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Detubulation Effects on the Action of Zinc on Frog Skeletal Muscle Action Potential

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Summary. Detubulation of the untreated fiber decreases the time constant of the action potential's foot (τ_f) and increases the maximal rate of rise of the spike ($\dot{V}_{max}$). Zinc at all concentrations, and regardless of whether the fiber is intact or detubulated, increases τ_f and decreases $\dot{V}_{max}$, and thus seems to decrease Na activation of the fiber. Detubulation was used principally to elucidate the localization and mechanism of the Zn^{2+}-induced retardation of the falling phase of the frog sartorius fiber action potential, which evidently results from a general depression of delayed rectification. At 50 to 1000 μM, Zn^{2+} not only prolongs repolarization of intact fibers (measured by increase in $t_{0.5}$, the half-time of the spike's fall), but also produces a marked hump early in this phase. Detubulation of zinc-free fibers reduces $t_{0.5}$ to about 80% of its intact value, and under Zn^{2+} treatment $t_{0.5}$ is increased but only to about 80% of that produced in the intact fiber, and the falling-phase hump is completely obliterated. Thus, detubulation decreases $t_{0.5}$ in Zn^{2+}-treated fibers not only by generally eliminating T-tubular participation in action potential generation, but also by subtracting a Zn^{2+}-induced retardation of tubular delayed rectification. Tubular delayed rectification must therefore be an intrinsic feature of normal excitation. These results are further analyzed by means of (i) Stanfield's findings about retardation of delayed rectification by Zn^{2+} and (ii) Adrian-Peachey's theory of T-tubule participation in action potential generation, which suggests that the Zn^{2+}-evoked repolarization hump signals onset of Zn^{2+}-altered active participation of T-tubules in determining the spike's shape.

Zinc ions considerably alter the course of the action potential and its excitatory conductances of both skeletal muscle (e.g., Mashima & Washio, 1964; Sandow *et al.*, 1964; Stanfield, 1975) and nerve (Begenisich & Lynch, 1974). In muscle fibers, Zn^{2+} in concentrations ranging from a few to 1000 μM produces progressively greater slowing of the repolari-

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zation phase, thus prolonging the spike by as much as 3× (Isaacson & Sandow, 1963; Taylor, Preiser & Sandow, 1972; Stanfield, 1973). Furthermore, for concentrations greater than about 250 μM, Zn^{2+} decreases both the rate of rise and the overshoot of the spike and increases the mechanical threshold (Sandow, Taylor & Preiser, 1965; Taylor *et al.*, 1972).

These effects in muscle are especially interesting since they cause various changes in excitation-contraction coupling. Spike prolongation produces twitch potentiation by as much as 3× (Isaacson & Sandow, 1963; Taylor *et al.*, 1972; Stanfield, 1973), and aequorin-monitored tests show that this potentiation is mediated by potentiated releases of activator Ca^{2+} (Lopez, Wanek & Taylor, 1976). The retarded rise of the spike and the increased mechanical threshold caused by the higher concentrations reduces the rate of tension rise at the very onset of the mechanical response (Taylor *et al.*, 1972; Sandow, 1973). The slowing of repolarization clearly results from depression of delayed rectification (Stanfield, 1975), and, though not yet directly demonstrated, the reduced rate produced on the rise of the spike seems to reflect depression of Na-activation by Zn^{2+} (but *see* Begenisich & Lynch, 1974).

The effects of Zn^{2+} on muscle are furthermore interesting since the localization of action of the ion—whether it is at the plasma membrane, the T-tubular membrane, or both—has not been resolved conclusively (Sandow & Isaacson, 1966; Stanfield, 1973). In the present work we have attempted to elucidate this problem by comparing the effects of Zn^{2+} on action potentials produced by intact fibers and by fibers detubulated by the glycerol treatment (Gage & Eisenberg, 1967; Howell, 1969). Our results indicate mainly that Zn^{2+} affects the excitatory responses of both plasma and T-tubular membranes, and especially as delayed rectification is involved, this indicates that the T-tubules as well as the plasma membrane perform this function. Our results also show that mere detubulation of fibers produces certain changes in the action potential not heretofore reported. A preliminary account of this work has appeared (Dass & Sandow, 1972).

