J. Membrane Biol. 53, 223–233 (1980)

The Journal of
Membrane Biology

Glycerol Treatment in Mammalian Skeletal Muscle

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Summary. (1) The effects of glycerol-treatment on the ultrastructure, tension, and electrical properties of rat sternomastoid muscle fibers are described. (2) The effect upon the ultrastructure of fibers differed from that previously reported for amphibian fibers, in that the sarcoplasmic reticulum, as well as the T-system, was disrupted. (3) Tension (tetanus and K-contracture) was abolished when preparations were returned to normal Krebs after exposure to a glycerol-Krebs solution (exposure periods were 1 hr in 200–350 mM-glycerol or 10–60 min in 350 mM-glycerol), although fibers had normal resting membrane potentials and action potentials. (4) Fibers treated for 1 hr with 350 mM-glycerol were detubulated when returned to normal Krebs. Specific membrane capacity was reduced and exogenous horseradish peroxidase (HRP) did not penetrate the T-system. (5) Fibers were not detubulated after treatment for 1 hr with 200 to 300 mM-glycerol or after treatment for 10 to 30 min with 350 mM glycerol. Specific membrane capacity and resistance were normal and HRP entered the T-system. (6) Ultrastructural disruption of the triad junction became progressively more extensive with increasing glycerol concentration used and may be responsible for uncoupling.

The primary aim of this investigation was to characterize, in a mammalian muscle preparation, the electrophysiological aspects of glycerol treatment, a procedure which has been shown to uncouple contraction from excitation in amphibian preparations (Eisenberg & Gage, 1967; Howell & Jenden, 1967; Gage & Eisenberg, 1969; Valdiosera, Clausen & Eisenberg, 1974). The secondary aim was to define, if possible, conditions of glycerol treatment which would yield mechanically-uncoupled mammalian fibers with normal membrane capacity, such as have been observed in amphibian preparations (Dulhunty & Gage, 1973; Chandler, Rakowski & Schneider, 1976). Finally, since investigation of the ultrastructure of the triad in such preparations may show alterations indicative of the nature of uncoupling and, at the same time, reveal something of the nature of the normal coupling process, the ultrastructure of both detubulated and non-detubulated glycerol-treated fibers has been investigated.

Materials and Methods

Glycerol Treatment

The experiments were performed on bundles of fast-twitch red fibers dissected from rat sternomastoid muscles (Dulhunty, 1979; Dulhunty & Dlutowski, 1979). Preparations were dissected in a normal Krebs solution at room temperature (~20 °C). The normal Krebs solution contained (mmol/liter): Na, 145; K, 3.5; Ca, 2.5; Mg, 1.0; Cl, 130.5; HCO_3, 25; glucose 11. The solution was bubbled with Carbogen (10% O_2, 90% CO_2) gas for 20 min before and during the experiment, giving a pH of 7.3. The procedure used for glycerol treatment was to rapidly change the bathing solution from the normal Krebs solution to a glycerol-Krebs solution for the period of treatment and then to change back to the normal Krebs solution. The glycerol-Krebs solution was prepared by adding the desired concentrations of glycerol to the normal Krebs solution.

Tension Recording

Tension was recorded with an RCA 5734 transducer. Bundles were electrically stimulated by external electrodes located on either side of the midpoint of the preparation. A supramaximal stimulus for the twitch was determined (normally 0.5 msec, 80 V) and used subsequently. For tetanic stimulation this was repeated at 100 Hz for 1–2 sec. Bundles were normally subjected to 1 tetanus every 5 min. The tetanic tension normally declined with time during control experiments at room temperature. A similar decline was

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0022-2631/80/0053-0223 $02.20

apparent during the 1-hr exposure to glycerol and was presumably not related to the presence of glycerol.

Electrophysiology

The passive electrical properties of fibers were measured using standard cable analysis techniques (Fatt & Katz, 1951; Hodgkin & Rushton, 1946). Two KCl-filled glass microelectrodes were inserted into the fiber. Membrane potential was recorded with one electrode and a current pulse injected into the fiber through the second electrode, using a current clamp circuit. The current electrode was kept at one point in the fiber, and the voltage electrode was inserted at a number of positions along the length of the fiber. The membrane potential response to a square current pulse was recorded for each electrode separation, normally between 20 and 800 μm. A Signetics microprocessor-based computing system was used to analyze the records. The time integral of the rising phase of the membrane potential response was measured electronically and sampled by the microprocessor system, along with the final amplitude of the response and the current pulse amplitude. Twenty to forty responses were sampled at each electrode separation and the average values stored and later used to calculate specific membrane resistance, R_m, and capacity, C_m (Adrian & Almers, 1973; Loo & Vaughan, 1976). R_m and C_m of control and of glycerol-treated preparations are denoted R_mc and C_mc, and R_mg and C_mg, respectively.

Electron Microscopy

Bundles were fixed by the addition of 2% glutaraldehyde to the Krebs solution bathing the preparation (Davey, 1973). Shortly thereafter, this solution was replaced by a similar fixative buffered with 5 mM-HEPES (N-2-hydroxyethylpiperazine-N'-2-ethane sulphonic acid) rather than with the bicarbonate buffer, and the preparations were transferred to glass vials. Fixation was continued overnight under refrigeration. The aldehyde fixed bundles were rinsed in HEPES-buffered Krebs solution, and small blocks suitable for embedding (and, where applicable, for HRP incubation – *see* below) were cut from them. The blocks were postfixed in 1% OsO_4 in unbuffered Krebs solution (2–20 hr, 0 °C), stained overnight in uranyl nitrate (0.5%, pH 5.2), dehydrated, and embedded in Spurr's (1969) resin. Micrographs of thin sections were obtained using a Philips EM201c or EM300.

Horseradish Peroxidase (HRP) Localization

HRP (Sigma Type VI, 2.5 mg/ml of Krebs solution) was used in some experiments to label the extracellular space (Eisenberg & Eisenberg, 1968). Aldehyde fixation was performed as described above, and the following incubation was performed immediately prior to osmium fixation. The blocks were infiltrated with diaminobenzidine (0.06% in 0.15M-Tris maleate buffer, pH 7.6) for 1 hr; then the reaction was initiated by adding 0.01% H_2O_2. The resulting mixture was changed at 15 min intervals for 1.5 hr. Finally the blocks were rinsed in several changes of the Tris-maleate buffer.

