Latency Relaxation: A Brief Analytical Review*

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In this report I review certain aspects of the research on the latency relaxation (LR), the minute relaxation of a stimulated muscle that occurs during the latter half of the latent period, i.e., just prior to the onset of contraction (*see* fig. 1, and, e.g., Sandow, 1944). The first part of my discussion will be historical, dealing with the early, mostly descriptive work on the LR, and then I shall present a more analytically oriented attempt to indicate the significance of the LR in relation to certain aspects of the response of a muscle to stimulation. I take pleasure in dedicating this article to Professor Ernst Fischer, as he terminates his formal work at the Medical College of Virginia and starts a new career in teaching abroad. My dedication takes on a special pertience because I began my studies on the LR in close relation to some of Professor Fischer's early research, and so I shall start by telling of this connection.

Professor Fischer published the research I have in mind just forty years ago (Fischer, 1926) and—as indicated in the title of his paper: "Die Zerlegung der Muskelzuckung in Teilfunktionen"—he delineated the behavior of the segments of an isometrically contracting frog sartorius muscle as a wave of activation coursed along its length after initiation at the spot where excitation was first set up. The main point of this study demonstrated that individual longitudinal segments of the isometrically contracting muscle shortened during the response by as much as 15%, and other segments, that either were not yet excited or were by then actually relaxing, were correspondingly stretched. I read this article in the early thirties when I was just beginning my study of muscle physiology, and I was greatly impressed by the fact that in an ordinary "isometric" contraction, the individual lengthwise elements of the muscle do not contract isometrically at all —in fact, each one not only shortens considerably during one phase of its response but it also in general lengthens markedly at some other phase.

This interested me very much for it showed that a normally occurring contraction of an indirectly stimulated muscle in the body, was not at all simple but was the resultant of the generally out-of-phase mechanical sequences of its many segments. And—even more pointedly to me at that time, since I was interested in studying basic mechanisms of contraction in an excized whole muscle, such as a frog's sartorius—Professor Fischer's findings plainly demonstrated that the prevailing methods for activating a contraction in an isolated muscle yielded a mechanical response which, as recorded either at the end or at any part of the muscle, could not possibly present correctly the basic dynamics of the elementary contractile unit. To some extent this difficulty could be lessened, as Professor Fischer's results suggested, by separating the stimulating electrodes to the opposite ends of the muscle, instead of placing them in the more conventional pattern, both together at one end. But it was clear that a more radical solution of the problem was needed, and this was found in what has come to be called "massive stimulation." In this procedure, first introduced by Brown and Sichel (1936) for work on single muscle fibers and later adapted by me for work on whole muscle (Sandow, 1947), the preparation is fully immersed in a physiological medium, and is flanked by a pair of long electrodes, originally made of bright silver or of silver-silver chloride, but now much more preferably made of platinum (*see* Sandow and Isaacson, 1966). When an electric stimulus, e.g., a short (0.2 msec) square-wave shock of adequate intensity, is applied to the electrodes of this massive stimulating system, the current passing through the medium from electrode to electrode necessarily passes simultaneously through all longitudinal elements of the intervening muscle tissue. Thus, they are all thrown into excitation at the same instant and they continue their responses quite synchronously (Sandow, 1948). In this way, the distortions due to propagation of a wave of activation are effectively eliminated. Another procedure for achieving practically the same result by means of a multi-electrode assembly has been introduced by the Hill school of muscle physiology in the "all-over" method of stimulation (Hill, 1949). It should be noted that even under either of these improved conditions the fundamental response of the contractile component is still affected by the presence of invetable series elastic material (Hill, 1949). But the record now made of the

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massively stimulated mechanical response, by connecting one end of the muscle to a myograph (the other being fixed), is at least free of the sort of propagative distortions so beautifully demonstrated by Professor Fischer in muscles stimulated in the more usual method by means of a pair of ordinary wire electrodes placed astride the muscle.

