

THE INFLUENCE OF MUSCLE LENGTH ON THE DEVELOPMENT OF FATIGUE IN TOAD SARTORIUS

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SUMMARY

1. Fatigue curves were obtained on muscles contracting at length (L) different from the optimal (L_0), and performing isometric tetani of fixed duration at regular intervals. Every sixth tetanus occurred at L_0 , the remainder took place at L . The parameters of the fatigue curve were compared with those of controls contracting always at L_0 . Fatigability was measured as the coefficient of exponential decrease of tension with time.

2. Fatigability varied linearly with length in shortened and lengthened muscles, but the slopes of the relations were significantly different for the two groups.

3. The tension values from single experimental muscles fell on two independent curves, one for those obtained at L_0 , another for data at L . The same muscle showed, therefore, two different fatigue processes which followed independent temporal evolutions.

4. Points 2 and 3 of this Summary imply that fatigue is a process dependent on the actual setting of the myofilaments during contraction and not distributed uniformly within the sarcomere.

5. It is proposed that fatigue results from local changes taking place at discrete reactive points in the myofilament. The experimental results are discussed in terms of this hypothesis and of the sliding model of contraction.

INTRODUCTION

In a previous paper (Aljure & Borrero, 1968) we presented an equation describing the development of fatigue in isolated toad sartorii performing isometric tetani of fixed duration at regular intervals. The equation was $T\% = (100 - b)e^{-kt} + b$ where b and k are constants for a given set of experimental conditions. In those experiments the two muscles of an animal, when submitted to the same procedures, behaved almost identically,

permitting the use of one as a control in experiments designed to establish the role of a given variable in the development of fatigue.

Reasons have been given for centring the study of the mechanisms of fatigue on the excitation-contraction coupling (ECC) or the contractile machinery rather than on the process of excitation (Eberstein & Sandow, 1963; Weber, Herz & Reiss, 1964; Aljure & Borrero, 1968). The dependence of fatigue upon the contractile activity of muscle can be studied ideally by comparing the development of fatigue in pairs of muscles acting under identical conditions for excitation and ECC but differing in the operation of the contractile mechanism. Advantage can be taken of the well-established dependence of extratension development on muscle length in isometric contraction (cf. Fenn, 1925). This is apparently due to changes in the operation of the contractile machinery, and is not associated with major alterations in the electrical behaviour of the cell membrane or in the ECC mechanism. It should be mentioned, however, that changes in resting length are associated with modifications in the rate of tension development during isometric contraction (Jewell & Wilkie, 1958, 1960), not explained on the basis of changes in the load-velocity curve of the contractile element or on the stress-strain relation of the series-elastic component, hence, probably related to some change in ECC.

This paper describes a systematic exploration of the effects of changing muscle length on the development of fatigue during a series of isometric contractions.

METHODS

The apparatus, solutions and procedures used have been described in detail and their limitations have been discussed (Aljure & Borrero, 1968). Briefly, curarized (final concentration of tubocurarine, 0.88 mg/l.) sartorii of *Bufo marinus* were stimulated at room temperature (21–25° C.) with square wave pulses of 1 msec duration, at 40 c/s, given every minute in trains of 2 sec duration. Contraction was isometric and tension was recorded with a strain-gauge transducer and ink writing polygraph. Between contractions the muscles were immersed in Ringer solution through which air was bubbled continuously; during tetani the muscles were exposed to air.

Muscle length was adjusted with the aid of a rack-and-pinion system. On the basis of the dimensions of the supporting apparatus and the distance between markers attached to its movable parts, it was possible to determine muscle length. Mechanical stops were placed so that length could be adjusted rapidly and in a reproducible way to either the length at which tension was maximum (L_0) or to another previously selected length (L).

L_0 was determined for each muscle by measuring extratension developed following a single stimulating pulse at successive adjustments of the rack-and-pinion system. Single pulses were used to avoid development of fatigue before the experimental period. Starting with a slack muscle, the length was gradually increased until the extratension went through a maximum and showed a very slight decrease; at this stage the length was carefully reduced until the maximum tension was reproduced. The limitations of this procedure are discussed under Results.