Results

The effect of glycerol treatment was more severe in fast-twitch mammalian fibers than in amphibian fibers. When red sternomastoid bundles were treated for 1 hr with 400 mM glycerol Krebs and then returned to normal Krebs, tension fell to zero within 10 min, and the resting membrane potential of most fibers fell to values less negative than −50 mV. The fibers appeared to be damaged and broken when viewed with a dissecting microscope. Fiber damage and depolarization were less severe when lower concentrations of glycerol were used (*see* Table 1, Dulhunty, 1979). When preparations were treated for 1 hr with 350 mM glycerol and returned to Krebs solution, many fibers had normal membrane potentials (more negative than −50 mV), although the number of such fibers varied considerably (30–90%) from one preparation to the next. Fibers on the surface of glycerol-treated bundles were often more depolarized than fibers deep within the bundles. Normal action potentials were recorded from fibers with resting potentials more negative than −60 mV. Occasionally, movement of a fiber could be observed through the dissecting microscope, but this was seldom sufficient to dislodge the microelectrodes. The experiments described below were done with glycerol concentrations between 200 and 350 mmol/liter.

Treatment for 1 hr with 350 mM glycerol

Tetanic tension fell by 10 to 30% (*see* Materials and Methods) during the 1-hr exposure to 350 mM glycerol at room temperature (Fig. 1*A*). When the bundles were returned to normal Krebs there was an initial increase in tetanic tension (5–10%), which then fell quickly to zero with a 50% time of about 8 min (Fig. 1*B*). Movement was sometimes seen following electrical stimulation, even though no tension response was recorded. It is possible that either a small fraction of the fibers in such cases were not uncoupled, or that the uncoupling was not complete in a number of fibers. This latter possibility is more likely, because a small length of activated myofibril would be unlikely to produce much tension at the ends of the fiber, since this would have to be manifest through the remaining length of unactivated and extensible sarcomeres in that myofibril. During the phase of tension reduction the shape of the tetanic response changed (Fig. 1*B*) and tension declined rapidly during the tetani to plateaus that were less than 50% of peak tension at their onset. The reduction in tetanic tension (Fig. 1*B*) was not due to failure of the action potential mechanism, since action potentials could be recorded from some surface fibers and most deep fibers. However, tension reduction may have been due to a change in activation kinetics so that a brief depolarization was no longer effective in releasing calcium from the sarcoplasmic reticulum.

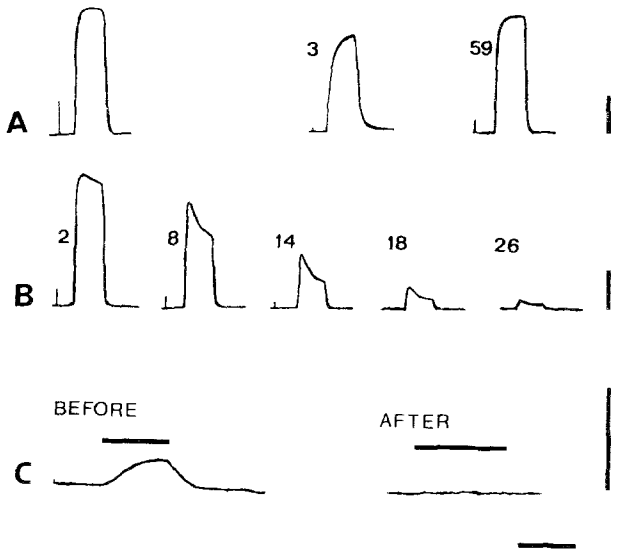

Fig. 1. Tension records during 350 mM glycerol treatment. (*A*): Twitch (small vertical deflections) and tetanic responses to electrical stimulation in normal Krebs (first record) and for the indicated times (in min) after the preparation was placed in 350 mM glycerol Krebs. Horizontal calibrations: 24 sec for twitches, 2 sec for tetani. (*B*): Tension records after return to normal Krebs for the indicated times (in min). Horizontal calibrations: 24 sec for twitches, 2 sec for tetani. Vertical calibrations: 1 kg/cm^2 applies to *A* and *B*. (*C*): Tension response to high external potassium ion concentration before and 30 min after a 1-hr glycerol treatment (same preparation as in *A* and *B*). The normal Krebs solution (3.5 mM K) was replaced by a high K solution (224 mM K) for the period indicated by the bar above the records. Horizontal calibration: 24 sec. Vertical calibration: 0.5 kg/cm^2

The ability of glycerol-treated fibers to contract in response to prolonged depolarization was investigated using potassium contractures. A normal K-contracture recorded before glycerol treatment is shown in Fig. 1*C*, together with a record made 30 min after the preparation had been returned to normal Krebs, at a time when its tetanic tension was zero. It is clear that an increase in external potassium concentration did not evoke a contracture in the glycerol-treated preparation.

The results of cable analysis experiments are listed in Table 1 (normal fibers) and Table 2 (glycerol-treated fibers). The edges of red fibers could not be clearly identified, and their diameters could not be measured. An average internal resistivity of 169 Ωcm was assumed (Hodgkin & Nakajima, 1969), and an equivalent diameter calculated. This assumption is reasonable, since the average calculated diameter of 12 normal fibers was 51 ± 2.6 μm (mean ± SE) which compares well with an average diameter of 51.8 ± 0.9 μm measured from frozen sections of 200 fibers (Dulhunty & Dlutowski, 1979). In both cases the measurements were made on muscles from animals weighing more than 300 g. The red fibers

Table 1. Passive electrical properties of fibers from four preparations[a]

Preparation code	Number of fibers *n*	Membrane potential *Vm* (mV)	Diameter *d* (μm)	Membrane resistance R_mc (Ωcm^2)	Membrane capacity C_mc (μF/cm^2)
21-2-1	6	76 (2)	50 (4)	1129 (105)	10.5 (1.0)
13-3-1	5	82 (1)	52 (5)	731 (50)	7.3 (0.8)
17-3-1	6	78 (2)	47 (1)	538 (63)	5.6 (0.2)
20-3-1	5	82 (1)	41 (1)	928 (73)	5.2 (0.9)

[a] Average values are listed and the SE is given in parentheses below each value.