The direct improvements resulting from recording a contraction having essentially perfect synchronization of response of all lengthwise units along each fiber were as follows: the peak tension output of the twitch was increased by about 30%, the rate of tension rise, especially at the start of a contraction, was increased several fold, the latent period was shorter, and the recorded LR was several times deeper (Sandow, 1945; Sandow and Kahn, 1952). These features of the massively stimulated contraction not only gave a truer account of the dynamical behavior of the basic contractile mechanism; but the great increase in the recorded depth of the LR very greatly reduced the difficulty of recording this minute relaxation against a background of extraneous base-line disturbances due to various amplifier noises and parasitic ambient mechanical vibrations. Hence, such improvements in myography as we now have in consequence of our present massive and all-over stimulation techniques owe much to Professor Fischer's indication in his 1946 paper that the tension course of an ordinarily stimulated muscle was a highly distorted variation of the behavior of the basic contractile component.

But, in my own case the indebtedness goes further, and this brings me to discuss the latency relaxation in detail. I first observed the LR late in 1941. It may be of interest that this occurred in consequence of my listening to some recorded music at home one evening and suddenly realizing that the crystal pickup unit of my phonograph (a piezoelectric device) might make a good mechano-electric transducer for myographic purposes. I immediately put my hunch to the test, for I removed the pickup arm from the phonograph and brought it to my laboratory where—quickly and rather crudely, to be sure—I set it up in connection with an oscilloscope as a myographic recorder. I soon found that not only did it have its expected use in myography (*see* Sandow, 1944), but, above all, it indicated unmistakably, though far from perfectly, something unexpected: the existence of a precontraction elongation of the muscle. I believed that evening that I had made the very first observation of this phenomenon. But later I recalled, and then definitely checked, that in his 1926 paper Professor Fischer, using a special elastometer, showed that a frog sartorius muscle undergoes a very small increase in extensibility during the latent period. Furthermore, Fischer stated in his paper that this was the equivalent of a precontraction elongation which had been observed for the first time by Rauh in 1922 by means of a simple, though extremely sensitive, optical lever system. I was, of course, rather disappointed that I was not the first to discover the LR. But I was also excited by the possibility that, by properly using my highly sensitive crystal pickup, I would have such a greatly improved technique over that available to Rauh, which enabled him only to detect the LR, that I could fruitfully develop studies of the LR and of the latent period as a whole in relation to the general problem of the mechanism causing activation of contraction. In the following I trace some features of this development. (Needless to say, the pickup arm of my phonograph was taken home in due time and reconnected to my phonograph, and it was replaced in my laboratory by an appropriately constructed piezoelectric myographic unit.)

It is interesting that the precontraction relaxation that Rauh had discovered did not immedately attract much attention. Probably this was due to the view held by some (*see* Rauh, 1922) that the effect was only an artifact, or to the feeling that, if real, it could hardly be studied in detail since the instruments available to muscle physiologists in the twenties could hardly do more than demonstrate its existence. The phenomenon, at any rate, was given a name, *Rausche Nase* (Rauh, himself, had called it *die Nase,* from the rather nose-like contour of the LR on his records). It is noteworthy that Professor Fischer did not refer to it in this fashion, and, in my 1944 paper, I gave reasons for discarding this term and replacing it with "latency relaxation." More substantively, however, attempts were made to account for this odd feature of a muscle's response, whether, e.g., it represented an active or passive increase in extensibility. Garten (in an appendix to Rauh, 1922) suggested that it represented a change in elastic coefficient caused by the heat which developed in an activated muscle. But this explanation was rejected by Fischer (1926; *see also* Abbott and Ritchie, 1951), and he concluded by stating: "Wir mussen vielmehr vorläufig einen noch unbekannten Umlagerungsprozess als Ursache dieses Phänomen ansehen." And, in essence, this view still holds although, as will be seen, we can now embellish our ignorance with new possibilities that are at least more alluring than those at first proposed.

For some twenty years following the earliest work on the LR there were only a few references to it (e.g., Schaefer and Göpfert, 1937; and Göpfert and Schaefer, 1941, who demonstrated the LR in mammalian muscle), but nothing further was done to elucidate its nature. Then, when there was a resumption of active interest in it, attempts were made to identify the material of the muscle that was responsible for producing it, and proposals were made that the source

