaded and the isometric tetanic contraction were also displayed. Muscle 19.

ured at respectively 70 ± 2, 100 and 137 ± 3 % of the time to peak isometric force. These ratios would probably be about 5 % larger, if the truly limiting force responses could be measured experimentally, i.e. at load clamps where delayed lengthening had just fused within the instantaneous response of phase 1.

In order to minimize activation effects in twitch contractions, this study was extended to tetanized cardiac muscle. Muscles were tetanized by imposing trains of high-intensity electrical stimuli in a bathing solution containing 10 mM-calcium chloride and 10 mM-caffeine (Henderson, Forman, Brutsaert & Sonnenblick, 1971). A representative load clamp during steady isotonic shortening of a heavily afterloaded tetanic contraction is illustrated in Fig. 2*A* along with the control afterloaded tetanus. As in twitch contractions, an abrupt lengthening (phase 1) accompanied the load step itself, and was again followed by delayed lengthening, i.e. a slow phase 2 and a faster phase 3. Isotonic lengthening was again followed by an abrupt fall in isometric force, despite the imposed load being maintained at the clamped level throughout the remaining part of the tetanus; subsequent recovery of isometric force and of isotonic shortening at the clamped load was always markedly delayed and free of oscillations. In Fig. 2*B* higher clamped loads which exceeded isometric tetanic force were imposed during steady isotonic shortening of heavily afterloaded tetanic contractions. Again, supra-isometric loads were held by the muscle for several milliseconds and this was again accompanied by a slow extension of the muscle. As in twitch contractions, P_0' force values were measured at different times and plotted in Fig. 6*B* along with a control tetanic isometric contraction (P_0). The ratio P_0'/P_0 for the four measurements shown in Fig. 6*B* was respectively 116·7, 117·0, 121·1 and 121·7 %. Again, these values were probably underestimated by about 5 % because of the procedure used.

For technical reasons we were unable to study the effects of increases of load during the early phase of isotonic shortening of afterloaded twitch and tetanic contractions; the small extent of isotonic shortening at these early times limited the length range available for isotonic lengthening during the clamps because of the presence of the mechanical stop in the electromagnetic lever system.

(*b*) *Dependence of* P_0' *on muscle length.* In Fig. 3, supra-isometric force potential, resulting from isotonic overloading, was studied as a function of initial length, i.e. at $l_{\text{max.}}$ and at 0·95 $l_{\text{max.}}$. Isotonic overloading during afterloaded isotonic contractions at the two different initial muscle lengths yielded a length–force relation which was considerably higher than that of the isometric conditions. Similar results were obtained during isotonic overloading of tetanized muscle set at different initial lengths.

Hence, for a given state of contractility in cardiac muscle, a family of length–force relations can be depicted depending on the instantaneous loading conditions, i.e. (*a*) at supra-isometric force levels from the shortest length obtained in the overloaded afterloaded twitches, (*b*) at intermediate force levels from the peaks of the ten isometric twitches at different initial muscle lengths, and (*c*) at infra-isometric force levels by measuring the shortest length reached by the two control afterloaded twitch contractions at $l_{\mathrm{max.}}$ and at 0·95 $l_{\mathrm{max.}}$; this latter phenomenon emphasizes the well known deactivation by shortening in cardiac muscle (Brady, 1968; Brutsaert & Sonnenblick, 1969; Edman & Nilsson, 1971; Brutsaert, Claes & Donders, 1972). At the time to peak force, maximal P_0'/P_0 ratios of 114·6 ± 1·8 and 113·9 ± 1·4 % (mean ± S.E. of mean, $n = 8$ muscles; muscles 2–9) were found at $l_{\mathrm{max.}}$ and 0·95 $l_{\mathrm{max.}}$ respectively; these P_0'/P_0 ratios did not differ significantly ($P > 0{\cdot}30$), indicating a proportional shift of the length–force relations for maximal force values between 0·95 $l_{\mathrm{max.}}$ and $l_{\mathrm{max.}}$.