Table 2. Passive electrical properties of fibers treated for 1 hr with 350 mM glycerol-Krebs and then returned to normal Krebs for 1 to 3 hr[a]

Preparation code	*n*	*Vm* (mV)	*d* (μm)	R_mg Ω cm^2	C_mg μF/cm^2	R_mg/R_mc[b]	C_mg/C_mc[b]
12-3-1	4	60 (6)	57 (9)	1565 (354)	2.4 (0.5)	2.1	0.32
16-3-2	9	68 (3)	62 (6)	812 (233)	2.7 (0.4)	1.5	0.48
17-3-2	9	68 (2)	57 (6)	1062 (134)	3.8 (0.6)	2.0	0.62
20-3-2	5	59 (2)	44 (5)	1089 (275)	1.9 (0.2)	1.17	0.36

[a] Specific resistance denoted R_mg, capacity C_mg; other abbreviations are defined in Table 1. Average values are listed and the SE is given in parentheses.

[b] Control resistance and capacity values (R_mc and C_mc), used in the calculation of the last two columns, were taken from Table 1 and were 13-3-1 for 12-3-1; 17-3-1 for 16-3-2 and 17-3-2; 20-3-1 for 20-3-2.

from younger animals (< 250 g) appeared to have a smaller diameter when observed under the dissecting microscope; the average calculated diameter of 11 fibers from these animals was 44.3 ± 1.2 μm. Normal membrane properties from two large rats (> 300 g) and two small rats (250 g) are given in Table 1. The membrane capacity of fibers from the older animals (21-2-1 and 13-3-1) is significantly greater than that measured from the younger animals (17-3-1 and 20-3-1), and the difference is greater than that predicted by the difference in diameter (Hodgkin & Nakajima, 1969; Dulhunty & Franzini-Armstrong, 1977). It is possible that the difference is related to an age-depen-

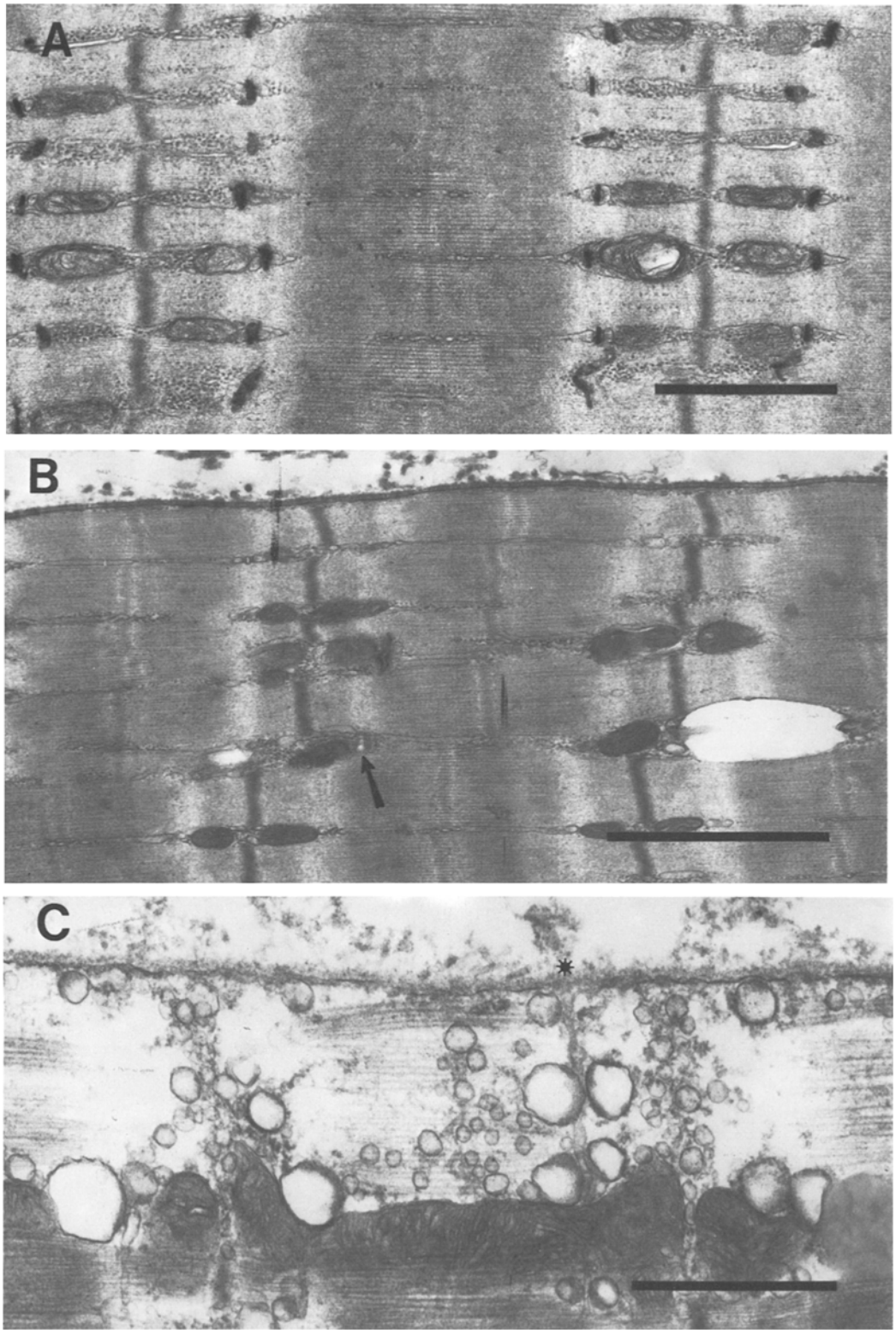

Fig. 2. The effect of 350 mM glycerol treatment on the ultrastructure of rat sternomastoid muscle fibers. (*A*): Control fiber soaked in a normal Krebs solution containing horseradish peroxidase (HRP) for 1 hr prior to fixation. Subsequent histochemistry produced the electron dense deposits where the HRP infiltrated the T-system. Scale bar represents 1 μm. (*B*): 350 mM glycerol treated fiber showing little signs of alteration as a consequence of the treatment. This fiber is an example of one soaked in an HRP Krebs solution after glycerol treatment. Note the comparative absence of labeled T-system. The large vacuole may be a swollen T-tube, as some T-tubes were enlarged (e.g., arrow). Scale bar represents 1 μm. (*C*): 350-mM glycerol treated fiber showing substantial vesiculation of the sarcoplasmic reticulum. This micrograph was selected to show an interesting, but rare T-tube open to the extracellular compartment (*) and clearly intact for some depth into the fiber, but not involved in any normal junctional contact with sarcoplasmic reticulum. Note also the electron density of the membranes of vesiculated reticulum in the positions where the triadic junctions would normally occur. Scale bar represents 1 μm