Materials and Methods

We used sartorius muscles of the frog, *Rana pipiens*, which were mounted horizontally at 1.2× body length in Ringer's solution in a Lucite trough. A pair of stigmatic platinum electrodes was used to externally stimulate selected superficial fibers with square wave shocks of 0.2 msec duration generated by an American Electronics Labo-

ratory Type 104 Stimulator. Action potentials were led-off by means of internal glass microelectrodes filled with 3 M KCl and having a resistance of 5–10 M, and were recorded by way of a Bioelectric Co. negative capacitance preamplifier and the Tektronix Type 565 oscilloscope. In every experiment the pick-up electrode was always 3 mm from the stimulus cathode. Each action potential was recorded simultaneously on a fast and a slow sweep to facilitate temporal analysis of all phases of the response.

Our standard Ringer's solution consisted, in mM, of: NaCl, 117; KCl, 2.5; $CaCl_2$, 1.8; and Tris buffer, 2; also present was 2×10^{-2} g/liter tubocurarine chloride. We disrupted the T-tubules by first exposing the muscles for an hour to the Ringer's medium containing 400 mM glycerol and then to a Ringer's medium (glycerol treatment, Eisenberg & Eisenberg, 1968) containing 5 mM each of $CaCl_2$ and $MgCl_2$ for 20 min, this time being sufficient to show typical detubulation effects in the action potentials of our muscles and in other electrical parameters, in general (Dulhunty & Gage, 1973). The concentration of Ca^{2+} and Mg^{2+} was increased to prevent reduction of the resting potentials which ordinarily occurs in the detubulated fibers (Eisenberg, Howell & Vaughan, 1971). Zinc, as $ZnCl_2$, was added to either the ordinary or the elevated $Ca^{2+} - Mg^{2+}$ Ringer's medium in various concentrations as needed. Control tests showed that the elevation in Ca^{2+} and Mg^{2+} in the Ringer's medium did not essentially alter the basic effects of zinc on the action potentials. Since zinc-induced changes are not easily reversible (Sandow & Isaacson, 1966), we studied the effects of each concentration of zinc (5 to 1000 μM) on separate muscles in either intact or detubulated condition. In a typical experiment the action potentials from 5–10 superficial muscle fibers were recorded successively in each of the following sequence of media: standard Ringer's, elevated $Ca^{2+} - Mg^{2+}$ Ringer's, and zinc Ringer's. Each medium was allowed to act for a 10-min period before any records were made. In detubulation experiments, first a few intact fiber responses were recorded in the zinc-free Ringer's media, then the muscle was exposed to the glycerol Ringer's medium, and subsequently the responses were recorded in the zinc-free and zinc-elevated $Ca^{2+} - Mg^{2+}$-Ringer's media. In view of the need to use separate muscles to investigate the action of zinc, the relevant data were necessarily restricted to unpaired variates and corresponding *t*-tests were used to check for statistical significance. In general, our results are presented as a mean ±SE (i.e., ±SD of the mean).

All procedures of our experiments were done at room temperature ranging from 20 to 22 °C.

Results

Zinc-Free Media

Comparison of Fig. 1*a1* and *a2* indicates that the action potentials of our detubulated fibers in normal Ringer's show typical, previously described features, i.e., absence of after potentials (Gage & Eisenberg, 1967) and decreased conduction time (i.e., increased conduction speed) (Hodgkin & Nakajima, 1972). But we observed several other changes also: detubulation decreases the time constant of the foot (τ_f) of the action potential, this being only minimally evident in the illustrated typical records of Fig. 1*a* (but *see* Table 1), and detubulation obviously increases

