The Dependence of Rigor Tension on Sarcomere Length in Vertebrate Muscle

It is no longer a matter of controversy that the active, isometric contractile tension produced by skeletal muscle is a function of the sarcomere length of that muscle. RAMSAY and STREET[1] first demonstrated that there was a linear decline in the tension when muscle was stretched from rest length, and GORDON et al.[2] were able to show that the tension was reduced to zero when the overlap of thick and thin filaments was also zero. This classic length-tension relationship has since been demonstrated for both actively contracting skinned[3] and glycerol-extracted[4] skeletal muscle fibres but has never been demonstrated for the tension produced in a muscle fibre when it passes from the relaxed to the rigor state. This then is the subject of this report.

Methods. We have chosen single, glycerinated[5] rabbit psoas muscle fibres as our experimental object because of the reversibility and ease with which such fibres can be induced into rigor (KCl 50 m*M*; *Tris*-maleate 10 m*M*; pH 6.5) and relaxation (rigor solution plus ATP 5 m*M*; $MgCl_2$ 5 m*M*; EGTA[6] 2 m*M*). Our experimental techniques involve simultaneous measurement of the fibre tension, dynamic stiffness (the tension oscillation produced in response to a 0.2%, 4 Hz length oscillation) and the sarcomere length (derived from the first order diffraction line of a 2 mW He-Ne laser) in a manner essentially the same as that previously described[7].

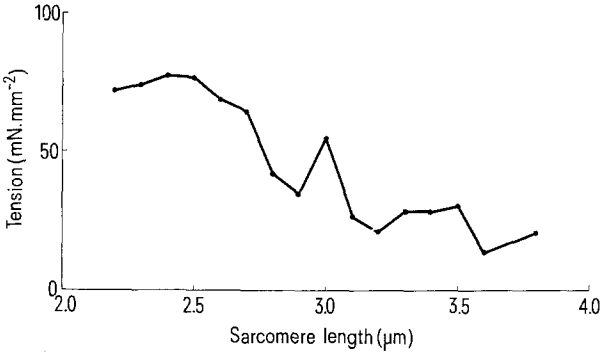

Fig. 1. Rigor tension (developed in excess of relaxed tension) from a single glycerinated psoas fibre plotted as a function of the sarcomere length. Each point represents the mean of 3 rigor tensions. The fibre was stretched in the relaxing solution and sarcomere lengths were determined from the first order diffraction lines of a He-Ne laser.

Fig. 2. The rigor tensions developed following the relaxing solution on 26 consecutive occasions. Preparation is a single glycerinated psoas fibre with a sarcomere length of 2.4 μm.

Variations in sarcomere length measurements include the width of the first order diffraction line at any point along the length of the fibre, (maximum ± 0.02 μm) as well as variations (± 0.15 μm) along the length of the fibre (about 1 cm) where measurements were taken every millimeter. Fibres which exceeded these requirements were discarded.

Results and discussion. Figure 1 demonstrates the typical effect of progressive stretch on the magnitude of the rigor tension where successive stretches were applied to a single muscle fibre in the relaxed state. From this figure it can be seen that there is little variation in the rigor tension at sarcomere lengths from 2.2 μm to about 2.6 μm, and then there is a dramatic drop in the tension as the sarcomere length is increased. This plateau and then the decline in the rigor tension cannot be attributed to the repeated cycling of the muscle fibres through the relaxed and rigor states, for this manoeuvre when performed without changing the sarcomere length produced no significant change in the tension (Figure 2). When the same kind of experiment was attempted on fibres where the initial sarcomere length was reduced before glycerination by electrical stimulation of the fibre bundle, we were unable to demonstrate a decrease in rigor tension at lengths below rest length similar to that seen in living fibres[2]. Instead, the progressive stretch produced little or no change in the rigor tension until a sarcomere length of about 2.5 μm was reached, and then the tension declined in much the same way as is illustrated in Figure 1. This suggests that some kind of irreversible damage occurs within the sarcomere (probably the thick filaments) when the sarcomeres are shortened to less than rest length and then stored in the glycerolextracting solution.