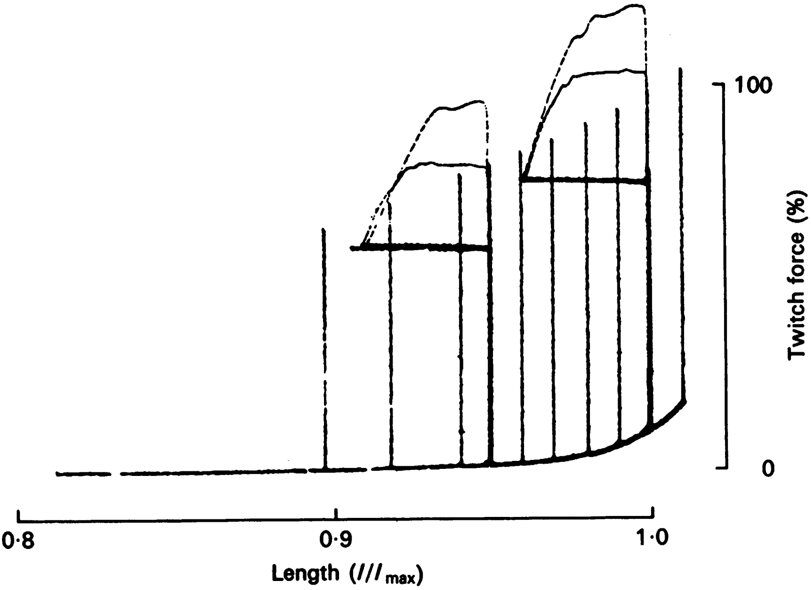

Fig. 3. Length–force relations of supra-isometric load clamps in twitch contractions at different initial muscle lengths. The length–force relations of an afterloaded isotonic and of two superimposed isotonically overloaded twitch contractions were recorded at the initial muscle lengths $l_{\mathrm{max.}}$ and 0·95 $l_{\mathrm{max.}}$, along with the resting and total force of ten control isometric twitch contractions at various lengths over the same length range (muscle 23).

2. *Supra-isometric force during isotonic relaxation of heavily afterloaded twitch contractions*

Supra-isometric forces were examined during isotonic relaxation of both control and load-clamped heavily afterloaded twitch contractions.

(*a*) *Control contractions.* Fig. 4*A* illustrates a series of superimposed afterloaded isotonic twitch contractions, i.e. from an isotonic twitch at preload only, then with different afterloads up to a full isometric twitch. In any afterloaded twitch, peak shortening occurred after peak force of the isometric twitch. The end of the isotonic force plateau of heavily afterloaded contractions clearly extended to beyond the superimposed control isometric twitch (Fig. 4*B*); during isotonic lengthening of the relaxing after-

