Reprinted from
PHYSICS IN MEDICINE AND BIOLOGY, VOL. 9, NO. 4, p. 559, October 1964

Muscle Twitch Tension—Influence of Electrical Stimulating Conditions at Different Temperatures

II. Results For Nitrate Ringer's

By ELIZABETH KELLY and WILLIAM J. FRY
Biophysical Research Laboratory,
University of Illinois,
Urbana, Illinois

(*Received* 13 *August* 1963)

§ 1. INTRODUCTION

THE first paper of this series (Kelly, Fry and Fry 1964) presents evidence that for temperatures above 18°C, the amplitude of 'peak' tension developed by an excised frog sartorius muscle in response to a single 'supermaximal' stimulus increases as the stimulus pulse duration is extended in the range 0·2 to 10·0 msec. It was noted in this paper that the single responses elicited by stimuli within this range of pulse durations satisfy all the usual criteria for *normal twitch*.

The results obtained on the effect of stimulus pulse duration on the amplitude of twitch tension, lead quite naturally to a consideration of the influence which the choice of stimulus pulse duration may have had on reported results on the effect of a particular class of chemical agents (nitrate, iodide, bromide, etc.) on the peak amplitude of twitch tension. Briefly, a number of investigators, including Kahn and Sandow (1950, 1955) and Hill and Macpherson (1954), have found that frog skeletal muscle, immersed in a solution (temperature range 17° to 25°C) in which certain anions such as bromide, nitrate or iodide are substituted for the usual chloride anion of Ringer's solution, will exhibit, in response to a 'supermaximal' stimulation, a twitch tension of approximately twice the amplitude of that developed in chloride Ringer's. However, in these same solutions, tetanus tensions exhibit essentially no increase. Other effects of such electrolytes on the muscle, such as changes in the latent period, are also found but will not be considered here. The cited investigators indicate that the fundamental effect of these anions is to cause an increase in the *duration* of the active state, with the consequence that the muscle has sufficient time to develop the increased twitch tension. It is of interest to note that the following two conditions are usually present in experiments in which large potentiation of twitch tension is reported as a result of substitution of some other electrolyte for chloride ion in Ringer's: (*a*) the stimulus pulse duration is short (when it is reported) and (*b*) the temperature is high—usually above 18°C.

§ 2. Instrumentation and General Procedure †

The sartorius muscle of the frog (*Rana pipiens*) was used in the studies. The excised muscle was mounted with its natural tendon attachments and a section of the attached bone at each end. A hole was drilled through each piece of bone and a small steel loop was employed to attach the bone directly to the measuring apparatus. For the isometric twitch tension studies, the muscle was stimulated once every two minutes with a single pulse delivered to a mass electrode by a constant current source. A photoelectric strain gauge transducer, an oscilloscope and a camera were used to record the isometric tension developed by the muscle.

The Ringer's solution contained, per litre, 6·7 g NaCl, 0·2 g KCl, 0·2 g anhydrous $CaCl_2$ and 0·1 g $NaH_2PO_4 \cdot H_2O$. It was buffered to pH 7·2 with 0·15 M sodium phosphate. The nitrate Ringer's solution was prepared according to the same procedure outlined for the chloride Ringer's, except that 9·78 g of $NaNO_3$ per litre of solution took the place of the NaCl. The curarized Ringer's consisted of a 1/50,000 w/v solution of tubocurarine chloride.

§ 3. Experiments and Results

3.1. *Effect of Nitrate Anion on Uncurarized Muscle*

In order to investigate the phenomenon of increased amplitude of tension observed under certain conditions of electrical stimulation, as described in Part I, under conditions of modified chemical environment, studies were made at temperatures above 20°c of the combined effect on the peak amplitude of the twitch tension of: (*a*) variation of the pulse duration of the stimulating current, and (*b*) substitution of nitrate Ringer's for chloride Ringer's. The first part of the experiments using chloride Ringer's was conducted as described in Part I. Briefly, the procedure consisted in recording the isometric twitch tensions in response to a series of graded electrical stimuli for each of a number of constant pulse durations. The initial stimuli were below threshold values and the final stimuli were so-called ' supermaximal '. Thus, the essential feature of the part of each experiment in chloride Ringer's was the ' maximal ' stimulation of the same muscle at a number of stimulus pulse durations in the range 0·2 to 10·0 msec.