Maximal or moderately supramaximal stimuli were used throughout. In adjusting the

voltage it was often found that once a stable level of extratension was reached, further increases in voltage produced unpredictable changes in extratension, frequently associated with slow relaxation. As the last phenomenon was generally linked with small repetitive discharges of the muscle, the stimulating voltage was usually adjusted under oscilloscopic control of the muscle action potentials. Stimuli clearly maximal, yet not producing secondary discharge, were selected.

In a typical experiment the control muscle was only stimulated at L_0 and the experimental muscle at L . In order to compare the development of fatigue, every sixth tetanus was carried out at L_0 in both muscles. During the interval between tetani both muscles were held at L_0 (cf. Fig. 1). In order to obtain working conditions differing for the two muscles only in the variable studied, care was taken to submit both of them throughout the experiment to departures from L_0 comparable in magnitude and duration. The experiments were continued until both muscles were near steady state, as judged by small variations of extratension in successive tetani. In general, this required about 1 hr of repeated tetanization.

Pairs of muscles from eighty-three toads (60–260 g) were used for this work; seventy-seven pairs of these muscles were employed for the study of relations between fatigability and muscle length; the remainder provided only collateral information. Muscle lengths ranged from 19.9 to 40.1 mm and the mean extratension developed at L_0 was 1967 g/cm² (± 67 s.e. of mean) in a group of twenty-five muscles taken at random (weights ranging from 111 to 310 mg). The study extended over a period of fourteen months and no evidence of seasonal variation was found.

RESULTS

Data were obtained from records like the one shown in Fig. 1A from which three separate graphs of the temporal evolution of extratension were derived, expressing tension as a percentage of the initial values at L_0 . One of the graphs corresponded to the control muscle, always contracting at L_0 , and the others to the experimental muscle, which contracted at either L or L_0 . From these graphs, plots of $\log (T\% - b)$ against t were obtained; the slope of each line (k) measured fatigability.

When measurements were taken at L_0 , muscles contracting at short lengths had much smaller fatigability than the controls (Fig. 1B) whereas in those stretched beyond L_0 , fatigability approached the control values (Fig. 1C). The temporal evolution of fatigue was almost identical for two muscles of the same animal contracting all the time at L_0 .

Active tension measured at L evolved following the equation reported for muscles contracting at L_0 , although there were important differences in the initial part of the curves and in the values of the parameters b and k (Fig. 1D, E). This last finding was puzzling because it implied that for a given muscle two different estimates of fatigability could be obtained, depending on whether the data at L or at L_0 were used.

In the study of the dependence of fatigue on muscle length each pair of muscles provided information for only one length; it was therefore necessary to pool results obtained from different animals. For the purpose of minimizing the influence of variability in muscle size, lengths were expressed as L/L_0 and ranged from 0.45 to 1.50. Greater lengths were avoided