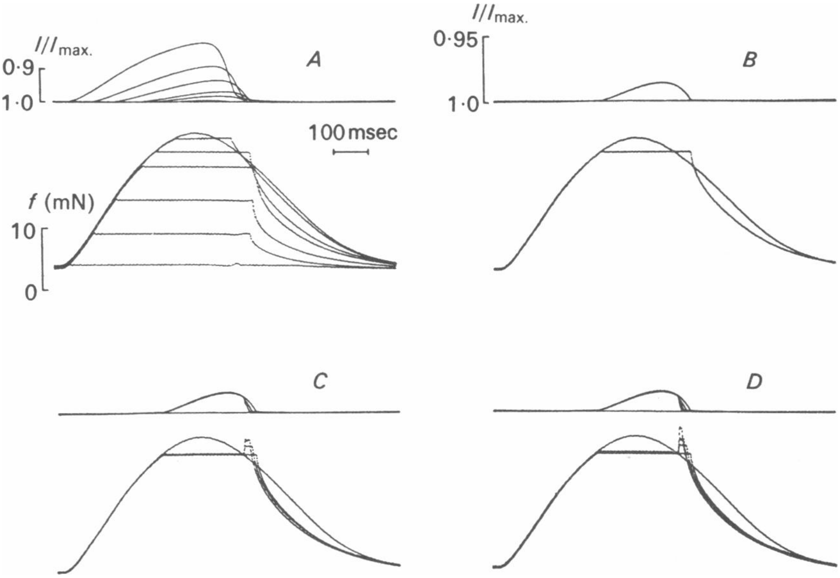

Fig. 4. Supra-isometric force during isotonic relaxation of heavily afterloaded twitch contractions. Panel *A*: time course of a series of afterloaded twitch contractions, and of a preloaded and an isometric twitch contraction, each test contraction being separated by an interpolated series of at least eight preloaded twitch contractions. Shortening (upper) and force development (lower) are shown. Panel *B*: a heavily afterloaded twitch contraction together with a control isometric twitch. Panel *C*: same as in panel *B*, with two superimposed load-clamped contractions. Load clamps were imposed during isotonic relaxation. The clamped supra-isometric loads were sustained for some time at the expense of delayed lengthening. Panel *D*: same as in panel *C* with an additional load-clamped contraction. Muscle 21. Force gains were the same for all panels. Length gain in panel *B* was the same for panel *C* and *D*, but was different for panel *A*.

loaded contraction, the load could still be carried at a time when the force in an isometric twitch had already fallen considerably. Isotonic lengthening during relaxation was always followed by an abrupt fall of subsequent isometric force, thus under-shooting the force levels of the isometric control contraction. This well known phenomenon of muscle behaviour (Jewell & Wilkie, 1960) has been illustrated in previous publications on cardiac muscle under similar conditions of load stabilization (Brutsaert *et al.* 1972). The isotonic force just before isometric relaxation averaged 110·6 ± 1·2 % (mean ± S.E. of mean, $n = 11$ muscles; muscles 8–18) of P_0 at 151 ± 2 % of the time to peak isometric force (point X in Fig. 6A).

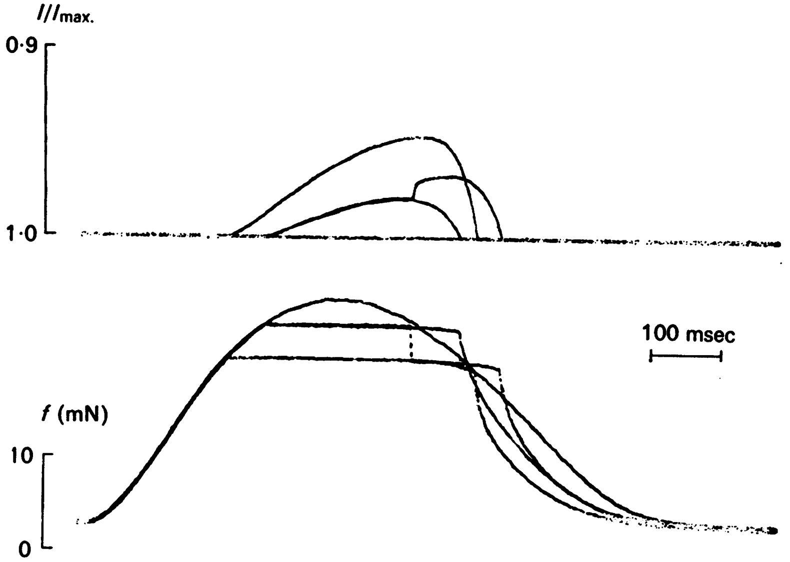

Fig. 5. Unloading steps during isotonic relaxation of afterloaded twitch contractions. Shortening (upper) and total force (lower) of a heavily afterloaded twitch contraction, which was suddenly unloaded just before the expected isotonic relaxation. The control afterloaded twitch contractions at loads corresponding to the load before and after the load step have also been represented along with the control isometric twitch. Muscle 15.

(*b*) *Loading steps*. The apparent ability of a muscle to sustain a load for longer times under isotonic conditions was investigated further by applying loading steps of various magnitudes during the time when isotonic force was higher than that in a control isometric twitch. The records obtained (Fig. 4C) were qualitatively similar to these obtained at earlier times (Fig. 1). Again, a maximal force response was obtained for load clamps where delayed lengthening was just separable from phase 1 (Fig. 4D). In all isotonic contractions, whether load-clamped or not, isotonic lengthening during isotonic relaxation was always followed by an abrupt fall of isometric force undershooting the fall of force of the isometric control contraction.