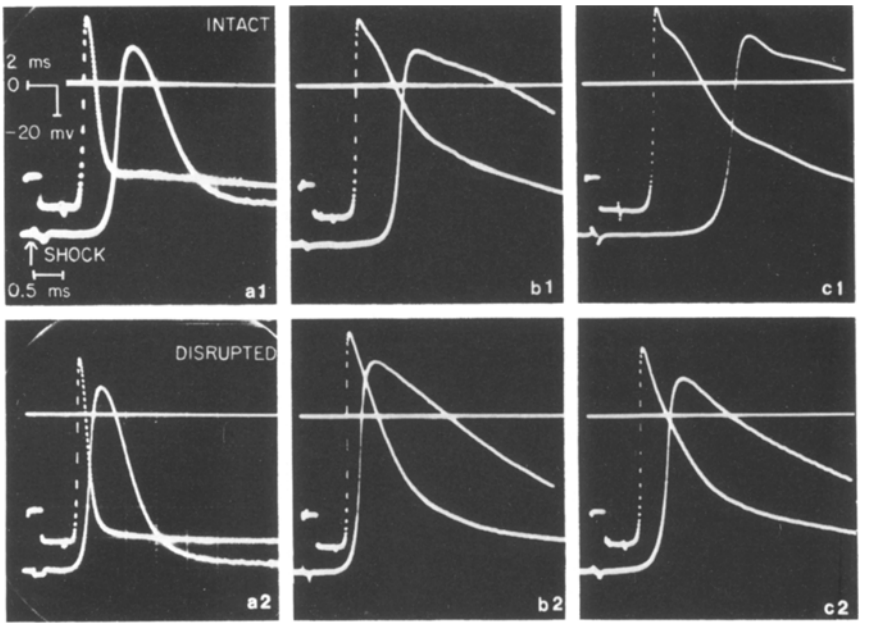

Fig. 1. Typical action potentials of surface fibers of the frog sartorius muscle, intact (upper panels) or detubulated (lower panels), under normal or zinc treated condition. Each response is recorded on both a slow (upper) and a fast (lower) sweep, with time and also voltage calibrations for all panels as given in *a1*. The calibration square wave at the start of each slow sweep represents 1.0 msec and 20 mV. The panels *a1* and *a2* are for normal fibers; *b1* and *b2*, 50 μM Zn^{2+}, and *c1* and *c2*, 1000 μM Zn^{2+}. In all cases the pickup electrode was 3 mm distant from the stimulating cathode. Temperature, 20–22 °C

the maximal rate of rise ($\dot{V}_{max}$) and of the rate of fall of the spike (represented by t 0.5, the 0.5 time of the fall). The mean values of these other changes, indicated by comparison of columns *a* and *b* of each of Tables 1, 2, and 3, show that detubulation decreases τ_f to 76% of the value of the intact fiber, increases $\dot{V}_{max}$ to 112% of the normal, and decreases $t_{0.5}$ to 79% of the normal. Our finding regarding τ_f is in contrast with Hodgkin and Nakajima (1972) who found no change on detubulation.

Zinc Media

General effects. Panels *b1* and *c1* of Fig. 1 show typical prolongations of the spike potentials of intact fibers produced by 50 and 1000 μM Zn^{2+}, respectively, which obviously result from slowing of the fall of the spike.

Table 1. Effects of detubulation and Zn^{2+} on the time constant of the action potential foot[a]

Zn^{2+} (μM)	Intact		Detubulated		Normalized effect of Zn^{2+}		Relative effect of detubulation dc^{-1}/ba^{-1}
	Normal a (msec)	Zn^{2+} b (msec)	Normal c (msec)	Zn^{2+} d (msec)	Intact ba^{-1}	Detubulated dc^{-1}	
5	0.120	0.118	0.088	0.086	0.983	0.977	0.994
10	0.131	0.126	0.095	0.095	0.962	1.000	1.040
50	0.138	0.138	0.108	0.086	1.000	0.796	0.796
500	0.127	0.155	0.095	0.121	1.220	1.274	1.044
1000	0.120	0.187	0.082	0.142	1.558	1.732	1.112
General mean ±SE	0.125 ±0.002		0.097 ±0.004	*Mean effect of detubulation* normal muscle: $ca^{-1}=0.757\pm0.026$ zinc treated muscle: $db^{-1}=0.729\pm0.028$			0.997 ±0.054
(n)	(12)		(12)				(5)
		$P\ll0.001$				$P>0.4$	$P>0.95$

[a] Each row presents data of two kinds of experiments. In one kind, intact muscles were used; in the other, different, detubulated muscles were used. For each type of experiment, the "normal" data give the control means for zero-Zn^{2+} medium; and the associated "Zn^{2+}" columns give the experimental means for these same muscles. Columns a, b, c, and d each give means of measurements made on action potentials of 5 or more surface fibers of 2 or more separate muscles. The coefficients of variance (SE/mean) of these means range from 0.000 to 0.192, with a mean $\pm SE=0.067\pm0.018$ ($n=20$). Each general mean ±SE is for the pooled data of its own column with n the number of the separate muscles used. The p value at the far left applies to the difference between the general means for columns a and c. The p value at the far right refers to the difference between the mean and 1,000. The appended data at the bottom of the table indicate that $\overline{c/a}$ is statistically equal to $\overline{d/b}$, and thus that $db^{-1}/ca^{-1}=dc^{-1}/ba^{-1}$.