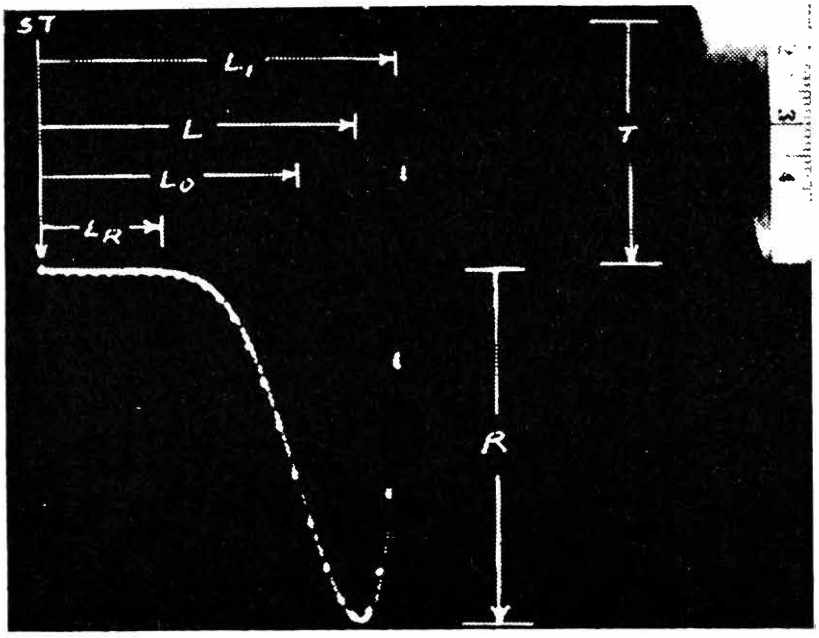
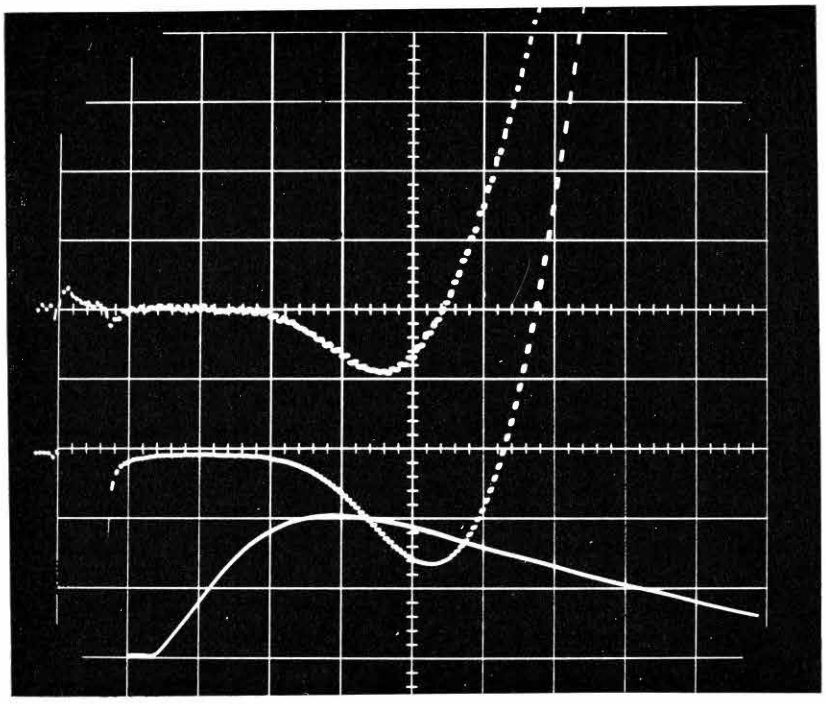


Fig. 1—Records of latency relaxation and associated changes in isometric twitch responses of the massively stimulated, curarized frog's sartorius muscle.

Upper photograph. Made at 13 C by piezoelectric transducer and cathode-ray oscillography (Sandow, 1952). *St* indicates instant of stimulation; the *L*'s indicate various latencies measured from the instant of stimulation: L_R, to onset of the LR; L_o, to point of inflection of the LR (at which contraction has onset, *see* Sandow, 1944); *L*, to end of the LR; and L_1, to onset of positive tension output above original resting tension level. *R* measures the depth of the LR, and *T*, the peak twitch tension recorded by optical myography. The dots superimposed on the LR record are timing signals at 0.2 msec intervals.