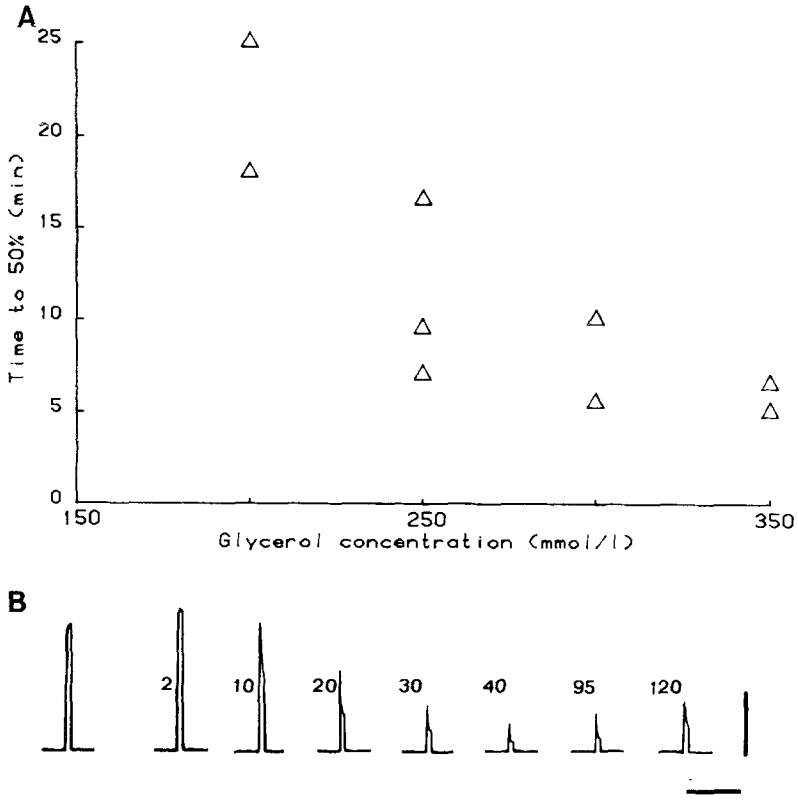

Fig. 3. The effect of glycerol concentration on the rate of tension reduction after glycerol treatment. In *A*, the time taken for tension to fall to 50% of its control value (i.e., tetanic tension recorded after 59 min in glycerol-Krebs) is shown on the ordinate. The concentration of glycerol used during the treatment is shown on the abscissa. Each symbol indicates a result from one bundle of fibers. In *B*, recovery of tension after glycerol treatment with 200 mM glycerol is illustrated: the first record shows tetanic tension after 59 min in 200 mM glycerol; the remaining records show tetanic tension after the indicated time (in min) after return to normal Krebs. Horizontal calibration bar indicates 24 sec, vertical calibration bar indicates 1 kg/cm^2

dent change in the area of T-system membrane in red sternomastoid fibers. Glycerol treatment had a similar effect on fibers from all animals, although smaller diameter fibers from younger animals often had more negative resting membrane potentials after treatment. Because of the variability in the specific capacity of control animals, the results are expressed as a fraction of control values taken from animals of a similar size.

The results in Table 2 indicate a significant fall in specific membrane capacity after glycerol treatment, and a smaller, but significant, increase in specific membrane resistance. The decrease in specific capacity and increase in specific resistance are indicative of a loss of membrane, which could be either surface or T-system membrane, or both. These results, by analogy with amphibian muscle data, suggest that the T-system may be electrically isolated from the surface membrane after the treatment.

To investigate this possibility, the ultrastructure of control and 350 mM glycerol-treated fibers was examined, and in some cases the accessibility of the T-system to HRP was tested. There was considerable variation in the effect of glycerol treatment on the appearance of the fibers and notably of the sarcoplasmic reticulum. In contrast to reports of little effect of glycerol treatment on the sarcoplasmic reticulum of frog muscle (Eisenberg & Eisenberg, 1968), the reticulum of 350 mM glycerol-treated fibers was often considerably disrupted (Fig. 2*C*). There was widespread damage to the T-system in all fibers, including those that otherwise looked unaffected (Fig. 2*B*), as evidenced by tubular swelling or the apparent absence of a tubular element between the adjacent terminal cisternae where a T-tube would normally join in the formation of a triad. When HRP was applied for up to 1 hr, 20 to 30 min after the glycerol treatment, very few T-tubes were labeled (Fig. 2*B*) compared to the virtual 100% labeling of control muscle (Fig. 2*A*). These observations are consistent with the interpretation that the reduction in specific membrane capacity as a consequence of 350 mM glycerol treatment results from the loss of the T-system membrane.

Treatment with 200 to 300 mM glycerol for 1 hr

Krolenko and Fedorov (1972) found that treatment of amphibian muscle with low concentrations of glycerol did not completely uncouple excitation and contraction. When red sternomastoid bundles were treated for 1 hr with 200, 250, or 300 mM glycerol Krebs and then returned to normal Krebs, tetanic tension fell to zero with a time course which depended on the concentration of glycerol used in the treatment (Fig. 3*A*). The time taken for tension to fall to 50%

