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DEUTERIUM OXIDE EFFECTS ON EXCITATION-CONTRACTION COUPLING OF SKELETAL MUSCLE

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SUMMARY

^2H_2O (99.8 %) Ringer's solution greatly reduces the twitch and tetanus of frog sartorius muscle and, as specially shown here, slows the onset features of the mechanical output of the twitch by: (a) increasing the time (L_R) from stimulus to start of latency relaxation; (b) slowing the development of the latency relaxation, and (c) greatly decreasing the rate of onset of tension development. These changes reflect effects of ^2H_2O on excitation-contraction coupling and they represent the critical direct effects of ^2H_2O on muscle since it does not depress either the action potential or the intrinsic myofibrillar contractility. The increase in L_R is attributed to slowed inward electrical propagation in the T-tubule. But the critical effect of ^2H_2O on frog muscle is to greatly depress mobilization of activator Ca^{2+}. The depression of the Ca^{2+} mobilization and of its effects on the activation of contraction evidently result from (a) a lowered rate of release of Ca^{2+} from the sarcoplasmic reticulum, as indicated by the slowed development of the latency relaxation, (b) a decreased amount of Ca^{2+} released in a twitch, and (c) a reduced speed of diffusion of the Ca^{2+} to the contractile filaments. The depressed mobilization of Ca^{2+} is apparently the essential cause of ^2H_2O's general depression of twitch and tetanus output.

INTRODUCTION

Deuterium oxide ("heavy water") depresses contraction of various vertebrate muscles as evidenced by reductions in both the amount and the speed of tension development (e.g. refs. 1–3). Heavy water does not produce these effects by acting directly on the contractility of the myofibrils since it does not decrease either the ATP-induced shortening of glycerinated fibers [2] or the ATPase activity (at pH 7.2) of "myosin B" extracted from rabbit muscle [4] (but see ref. 3, which evidently dealt

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with myosin ATPase). And at least in crayfish muscle, ^2H_2O actually enhances contractions induced by Ca^{2+} acting directly on the myofibrils and by caffeine (ref. 5 and our own caffeine results). Furthermore, ^2H_2O does not inhibit production of the action potential of muscle (refs. 1–3 and 6, and our own results) or of nerve [7, 8]. These results suggest that the prime effect of ^2H_2O on muscle must be to depress some step(s) of excitation-contraction coupling that normally serve to couple the depolarization of the membrane to the activation of myofibrillar contraction [2, 5, 9].

This inference is especially supported by Kaminer and Kimura's finding [9] that ^2H_2O-treated barnacle fibers, sufficiently depolarized to activate a relatively small contraction, produced no Ca-transient, as monitored by aequorin [10], and consequently did not contract. However, these results, appraised in terms of the tension/membrane depolarization relations for crustacean fibers (e.g. refs. 5 and 11), probably signify that the ^2H_2O did not completely suppress the capacity to release activator Ca^{2+}, but merely made this capacity dependent on super-normal amounts of depolarization (i.e., the heavy water raised the mechanical threshold) [5]. Thus, in the barnacle fibers, ^2H_2O seems to act by directly depressing depolarization-dependent release of free Ca^{2+}. Furthermore, a recent report [12] questions Kaminer and Kimura's results on the ground that misleading effects can be obtained from some impurity in commercially provided ^2H_2O, which can be removed by redistillation of the product. However, the barnacle findings are not changed by such redistillation (personal communication from Dr. B. Kaminer), nor are our results (see later). Moreover, the reported spurious effects [12] dealt with Ca-uptake by isolated (rabbit) sarcoplasmic reticulum, and consequently might be significant regarding relaxation of muscle but not for excitation-contraction coupling, which is primarily concerned with the release of Ca^{2+} from the sarcoplasmic reticulum.

Our report deals with ^2H_2O effects especially on the various mechanical aspects that comprise the onset features of contraction in frog skeletal muscle, i.e., the latent period and its constituent element, the latency relaxation, and the earliest signs of tension rise that collectively reflect certain steps of excitation-contraction coupling [13–15]. We confirm and extend the results that ^2H_2O's prime effects are on certain intermediate steps of excitation-contraction coupling, and we show that these effects are consistent with the previously made proposals [13–15] regarding the significance of the onset features in delineating certain steps of excitation-contraction coupling, especially those determining mobilization of the free Ca^{2+} that activates contraction. We propose furthermore, that the overall effects of heavy water on frog skeletal muscle are caused mainly by its capacity to greatly reduce this Ca^{2+} mobilization.