We noticed that the rigor tensions in those fibres which had been glycerinated at lengths shorter than rest length appeared to conform to the pattern seen in the sarcomere length-active tension curve[2], i.e. the shorter the sarcomere below rest length, the smaller the rigor tension. The experiments summarized in this figure were performed as follows: the sarcomere length of each fibre was measured and the mean of the first 3 rigor tensions at that length was recorded. The fibre was then discarded and a new fibre measured. Thus, these experiments (unlike the one represented in Figure 1) involved no stretching of fibres, and only in some cases initial shortening prior to glycerination. Each data point represents the mean of those experiments whose sarcomere lengths fell into a 0.1 μm interval. The error bars represent the standard errors of the means. 51 different fibres are represented of which 15 had initial sarcomere lengths shorter than rest length. Since these results were obtained from many fibres, an additional error is introduced, namely, the error resulting from the measurement of the fibre diameter. All cross-sectional areas were estimated from fibre diameters

[1] R. W. RAMSAY and S. F. STREET, J. cell. comp. Physiol. *15*, 11 (1940).
[2] A. M. GORDON, H. E. HUXLEY and F. J. JULIAN, J. Physiol., Lond. *184*, 170 (1966).
[3] D. C. HELLAM and R. J. PODOLSKY, J. Physiol., Lond. *200*, 807 (1969).
[4] P. WARD, C. EDWARDS and E. BENSON, Proc. natn. Acad. Sci., USA *53*, 1377 (1965).
[5] E. ROME, J. molec. Biol. *65*, 331 (1972).
[6] EGTA: ethyleneglycol-bis-(β-amino ethyl ether)*N*, *N*′-tetra-acetic acid.
[7] C. DOS REMEDIOS, R. MILLIKAN and M. MORALES, J. gen. Physiol. *59*, 103 (1972).

measured at several points along the length of the fibre. It was assumed that the fibres had a circular cross-sectional profile, but this was rarely so. Nevertheless, within this framework of uncertainty, the linear regression of the experimental points in Figure 3 gave a slope of -55.7 with a correlation coefficient of 0.93. It is significant that the intercept of this slope with the abscissa occurs at 3.7 μm sarcomere length which compares favourably with the value of 3.8 μm that would be expected on the basis of PAGE and HUXLEY'S[8] electron microscope data. The sarcomere length at which the maximum tension was obtained was 2.5 μm which is in close agreement with the expected value of 2.4–2.5 μm. The expected plateau between the sarcomere lengths 2.2 and 2.6 μm (Figure 1) does not appear in Figure 3, possibly because of the sources of error mentioned above, particularly errors inherent in the measurement of rest length. The slope between these points is small, however, clearly smaller than that below 2.2 μm and above 2.6 μm. Variability in the region of the expected plateau and at sarcomere lengths shorter than rest length was so great that no attempt was made to calculate regressions in this area. Nevertheless, a rapid decline in the rigor tension at sarcomere lengths less than 2.2 μm is demonstrated. This feature agrees, at least qualitatively, with the active length tension diagram[1,2], and adds weight to our above mentioned suggestion that the glycerination procedure in some way results in a partial, yet irreversible, loss of function in those fibres which were glycerinated at shorter than rest length.