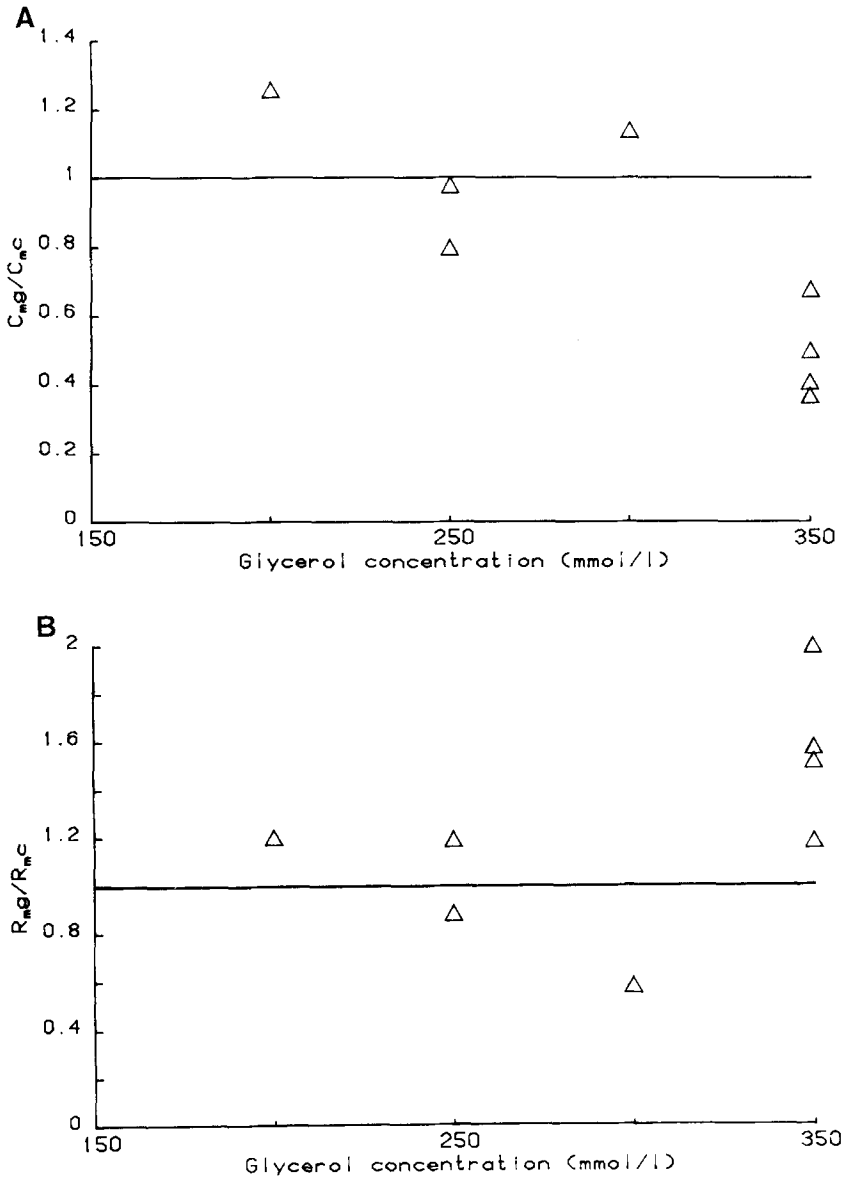

Fig. 4. The effect of glycerol concentration on passive electrical properties recorded 1 to 3 hr after preparations were returned to normal Krebs solution. In *A*, the average membrane capacity of fibers from each glycerol-treated bundle, C_mg, is normalized to the average membrane capacity of normal fibers, C_mc, (ordinate), and plotted against glycerol concentration (abscissa). The line indicates no change in membrane capacity. In *B*, the average membrane resistance of fibers from each glycerol-treated bundle, R_mg, is normalized to the average normal membrane resistance, R_mc, and plotted against glycerol concentration. The line indicates no change in membrane resistance

of control (where the control tetanus was recorded after 59 min in the glycerol-Krebs solution) was 18 to 24 min following treatment with 200 mM glycerol and 6 to 8 min after treatment with 350 mM glycerol. Uncoupling of excitation and contraction was not always complete when 200 mM glycerol was used; e.g., in the experiment shown in Fig. 3*B*, the tetanic tension fell in the usual way during the first hour after treatment with 200 mM glycerol Krebs and then recovered slightly over the subsequent 60 min. Complete recovery of tension was never observed.

Passive electrical properties of fibers were recorded 1 to 3 hr after the bundles (used to obtain the data shown in Fig. 3*A*) had been returned to normal Krebs. Fibers with normal resting potentials were selected for analysis. In Fig. 4*A*, C_mg/C_mc (*see* Materials and Methods) is plotted against the concentration of glycerol used in the treatment. Specific membrane capacity was essentially unaltered by treatment with 200, 250, or 300 mM glycerol Krebs. Thus the 30 to 60% reduction in C_mg after treatment with 350 mM glycerol is in marked contrast to the results obtained with lower glycerol concentrations. In Fig. 4*B*, R_mg/R_mc (*see* Materials and Methods) is plotted against the glycerol concentration used in the treatment. Membrane resistance is obviously a more variable parameter than membrane capacity. However there is not a consistent change in membrane resistance after treatment with low glycerol concentrations.

The unaltered membrane properties of fibers treated with 200, 250, or 300 mM glycerol suggests

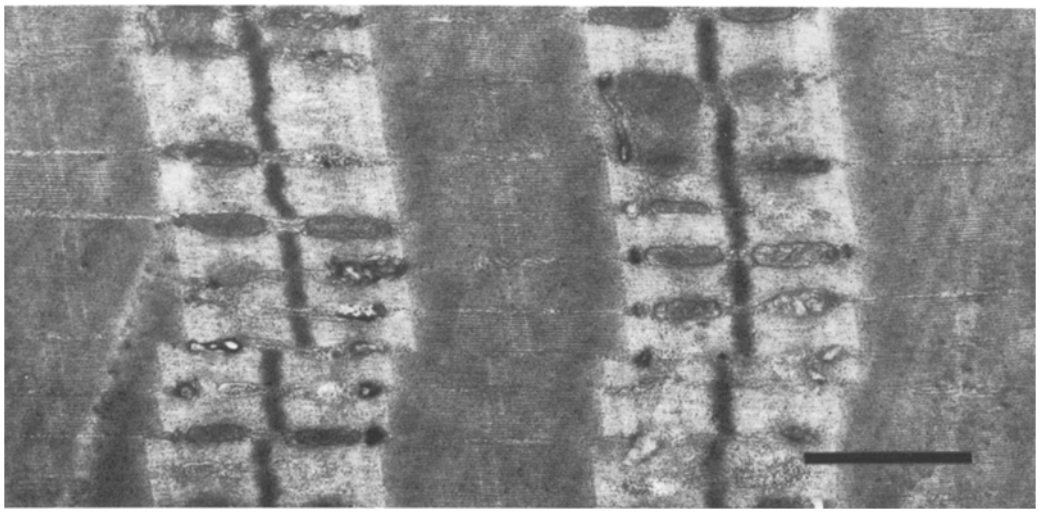

Fig. 5. 200 mM glycerol-treated fiber soaked in normal Krebs solution containing HRP. Comparison with a control fiber shown in Fig. 2*A* shows a slight reduction in the number of T-tubes seen in a similar area (*see also* Fig. 6). It is evident that most T-tubes are labeled with HRP. There are no obvious signs of disruption of the sarcoplasmic reticulum such as that frequently seen when the glycerol concentration used for the treatment was high (*see* Fig. 2*C*). Scale bar represents 1 μm