(*c*) *Unloading steps.* Appropriate unloading of the muscle at the onset of or during isotonic relaxation of heavily afterloaded twitch contractions made it possible to extend this investigation to later times during isotonic

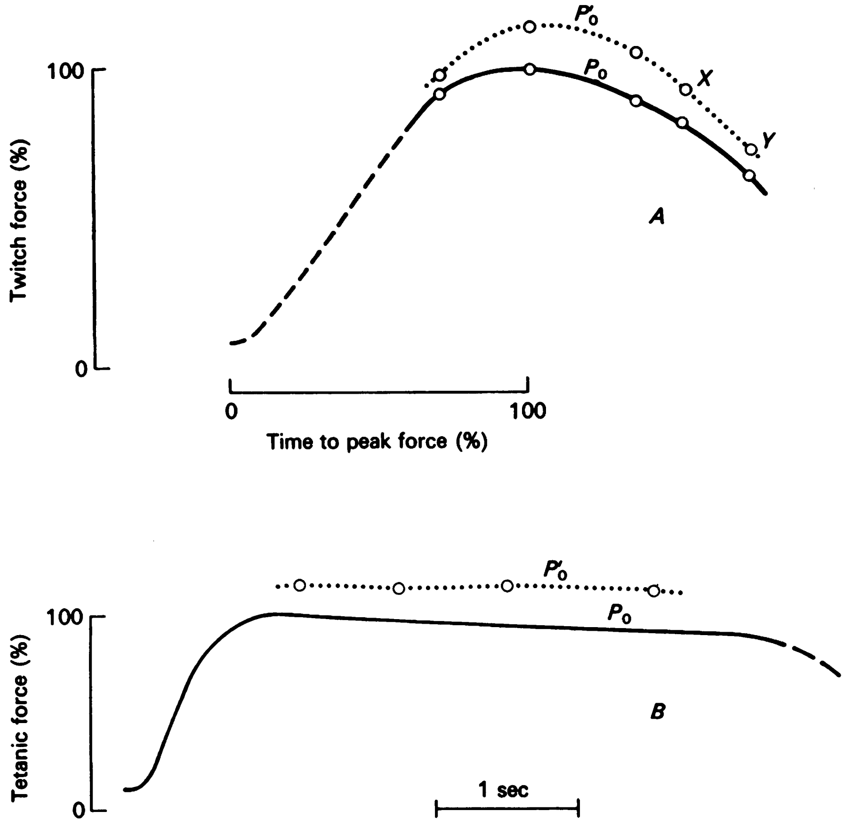

Fig. 6. Time course of maximal force potential (P_0') in twitch and tetanic afterloaded contractions. Panel *A* (twitch): mean values of force development in control isometric contractions (P_0) and force potential (P_0') measured in heavily afterloaded contractions. These values are respectively: (mean ± S.E. of mean; $n = 11$ muscles) 92·1 ± 0·7 and 97·6 ± 0·8 % of peak twitch force at 70 ± 2 % of the time to peak twitch force; 100 and 115·2 ± 1·4 % of peak twitch force at 100 % of the time to peak twitch force; 90·3 ± 0·7 and 106·2 ± 2·6 % of peak twitch force at 137 ± 3 % of the time to peak twitch force; 83·1 ± 1·0 and 91·1 ± 0·5 % of peak twitch force at 151 ± 2 % of the time to peak twitch force; and ($n = 4$ muscles) 65·4 ± 2·4 and 74·0 ± 1·7 % of peak twitch force at 175 ± 4 % of the time to peak twitch force. Muscle characteristics: see Table 1. Panel *B* (tetanus): time course of P_0 and P_0' of an isometric tetanus. Muscle 24. Time scales for twitch and tetanus are in percent and absolute values respectively.