The values of τ_f were obtained as described in Hodgkin and Nakajima (1972).

Additional changes not previously mentioned (*cf.* Taylor *et al.*, 1972) are increased conduction time, slower rise of the foot (especially evident in *c1*), and, most interesting, a shoulder shortly after the start of repolarization. As generally illustrated in panels *b1* and *c1*, the shoulder divides the repolarization phase into three fairly distinct parts: (i) an initial fall of potential from crest of about 5–12 mV at a rate definitely lower than occurs in the untreated muscle; (ii) the shoulder itself involving an additional still slower decrease in potential of about 10 mV; and, finally, (iii) a quasi-exponential fall to the resting potential, with a faint indication of a transiently reduced rate of fall at about −40 mV, which is

Table 2. Effects of detubulation and Zn^{2+} on the maximal rate of rise of the action potential[a]

Zn^{2+} (μM)	Intact		Detubulated		Normalized effect of Zn^{2+}		Relative effect of detubulation dc^{-1}/ba^{-1}
	Normal a (V/sec)	Zn^{2+} b (V/sec)	Normal c (V/sec)	Zn^{2+} d (V/sec)	Intact ba^{-1}	Detubulated dc^{-1}	
5	549	525	641	605	0.96	0.94	0.979
10	484	542	592	633	1.12	1.07	0.955
50	514	556	680	610	1.08	0.90	0.833
500	554	476	589	465	0.86	0.79	0.919
1000	566	354	618	463	0.62	0.75	1.20
General mean ±SE	533 ±10		624 ±11		*Mean effect of detubulation* normal muscle: $ca^{-1}=1.12\pm0.04$ zinc-treated muscle: $db^{-1}=1.14\pm0.04$		0.977 ±0.061
(n)	(12)		(12)				(5)
		$P\ll0.001$				$P>0.7$	$P>0.95$

[a] The organization of the data is the same as described for Table 1. The mean coefficient of variance of these data $=0.061\pm0.012$ ($n=20$). The raw data were obtained from the same records used for Table 1.

reminiscent of the initiation, though at a later time, of the negative after potential of the normal action potential. The shoulder is clearly displayed in records of 50 and 500 μM Zn^{2+} spikes reported earlier by our laboratory (Taylor *et al.*, 1972), and it seems detectable in a 5 μM Zn^{2+} record of Edman, Grieve and Nilsson (1966) and in a 100-μM record obtained by Stanfield (1973), but none of these reports made any mention of it. However, a 500-μM record of Mashima and Washio (1964) includes no shoulder.

After glycerol treatment, the action potentials in both 50 and 1000 μM Zn^{2+} (Fig. 1) show reduced conduction time, faster timing of the foot, slightly increased rate of rise (compare spacing of the switching pulses), and, most strikingly, they are qualitatively different since the shoulder is completely absent, so that the entire fall from crest is quasi-exponential, with a general, much faster rate of fall than occurs in the intact fiber.

Detailed effects. The effects of zinc on each of the main parameters must be considered in terms of a comparison of the changes in the intact *vs.* the detubulated fiber. Table 1 shows that with increasing concentration of Zn^{2+}, τ_f increases in both intact and detubulated fibers

Table 3. Effects of detubulation and zinc on the half-time of fall of the action potential[a]

Zn^{2+} (μM)	Intact		Detubulated		Normalized effect of zinc		Relative effect of detubulation
	Normal *a* (msec)	Zn^{2+} *b* (msec)	Normal *c* (msec)	Zn^{2+} *d* (msec)	Intact[b] ba^{-1}	Detubulated dc^{-1}	dc^{-1}/ba^{-1}
5	0.542	1.551	0.480	1.055	2.86	2.20	0.769
10	0.585	2.293	0.464	1.502	3.92	3.24	0.827
50	0.554	3.014	0.451	2.025	5.44	4.49	0.825
500	0.549	3.323	0.450	2.100	6.05	4.67	0.772
1000	0.627	3.924	0.462	2.365	6.26	5.12	0.818
General mean	0.571		0.471	*Mean effect of detubulation*			0.802
±SE	±0.016		±0.011	normal muscle: $ca^{-1}=0.792\pm0.026$			±0.013
(*n*)	(15)		(15)	zinc-treated muscle: $db^{-1}=0.648\pm0.014$ $P<0.001$			(5)
		$P\ll0.001$		$db^{-1}/ca^{-1}=0.818$			$P<0.001$[c]

[a] The organization of the data is the same as described for Table 1. The mean coefficient of variance for these data $=0.59\pm0.011$ ($n=20$). The raw data were obtained from the same records used for Tables 1 and 2.