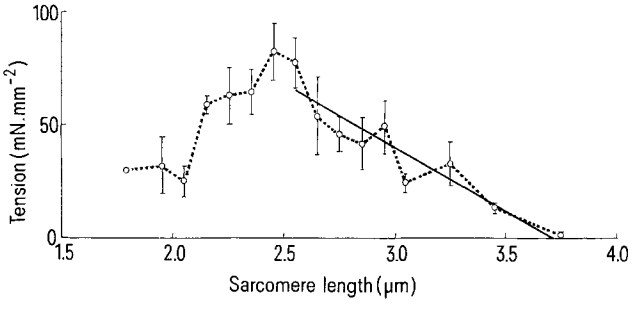

Fig. 3. Relation between the sarcomere length and rigor tension for 51 different single psoas fibres measured at their glycerinated length. Experimental points represent the means (± standard errors of the means) which have been joined by the broken line. The unbroken line represents the linear regression through the data points between 2.6 and 3.7 μm sarcomere lengths. The slope of this regression is -55.7 with a correlation coefficient of 0.93. The data represented in this figure were not obtained from stretch experiments such as in Figure 1. The experimental point at 1.8 μm sarcomere length was obtained from a single fibre. All other points represent the mean tension of more than one fibre.

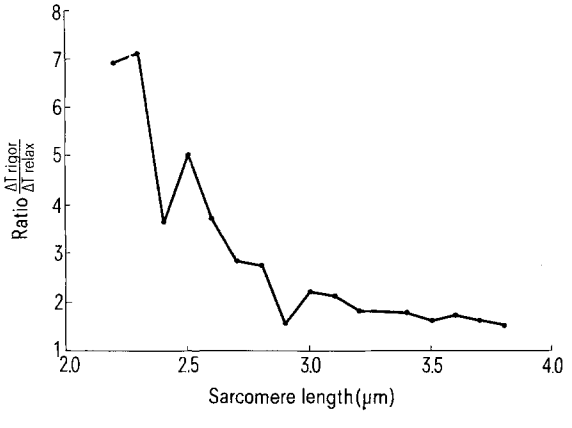

Fig. 4. The magnitude of the tension oscillations in rigor and relaxation in response to a constant sinusoidal length oscillation is plotted against sarcomere length. These data were obtained from a single glycerinated psoas fibre in the same experiment as shown in Figure 1.

The relationship between sarcomere length and dynamic stiffness is shown in Figure 4. Here, the sinusoidal length oscillation of constant magnitude produced markedly different oscillations in the tension in the two states. These data, which are in quantitative agreement with published data[7,9,10], were obtained in the same experiment as the data in Figure 1 and are consistent with the effects of a decrease in filament overlap as the sarcomere length is increased. The departure from linearity is due to an increase in the passive tension in these two states resulting from the progressive stretch.

It is not a novel observation that glycerinated[9,11] or living[7] muscle fibres develop a significant tension when rigor is induced from the relaxed state, and that this change in tension is accompanied by a substantial increase in the dynamic elastic modulus. However it has not previously been demonstrated that the magnitude of the rigor tension closely mirrors that of the active length-tension relationship. Our particular interest in the rigor length-tension relationship stems from proposals we have made[10] concerning the elementary chemical and mechanical stages of the contraction cycle where we envisage the rigor state as being part of the continuing cycle that normally occurs during active tension development. Two stages of this cycle are represented in our experiments. The first stage is relaxation where myosin (M) and actin (A) are dissociated in the presence of ATP (i.e. M-ATP + A) thus producing a low dynamic stiffness and low tension. This stage would predictably be much less dependent on the sarcomere length (i.e. the degree of filament overlap) than the rigor state where ATP is absent and the myosin and actin components are free to interact (i.e. M-A, no ATP). The manoeuvre of alternating the relaxing and rigor solutions should result in either the simple desorption of the ATP to produce the rigor state[11] or alternatively the myosin may hydrolyse the ATP (M-ATP + A) to ADP + Pi (M-ADP + A) followed by mechanical union with actin and desorption of the nucleotide (M-A). WHITE[9] argues convincingly that the ATP bound to myosin is hydrolyzed rather than desorbed during the transition from relaxation to rigor, and if this is so then we might predict that the relaxation-rigor cycles performed in the present experiments would reflect some of the properties of cycles of mechanical events experienced by the fibre during active contraction. One such property would be a similarity between the length-tension curves for both active and rigor tensions.