METHODS

We have used frog sartorius muscles in oxygenated standard frog Ringer's solution made up with either H_2O or 99.8 % ^2H_2O (Bio-Rad Laboratories, Rockville Centre, N.Y.) and containing $2 \cdot 10^{-5}$ g per ml of (+)-tubocurarine chloride to ensure only direct stimulation of the muscles. Both media were generally adjusted (by use of a pH meter) to pH 7.2 with Tris · HCl buffer. In some cases the ^2H_2O-Ringer's solution was set at pH 6.8 to allow for the difference of $pH = pH$ reading + 0.4 [16], but this made no significant difference in our present results. Control

experiments with glass redistilled-^2H_2O yielded both mechanical and electrical results with no detectable difference from those involving the substance as received from the supplier. The muscles were maintained in the Ringer's bath at 20 ± 0.1 °C in a massive stimulation chamber and they were stimulated under an initial tension of 1–2 g with slightly supramaximal, massive, 0.3 ms square-wave shocks. The isometric tension output was recorded by the RCA 5734 transducer tube and appropriate cathode-ray oscillography so as to yield records as shown in Fig. 1. We recorded action potentials at room temperature (21–23 °C) by means of conventional internal electrode techniques. Stimuli were applied to a surface fiber of the sartorius by means of the tips of a pair of Pt electrodes, with the cathode directed toward the pick-up electrode, which was always 3 mm from the cathode. In general, we used methods that are standard for our laboratory as described in detail elsewhere (e.g., ref. 17).

RESULTS

Fig. 1 shows, typically, that the ^2H_2O Ringer's solution caused, as in earlier work [1–3], a very great reduction in peak twitch tension and a much smaller decrease in tetanus output. In general, the twitches and tetani were reduced, respectively, to 5–10 % and 10–75 % of normal. Note also that the rate of tension development of both twitch and tetanus was greatly reduced.

Our particular interest is in the onset features* as typically represented in Figs. 1a and b. It is directly obvious that: (a) L_R, L_0, L, and L_1 are all increased; (b) R is hardly altered; and (c) there is a considerable decrease in the general rate of onset tension development. To definitely specify this onset rate we shall use the value of dP/dt (or $\dot{P}$) achieved by the muscle at the instant when it has just reversed the latency relaxation, i.e., at the time L_1. We have so chosen this parameter, i.e., $\dot{P}_{ON}$, since it specifies the rate of tension development occurring at just about the time excitation-contraction coupling has completely run its course, and it therefore directly measures the general effectiveness of such a coupling. Furthermore, at the time of $\dot{P}_{ON}$, the tension of the muscle is zero and therefore the rate of tension development is free of complicating effects that might arise from a stretch of any series of elasticity in the muscle or the recording system. For 6 different muscles tested as indicated in Figs. 1a and b the average values of the various onset parameters are given in Table I. The heavy water obviously causes statistically high significant absolute increases in all the temporally defined onset features. R, however, suffers only a possible significant slight decrease. There are also changes in kinetics of the contraction and relaxation periods of the tetanus but these will be dealt with elsewhere. Finally, Fig. 1 confirms that the shape of the action potential is not drastically affected, though there is an obvious reduction in conduction velocity, as previously reported by others [e.g. refs. 7 and 8]. We mention, in passing, that ^2H_2O in our tests, as in others, [7, 9] also slightly reduced the rate of both the rise and fall of the spike, the latter being

* Abbreviations: Time parameters, all measured in ms from the start of the shock: L_R, to the beginning of the latency relaxation; L_0, to the latency relaxation's point of inflection, and L, to its deepest point; L_1, to the instant at which the rising tension crosses the zero tension base-line (L_0, L, and L_1, indicate variously defined latencies for development of positive tension, and L_1 measures the conventionally defined latent period); R, the depth of the latency relaxation; P, tension output; P_{ON}, the onset rate of tension development, as measured at L_1.