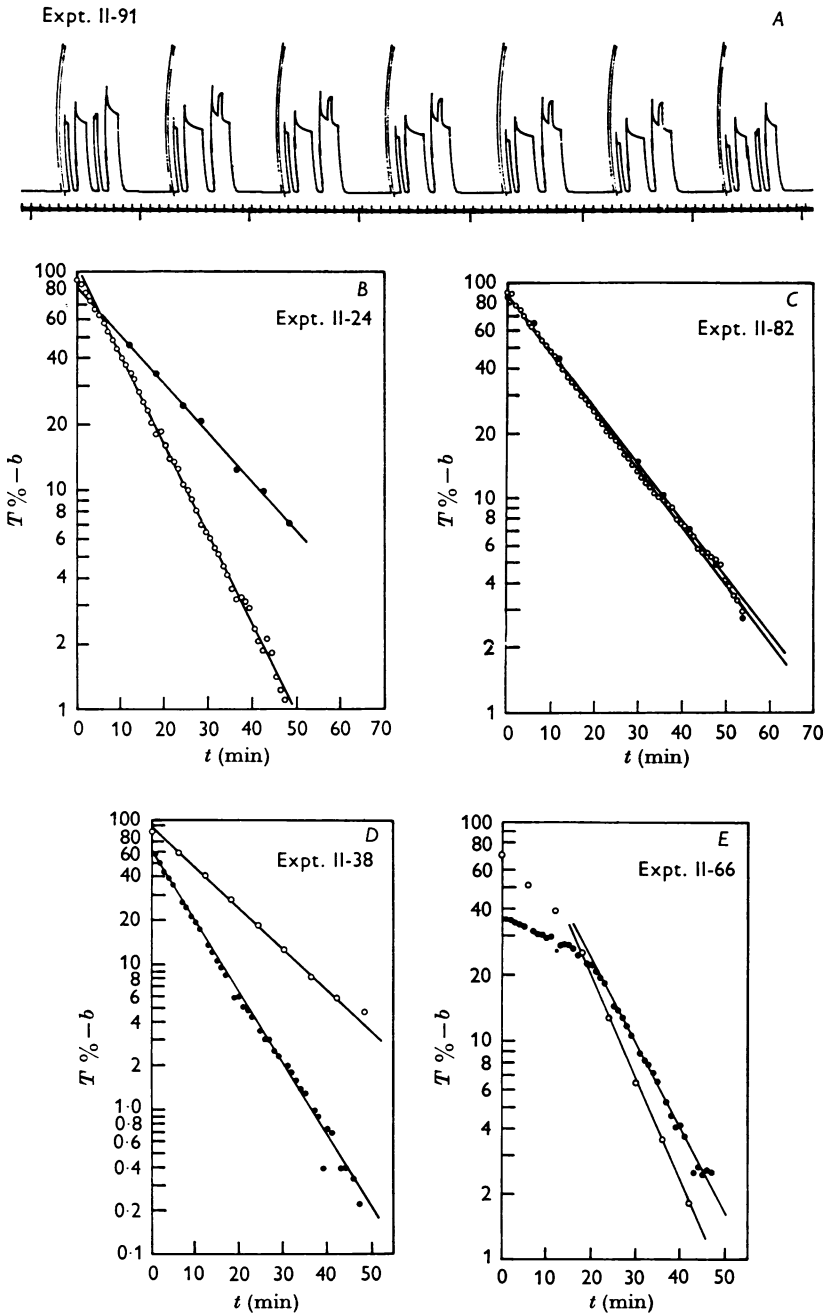


Fig. 1. For legend see opposite page.

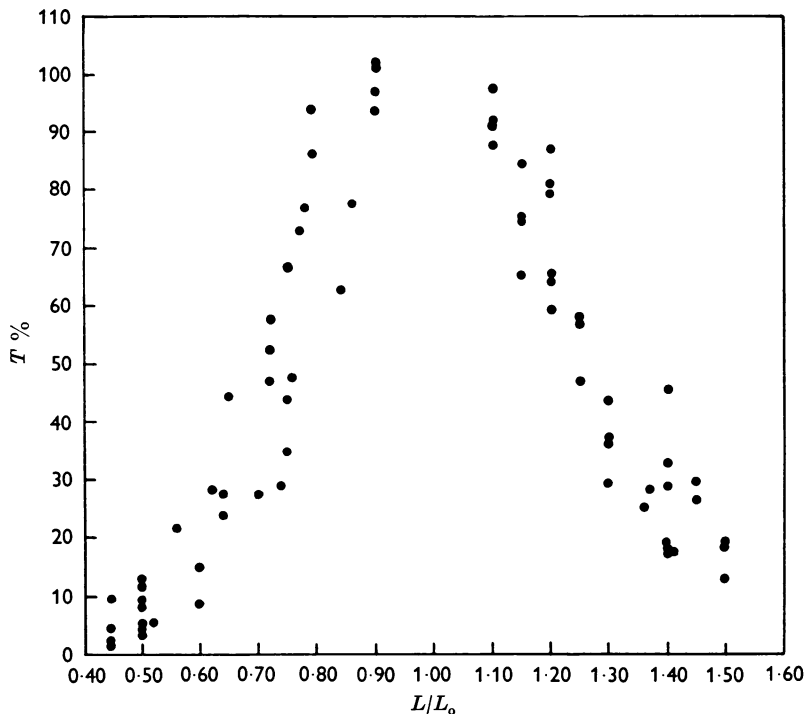


Fig. 2. Length-active tension diagram. Length is expressed as L/L_0 . Active tension is calculated as percentage of the maximum tetanic tension developed by the muscle at L_0 , right at the start of the fatiguing period. Results from seventy-four experiments are included. Each muscle contributed information for only one length. $L/L_0 = 0.62$ is a point of inflexion as judged by the fact that linear regression equations for the regions with abscissae 0.45 to 0.60 and 0.64 to 0.90 cross at that point.