The second part of each experiment consisted in immersing the muscle in a nitrate Ringer's bath which was isotonic to the standard Ringer's, and after fifteen minutes' recording the isometric twitch tension developed in response to a series of graded electrical stimuli, employing the same stimulus pulse durations applied when the muscle was in the chloride Ringer's. In addition to the experiments in which different stimulus pulse durations were employed for each muscle, a series of experiments

† Part I should be consulted for further details on instrumentation and procedures.

were also performed to determine the effect of nitrate Ringer's on the amplitude of developed tension, with each individual muscle stimulated throughout with a constant pulse duration of either 0·2, 2·0, 4·0, 6·0, 8·0 or 10·0 msec. This latter technique enjoys the advantage of limiting fatigue because of the smaller number of total contractions required of the muscle in any one experiment, as compared with the number required when various pulse durations are applied.

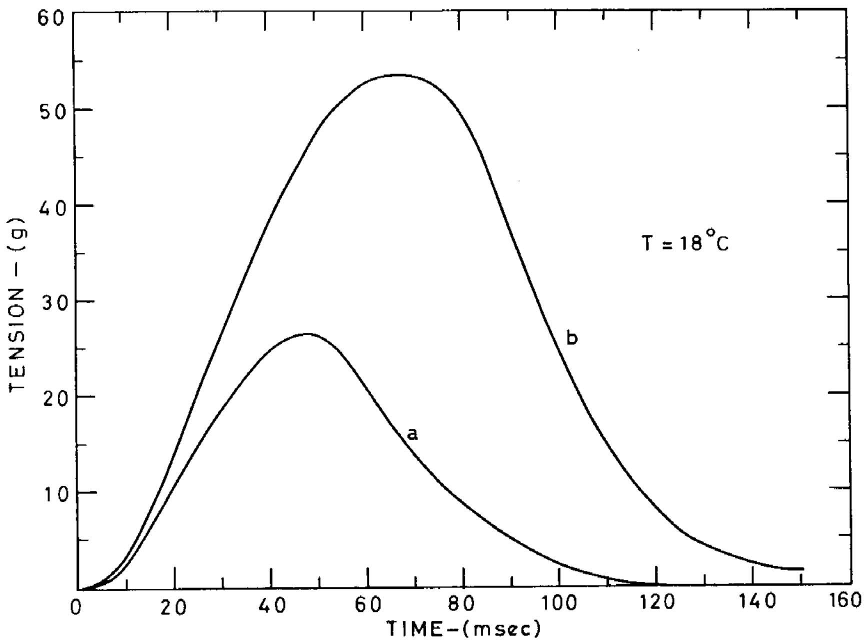

Fig. 1. Maximum isometric twitch responses of an excised, uncurarized frog sartorius muscle at 18°c reproduced from fig. 7 of Hill and Macpherson (1954); (*a*) chloride Ringer's, $TL_0/M=0{\cdot}72$; (*b*) 9·5 min after substitution of 67% iodide Ringer's, $TL_0/M=1{\cdot}40$.

Table 1 summarizes representative measurements obtained by us from experiments of the type just described and also lists for comparison purposes the results of other investigators. Two features of these data are significant. In our experiments, (1) the magnitudes of the tension in chloride Ringer's are considerably greater than the average values reported by the other investigators, and (2) when the full tension response of the muscle in chloride Ringer's is approached, as a result of the application of a long duration stimulus pulse, no large increase in amplitude of tension is found after substitution of nitrate Ringer's for chloride Ringer's.

Fig. 1 is a duplication of the two traces from fig. 7 of Hill and Macpherson's (1954) publication on the effect of various anions on the duration of the active state. The two traces represent the peak twitch response of the frog sartorius muscle (*a*) in chloride Ringer's and

Table 1. Excised frog sartorii[a]—single responses.