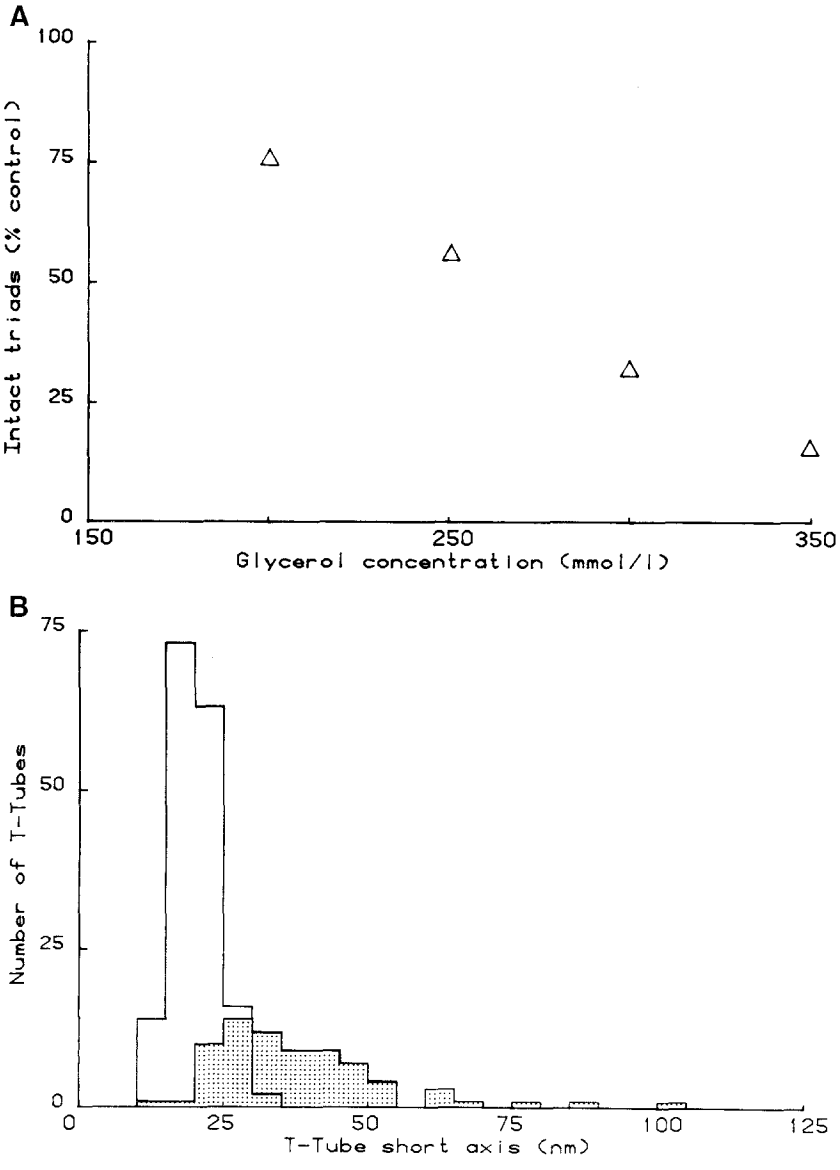

Fig. 6. The effect of glycerol treatment concentration on the integrity of the triad. (*A*): the incidence of intact triads at sites at which they are likely to be found in longitudinal sections, i.e., in the interfibrillar spaces adjacent to *A–I* boundaries. The results are expressed as a fraction of control values (ordinate) in relation to the concentration of glycerol used in the treatment (abscissa). A sample of at least 250 sites was used for each concentration. (*B*): The distribution of T-tube size, measured as the short axis (parallel to the longitudinal axis of the fibers) in control (unshaded) and 200 mM glycerol-treated (shaded) fibers

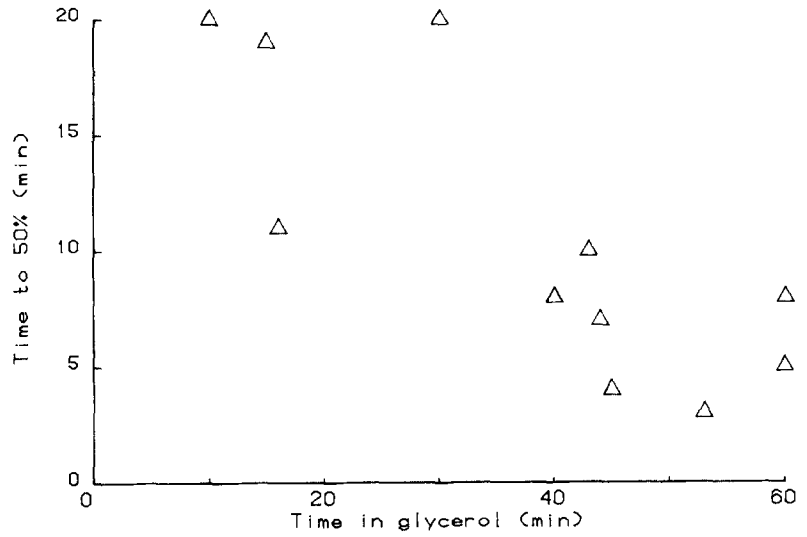

Fig. 7. The effect of length of exposure to 350 mM glycerol-Krebs on the rate of tension reduction when the bundles were returned to normal Krebs. The time taken for tension to fall to 50% of its control value (measured at the end of the exposure to 350 mM glycerol-Krebs) is plotted on the ordinate. The time for which the preparation was exposed to 350 mM glycerol-Krebs is indicated on the abscissa