Legend to Fig. 1.

Fig. 1. *A*, upper trace, sample of tension records in one experiment in which the muscles were lengthened to $1.50 L_0$. Cycles of operation are repeated every minute and include successively calibration signal, contraction of the control muscle at L_0 , passive distension of the control muscle and, in all but the first and last cycles presented, contraction of the experimental muscle during stretch at L . In the first and last cycles shown the control muscle is handled as described, whereas the experimental muscle contracts at L_0 and afterwards is passively distended to L . Lower trace, time; downward deflexions every one minute. *B* to *E*, fatigue curves for shortened and lengthened muscles. Abscissa, time in min. Ordinate, extratension (T) % minus b in a logarithmic scale. Data obtained at L_0 appear in *B* for a muscle shortened to $0.64 L_0$ (filled circles) and in *C* for one lengthened to $1.50 L_0$ (filled circles); in both, open circles represent controls. *D* is a comparison of fatigue measurements obtained in one muscle at L_0 (open circles) with those obtained at $L = 1.25 L_0$ (filled circles). *E* was obtained from another muscle which contracted either at L_0 (open circles) or at $L = 0.75 L_0$ (filled circles).

because of the danger of irreversible changes in the muscles. When lengths differed from L_0 , initial extratension decreased (Fig. 2) according to the length-tension relation (cf. Fenn, 1925).

The fatigability of different muscles was compared by using the ratios of experimental to control values (k_E/k_C), additional subscripts indicating whether the data for the experimental muscles were obtained at L or at L_0 .

Figure 3*A* shows the data at L_0 obtained in fifty-five experiments. For shortened muscles there was a significant correlation between fatigability and muscle length. The dependence between the two variables was much smaller for $L/L_0 > 1$; in fact, the correlation coefficient was not statistically significant (Table 1).

Figure 3*B* is a summary of the data at L obtained on the same muscles used for Fig. 3*A*. Only forty-two experiments appear because some of the extreme cases of lengthening and shortening were excluded due to the difficulty of accurately measuring extratensions; these were very low at the start and further decreased in the process of fatigue. The data followed a regularly decreasing function with a change of slope at about $L/L_0 = 1$. The correlation coefficient between fatigability and muscle length was statistically significant for lengthened but not for shortened muscles (Table 1). This graph is very different from that of Fig. 3*A*; thus, for shortened muscles fatigability was lower than that of the controls if measured on data at L_0 and greater if calculated from values at L . For muscles contracting at lengths beyond L_0 , fatigability was lower than that of the controls, as judged by either criterion, but the data at L were markedly lower than those at L_0 .

Fatigability of shortened muscles was significantly different from that of lengthened muscles (compare equations (1) with (2) and (3) with (4) in Table 1). Fatigability at L_0 was significantly different from that at L (compare equations (1) with (3) and (2) with (4) in Table 1).

In the plot of Fig. 2 each muscle gave data for only one length. Nevertheless, the graph is similar to those obtained in the frog, on single whole muscles (see, for example, Aubert, Roquet & Van der Elst, 1951), on single fibres (Ramsey & Street, 1940) and on individual sarcomeres (Gordon, Huxley & Julian, 1966). This agreement and the rather small dispersion of the data give confidence in our measurements of L_0 . It has been shown on mammalian muscles that the length-tension diagrams obtained with twitches and with tetani are not identical (Bahler, Fales & Zierler, 1967); therefore the abscissae of Figs. 2 and 3 may be somewhat in error. Because the maximum of Fig. 2 corresponds approximately to abscissa 1.00, the error, if present, must be small and would not change substantially the curves of fatigability against length. For this reason and the fact that