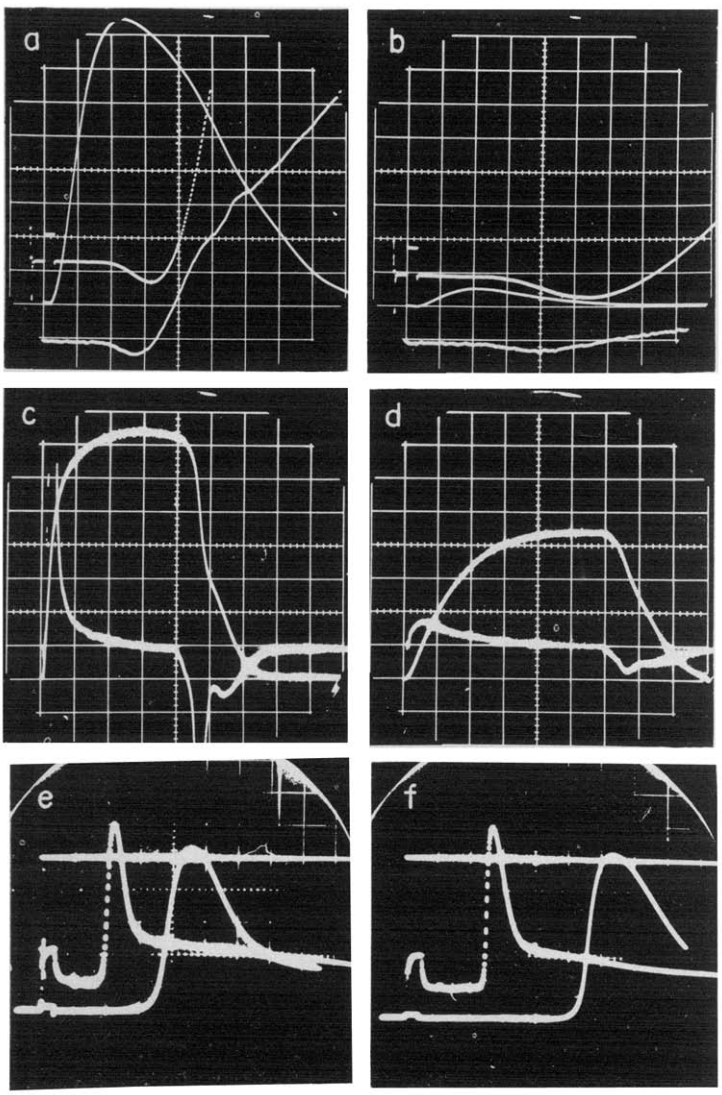

Fig. 1. Typical effects of 99.8 % ^2H_2O Ringer's solution after 20 min action on the isometric twitch and tetanus and on the action potential of a frog sartorius muscle at 20 °C. (a) Normal twitch. The large peaked trace is the tension output, P, of the entire twitch (calibration: horizontal, 20 ms/square; vertical 5.0 g/square); the shock (not shown) occurred at start of the trace. The two other traces (impressed on the electronically switched sweep of the second beam of the oscilliscope) record the onset features of the same twitch, with horizontal calibration of 1 ms/square for both traces. The upper of these traces, shows first, at the left, the shock (as a 0.3 ms square wave) and then the mechanical changes (vertical calibration, 100 mg/square), including in sequence: (i) the period of mechanical quiescence, following the shock L_R, of about 1.4 ms; (ii) the latency relaxation achieving a depth, R, of about 50 mg; and finally (iii) the phase of very rapid rise of the contraction period. The lower trace on the fast sweep (i.e., the lowest of the 3 recorded traces) is the time derivative, dP/dt, of the upper trace, with calibration 0.19 g · ms per square. (b) ^2H_2O twitch, with the 3 types of traces and their calibrations as in (a). (c) Normal and (d) ^2H_2O tetani. Calibrations: horizontal, 100 ms/square and vertical, 10 g/square. Each record includes the derivative as well as the direct

TABLE I

EFFECTS OF 99.8 % ^2H_2O ON ONSET FEATURES OF THE ISOMETRIC TWITCH OF FROG SARTORIUS MUSCLES AT 20 °C

Each below mean ±standard error was calculated from values obtained in separate experiments on 6 different sartorius muscles, first tested 2–3 times in H_2O medium, and then similarly in ^2H_2O medium after at least 10 min exposure to allow full development of the experimental changes. The *P* values of the percentage relative changes were each obtained by means of the *t*-test for the unpaired variates as averaged in the *C* and *E* values.