[b] These normalized effects are much larger than usually reported (*cf.* Taylor *et al.*, 1972; Stanfield, 1973). This may be due to our having obtained the records in high Ca/Mg media, but no tests were done to prove this. The large magnitude of the present results is not caused by the use of $t_{0.5}$ (as against D_{25}, see Taylor *et al.*, 1972). But they are mutually completely consistent and therefore acceptable in the present investigation.

[c] This *P* value indicates that the mean value 0.802 is significantly different from 1.0.

(columns *b* and *d*). However, these normalized increases are essentially proportionately the same, as shown especially by the mean of the last column of the table which proves statistically that the general ability of zinc to increase τ_f is the same in both detubulated and intact fibers, i.e., $dc^{-1}/ba^{-1}=1$. Furthermore, the mean effect of detubulation is the same in both normal and zinc-treated muscles (i.e., $db^{-1}/ca^{-1}=1$, statistically, as should be true, anyway, since $db^{-1}/ca^{-1}=dc^{-1}/ba^{-1}$ algebraically).

Table 2 demonstrates that $\dot{V}_{max}$ behaves in all essentials in exact parallel to τ_f. Thus, as the concentration of Zn^{2+} is increased, proportionate decreases in $\dot{V}_{max}$ occur in both intact and detubulated fibers. That is, zinc generally decreases $\dot{V}_{max}$ to the same extent whether or not the fiber is detubulated (i.e., $dc^{-1}/ba^{-1}=1$, essentially); or, equivalently,

detubulation causes the same relative increases in $\dot{V}_{max}$, whether or not the muscle is treated with zinc (i.e., $db^{-1}/ca^{-1}=1$, also).

Table 3 indicates that Zn^{2+} at each of its concentrations causes a smaller prolongation of the spike in the detubulated muscle than it does in the intact muscle (*compare* dc^{-1} and ba^{-1}), and that the mean ratio of these values is essentially constant at 0.802 ± 0.013. Furthermore, this effect holds quantitatively even in the absence of Zn^{2+}, for the mean of the ratios ca^{-1} is 0.792 ± 0.026, obviously statistically the same as the above value. This specific ratio appears in still another feature of the data of Table 3. Note first that, as shown by the mean value of ca^{-1}, detubulation of the zinc-free muscle reduces $t_{0.5}$ to 79.2% of its value in the intact muscle. Second, note that the mean ratio db^{-1} indicates that detubulation of the zinc-treated muscle decreases $t_{0.5}$ to 64.8% of its value in the intact muscle. Thus, the effect of Zn^{2+} on the capacity of detubulation to reduce $t_{0.5}$ may be given by the mean of $db^{-1}/ca^{-1} = 81.8\%$, which is statistically the same as the two other values (80.2 and 79.2%) given above. But this is to be expected directly since algebraically $db^{-1}/ca^{-1} = dc^{-1}/ba^{-1}$. Thus, no matter whether Zn^{2+} is present or not, detubulation always reduces $t_{0.5}$ to about 80% of its corresponding value in the intact muscle. This result is strikingly different from the behavior of τ_f and $\dot{V}_{max}$, since, as shown by Tables 1 and 2, detubulation has no effect on their normalized effects of Zn^{2+}, and yet regardless of whether or not Zn^{2+} is present, detubulation reduces τ_f and increases $\dot{V}_{max}$.

Discussion

As indicated in Tables 1, 2, and 3, detubulation always speeds up the three action potential parameters we have studied, whether or not zinc is involved. Though the conduction speed was not studied in detail, Fig. 1 shows that it is similarly affected. The general similarity of these hastening effects suggests that they have a common basis, and we infer that this is the reduction in fiber capacitance, C_M, resulting from detubulation (Gage & Eisenberg, 1969). Such a change would be involved directly in affecting τ_f (Hodgkin & Nakajima, 1972, hereafter designated HN) and indirectly in determining $\dot{V}_{max}$ and $t_{0.5}$ (Hodgkin & Huxley, 1952). However, we cannot discuss this inference quantitatively since we did not develop our experiments as did HN (because our original purpose was merely to study the repolarization phase, and then, owing to certain logistic factors, the unexpected findings about τ_f, and $\dot{V}_{max}$ could not be

fully pursued) to include measurements of the membrane capacitance, especially its high frequency value, C_f, characterizing the foot of the action potential.[1] However, there still remains a discrepancy between our and HN's measurements of the effect of detubulation on τ_f. But a species difference may be involved since their muscles were obtained from *R. temporaria*, and ours from *R. pipiens*. Further experiments are obviously needed in this regard.