Investigator	Pulse duration (m sec)	Tempera-ture (°c)	Curarized	Ringer	Peak tension (g): Chloride Ringer	Peak tension (g): Exp. Ringer	TL_0/M[b] (kg/cm²)/(g/cm³): Chloride Ringer	TL_0/M[b] (kg/cm²)/(g/cm³): Exp. Ringer	Increase of tension in exp. Ringer (%)
Kahn and Sandow (1950)	0·1[c] to 0·2	25·0	Yes	Nitrate	Implicated average of 25[d]	Not given	Not given	Not given	325
Hill and Macpherson[e] (1954)	0·3[e]	18·0	No	Nitrate	Not given	Not given	Not given	Not given	150
	0·3	17·0	No	Nitrate	23·1	48·1	Not given	Not given	108
	0·3	18·0	No	67% iodide	27·0	53·0	0·72	1·40	96
Kahn and Sandow (1955)	0·2	25·0	No	Nitrate	⅓ tetanus[f]	Not given	Not given	Not given	82[g]
Present paper[h]	0·2	21·6	No	Nitrate	38·0	82·5	1·09	2·36	117
	2·0				66·5	83·0	1·90	2·37	25
	0·2	21·4	No	Nitrate	36·5	62·8	1·27	2·18	72
	2·0	21·4	No		56·5	68·2	1·96	2·37	21
	2·5	21·4	No		59·0	68·2	2·05	2·37	16
	0·3	18·0	No	Nitrate	63·1	103·5	2·60	4·26	64
	10·0	18·0	No		95·0	103·5	3·91	4·26	9
	8·0	23·2	No	Nitrate	53·0	59·3	2·17	2·42	11·9
	8·0	23·0	No	Nitrate	65·0	68·3	2·11	2·22	5
	10·0	23·6	No	Nitrate	100·1	107·0	2·87	3·07	6·9

Notes

a. Kahn and Sandow (1955) and our data obtained on *Rana pipiens*; remaining referenced authors did not indicate frog species.
b. In the formula TL_0/M, T = twitch tension (kg), L_0 = maximum length of intact muscle (cm), and M = muscle mass (g).
c. Given in Hill and Macpherson (1954).
d. Average value Sandow (1944), p. 225.
e. Each line of data refers to figs. 5, 6, 7 respectively of the reference; pulse duration is given in the reference for fig. 5, i.e. 0·3 m sec, assumed to be the same value for figs. 6 and 7.
f. P. 169.
g. Fig. 14.
h. The individual groups of data refer to a single muscle in each case.

(*b*) 9·5 minutes after substitution of 67% iodide Ringer's for the chloride Ringer's. It is of interest to compare these two traces with those representing the responses of a frog sartorius muscle studied by us. Fig. 2 shows the peak tension responses in chloride Ringer's to stimuli of 0·3, 2·0, 6·0, and 10·0 msec durations respectively. Comparison of fig. 2*a* with fig. 1*a* reveals that in our investigations the tension amplitude and maximum time rate of rise of tension in chloride Ringer's are considerably greater than those obtained by Hill and Macpherson, although in both cases the muscles were stimulated with single pulses of

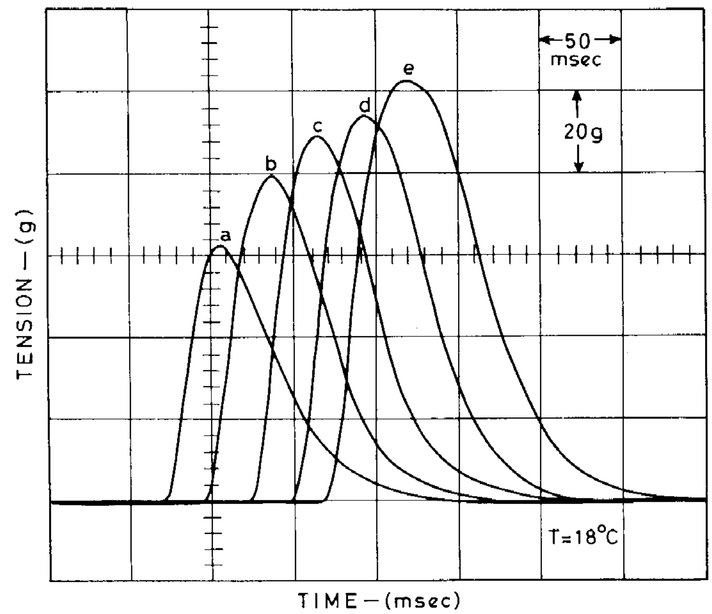

Fig. 2. Isometric responses to single 'supermaximal' stimuli from a mass electrode of an excised, uncurarized frog sartorius muscle at 18°c: (*a*) chloride Ringer's, stim. dur. 0·3, $TL_0/M=2{\cdot}6$; (*b*) chloride Ringer's, stim. dur. 2·0, $TL_0/M=3{\cdot}3$; (*c*) chloride Ringer's, stim. dur. 6·0, $TL_0/M=3{\cdot}7$; (*d*) chloride Ringer's, stim. dur. 10·0, $TL_0/M=3{\cdot}9$; (*e*) nitrate Ringer's, stim. dur. 0·3, $TL_0/M=4{\cdot}3$.