Features	Ringer's medium		Relative change	
	H_2O (Control, *C*)	^2H_2O (Experimental, *E*)	$(E-C)/C$ (%)	*P*
L_R (ms)	1.38±0.07	2.09±0.22	51±23	< 0.05
L_0 (ms)	2.58±0.15	4.11±0.08	59±17	< 0.01
L (ms)	3.05±0.11	5.02±0.17	65±20	< 0.0125
L_1 (ms)	3.71±0.15	7.03±0.32	89±35	< 0.05
L_0-L_R (ms)	1.20±0.17	2.02±0.23	68±27	< 0.05
L_1-L_0 (ms)	1.13±0.21	2.92±0.32	158±38	< 0.005
L_1-L (ms)	0.66±0.19	2.01±0.36	205±41	< 0.0025
R (mg)	−59.4 ±7.3	−45.7 ±5.1	−23±9	< 0.1
$\dot{P}_{ON}$ (g/ms)	0.37±0.04	0.07±0.01	−81±4	< 0.0005

great enough to cause an approx. 30 % increase of the spike's duration as measured at −20 mV. All of the effects described above were fully produced after only about 5 min exposure to the ^2H_2O-Ringer's solution and tended to reverse quite as rapidly after replacing the muscle in a normal Ringer's solution. The variations in the time parameters are interesting in terms of their relative changes as given in the fourth column of Table I; clearly, the relative changes of L_R, L_0, L, and L_1 tend to increase slightly with the passage of time during the onset period. But this tendency becomes particularly striking when the calculations are not made in terms of the direct parameter values themselves, but rather by means of certain differences of the intervals between them. Thus, ^2H_2O increases the interval L_0-L_R by 68 %, but the increase of L_1-L_0 is 158 % and of L_1-L, 205 %. L_R is also an interval, i.e., between the instants of of stimulation and of the start of the latency relaxation, and its increase by ^2H_2O is only 51 %. Now note that as these various intervals appear later and later during the onset period, their relative increases become larger; and this is especially striking when we compare the increase for L_1-L (205 %) or for L_1-L_0 (158 %) with that for L_0-L_R (68 %). These results indicate that ^2H_2O seems to affect differently a series of separate steps of the onset mechanisms and that the effects (always an increase in the duration of the steps) become larger the later the step is in the onset period. It will

myogram. (e) Normal and (f) ^2H_2O action potentials. In each record the action potential is recorded on both a slow (2 ms/square) and a fast (0.5 ms/square) sweep. Common voltage calibration: 20 mV/square. Horizontal line indicates zero potential level for slow-sweep action potential; fast-sweep potential shifted one square downward. The small square-wave deflection on the fast sweep is the shock artifact. Conduction distance to pick-up electrode was 3 mm for both normal and ^2H_2O tests and therefore the longer ^2H_2O conduction time reflects a smaller conduction velocity of the spike in ^2H_2O.

be seen later that these steps can be related to the sequence of links of excitation-contraction coupling and that the several heavy water effects can be used to elucidate the means by which this coupling occurs.

In further support of the view that ^2H_2O does not depress twitch and tetanus output of frog muscles by a direct action on the contractile filaments, we have shown (Sandow, A. and Sphicas, E. C., unpublished results) that ^2H_2O does not significantly change either the rate of development or the amount of the contracture evoked in frog sartorii by 10 mM caffeine. Our caffeine results have a double significance since production of the caffeine contracture must involve two main mechanisms: (a) the direct action of the drug on the sarcoplasmic reticulum of intact muscle to cause release of activator Ca^{2+} [18], and (b) the activation of contraction by the released Ca^{2+}. Thus, our caffeine results indicate, first, that even though ^2H_2O depresses the depolarization-dependent release of Ca^{2+}, as caused normally by the exogenous current used in the crayfish and barnacle experiments [5, 11], or by the action potential in e.g., our experiments, it has no obvious effect on the chemically dependent mechanism by which caffeine causes release of the activator Ca^{2+}. And this is also indicated in that ^2H_2O does not alter chemically induced contraction of smooth and heart muscle [2]. This distinction is of considerable interest in itself. But we presently stress that, given an unchanged caffeine-induced release of Ca^{2+} in the presence of ^2H_2O, then the occurrence also of an essentially normal mechanical output signifies, in harmony with the previously cited evidence [2, 4, 5], that ^2H_2O does not depress the ability of the contractile filaments to be activated to contract by Ca^{2+}.