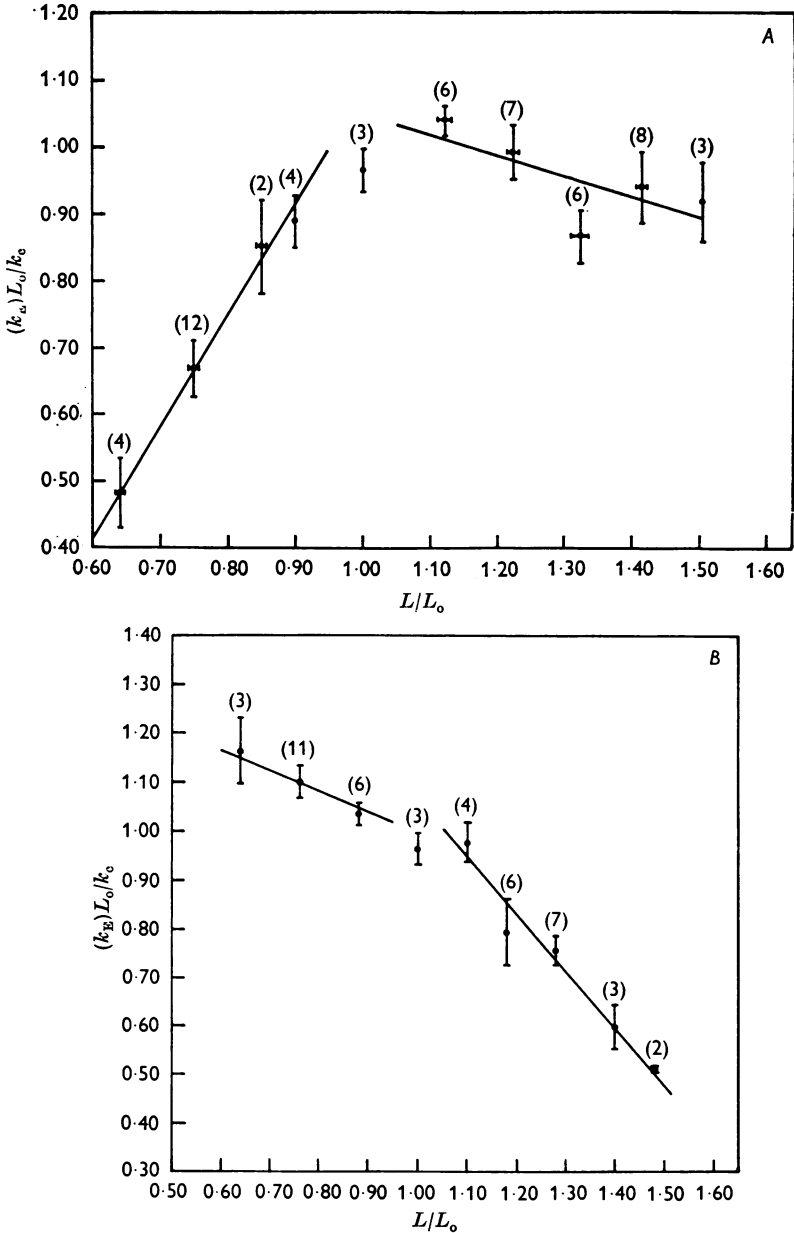


Fig. 3. Correlation between fatigability and muscle length. Abscissae, L/L_0 . Ordinate, ratio of values of k for experimental muscles and for controls. Each point is the average of several experiments (number in parentheses). The bars represent plus or minus one standard error of the mean. The regression lines were calculated by least squares excluding the muscles in which $L = L_0$. A, data at L_0 from fifty-five experiments; B, data at L from forty-two of the experiments included in A.

pertinent data for the toad are not available, no attempt has been made to correct the values of L/L_0 .

Stretching of the muscles did not affect fatigability because in six experiments in which both muscles contracted always at L_0 but one of them underwent periodic stretches to lengths ranging from 1.32 L_0 to 1.53 L_0 , mean values of k did not differ significantly ($\bar{k} = -0.09650 \pm 0.00511$ (S.E.) sec^{-1} for stretched muscles; $\bar{k} = -0.08930 \pm 0.01019$ (S.E.)

TABLE 1

			s.e. of estimate	r	P
Data at L_0					
$L/L_0 < 1$	$y = -0.588 + 1.6684x$	(1)	0.035	0.7940	≤ 0.01
$L/L_0 > 1$	$y = 1.363 - 0.3127x$	(2)	0.121	0.2933	> 0.10
Data at L					
$L/L_0 < 1$	$y = 1.413 - 0.4136x$	(3)	0.077	0.3247	> 0.10
$L/L_0 > 1$	$y = 2.250 - 1.1840x$	(4)	0.120	0.7559	≤ 0.01

y stands for k_E/k_C and x for L/L_0 . r is the correlation coefficient and the significance of its difference with respect to zero was established by the t test. The coefficients of equations (1) and (2) differ significantly from one another; the same is true for the pairs of equations (3) and (4), (1) and (3) and finally for (2) and (4). In all cases $P \leq 0.01$ as calculated by the F test (cf. Johnston, 1960).

sec^{-1} for unstretched muscles; $P > 0.5$). Furthermore, in the main series, where the stretch varied from experiment to experiment, there was no systematic change in the values of k for the controls, except perhaps for small decreases when $L/L_0 > 1.40$.