0·3 msec duration at 18°c. It should be noted that the TL_0/M value for fig. 2*a* is 2·6 and for fig. 1*a* is 0·72. Further, the greatest twitch tension amplitude indicated by Hill and Macpherson for the muscle in *iodide* Ringer's ($TL_0/M=1{\cdot}4$) is considerably below the tension amplitudes obtained by us for the muscle in *chloride* Ringer's ($TL_0/M=2{\cdot}6$ to 3·9). Fig. 2*e* reproduces the twitch response of the muscle after nitrate Ringer's had been substituted for the chloride Ringer's. (Only the response to the 0·3 msec duration stimulus in nitrate Ringer's is shown since, in general, an increase in the duration of the stimulus in nitrate Ringer's results in only a minor increase in the amplitude of twitch tension.) It is of interest that despite the high tension output of the muscle in chloride Ringer's, a small (9%) increase in the amplitude of the twitch tension occurred when the muscle was immersed in nitrate Ringer's. The magnitude of this increase is of the same order as that found by Ritchie (1954) and Hill and Macpherson (1954) when nitrate Ringer's is substituted for chloride Ringer's for sartorii muscles at 0°c. The postulated mechanism of previous authors—namely, an increase in the duration of the active state —to explain the usually observed effect of nitrate Ringer's and similar solutions on the amplitude of twitch tension of frog skeletal muscle, should be re-examined in view of the data presented here.

3.2. *Effect of Nitrate Anion on Curarized Muscle*

A limited number of experiments were performed to investigate the effect of nitrate Ringer's solution on the amplitude of twitch tension of curarized muscles. As indicated in Part I, such preparations are of interest because, in contrast to the uncurarized preparations, these do not exhibit the increased amplitude of twitch tension to increased stimulus pulse duration and, in addition, their peak tension is low compared with an uncurarized muscle which is adequately stimulated. The substitution of nitrate Ringer's for chloride Ringer's in the curarized preparation does result in an increase in peak twitch tension which is of the order of 100% or more. However, the level of the peak tension in nitrate Ringer's is never greater than the peak tension of the same muscle in chloride Ringer's when it is *uncurarized* and *adequately* stimulated. Table 2 lists the results of a typical experiment.

Table 2.

Pulse duration (single stimulus) (msec)	Temperature (°c)	Ringer's	Peak tension (g)	TL_0/M $(kg/cm^2)/(g/cm^3)$
0·2	24·5	Chloride	31·8	0·94
10·0	24·5	Chloride	83·5	2·47
0·2	24·5	Cur. Chloride	30·5	0·90
10·0	24·5	Cur. Chloride	28·5	0·84
0·2	24·5	Cur. Nitrate	71·2	2·10

The order of listing the data in the table indicates the sequence in which the measurements were made.

§ 4. Discussion

In the investigations reported here, the effect of nitrate Ringer's on the peak tension developed at high temperatures in response to a single stimulus is essentially the same (i.e., only small potentiation) as that found at low temperatures by other investigators (Ritchie 1954, Hill and

Macpherson 1954), provided the muscle is adequately stimulated at the high temperature. The observation that a significant increase in peak tension in nitrate Ringer's is evident only if the muscle previously exhibits a relatively low amplitude tension response in chloride Ringer's, due to either curarization or to the configuration of stimulating conditions, would appear to be related either to the question of *what percentage of the fibres*, in an intact muscle, are responding in a curarized preparation at high temperature (or in a muscle stimulated with a short pulse), or to the question of whether curarized *single muscle fibres* (or uncurarized single fibres stimulated with pulses of short duration) at high temperature respond to single electrical stimuli with the same amplitude of developed tension as uncurarized fibres adequately stimulated.