relaxation. Fig. 5 demonstrates that the clamped load was held for a longer time than in the control afterloaded contraction. After an initial fast shortening at the time of the unloading step itself, due to the series compliance, the smaller load could be sustained for about 40 msec at a time when the force in the control isometric twitch continued to fall considerably lower. Sustainment of such supra-isometric loads was accompanied by some shortening followed by a delayed isotonic relaxation. In such unloaded clamped contractions, the mean supra-isometric force value just before isometric relaxation was 113·3 ± 2·4 (mean ± S.E. of mean, $n = 4$ muscles; muscles 13–16) % of P_0 at 175 ± 4 % of the time to peak isometric twitch force (point Y in Fig. 6A). Similar supra-isometric forces during isotonic relaxation were also determined at still later times

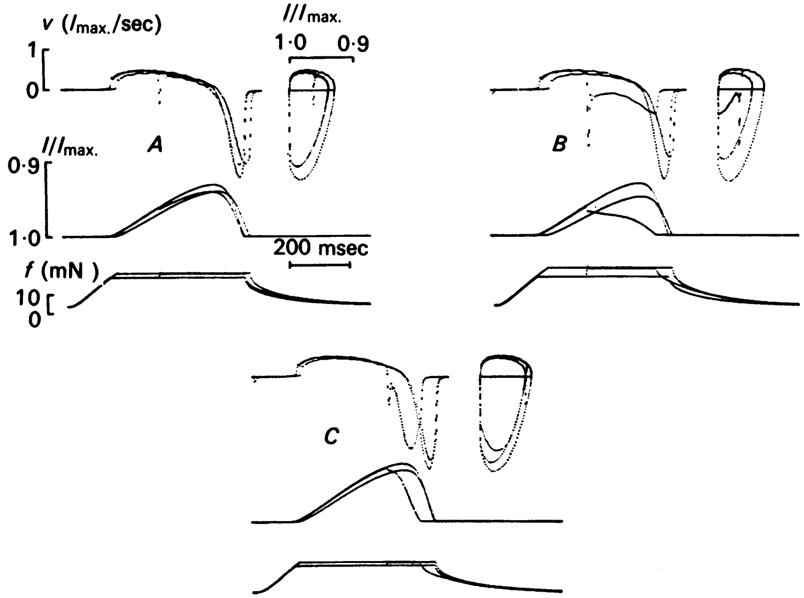

Fig. 7. Time independence of the force–velocity–length interrelation during isotonic shortening of twitch contractions. Effects of a sudden increase of load on the time course of shortening (middle), velocity (upper), total force (lower) and on velocity as a function of instantaneous length (upper right corner of each panel) of afterloaded twitch contractions. Calibrations are the same for all panels. Muscle 21. Panel A: effects of a small step increase of load during isotonic shortening of an afterloaded twitch contraction. Both control afterloaded contractions at loads corresponding to the load before and after the clamp are also shown. Panel B: effects of a large step increase of load during isotonic shortening at the same instant as the load clamp shown in panel A. Panel C: a small step increase of load, with the same amplitude as in panel A, was imposed late during isotonic shortening.

during relaxation, when either unloading steps of larger magnitude or successive unloading steps were imposed.

3. *Time independence of the force–velocity–length interrelationship*

Imposing abrupt alterations of total load on a shortening muscle has provided evidence for a close interrelationship between force, instantaneous length during shortening and shortening velocity. It is independent of time over a major portion of external shortening (Brutsaert *et al.* 1971; Brutsaert, 1974). The load clamp experiment which has led to this observation is illustrated in Fig. 7*A*. In the load-clamped contraction, the same shortening velocity on the velocity–length record was reached at the same length and total load as in the control contraction despite marked differences in time on the shortening-time tracings. However, load clamps of larger magnitudes (Fig. 7*B*) or load steps of the same amplitude but at later times during isotonic shortening (Fig. 7*C*), failed to reveal matching of the velocity–length pathways independently of time. For an appropriate magnitude of such clamps, after the clamp step with concomitant fast lengthening, subsequent shortening eventually disappeared; instead, further increasing the load clamp amplitude resulted in delayed lengthening (Fig. 7*B*, *C*), which for an appropriate amplitude proceeded in two phases similar to the delayed lengthening illustrated in Fig. 1*A*.