The effects of ^2H_2O on the general tension output and the onset features of the frog sartorius muscle are similar to those produced by strongly hypertonic Ringer's media [19]. This raises the question whether the ^2H_2O produces osmotic effects as do hypertonic solutions. This is inherently improbable since the treated muscles exchange their normal H_2O for ^2H_2O and therefore should behave osmotically in relation to the essentially isosmotic external ^2H_2O medium rather as they did under normal conditions when the common solvent internally and externally was H_2O. Furthermore, as already mentioned the effects of ^2H_2O seem localized on excitation-contraction coupling processes, whereas those of hypertonicity are essentially on the contractile system (e.g. ref. 20). However, to test for osmotic effects we have studied the weight changes of sartorii after exposure to the ^2H_2O Ringer's medium, and have found that after as much as 15–50 min exposure, 6 muscles, far from losing weight as if exposed to a hypertonic medium, actually showed an average 7.9 ± 0.5 (S.E.) increase in weight. Thus, all the ^2H_2O effects of interest, for all their similarity to the hypertonic effects, must result from some specific chemical influence of ^2H_2O. As for our finding of the small increase in volume, the cause of this is obscure though it may reflect the fact that the density of ^2H_2O (at 20 °C) is about 1.10 that of H_2O [16] and thus may have altered relative osmolalities of the internal and external media of our ^2H_2O-treated muscles. In any case, the increase in weight of the ^2H_2O muscle is so small that we are neglecting any small, possible effect this may have caused on their response mechanisms.

DISCUSSION

The main interest of our work lies in the general finding that heavy water

considerably slows development of the onset features of the twitch of frog skeletal muscle. The overall magnitude of this effect is indicated by the 89 % increase in L_1 (Table I), i.e., of the conventionally defined latent period of the twitch. Since L_1 essentially measures the time for excitation-contraction coupling to run its course [21], the increase in L_1 clearly signifies that ^2H_2O greatly slows the development of this coupling, and thereby, as will be shown in the following, depresses it (but not "blocks" it, as erroneously ascribed [5] to our earlier description of the effects of heavy water [22]).

Some details of these effects are suggested on the basis of previous work of this laboratory (e.g. refs. 13, 14 and 22) which indicates: (a) that L_R measures the time taken by the action potential to develop at a given spot of the plasma membrane and for the consequent electrical signal, moving inward in the T-tubules, to act by way of the triadic junctions to cause release of activator Ca^{2+} from the lateral vesicles of the sarcoplasmic reticulum; (b) that the latency relaxation is a mechanical sign of the process by which the lateral vesicles release their pulse of activator Ca^{2+}; and (c) that L_0, L, and L_1 indicate successive instants in the first few ms of the development of positive tension [13, 14, 23]. This interpretation of the mechanical onset features is not fully established, the significance of the latency relaxation being especially questioned by claims (e.g. ref. 19) that it represents a precontractile response of the myofilaments. However, our interpretations or hypothesis of the onset features has much strong evidence in its favor (e.g. refs. 13, 14, 21, 22, 24 and personal communication), and we base our attempt to explain our ^2H_2O effects by means of this hypothesis, as much to test it as to elucidate the action of the heavy water.