Lower photograph. Record made at 20 C by the RCA Type 5734 mechanoelectronic transducer tube and multi-channel cathode-ray oscillography. The twitch output is recorded on the lowest trace, with one horizontal box equalling 10 msec, and one vertical, 10 g. The middle and uppermost records present the earliest part of the mechanogram on the two traces of the electronically switched second beam of the oscilloscope, the lower of these giving the LR in direct form, and the upper as the time derivative (rate) of the direct tension change. For both of these traces, one horizontal box equals 0.5 msec, and the direct LR tension calibration is 50 mg for one box. Note that the direct and derivative LR traces first record the duration of the shock (0.3 msec).

was the contractile material (Sandow, 1944) or, as cogently discussed by Hill (1951), some parallel structure such as the sarcolemma. Although evidence then available could not definitely resolve this issue, the presence of the LR and the study of its variations under a variety of conditions provided new definite criteria for measuring the latent period, which permitted determination of certain kinetic features of the earliest phases of activation of a muscle's response (Sandow, 1944, 1945, 1947; Abbott and Ritchie, 1951; Hill, 1951) and indicated some underlying aspects of excitation-contraction coupling (Sandow, 1952, 1965; Sandow, et al., 1964; Sandow and Preiser, 1964; Sandow, Taylor and Preiser, 1965). Since in this article I do not intend making a comprehensive review of work on the latent period, the interested reader can refer to the listed papers for details of the indicated applications of the observations regarding latency phenomena.

I wish now to discuss the latest attempts to give significance to the LR. In the period since the aforementioned proposals were made about the nature of the LR, some new and highly important functional systems of muscle have been discovered—the sliding filament mechanism of contraction, and the internal membrane systems concerned with excitation-contraction coupling—and it is interesting to speculate about them in relation to the LR.

As is well known (H. E. Huxley, 1960, 1965), the sliding filament mechanism involves two sets of interdigitating filaments in each sarcomere; the one, thin and made up of actin, and the other, thick and made up of myosin. These engage in contraction when the heavy meromyosin side-branches of the thick filaments are activated by excitation-contraction (E-C) coupling to form cross-bridge attachments to the actin filaments, and then move, and make and break cyclically, so as to force the thin filaments further into the spaces between the thick ones, thereby tending to make the sarcomeres shorten and set up contraction in the fiber as a whole. A. F. Huxley (1957), impressed with the view (Hill, 1951) that the LR must arise in a structure parallel to the contractile system, suggested that this condition could be satisfied if either, but not both, the actin or the myosin filaments had special connections so arranged as to make each part of an element running continuously between the two neighboring Z lines of a sarcomere and under some tension in the resting fiber. The LR could then be conceived to arise as a relaxation of this tension that begins just prior to the structurally parallel formation of the cross-bridges and the resultant development of positive tension. At the time this scheme was postulated it was thought that the inner ends of the actin filaments were joined together by the so-called S filaments, and so there seemed to be a factual basis for the particular supposition that it was the actin filaments that produced the LR as described above. But there is now no visual evidence for the existence of the S filaments, nor of equivalent ones that were imagined to join the myosin filaments to the Z lines, and so there is at present no morphologically evident basis for explaining the LR as suggested by Huxley. But, as discussed by Podolsky (1964), certain physiological evidence suggests the existence of S filaments, and so there is still a possibility that the LR reflects a very early relaxation of a specially continuous type of thin filament.

If we disregard the view that the LR must take place in an element parallel to the contractile mechanism, it could be supposed that even in the resting state some cross-bridges exist and create some tension, and that the LR is produced when some very early event of E-C coupling breaks these cross-bridges. But this mechanism is inherently quite improbable, and certain features of it, which need not be detailed here, are in contradiction to experimental results. I, therefore, feel it does not merit further discussion.