Our results that zinc (from 250 to 1000 μM, applied externally) reduces $\dot{V}_{max}$ in both intact and disrupted fibers are similar to earlier findings (Taylor *et al.*, 1972), and they can be most simply explained by assuming that Zn^{2+} depresses Na-activation (Taylor *et al.*, 1972). Thus, both our experimental facts and corresponding inference contradict Stanfield's (1975) assertions that external Zn^{2+} affects neither the kinetics of the spike's rise nor Na^+-activation. Furthermore, it has been demonstrated that injected Zn^{2+}, at higher concentrations than ours, depresses Na-activation of the squid giant axon (Begenisich & Lynch, 1974) and of the node of Ranvier (Fox, Rojas & Stampfli, 1974). In view of the very low permeability of the muscle fiber to Zn^{2+} and other surface features of its action in potentiating the twitch (Sandow & Isaacson, 1966), we judge that in our tests it had acted externally to affect $\dot{V}_{max}$. Hence we conclude that, probably depending on type of fiber, both external and internal sites of the activated Na^+ channel must be accessible to the action of Zn^{2+}.

The effects of detubulation on τ_f and $\dot{V}_{max}$ raise a question regarding the absence in Adrian and Peachey's (1973, hereafter designated AP) theory of the muscle fiber action potential of any overt expression of changes owing to tubular elements during the rising phase of the spike. Our results clearly show that detubulation speeds up both τ_f and $\dot{V}_{max}$, and thus seem to be at variance with the theoretical delineation of these features of the model as discussed by AP.

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1 According to the formulation of HN [*see also* Nakajima & Hodgkin, 1970, and Jack, Noble & Tsien, 1975, Eq. (6.16)], τ_f and C_f are involved inversely and this seems to contradict our inference that reduction in membrane capacitance could account for our result that detubulation decreases τ_f. But the HN formula involves θ (θ=velocity of the action potential). Unfortunately, there seems to be no accepted simple expression for θ in terms of the basic electrical features underlying propagation of the action potential (Jack *et al.*, 1975). But an approach to this expression may be made in terms of the theory of θ proposed by Rushton (1937) or by Offner, Weinberg and Young (1940). Use of their expressions for θ in the HN relation for τ_f proves in either case that τ_f is proportional to $C_f R_{Mf}$ where R_{Mf} is the membrane resistance ($\Omega\,cm^2$) appropriate to the fiber during the foot. It is now obvious that τ_f is actually directly proportional to the membrane time constant for the foot and thus directly proportional to C_f.

Although the repolarization phase is speeded up by glycerol treatment, even in presence of Zn^{2+}, this effect is quantitatively different from that found for τ_f and $\dot{V}_{max}$ (*cf.* the dc^{-1}/ba^{-1} values of Tables 1, 2, and 3). The special finding here can be most usefully described by noting that Zn^{2+} at any concentration causes a smaller normalized increase in $t_{0.5}$ in the detubulated fiber than it does in the intact one. Moreover, the Zn^{2+}-induced repolarization hump is missing in the detubulated spike. Obviously, these effects must be attributed to the elimination of the T-tubules which results from glycerol treatment, and it might be supposed that they could be caused by the large reduction in the fiber low-frequency capacitance produced by detubulation (HN). But if this were the only factor, it would be difficult to explain, in the detubulated fiber, both the smaller increase in $t_{0.5}$ when Zn^{2+} is present and the qualitatively distinct elimination of the hump. Thus something other than, or in addition to, the decreased capacitance must occur to account for these special changes. Hence, we turn to the good evidence that the general prolongation of the spike's falling phase by zinc is caused by greatly depressing both the rate and amount of delayed rectification (Stanfield, 1975). Noteworthily, this work (*see also* Stanfield, 1973) left open the question of the location of these depressive effects. However, our result that Zn^{2+} prolongs the repolarization phase to a lesser extent in the detubulated fiber than in the intact one, indicates that a moiety of the depressed delayed rectification due to Zn^{2+} is removed when the T-tubules are made inoperative. Thus we conclude that zinc's inhibition of delayed rectification occurs at both the T-tubules and the plasma membrane.