It is now known that the T-tubule signal is essentially like the action potential in the plasma membrane, i.e., an active process (e.g., ref. 25). Hence, the fact that ^2H_2O decreases the conduction speed of the action potential suggests that it also slows conduction of the tubular action potential. Thus, the ^2H_2O-induced increase in L_R can be attributed to the decreased speed of conduction of the electrical signal in the T-tubule. However, it seems unlikely that this increase could be the effect of ^2H_2O on excitation-contraction coupling that by itself causes the general depression of the twitch and tetanus outputs. For, the ^2H_2O spike, though conducted more slowly, suffers little change in shape (Figs. 1e and f) and this should also occur in the tubules [25]. Thus the depolarizing signal impressed on the lateral vesicles at the triadic junctions would not be critically different from the normal and hence would be unable in itself to alter the release of the activator Ca^{2+} so as to greatly, if at all, depresss contraction. A similar conclusion derives from the work on the barnacle fiber [9], since ^2H_2O produced no significant change in the membrane depolarization resulting from the exogenously applied current, and yet Ca^{2+}-release and contraction were depressed, thus indicating that some other process than the electrical one had been depressed by the ^2H_2O. We can infer furthermore, that the ^2H_2O-induced drastic mechanical effects in our tests are not essentially dependent on the action potential changes since the somewhat prolonged spike, as in other such cases in H_2O muscles (e.g. refs. 15 and 17), should cause potentiation, if anything, and depression, of the twitch. However, as indicated by the increase in L_R, heavy water does delay the start of release of Ca^{2+} and, as will be seen later, this may aid in depressing activation of contraction.

The indication that the changes in the action potential cannot account for the

critical effects of ^2H_2O suggest that these effects occur at the immediately following part of excitation-contraction coupling, which brings about the actual mobilization of the free Ca^{2+} for activation of contraction. We will consider this mobilization to consist of two major steps: (a) the release of Ca^{2+} from the sarcoplasmic reticulum and (b) the diffusion of the Ca^{2+} to the troponin on the thin filaments where the immediate, intrinsic effect of Ca^{2+} as activator of contraction occurs [26]. Our results on the onset features suggest that both these processes are affected by ^2H_2O. Regarding the first, we note again that ^2H_2O can suppress a depolarization-dependent Ca^{2+} release in the barnacle fiber. This suggests, in terms of our hypothesis [13, 14] that the latency relaxation signals the release of Ca^{2+} from the sarcoplasmic reticulum, that the ^2H_2O should produce some depressive effect on the latency relaxation, and we infer that this is the slowed development of the latency relaxation we observe in our ^2H_2O-treated muscles.

Various lines of evidence [13, 14, 23] suggest that positive tension development actually begins at L_0, so that the development of the latency relaxation during the interval L_0-L_R must occur free of any masking by the rise of tension. Hence we take the value of this interval to indicate the general kinetics of Ca^{2+} release, and, therefore, use the reciprocal of it to measure the average rate of release of Ca^{2+} by the sarcoplasmic reticulum. Using the data of Table I, $1/(L_0\text{-}L_R)$ is 0.83 normally and 0.50 under ^2H_2O, and we therefore conclude that the heavy water reduces the rate of Ca^{2+} release and thus the rate of its mobilization, to $0.50/0.83 = 0.60$ of the control value. (This factor is also obtained by using the change in the relative interval values for L_0-L_R given in Table I). Furthermore, it has been found (Homsher, E., personal communication) that ^2H_2O reduces the twitch activation heat of frog semitendinosus muscle to 58 % of normal, suggesting "that the total amount of Ca^{2+} released in the presence of ^2H_2O is less than that in normal Ringer's medium". Hence we assume that the decreased rate of Ca^{2+} release as indicated by the reduced rate of development of the latency relaxation has associated with it a reduction in the total amount of Ca^{2+} released during excitation-contraction coupling.

An integral part of the above analysis is that ^2H_2O depresses the depolarization-dependent release of Ca^{2+} from the sarcoplasmic reticulum. Contrary results seem to have been found for crayfish muscle [5] in experiments on stripped fibers whose reticulum was depolarized by replacing the 200 mM propionate of the normal medium with equivalent chloride. In these experiments, however, the replacement involved only the full 200 mM interchange of the two anions and it is possible that this obliterated a repressive effect of ^2H_2O that would be revealed with an interchange of a smaller concentration of the anions and, therefore, a less pronounced depolarization of the sarcoplasmic reticulum. In any case, the finding that heavy water reduces the activation heat of frog muscle (Homsher, E., personal communication) attests to the validity of our conclusion of a depressive effect of ^2H_2O on the sarcoplasmic reticulum. Furthermore, it is possible that our findings and the seeming contrary results [5] are both correct and that we are confronted here with a species difference.