DISCUSSION

1. *Supra-isometric force potential in cardiac muscle*

Although it has not yet been possible, for technical reasons, to explore the time course of maximal force potential in the very early phase of the contraction, at somewhat later times maximal force potential during isotonic overloading rose faster than force development of a control isometric twitch; it reached peak values which were substantially higher than peak force of both control isometric twitch and tetanic contractions and it could be extended over a major portion of twitch relaxation. Depending on the *instantaneous* loading conditions, i.e. (1) isometric, (2) afterloaded isotonic and (3) load-clamped afterloaded isotonic, different length–force relations were obtained; hence, a family of active length–force curves can be depicted for a given state of contractility. These effects must be distinguished from the alterations of the length–force relation that depend on the *long-term* effects of the loading conditions in preceding contractions (Parmley *et al.* 1969; Kaufmann *et al.* 1971; Jewell & Rovell, 1973; Brutsaert, 1974).

Supra-isometric forces have previously been examined in skeletal muscle during tetanic contractions (Katz, 1939; Hill, 1970; Flitney & Hirst, 1975;

Edman, Elzinga & Noble, 1976). Our findings resemble Hill's results (Hill, 1970, p. 66) where the tension plateau produced by a stretch was on the average 14% higher (compare our Fig. 6) than the isometric tension of a contraction at the same length; this excess of tension was ascribed by Hill to the contractile machinery getting locked in a strained condition. However, whereas in skeletal muscle supra-isometric forces were sustained throughout the stimulation period, this was clearly not the case for cardiac muscle: even when tetanized, sustainment of supra-isometric loads at the expense of a slow delayed isotonic lengthening is limited as this latter slow lengthening (phase 2) leads to a final faster isotonic lengthening (phase 3); this faster yielding is always followed by an abrupt fall in isometric force reflecting a breakdown of mechanical activity. Though obtained by changes of load rather than length, this maximal supra-isometric force potential during isotonic overloading resembles in some way the responses obtained by stretching isometrically contracting cardiac muscle (Brady, 1965, 1968, 1971).

2. *Nature of the delayed lengthening during isotonic overloading*

Isotonic overloading resulted in three step yielding of the muscle, consisting in an initial fast lengthening (phase 1), a second slower viscous-like delayed lengthening (phase 2) followed by a final faster isotonic 'give' (phase 3). Distinct phases 2 and 3 were always present in tetanic contractions, but in twitch contractions they could only be clearly distinguished at infra-isometric force levels and at lower temperatures (Housmans & Brutsaert, 1976). The initial fast lengthening of the muscle accompanying the load step itself was proposed to represent instantaneous extension of undamped series compliance. Series compliance in cardiac muscle includes damaged tissue adjacent to the clip, branching, spiral arrangement of the fibres and shearing through Z-lines and intercalated disks (Muir, 1965; Abbott & Gordon, 1975; Julian & Sollins, 1975; Krueger & Pollack, 1975). Besides these non-crossbridge sources of series compliance, extension of the more compliant portion of the crossbridges could also take part in this initial fast lengthening, thereby dragging strongly attached crossbridges to higher force producing positions (Simmons & Jewell, 1974) and detaching loosely bound crossbridges, so that larger loads could be sustained by an even smaller number of attached crossbridges (Huxley & Simmons, 1971). The subsequent slower phase 2 and 3 lengthening at a constant load might possibly reflect back rotation of attached crossbridges to their first point of attachment, and then finally detachment (Housmans & Brutsaert, 1976). The same sequence of crossbridge events could be considered as the mechanical basis for the delayed lengthening during load clamps exceeding isometric force, and, hence, as the mechanism underlying the ability of cardiac muscle to resist supra-isometric loads. Maximal supra-isometric force responses would then represent the maximal force potential generated by crossbridges that still remained attached immediately after the load step. Because of detachment of loosely bound crossbridges already during phase 1, the experimentally measured maximal force levels (P'_0) slightly underestimated the true 'maximal force potential' of all crossbridges attached at any time for a given state of contractility. From the confirmation of a supra-isometric force potential during steady-state tetanic contractions it would follow that these supra-isometric forces do not simply result from recruitment of additional crossbridges due to supplementary calcium release induced by the load clamp procedure itself or by the concomitant fast lengthening (Endo, 1972; Ridgway & Gordon, 1975; Steiger, Brady & Tan, 1976) or due to changes of action potential duration (Kaufmann *et al.* 1971).