The dependence of fatigability on length was studied down to $L/L_0 = 0.45$, but in the experiments in which length was very small the correlation broke down, probably due to the appearance of delta state (Ramsey & Street, 1940). In fact, for markedly shortened muscles the initial fall of tension was much faster than in the controls. The drop of tension in the first 6 min (ΔT) was measured and a graph of

$$100 \times \frac{\Delta T_{\text{control}} - \Delta T_{\text{experiment}}}{\Delta T_{\text{control}}} \text{ against } L/L_0$$

presented an abrupt change of magnitude and sign of the slope at $L/L_0 = 0.62$, indicating that an additional factor had come into play. On this basis, sixteen experiments performed with $L/L_0 < 0.62$ were discarded. A modification of behaviour is also shown in the length-tension diagram of Fig. 2 which, similarly to Ramsey & Street's (1940), changes slope in the region of $L/L_0 = 0.60$ to 0.64.

In the present series of experiments the initial part of the fatigue curves at both L_0 and L is non-exponential as has been observed previously (Aljure & Borrero, 1968). At L_0 the duration of the non-exponential phase

did not depend on muscle length, whereas at L it increased regularly from zero for the most shortened muscles, to values exceeding those of the controls, for the most elongated. For the latter, fatigue was hardly recognizable in the non-exponential phase of the data at L .

DISCUSSION

Fatigue in an isolated muscle could be due either to failure in the ECC mechanism or to disruption in the operation of the contractile machinery itself, failure of the excitable component being probably of minor importance. In particular, disturbances in the handling of Ca^{2+} , depletion of energy sources and accumulation of metabolites could be implied. The changes in fatigability associated with variations in muscle length, reported above, could arise from the known influences of muscle length on tension development, phosphorylcreatine (PC) splitting and rate of heat production, or from yet unknown mechanisms.

Active tension does not seem to be an important factor of fatigue since reducing the initial tension to similar fractions of the maximum by adjusting muscle length to values above and below L_0 lengthened and shortened muscles fatigued at very different rates. Furthermore, in the case of the data at L , fatigability of the most shortened muscles, that were developing very little tension, even exceeded that of the controls.

It is unlikely that our results could be explained by depletion of energy sources because the relations found between PC splitting or rate of heat production with muscle length (Abbott, 1951; Aubert, 1956; Infante, Klaupiks & Davies, 1964; Sandberg, 1965) are entirely different from those relating fatigability and muscle length. The statement about PC splitting applies to both total splitting and to the component, independent from tension, that varies with length (Sandberg, 1965).

The finding of different values of fatigability on the same muscle, depending on whether data at L_0 or at L are used for the calculations, indicates that neither value is a complete measure of this phenomenon. It also implies that 'fatigue' is not distributed uniformly throughout the contractile structure and that, therefore, it cannot be due entirely to factors affecting evenly the sarcomere as would be probably the case with diffuse depletion of energy sources or accumulation of metabolites. It may be inferred that fatigue derives, at least in part, from local changes taking place at discrete points within the sarcomere.

According to current views about ECC (Sandow, 1965) both liberation and removal of calcium have generalized effects on the sarcomere. Thus it seems unlikely that alteration of these mechanisms are major factors in the development of fatigabilities that depend on length, yet follow inde-

pendent temporal evolutions when a single muscle contracts alternatively at two different lengths. A similar argument applies to the postulated effect of calcium trapping (Weber *et al.* 1964).