We now consider the internal membrane system as a second recent major development of our knowledge of how muscles work that may have some bearing on the mechanism of the LR. This set of structures is composed of two distinct parts, the longitudinally oriented sarcoplasmic reticulum (SR) and the transverse (T) tubules. These ramify throughout the interior of the fiber, but combine to form special complex units which are in regular register with certain parts of the striations. In the skeletal muscle of the frog these units are the triads which are composed of a central T tubule flanked by a pair of lateral sacs of the SR, and which closely encircle each myofibril at the level of each Z band (*see*, e.g., Peachey, 1965a and H. E. Huxley, 1964). As recently reviewed (Sandow, 1965), the function of these structures in E-C coupling seems, in outline, to be as follows: the action potential, running its course essentially along the surface membrane of the fiber, produces an inward moving signal in the radially oriented T tubules which, on arriving at each triad, activates the two flanking lateral sacs of the SR to release some of their stores of bound calcium. The free Ca^{2+} diffuses into the neighboring myofibrils, first into the I bands near the Z lines and then toward the region of cross-bridges in the A bands where it activates the sliding filament mechanism of contraction. It is noteworthy that A. F. Huxley (1957) proposed that, at least in frog fibers, there seems to be a longitudinal spread of activation in each sarcomere, which starts at the level of the Z lines and moves through the I bands to the region of overlap in the A bands. As one of the possibilities of a mechanism for such a process, Huxley suggested the diffusion of a

substance, and this is now given a specific form in the diffusion of Ca^{2+} through the myofibrils as indicated above.

It is obvious that in this E-C coupling sequence the actions of the T tubules and the lateral cisternae, and the diffusion of the Ca^{2+} to the cross-bridges, must occur during the latent period, and it is therefore reasonable to ask whether the LR might not be an expression of some feature of these processes. There seems to be no reason why the diffusion of Ca^{2+} should produce any mechanical effect such as an LR. And propagation of the E-C coupling signal down the T tubules can also be ruled out, since this occurs very early in E-C coupling (it may even occur in part almost in synchrony with the action potential, *see* Sandow and Isaacson, 1966), and thus very likely takes place during the earlier part of the latent period before the LR develops. We are thus left with the possibility that the Ca-releasing action of the SR in E-C coupling is responsible for producing the latency relaxation.

In elaboration of this idea, it should be noted that the SR makes up a large part, 15%, of the volume of the fiber (Peachey, 1965a), and it is arranged in longitudinal columns within the fibers thus forming, in conjunction with the triadic element of the T tubules, a set of essentially continuous axially oriented mechanical systems which envelop and are in parallel with the myofibrils. Now, release of Ca^{2+} from the lateral cisternae could set up an osmotic change in the SR, i.e., the release of Ca^{2+} from the lateral sacs (especially if accompanied by some anion) should cause the osmotic pressure to decrease in the SR and cause water to flow out of it. Thus, tension within the initially stretched muscle should decrease and give rise to the latency relaxation. This decrease in tension should, furthermore, be detectable not only as the usually observed axially trecorded LR, but also as a transverse LR, if the postulated decrease in osmotic pressure results in essentially isotropic changes in shape of the SR. Such a transverse LR has, in fact, been observed (Sandow, 1947). Thus, there is the following presumptive evidence that the LR reflects a decrease in tension of the SR as the lateral sacs of this structure release Ca^{2+} during E-C coupling: 1) the LR occurs after a brief delay following excitation, but before contraction begins, and thus at a time when the SR should be releasing Ca^{2+}; 2) an osmotically produced decrease in tension of the SR may conceivably occur in consequence of the release of Ca^{2+} by the SR; and 3) the general argument of Hill (1951) that the LR must occur in a structure parallel to the contractile component is satisfactorily met by making the reticulum responsible for producing the LR. In accordance with this interpretation of the LR, we should note that certain of its parameters may serve as temporal signposts of the release and longitudinal spread of the activator Ca^{2+} in the myofibrils. Unfortunately, such signposts must involve some ambiguity because our present knowledge (Sandow, 1965) indicates that it takes some time, of the order of several milliseconds, for the activating signal in the T tubules to spread inward into the depths of the fiber. Hence, the series of reticular elements of the various triads connected to a particular T tubule will be asynchronously activated to release their Ca^{2+} and produce their supposed LR response, and there will then also be an asynchrony in the onset of tension in the corresponding myofibrils. It seems reasonable to suppose, however, that at least the temporal features of the LR represent the essential timing of the events of interest occurring in a thin layer of myofibrils which lie just inside the sarcolemma and are the first to respond. Therefore, the assumption is made in the following that L_R effectively marks the instant at which the Ca^{2+} is released

from the reticulum of this relatively superficial layer and begins its longitudinal diffusion in the neighboring myofibrils, and L_o signifies the earliest moment at which the Ca^{2+} has reached the nearest overlap spot in sufficient concentration (*see* Sandow, 1965) to activate contraction. The justification for choosing L_o for indicating the onset of contraction and not L or L_1, will be found elsewhere (Sandow, 1944).