Further aspects of the effect of Zn^{2+} in our experiments, especially concerning the repolarization hump, can be adduced by means of AP's theory of the muscle fiber's action potential, particularly an interesting feature of it, namely (Peachey, 1973), that the cycle of conductance changes in the T-tubules involves a positive current flow from the external medium into the T-tubules, which at the start passes through the open Na-channels of the T-tubular membrane and later helps to recharge the tubular capacity as it repolarizes. To complete its circuit, this current must pass through the sarcoplasm and outward across the surface membrane, thus tending to depolarize it. Should this occur during the period when delayed rectification is the predominant conductance feature of the surface membrane, it will oppose and retard repolarization of the action potential as recorded conventionally by an internal electrode. Peachey (1973) states that "Perhaps the most important aspect of this

observation is that it gives us a method for obtaining with a microelectrode a record that reveals the activity of the T-system."

Several such revelations regarding our results of this "AP tubular effect," (APTE, our term) are as follows. According to the AP calculations for a 100-μm diameter fiber at 20 °C (conditions approximated in our tests) the APTE should become evident about 0.5–1 msec after the onset of the action potential, and this is just when our action potentials begin their falling phase (*see* Fig. 1). Thus the APTE in the zinc-free tests could account for the relative slowness of repolarization that occurs in the intact, as against the disrupted, fiber. Furthermore, in the zinc-treated muscles the increase in $t_{0.5}$ would be due not only to the direct depressive effects on delayed rectification of the surface, but also to the incidental APTE here exaggerated by the zinc-induced slowing of tubular delayed rectification.

A second application of the APTE concerns the timing of the shoulder's appearance in the zinc-treated intact fiber potentials. We infer that the part of the repolarization phase that precedes the shoulder occurs so early that it indicates the effect of zinc on mainly, or possibly only, the plasma membrane. This is supported by the fact (*see* Fig. 1) that the prolongation of this part of the spike is about the same in both the detubulated and intact zinc-treated fibers. The appearance of the shoulder about 1.0–1.5 msec after onset of the spike must then signal the start of the APTE as affected by the action of zinc on the Na-activation of the T-tubules, such a delay being expected theoretically since the depolarizing effect of the APTE should appear soon after onset of the spike (AP).

As a final correlation of our results with the AP theory, note first (Fig. 1) that the tubular contribution to the falling phase of our recorded action potentials must be represented by the difference between the intact and detubulated fiber's generation of this phase. By using the values of $t_{0.5}$ (Table 3) to estimate this difference quantitatively, it appears that the tubules, whether exposed to Zn^{2+} or not, probably contribute about 20% of the fiber's total delayed rectification. Internal evidence in the AP (1973) presentation (*see* especially their Table 1*B*) indicates that the theoretical contribution of the tubular delayed rectification would be 18.6% of the fiber's total. That these two values are so close may be a coincidence. But, in so far as our rough procedure makes sense, the above accord lends support to the feature of the AP theory dealing with involvement of the tubules in generation of the muscle action potential. Clearly, a rigorous theoretical treatment of this point would require that the rather formidable sort of analysis used by

AP be applied to the various alterations caused by Zn^{2+} in the generation of the action potential.

In conclusion, the most important result of our work is that the action of Zn^{2+} to prolong the falling phase of the action potential by depressing delayed rectification takes place at the T-tubular membrane as well as at the plasma membrane. The impact of this on the role of the action potential in excitation-contraction coupling, in general, and particularly in potentiated twitches such as produced by Zn^{2+}, has yet to be determined. However, our results help to clarify questions that have been raised (e.g., Adrian, Chandler & Hodgkin, 1970) whether the tubules have delayed rectification, and they refute the view (Ildefonse, Pager & Rougier, 1969) that detubulation eliminates all delayed rectifier activity. Furthermore, our results are completely consistent with the AP assumption placing such activity in the T system as well as in the plasma membrane and with the clear results of Kirsch, Nichols and Nakajima (1977) that the T-tubules possess delayed rectifier channels.

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