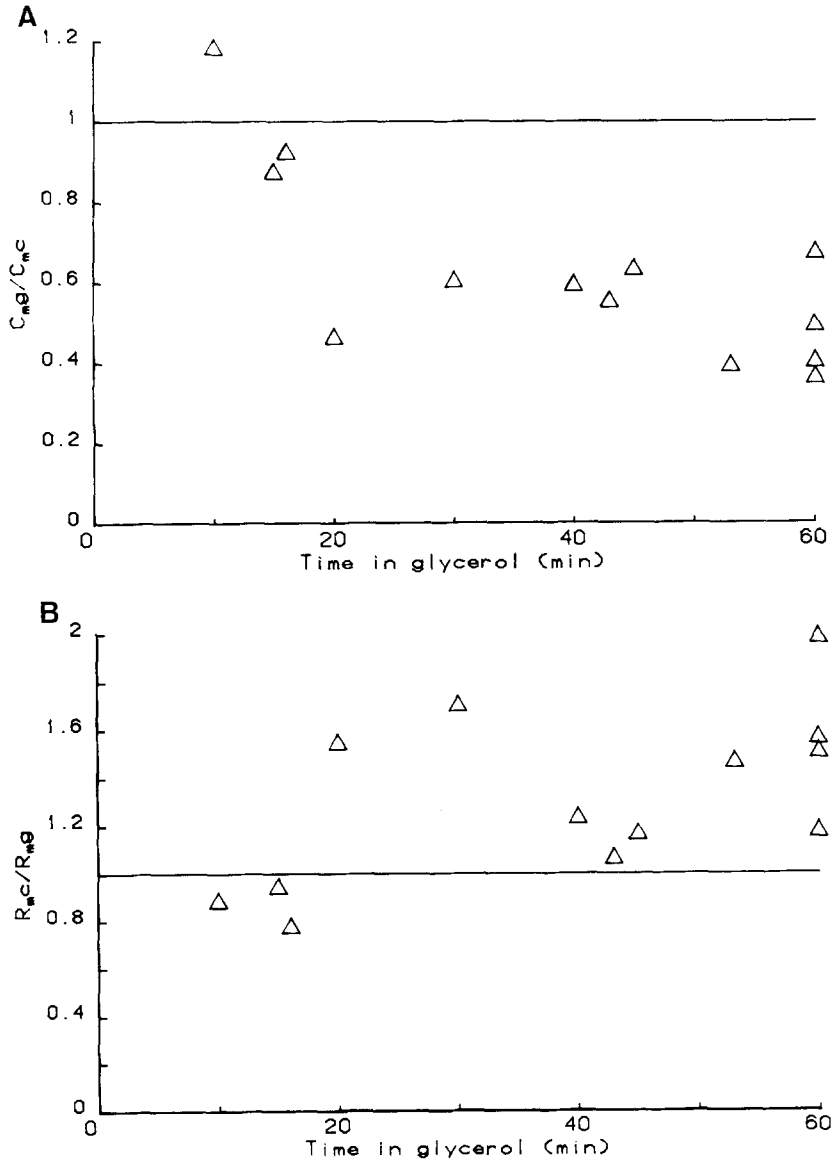

Fig. 8. Effect of length of exposure to 350 mM glycerol on passive electrical properties recorded 1 to 3 hr after the preparations were returned to normal Krebs. The ordinates are the same as in Fig. 3. In *A*, the normalized specific membrane capacity, C_mg/C_mc, is plotted against duration of exposure to 350 mM glycerol Krebs. In *B*, the normalized specific membrane resistance is plotted against the duration of exposure to 350 mM glycerol Krebs. The lines indicate no change in membrane properties

that the T-system is not substantially disrupted or electrically isolated from the surface membrane by these procedures. These suggestions were investigated by ultrastructural examination of the appearance of the T-system and its relation to the sarcoplasmic reticulum within the triad, and by soaking preparations uncoupled by a 200 mM glycerol treatment in a Krebs solution containing HRP.

Following 200 mM glycerol treatment the T-system of HRP soaked fibers was well labeled and there was no gross disruption of the structure of the T-tubes or of the triad (Fig. 5). Taken together with the electrophysiological results, these observations argue against T-system isolation as the mechanism of uncoupling after treatment with low glycerol concentrations. On the other hand, there was some suggestion that the absolute number of T-tubes seen in an area of section had declined somewhat. This appearance was quantified, both for 200 mM glycerol treatment and for the other concentrations used in this study in the following way: micrographs of longitudinal sections were scanned along the interfibrillar spaces; adjacent to each *A–I* boundary, the presence or absence of an intact triad was noted. The data obtained from at least 250 sites for each concentration are displayed graphically in Fig. 6*A*. There is a progressive decline in the number of intact triads as the glycerol concentration used increases. There was also an increase in the diameter of T-tubes of treated fibers (Fig. 6*B*), suggesting that there was a redistribution of the T-system membrane, without its isolation, after treatment with low glycerol concentrations.

Treatment with 350 mM glycerol for periods less than 1 hr

The results of treatment of sternomastoid preparations with low concentrations of glycerol (*see* above) are similar to the effects of brief treatment with 400 mM glycerol described in amphibian muscle (Dulhunty & Gage, 1973). It was interesting to look at the effect on red sternomastoid fibers of brief treatment with 350 mM glycerol. Tetanic tension fell to zero after periods as brief as 10 min; however, the time course of tension reduction was faster when the treatment period was longer (*see* Fig. 7). When the treatment period was less than 10 min, tetanic tension fell; however, considerable movement of the preparation was apparent following external stimulation, and movement of single fibers was observed following an action potential evoked by passing depolarizing current through an intracellular microelectrode.

The effect on passive electrical properties of different treatment times is illustrated in Fig. 8. The membrane properties were essentially normal when treatment was for times less than 30 min. There was a progressive decrease in membrane capacity (Fig. 8*A*) and increase in membrane resistance (Fig. 8*B*) as the treatment time was increased from 30 min to 1 hr. This result is similar to that reported for amphibian muscle fibers (Dulhunty & Gage, 1973).

Discussion

It is apparent that the electrophysiological effects of glycerol treatment in mammalian muscle fibers observed in this study are similar to those reported for amphibian preparations (Eisenberg & Gage, 1967; Howell & Jenden, 1967; Gage & Eisenberg, 1969; Dulhunty & Gage, 1973; Valdiosera et al., 1974). The specific membrane capacity of fibers from the four preparations treated for 1 hr with 350 mM glycerol varied from 1.8 to 5.0 $\mu F/cm^2$ (Table 2), so that the degree of detubulation must have varied considerably. If the geometry of the (exterior) surface membrane (folds and caveoli; Dulhunty & Franzini-Armstrong, 1977), is similar in mammalian and amphibian preparations, then fibers with a specific membrane capacity less than 2 $\mu F/cm^2$ were almost completely detubulated. Some T-system membrane must have remained continuous with the surface membrane in fibers with higher specific membrane capacities. Two variations of glycerol treatment were effective in uncoupling excitation and contraction without detubulation. Preparations treated for 1 hr with low glycerol concentrations (200 to 300 mM glycerol) or treated briefly (10 to 30 min) with 350 mM glycerol did not respond mechanically to electrical stimulation or depolarization in high potassium solutions, but had normal membrane capacity and resistance. The site of uncoupling in these preparations must have been internal to the surface membrane; either the triad, the sarcoplasmic reticulum, or the myofibrils was affected by the treatment.