There is a considerable amount of evidence about the variations in all the different parameters of the LR in the frog sartorius muscle as a function of muscle length (Sandow, 1944; Abbott and Ritchie, 1951; Guld and Sten-Knudsen, 1960, fig. 2), and pH and temperature (Sandow, 1947). In view of the preceding discussion (and other considerations, too) it is at present unclear how these various findings can be used to test the hypothesis that the LR has its source in the reticulum, and much more work is needed to settle the various problems which are posed by such tests. For the present, however, we turn again to the results presented in fig. 2 and note the following. Increase in length causes in general oppositely directed temporal variations in L_R, and in L_o, L and L_1 which measure, each in its own fashion, the latent period for positive tension development. This difference is consistent with the hypothesis for it might be expected that, since the LR and the positive tension development are supposed to occur in such structurally and functionally different components of the fiber, they might then show qualitative differences in the way their kinetics are altered by changes in muscle length.

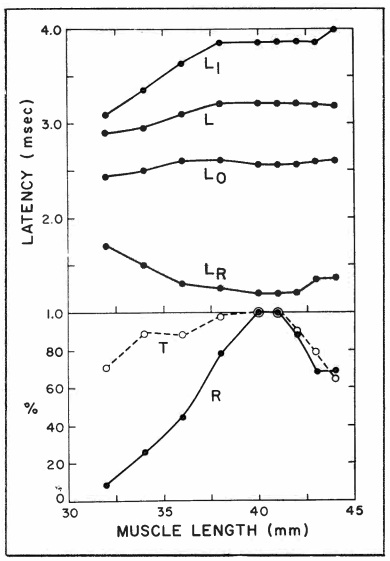


Fig. 2—Changes in latencies and tension outputs of an isometric massively stimulated curarized frog's sartorius muscle (in situ length, 33 mm) at 20 C as a function of the length of the muscle. Symbols of the various parameters are defined in figure 1.

Particular problems are posed by the specific nature of the effects of muscle length on L_R as against those on the various positive tension L's. It is not easy to see how increase in muscle length should alter the supposed behavior of the SR so as to decrease the time L_R. It is conceivable, moreover, that this decrease in L_R is, at least in part, a consequence of the decrease in transverse size due to stretching the fiber, and thus of some change in the timing of the T tubular function in E-C coupling. More meaningful, however, is the stretch-induced increase in the L's for positive tension, especially in relation to the associated decrease in L_R. Now, Guld and Sten-Knudsen (1960)—who have obtained results on single fibers like those on whole frog sartorii reported earlier (Sandow, 1944; Abbott and Ritchie, 1951) and now confirmed by the new experiments of fig. 2—ascribed the increased latency for positive tension development that is produced in stretched fibers to the greater time required for an activation process (in the general sense as proposed by A. F. Huxley (1957), but whose detailed nature they did specify) to spread longitudinally over the greater distance in the stretched sarcomeres from Z line to filament overlap region. Guld and Sten-Knudsen's analysis of their results indicated that the activation process moves longitudinally in the myofibril at 22 C with a velocity of 0.4 mm/sec. I agree with their general interpretation, and suggest, furthermore, as already indicated in the foregoing, that the essential process is the longitudinal diffusion of Ca^{2+}. As discussed elsewhere, (Sandow, 1965) the processes of E-C coupling by which Ca^{2+} is made available to activate contraction, in, e.g., a frog skeletal muscle fiber, include both radial and longitudinal diffusion through the myofibril until the Ca^{2+} reaches the overlap region of the filaments in the A band, where it activates contraction. A condensed analysis of the radial diffusion kinetics of the Ca^{2+} has been given in the previously cited work (Sandow, 1965), and our concern now is only in the longitudinal kinetics. This is a special problem in diffusion theory which will not be discussed in detail here. But, if we recall that stretch of a sarcomere causes an increase in width of only the I band (Huxley, 1960), it is obvious that the longitudinal path and associated time for diffusion of the Ca^{2+} from the neighborhood of the Z line, where it is liberated, to the region of overlap in the A band, where it acts, should be greater, the more the sarcomere is stretched. And furthermore, a measure of the time required for this to occur, as previously suggested, would be the difference in time between L_o and L_R, i.e., $L_o - L_R$. This postulated mechanism might be questioned by positing that stretch of a muscle would result in some displacement and distortion of shape of the lateral sacs that would tend to reduce or even eliminate the increase in diffusion distance with stretch. But this does not seem likely, because the detailed structure of the relatively sparse and irregularly subdivided longitudinal tubules and fenestrated collar of the SR (Peachey, 1965a and b) suggests that these elements would be pref-