Indeed, the rigor state is accepted[12] as being the 'final' step in the active mechanical cycle of cross-bridge action on the basis of three-dimensional reconstructions of actin and myosin molecules interacting in the absence of ATP,

[8] S. PAGE and H. E. HUXLEY, J. Cell Biol. *19*, 369 (1973).
[9] D. C. S. WHITE, J. Physiol., Lond. *208*, 583 (1970).
[10] C. DOS REMEDIOS, R. YOUNT and M. MORALES, Proc. natn. Acad. Sci., USA *69*, 2542 (1973).
[11] H. J. KUHN, Experientia *29*, 1086 (1973).
[12] H. E. HUXLEY, Nature, Lond. *243*, 445 (1973).

.e. in the state of rigor. We have demonstrated a logical extension of the classic length tension relationship to the 'contraction' due to the rigor state, thereby drawing attention ot its dependence (amongst other things[7,9]) on the sarcomere length.

[13] We thank Dr. DARCY GILMOUR for discussions and comments. This work was supported by grants from the Australian Research Grants Committee and The National Heart Foundation of Australia.

Zusammenfassung. Bei Glycerin-extrahierten Fasern des Kaninchen-Psoas-Muskels wurde ein der normalen isometrischen Kontraktion ähnlicher Zusammenhang zwischen Sarkomerenlänge und -spannung für die Kaliumkontraktur festgestellt.

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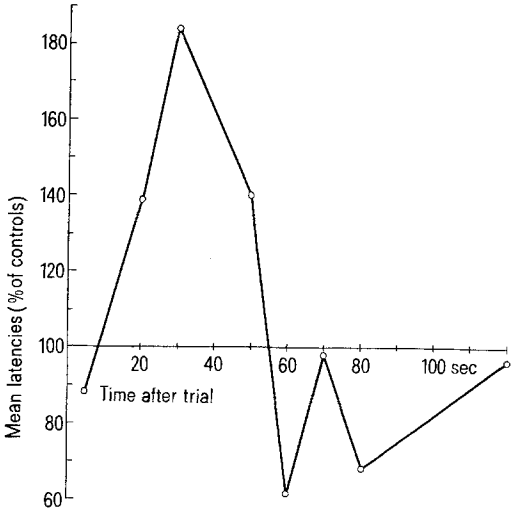
Facilitation of Learning by Reward of Post-Trial Memory Processes

A wide variety of treatments administered during a critical period following a learning experience have been shown to exert an effect on subsequent performance of the learned task. For example, memory can be disrupted by posttrial electroconvulsive shock[1-3], cortical and hippocampal spreading depression[4-6], drugs[7-9], brain stimulation[10-15], and other treatments; or can be facilitated by drugs[7,16,17] and by stimulation of the reticular formation[18,19] and perhaps other brain structures[20].

The critical post-trial period during which memory can thus be influenced has traditionally been considered to represent a period of memory 'consolidation' which is still widely thought to be coded in terms of active electrophysiological processes. Since various studies have shown that electrical activity of the brain can be directly controlled by operant conditioning procedures[21-27], we have hypothesized that memory consolidation per se can be brought under control of reward, and in the present study provide evidence that it can be reinforced by access to food. This hypothesis rests on the proposition that the 'period of consolidation', as defined by post-trial manipulations, reflects a labile, dynamic process which can be considered as a 'response', and thus susceptible to contingencies of reinforcement.



Effect of food reward on passive avoidance learning. Abscissa represents times after the conditioning trial when 1 min access to food was given to the experimental groups. The step-down latencies are expressed in terms of mean percent of each control group on the ordinate. (e.g. the 30 sec reward group remained on the platform 84% longer than its control group).