An analysis of Kahn and Sandow's (1950) publication on the effect of nitrate Ringer's on muscle is of interest because this paper apparently had a strong influence on the direction of subsequent research in this field. However, before discussing this publication, mention should be made of the work of Chao (1934 a, 1934 b) who expended considerable effort to determine the effects of various electrolytes on muscle contractile properties. His later investigations (1935, 1937) were concerned with the problem of devising more sensitive techniques of stimulation in order to confirm both his own previous conclusions and those of a number of other investigators, namely, that certain electrolytes sensitize muscle fibres so that they exhibit increased ' irritability ' or decreased threshold. Chao (1935, 1937) used a so-called submaximal stimulus, i.e. a stimulus level yielding a constant response in chloride Ringer's (responses of one-third to one-half maximal), as a " more sensitive and more convenient " choice than the ' threshold ' stimulus choice commonly used by other investigators. He found that the effectiveness of various Na-salt solutions in decreasing the stimulation threshold of muscle fibres depends on the nature of the anions, and that the magnitude of the effect follows the lyotropic anion order Cl, Br, NO_3, I, CNS.

In their 1950 publication, Kahn and Sandow, in addition to their observations on the effects of nitrate ion on the latent period and on latency relaxation of curarized skeletal muscle, indicate that nitrate ion substitution for chloride ion results in an increase of approximately 325% in the amplitude of the peak twitch tension. The electrical stimulation pulse durations applied were 0·1 to 0·2 msec (as indicated by Hill and Macpherson 1954) and the shocks were supermaximal. No data are provided in the publication on the magnitude of stimulus current density employed nor on the tension output of the muscle. In comparing their results on augmented twitch tensions with those of Chao, Kahn and Sandow discuss two features:

(*a*) Chao, using submaximal stimuli, found that nitrate Ringer's caused a reduction in rheobase for electrical stimulation and this change in sensitivity of the fibres resulted in mechanical augmentation of the isotonic contractions due to a recruitment of fibres. With respect to this first feature, Kahn and Sandow dismiss the possibility that the augmentation of tension in their own studies could be caused by the same phenomena, because slightly supermaximal shocks were used and "under these conditions, irrespective of the lowered threshold of the NO_3-treated muscle, no recruitment of fibres is possible ". On the basis of the results presented in Part I, it is now evident that the so-called ' supermaximal ' shocks used by Kahn and Sandow were not capable of eliciting the maximum response of the muscle.

(*b*) Kahn and Sandow duplicated some of Chao's work with submaximal stimuli, i.e., using stimulus levels chosen to produce 50% of maximal response and found, as did Chao, an increase of tension in nitrate Ringer's. In addition, they found that the action potential as recorded by a gross electrode is " much larger in the nitrate medium, thus indicating recruitment of fibres made possible by the nitrate induced increase in excitability. But the mechanical response of these muscles is very much larger than would be expected if only recruitment of mechanically normal fibres were involved. Hence, our results in general prove that the nitrate ion does not merely reduce the threshold, but—and this is the essential new finding in our research—it also can somehow modify the events of the latent period and increase the contractile strength of each excited fibre ". In considering this second feature in the light of the results presented in Part I, it is obvious that the large mechanical response in the nitrate Ringer's solution was to be expected, since the muscle under study was a *curarized* preparation, at a *high temperature* stimulated to provide ' 50% of maximum contraction response '. This particular set of conditions would yield a contraction response in chloride Ringer's far below 50% of maximum muscle capability (i.e., the response of an uncurarized muscle appropriately stimulated), and consequently the increase in the mechanical response in nitrate Ringer's solution would be quite impressive. It is of considerable interest, therefore, that the conclusions of this 1950 publication of Kahn and Sandow on the effect of nitrate ion on skeletal muscle were based on two premises which are now open to question. A number of papers on the effect of various electrolytes on the contractile properties of skeletal muscle followed Kahn and Sandow's 1950 paper (Kahn and Sandow 1951, Sandow and Kahn 1952, Ritchie 1954, Hill and Macpherson 1954, Lammers and Ritchie 1955, Kahn and Sandow 1955). In many of these later papers interpretation of the data was influenced considerably by the concept of the supermaximal stimulus.

These studies were aided by a contract (NR 101-075) between the Office of Naval Research, Department of the Navy, and the University of Illinois. Grateful acknowledgment is made to Stephen V. Hall for his assistance in carrying out the experiments.