erentially stretched by increase in length of the sarcomere, thus leaving the lateral cisternae in their essentially original placement opposite the portions of the I band close to the Z line.

Turning now to the results presented in fig. 2 (which were obtained from massively stimulated frog sartorii and therefore are preferable to those previously obtained (Sandow, 1944) by wire electrode stimulation), we see that the interval $L_o - L_R$ is in fact increased with increase in length of the muscle, and thus it increases with lengthening of the sarcomere, or, more precisely, with increase in the distance of longitudinal diffusion of the activator, this distance being, in essence, half the width of the I band, i.e., 0.5 I. In connection with the results of fig. 2, the average sarcomere lengths corresponding to the muscle lengths of 32 and 40 mm were very likely about 2.3 and 2.9 μ, respectively, and thus the increase in the 0.5 I distance was 0.3 μ. And the corresponding increase in $L_o - L_R$ was from about 0.7 to 1.4 msec, i.e., 0.7 msec. Thus, an apparent velocity for longitudinal diffusion of activator Ca^{2+} would be $0.3\mu/0.7$ msec $= 0.43\mu$/msec, and this is in quite good agreement with the value, 0.4 mm/sec, i.e., 0.4 μ/msec, that Guld and Sten-Knudsen (1960) found from a similar analysis of their results obtained on single muscle fibers at 22 C.

It is obviously of great interest to determine whether this apparent speed of longitudinal movement of an activating influence could be accounted for by the diffusion of Ca^{2+} along the length of one-half of an I band. Rough calculations suggest that this is the case, but the details will be omitted here. For the present, however, the main point of our analysis regarding the significance of the increase in the time interval $L_o - L_R$ with stretch of the muscle is that it suggests that the time lapse between the beginning of the LR and the earliest instant of tension production may be accounted for by the time taken for the diffusion of a substance, which I assume to be Ca^{2+}, from a point in the sarcomere near the Z line to the region of overlap of the sliding filaments. This is consistent with the view that the LR reflects a mechanical change of the stimulated fiber which is produced in consequence of the release of Ca^{2+} from the lateral sacs of the reticulum. Much more must be done, however, to test the proposed hypothesis. Thus, on the basis of the hypothesis, the ability of a muscle to produce an LR should be related to the structure of its internal membrane systems, particularly of the SR. Indicative in this connection are the peculiarities and the relative difficulty of recording the LR in certain invertebrate muscles (Lowy and Sten-Knudsen, 1963). But, before anything definite can be concluded in this regard it will be necessary to know much more about the SR, or, more generally, of the system regulating Ca flux of these muscles (but *see* Hanson and Lowy, 1961). It is also interesting that, in the amphibia, the slow muscles differ from their fast counterparts in respect to both the T tubules and the SR (Peachey and Huxley, 1962; Page, 1965) and it will be of great importance to see if the slow muscles produce corresponding variations in their LR. Finally, it should be noted that in some muscles the T tubules and hence the corresponding lateral sacs of the SR make contact with myofibrils at the level of the boundary of the A and I bands (e.g., A. Huxley, 1959; Andersson-Cedergren, 1959) and, therefore, stretch of such fibers should not cause the increase in tension latency found in frog fibers. Clearly, there are several types of experiments yet to be performed which should indicate whether the hypothesis regarding the source of the LR in the behavior of the sarcoplasmic reticulum is correct.

In conclusion, it is worth emphasizing that the LR is an undoubted feature of the response of many different types of muscles. It is provocative that there is at present no definitive explanation of it although this review indicates that either the thin member of the sliding filaments or the sarcoplasmic reticulum may provide the structural basis for producing the LR. Further research should resolve this problem and in so doing increase our comprehension of the mechanisms, in general, by which muscles respond to stimulation.

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