Materials and methods. The subjects were albino mice of the inbred C3H/He/Gif COB strain, outbred from Charles River Mouse Farms ICR COBS. They were kept in groups of 20 animals per cage with ad libitum access to food and water. They were kept at all times on a reversed 06.00–18.00 h 12 h light/dark cycle.

The passive avoidance step-down equipment consisted of a box with 50 × 50 cm high walls with a grid floor made of 6 mm diameter stainless steel bars spaced 13 mm apart (7 mm interbar distance). In the middle of the box was a 1 cm high, 67 mm diameter round wooden platform. Fitted over this platform was a removable 20 cm long, 68 mm diameter plastic tube. The electric foot shock across the grid consisted of a scrambled 1 sec duration

[1] S. L. CHOROVER and P. H. SCHILLER, J. comp. Physiol. Psychol. *61*, 34 (1966).
[2] J. A. DEUTSCH, in *The Physiological Basis of Memory* (Ed. J. A. DEUTSCH; Academic Press, New York 1973), p. 113.
[3] A. A. SPEVAK and M. D. SUBOSKI, Psychol. Bull. *72*, 66 (1969).
[4] H. H. AVIS and P. L. CARLTON, Science *161*, 73 (1968).
[5] J. BURES and O. BURESOVA, J. comp. Physiol. Psychol. *56*, 268 (1963).
[6] R. A. HUGHES, J. comp. Physiol. Psychol. *68*, 637 (1969).
[7] J. A. DEUTSCH, in *The Physiological Basis of Memory* (Ed. J. A. DEUTSCH; Academic Press, New York 1973), p. 59.
[8] M. E. JARVIK, A. Rev. Psychol. *23*, 457 (1972).
[9] D. QUARTERMAIN, B. S. MCEWEN and E. C. AZMITIA, Science *169*, 683 (1970).
[10] R. L. BRUNNER, R. R. ROSSI, R. M. STUTZ and R. G. ROTH, Psychon. Sci. *18*, 159 (1970).
[11] R. P. KESNER and R. W. DOTY, Expl Neurol. *21*, 58 (1968).
[12] J. H. MCDONOUGH and R. P. KESNER, J. comp. Physiol. Psychol. *77*, 171 (1971).
[13] A. B. ROBINS and G. T. THOMAS, Psychon. Sci. *12*, 291 (1968).
[14] A. ROUTTENBERG and N. HOLZMAN, Science *181*, 83 (1973).
[15] E. J. WYERS, H. V. S. PEEKE, J. S. WILLISTON and M. J. HERZ, Expl Neurol. *22*, 350 (1968).
[16] R. G. DAWSON and J. L. MCGAUGH, in *The Physiological Basis of Memory* (Ed. J. A. DEUTSCH; Academic Press, New York 1973), p. 78.
[17] J. L. MCGAUGH, A. Rev. Pharmac. *13*, 229 (1973).
[18] V. BLOCH, Brain Res. *24*, 561 (1970).
[19] A. DENTI, J. L. MCGAUGH, P. W. LANDFIELD and P. G. SHINKMAN, Physiol. Behav. *5*, 659 (1970).
[20] C. K. ERICKSON and J. B. PATEL, J. comp. Physiol. Psychol. *68*, 400 (1969).
[21] A. H. BLACK, in *The Psychology of Learning and Motivation* (Ed. G. H. BOWER; Academic Press, New York 1972), vol. 6, p. 47.
[22] E. E. FETZ, Science *163*, 955 (1968).
[23] E. E. FETZ and D. V. FINOCCHIO, Science *174*, 431 (1971).
[24] S. S. FOX and A. P. RUDELL, Science *162*, 1299 (1968).
[25] J. OLDS, 23rd Int. Congr. Physiol. Sci., Tokyo 1965, p. 372.
[26] J. P. ROSENFELD, A. P. RUDELL and S. S. FOX, Science *165*, 821 (1969).
[27] W. WYRWICKA and M. B. STERMAN, Physiol. Behav. *3*, 703 (1968).