A tentative hypothesis to explain the relations between fatigability and muscle length can be constructed on the following basis: tension development is due to the activity of the actin-myosin bridges included in the zone of overlap (A. F. Huxley, 1957; H. E. Huxley, 1960). Fatigue derives

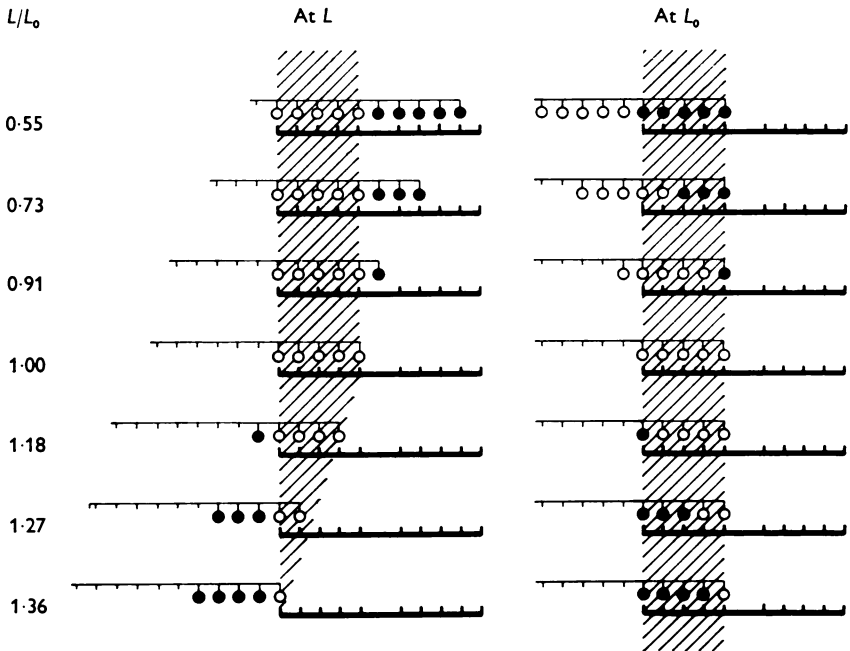


Fig. 4. Schematic representation of the relative positions of the reactive points involved in contraction at L (left-hand side) and at L_0 (right-hand side). Shaded, zone of overlap between thin filaments and the corresponding half of the thick filaments. Open circles, points of high fatigability due to their participation in most tetani. Filled circles, points of low fatigability. To test the hypothesis, 'fatigabilities' were computed as $k = \alpha N_1 + \beta N_2$, N_1 and N_2 being the number of points of low and high fatigability, respectively: β was taken as $1/3 \alpha$, based on the previously reported relation between k and mean rate of stimulation (Aljure & Borrero, 1968).

from local changes taking place at the reactive points of the thin filaments included in the zone of overlap and is a function of the frequency of their participation in contraction (Aljure & Borrero, 1968). These points are of two kinds: some that in most contractions are located within this zone and some brought to it only in a few tetani. Fatigability measured at a given length would depend upon number and kind of points operating at that length.

Applying this hypothesis to the data derivable from Fig. 4, 'fatigability' was calculated for different muscle lengths at both L and L_0 . The numerical values obtained followed relations similar to those of Fig. 3. The only gross disagreement with the experimental findings consisted in the prediction of rather low fatigabilities for elongated muscles at L_0 . A similar analysis carried out assuming that the points undergoing fatigue are located on the myosin filament conflicts with all the results at L_0 .

The hypothesis proposed does not exclude the existence of other fatiguing processes, nor does it clarify the mechanism of the events taking place at the reactive points during fatigue. The suggested independence between reactive points does not conflict with the possibility of these points having access to a common energy pool. Fatigue would depend on local, perhaps structural changes. Recovery, and the attainment of a steady state (Aljure & Borrero, 1968) would result from relations between general energy supply and these local changes; exchanges between neighbouring reactive points would be at least limited.

The non-exponential phase of the fatigue curve most probably depends on factors different from those considered in this paper. The following comments seem pertinent. Active tension in a muscle contracting at L depends on the difference between L and L_0 , and should vary if either term changes. Increases in L_0 associated with stretch or contraction have been reported for muscles working at standard or higher lengths (Aubert *et al.* 1951; Reichel, 1960). In a study yet unpublished we have confirmed these reports and found that in the course of fatiguing procedures similar to those employed in this work, there are early and progressive increases in L_0 , even for muscles contracting at short lengths. These changes would tend to slow down the fall in active tension of lengthened muscles and to speed up that of shortened. It should be mentioned, however, that the magnitude and evolution of the changes of L_0 we have encountered could only explain a small fraction of the difference in fatigability between experimental and control muscles.

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