As for the diffusion of the released Ca^{2+} to its target sites on the thin filaments, this should be slowed in our treated muscles since the viscosity of ^2H_2O at the temperature of our experiments is about 25 % greater than that of H_2O [16]. Assuming that, in the myosplasm, the diffusion coefficient of released Ca^{2+} (probably in association with some anion) is inversely proportional to the viscosity of the myoplasmic medium,

then the ^2H_2O would decrease the speed of diffusion of the Ca^{2+} to 0.80 (i.e., 1/1.25) of that in the H_2O-myoplasm, and thus correspondingly retard the arrival of the Ca^{2+} at the thin filaments. Moreover, the ^2H_2O -induced reduction of the amount of Ca^{2+} released would also depress mobilization of Ca^{2+}, since, for a given diffusion speed, it would decrease the rate at which the actual Ca^{2+} concentration rises at the critical myofilament sites.

It is now evident that ^2H_2O should decrease the effectiveness of Ca^{2+} mobilization by reducing (a) the rate of release of the Ca^{2+}, (b) the amount of it released, and (c) the speed of its diffusion. These depressive changes are sequentially produced and their effects must therefore be cumulative. Clearly, it is for this reason that the relative changes of the successive intervals given in Table I become greater, as previously mentioned, the later the interval appears during the onset period. To obtain the net, cumulative consequence of the individual effects on the rate of Ca^{2+} mobilization we assume, to a first approximation, that this is given by the product of their separate actions, i.e., $0.60 \times 0.58 \times 0.80 = 0.28$. This factor is a theoretical index of the ^2H_2O-induced total decrease in rate of mobilization of Ca^{2+} and it should therefore indicate, at least roughly, the decrease to be expected in the experimental onset rate of tension development. Measures of this tension rate are given by the value of $\dot{P}_{ON}$ or, alternatively, by $R/(L_1\text{-}L)$ which is the average rate during the interval $L_1\text{-}L$. Using the data of Table I, it is evident that ^2H_2O decreases these respective rates to 0.19 and 0.25 of their normal values. These experimental values are not too different from our theoretically predicted value of 0.28. We therefore conclude that the above analysis is essentially valid and thus presents to a first approximation the critical effects of ^2H_2O on excitation-contraction coupling of frog muscle. An important consequence of this is that the effects of ^2H_2O on excitation-contraction coupling, as interpreted by our analysis, present evidence in support of our proposals [11, 12] that the interval L_R measures the time for the initial, depolarizing electrical events of the coupling to run their course, and that the latency relaxation itself signals the mechanism involved in the actual release of activator Ca^{2+} by the sarcoplasmic reticulum.

The depressive effects of ^2H_2O on excitation-contraction coupling suggest that they would produce an increase in the mechanical threshold, or, indeed, a general shift of the tension/depolarization relation to greater values of depolarization. Such an effect has been found in crayfish fibers [5], though the attempt to explain it has been based on the conclusion, which we question (as explained above), that ^2H_2O has no effect on the release of activator Ca^{2+} from the crayfish fiber's sarcoplasmic reticulum. At any rate, in preliminary experiments (Homsher, E., personal communication) it has been found that heavy water does raise the mechanical threshold of our frog fibers, and we infer that this results especially from the depressive effects of the ^2H_2O on the mobilization of Ca^{2+}.

Two questions remain for discussion. First, it is possible that ^2H_2O barely affects the experimentally observed value R because of the way in which it alters the rates of latency relaxation development and tension onset. These two processes, in general, tend to overlap somewhat so that oncoming tension development tends to mask the expression of the latter portion of the latency relaxation [13, 14, 23]. In the normal muscle the rate of onset of tension is on the average about 10 times faster than the rate of development of the latency relaxation. But in the ^2H_2O muscle

tension onset is slightly slower than latency relaxation development; hence, the masking effect would be less than in the normal case and the value of R relatively large. It is thus possible that in terms of potentially expressible latency relaxations, the normal one, substantially reduced by the relatively large masking effect, would have been considerably larger than the ^2H_2O-affected one than is indicated by the observed values of R of the two latency relaxations. We thus infer that ^2H_2O does decrease the depth of the latency relaxation, and that this would signify a reduction of total Ca^{2+} mobilized for activation of contraction, as experimentally indicated by the results on activation heat (Homsher, E., personal communication).