In contrast to reports on glycerol-treated amphibian muscle there was a significant vesiculation of the sarcoplasmic reticulum in glycerol-treated mammalian preparations, indicating probable species variation in the glycerol and/or water permeability of the sarcoplasmic reticulum membranes. The implication of this observation on the mechanism of glycerol treatment in the two preparations is unclear. However two factors suggest that, as has been proposed for amphibian muscle (Dulhunty & Gage, 1973), the uncoupling is associated with disruption of the triad. First, there is little evidence of disruption of the myofibrils, or of the sarcoplasmic reticulum other than at the triad junction, in uncoupled preparations where

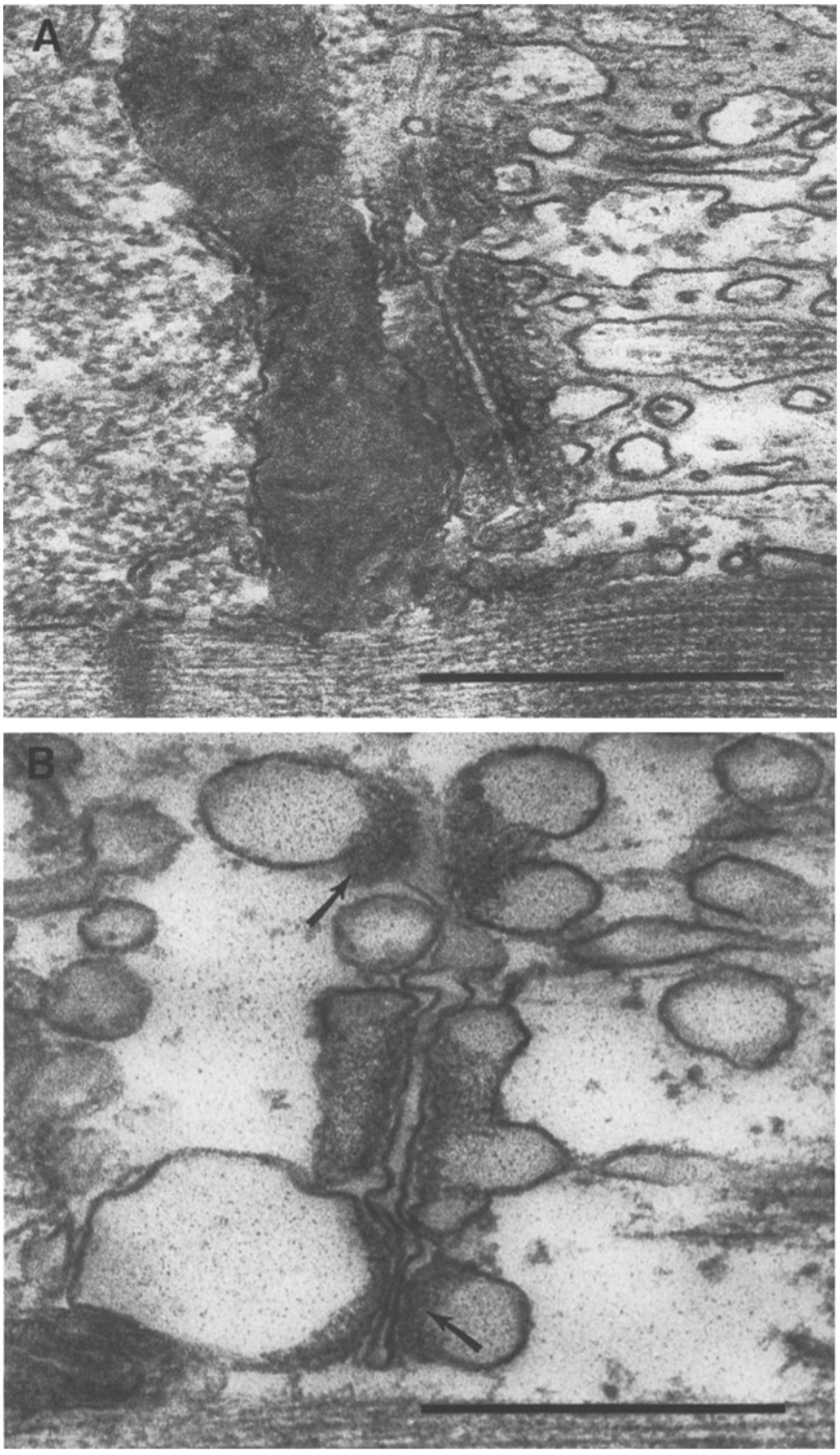

Fig. 9. Dense material involved in the triad junction. (*A*): Triad from a control fiber, showing junctional feet, and dense-staining material within the terminal cisternae. Scale bar represents 0.5 μm. (*B*): Triad from a 250 mM glycerol-treated fiber. Note the dense material associated with the junctional surface of the terminal cisternae (arrows) and the almost complete absence of junctional feet. Scale bar represents 0.5 μm

there was no detubulation (Fig. 5). Second, the triad is disrupted by glycerol treatment to a degree dependent upon the glycerol concentration used (Fig. 6*A*). An apparent conflict is that the tension fell to zero after treatment with 200 mM glycerol, while 75% of the triads remained intact (Fig. 6*A*). It is possible that the apparently intact triads were subject to mechanical forces sufficiently large to cause uncoupling before physical disruption of the triad was observed.

An interesting question arising from this work

is the function of the dense-staining material within the terminal cisternae (Fig. 9*A*). It has been suggested on ultrastructural (e.g., Costantin, Franzini-Armstrong & Podolsky, 1965) and biochemical grounds (Meissner, Conner & Fleischer, 1973) that this material is a calcium binding agent. In the vesiculated terminal cisternae it was observed on the surface that would have been involved in the T-tube-reticulum junction (Figs. 2*C* and 9*B*). In fact, the close association between the dense staining material and the junctional reticulum is more obvious in vesiculated reticulum than in normal reticulum where it appears to diffusely fill the terminal cisternae. This material is in a position critically affected by mechanical strains to the T-tube-reticulum junctions. In addition, its location suggests it might normally be a part of the junction, and consequently, it could play an important role in excitation-contraction coupling, a suggestion supported by changes in density of this material which occur during repetitive stimulation (Eisenberg & Gilai, 1979). Conceivably, disruption of this material is responsible for the uncoupling brought about by glycerol treatment.

This work was supported by the Australian Research Grants Committee, the Australian National Health and Medical Research Council, and the Muscular Dystrophy Associations of America. Our thanks go to Miss Judith Furby and Miss Monica Dlutowski for technical assistance.

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Received 23 October 1979; revised 11 December 1979