3. *Isotonic relaxation of mammalian cardiac muscle*

The initial slow lengthening at supra-isometric force levels during isotonic relaxation reflected fused phase 2 and 3 delayed lengthening and might thus also be interpreted in terms of crossbridge mechanisms. An increasing number of detaching crossbridges would leave a decreasing number of attached crossbridges to resist the same load for some time by back rotation and eventual detachment, thus allowing for supra-isometric forces to be resisted and for sliding of actin and myosin filaments back to their original position. Back rotation of crossbridges and sliding of the filaments ought not necessarily to be considered as two successive events of the entire muscle owing to possible spatial and temporal heterogeneity of relaxation in different regions along the muscle. Detachment of crossbridges is substantiated by the abrupt fall of force during subsequent isometric relaxation.

The small amount of shortening, which postponed isotonic relaxation in heavily afterloaded twitch contractions that were unloaded at the onset of the expected control isotonic relaxation, might then be interpreted as a pivoting movement of attached crossbridges, i.e. a forward rotation lifting the diminished load followed by back rotation and final detachment of the crossbridges, again allowing for supra-isometric force levels to be maintained at that time.

4. *Time independence of the force–velocity–length interrelationship in cardiac muscle*

Previous studies (Brutsaert & Sonnenblick, 1969; Brutsaert *et al.* 1971; Brutsaert, 1974) have shown that the velocity of shortening for a given total load is a unique function of instantaneous length during external shortening, regardless of the initial muscle length and regardless of time from the onset of external shortening till just prior to peak shortening. Although this time independent force–velocity–length interrelationship was originally described in the isolated papillary muscle preparation and ascribed to a complex interaction of multiple activating, inactivating and viscous loading factors (Brutsaert, 1974; Brady, 1974), this concept was recently confirmed in cardiac cellular preparations with non-functional or partially skinned sarcolemma which were directly activated by iontophoretically released calcium ions, thus bypassing any significant control from the sarcoplasmic reticulum (De Clerck, Claes & Brutsaert, 1977). This would imply that a time independent force–velocity–length relationship could, instead, be a property of the actomyosin interaction. Time independence of velocity–length relations after abrupt alterations of load might then reflect structural changes in attached crossbridges similar to those discussed above.

Load clamped contractions, with large loading steps during the early part of external shortening or with small loading steps late in the isotonic phase, failed to demonstrate time-independent matching of the velocity–length pathways of the clamped and of the control contraction. The concomitant delayed lengthening might then again reflect back rotation and forcible breaking of crossbridges. Load clamps which revealed a time independent force–velocity–length relation during a major portion of external shortening, hardly showed any visible yielding; this might be attributed to the fact that the adjustment of attached crossbridges to the new load is dominated by a continuing gain of attaching crossbridges. Time-dependence in the earliest part of the contraction (Brutsaert, 1974) might then be mainly determined by the dominant effect of rising activation. This view is indirectly substantiated by conditions of delayed activation, as e.g. after the addition of caffeine (Henderson, Claes & Brutsaert, 1973) or after the substitution of calcium by strontium (Brutsaert & Claes, 1974) in mammalian cardiac muscle or as in frog heart muscle (Henderson & Brutsaert, 1974), whereby the phenomenon of time independence was proportionally retarded. Once activation for a given state of contractility is fully developed and enough force is generated to carry a given load, large load clamps would be required for yielding of attached crossbridges to predominate over continuing binding of additional crossbridges to fully activated sites; only small increases of total load would be required to reveal this yielding effect at later times, when attachment of additional crossbridges would become less probable. In conclusion, the time-independent force–velocity–length interrelationship in mammalian cardiac muscle after moderate load clamps can thus at least in part be explained by some degree of tenacity of attached crossbridges, being overwhelmed at early times by the dominant effects of activation when additional cross bridge attachment is still proceeding and balancing the diminished degree of activation during late isotonic shortening.

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