The second remaining question concerns the mechanism by which ^2H_2O so sharply reduces especially the twitch peak output, if, as we show, its direct action is only to curtail the mobilization of activator Ca^{2+}. We hypothesize that two effects may be involved. (a) ^2H_2O, though greatly reducing the rate of Ca^{2+}-mobilization, may have little effect on the uptake of Ca^{2+} by the reticulum of intact muscle, since at the internal pH of muscle it seems to hardly alter the uptake of Ca^{2+} by isolated reticulum [12] and also because it has essentially no effect on the decay of the active state of skeletal muscle contraction [3]. Thus, in the ^2H_2O muscle removal of activator Ca^{2+} relative to its mobilization for contraction, would be much faster than it is normally so that retention of the amount of Ca^{2+} actually available for full activation of contraction would be diminished. (b) ^2H_2O evidently reduces the total amount of Ca^{2+} released during excitation-contraction coupling of the twitch. Both these processes would obviously tend to decrease the ability of the muscle to develop tension in electrically activated contractions and thus produce the diminished twitch (and tetanus) outputs so characteristic of the ^2H_2O-treated frog muscle.

Our discussion of the above two questions presents, in effect, hypotheses for possibly explaining the mechanisms that may be involved. Further work is planned to test each of these hypotheses.

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REFERENCES

1 Goodall, M. C. (1957) Yale J. Biol. Med. 30, 224
2 Kaminer, B. (1960) Nature 185, 172–173
3 Svensmark, O. (1961) Acta Physiol. Scand. 53, 75–84
4 Hotta, K. and Morales, M. F. (1960) J. Biol. Chem. 235, PC61–PC63
5 Eastwood, A. B., Grundfest, H., Brandt, P. W. and Reuben, J. P. (1975) J. Membrane Biol. 24, 249–263
6 Jenerick, H. (1964) Am. J. Physiol. 207, 944–946
7 Garby, L. and Nordqvist (1955) Acta Physiol. Scand. 34, 162–168
8 Spyropoulos, C. S. and Ezzy, M. E. (1959) Am. J. Physiol 197, 808–812
9 Kaminer, B. and Kimura, J. (1972) Science 176, 406–407
10 Ashley, C. C. and Ridgway, E. B. (1970) J. Physiol. 209, 105–130
11 Edwards, C., Chichibu, S. and Hagiwara, S. (1964) J. Gen. Physiol. 48, 225–234
12 Huxtable, R. and Bressler, R. (1974) J. Membrane Biol. 17, 189–197

13 Sandow, A. (1966) Med. Coll. Virginia Quart. 2 (2), 82–89
14 Mulieri, L. A. (1972) J. Physiol. 223, 333–354
15 Sandow, A. (1973) J. Mechanochem. Cell. Motility 2, 193–207
16 Thompson, J. F. (1963) Biological Effects of Deuterium, p. 3, Pergamon Press, New York
17 Taylor, S. R., Preiser, H. and Sandow, A. (1972) J. Gen. Physiol. 59, 421–436
18 Weber, A. and Herz, R. (1968) J. Gen. Physiol. 52, 750–759
19 Hill, D. K. (1968) J. Physiol. 199, 637–684
20 Gordon, A. M. and Godt, R. E. (1970) J. Gen. Physiol. 55, 254–275
21 Sandow, A. (1965) Pharmacol. Rev. 17, 265–320
22 Sandow, A., Sphicas, E. C. and Pagala, M. K. D. (1974) Fed. Proc. 33, 383
23 Sandow, A. (1944) J. Cell. Comp. Physiol. 24, 221–256
24 Herbst, M. (1975) Habilitationsschrift, Physiologisches Institut der Universität Hamburg
25 Adrian, R. H. and Peachey, L. D. (1973) J. Physiol. 235, 103–131
26 Ebashi, S., Endo, M. and Ohtsuki, I. (1969) Q. Rev. Biophys. 2, 351–384