Summary

An investigation was pursued to correlate the findings of previous investigators on the effect of solutions, such as nitrate Ringer's, on the amplitude of twitch tension at high temperature, with values of the parameters which they employed for electrical stimulation. It was observed that a muscle stimulated in chloride Ringer's by a single stimulus, which elicited the *maximum* response capability of the muscle for the specific environmental conditions, did not show any large increase in amplitude of tension in nitrate Ringer's. An increase in the amplitude of twitch tension in nitrate Ringer's was observed only when the peak twitch tension of the muscle in chloride Ringer's was inadequate, due either to the choice of stimulus pulse duration or to curarization.

Résumé

On a entrepris un travail tendant a relier des résultats de chercheurs antérieurs relatifs à l'effet de solutions (telles que " azotate " de Ringer) sur l'amplitude de la tension de secousse à une haute température avec les valeurs des paramètres employes par les chercheurs antérieurs pour la stimulation électrique. On a observé qu'un muscle qui a été stimulé dans du " chlorure " de Ringer par un stimulus unique permettant d'obtenir le pouvoir *maximum* de réponse pour le muscle aux conditions spécifiques ambiantes, n'avait pas montré une grande augmentation de l'amplitude de tension dans l' " azotate " de Ringer. On n'a observé une augmentation de l'amplitude de la tension de secousse dans l' " azotate " de Ringer que si la tension " de crête " de secousse pour le muscle en " chlorure " de Ringer était insuffisante soit à cause du choix de la durée de l'impulsion stimulante soit dû à la curarization.

Zusammenfassung

Die vorliegende Arbeit ist eine Fortsetzung der Untersuchung zwecks Korrelation der Ergebnisse früherer Forscher über den Einfluss von Lösungen, wie z.B. Ringer's Nitratlösung, auf die Amplitude der Zuckungsspannung bei erhöhper Temperatur mit Werten von Parametern für elektrische Anregung von früheren Forschern benutzt. Es wurde beobachtet, dass ein Muskel, welcher in der Ringerschen Chloridlösung mit einem Einzelstimulus stimuliert wurde, der für die spezifischen Umweltverhältnisse eine *maximale* Anregungsfähigkett des Muskels hervorrief, keine grosse Vergrösserung der Spannungsamplitude in Ringerscher Nitratlösung zeigte. Eine Vergrösserung der Zuckungsspannungsamplitude konnte nur dann in Ringerscher Nitratlösung beobachtet werden, wenn die Spitzenzuckungsspannung des Muskels in Ringerscher Chloridlösung entweder infolge der Wahl der Reizimpulsdauer oder infolge der Kurarisierung unzureichend war.

Резюме

Продолжалось исследование, целью которого является коррелирование результатов, найденных другими авторами для влияния растворов вроде нитратного раствора Рингера на амплитуду напряжения судороги при повышенной температуре, с употребленными этими авторами параметрамиы электрической стимуляции. Было замечено, что мышца, стимулированная в хлоридном растворе Рингера единичным стимулом, который вызывал *таксимальную* слособность мышцы к реакции для данных условий окружающей среды, не показывал большого увеличения амплитуды напряжения в нитратном растворе Рингера. Увеличение амплитуды напряжения судороги в нитратном растворе Рингера наблюдалось лишь в случае, когда пиковое напряжение судороги мышцы в хлоридном растворе Рингера было недостаточным вследствие или выбора продолжительности стимулирующего импульса или же кураризации.

References

Chao, I., 1934 *a*, *Am. J. Physiol.*, **109**, 550.
1934 *b*, *Ibid.*, **109**, 561.
1935, *J. Cell. and Comp. Physiol.*, **6**, 1.
1937, *The Science Reports of Natl. Tsing Hua Univ.*, Ser. B, **11**, 183.
Hill, A. V., and Macpherson, L., 1954, *Proc. Roy. Soc.* B, **143**, 81.
Kahn, A. J., and Sandow, A., 1950, *Science*, **112**, 647.
1951, *Fed. Proc.*, **10**, 71.
1955, *Ann. N. Y. Acad. Sci.*, **62**, 137.
Kelly, E., Fry, W. J., and Fry, F. J., 1964, *Phys. in Med. and Biol.*, **9**, 371.
Lammers, W., and Ritchie, J. M., 1955, *J. Physiol.*, **129**, 412.
Ritchie, J. M., 1954, *J. Physiol.*, **126**, 155.
Sandow, A., 1944, *J. Cell. and Comp. Physiol.*, **24**, 221.
Sandow, A., and Kahn, A. J., 1952, *J. Cell. and Comp. Physiol.*, **40**, 89.