

**Frog Skeletal Muscle Fibers: Changes in Electrical  
Properties after Disruption of Transverse Tubular System**

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*Abstract. In muscle fibers which have been exposed for 1 hour to a Ringer solution containing 400 millimolar glycerol and then returned to plain Ringer solution, the transverse tubular system is disrupted. At the same time the membrane capacitance is markedly reduced and hyperpolarizing current pulses no longer produce a slow, progressive increase in potential (creep). The large capacitance of muscle and the phenomenon of "creep" must both depend on an intact transverse tubular system.*

The values of the membrane capacitance of nerve and muscle are strikingly different, being of the order of 1  $\mu\text{farad}/\text{cm}^2$  in nerve (1) and 4 to 8  $\mu\text{farad}/\text{cm}^2$  in skeletal muscle of frogs (2, 3). The higher membrane capacitance of muscle fibers has been

attributed to the presence of two components, the surface membrane and the membrane of the transverse tubular system (3, 4). From measurements of impedance in frog skeletal muscle (3) the surface membrane appears to have a capacitance of 2.6  $\mu\text{farad}/\text{cm}^2$  and

the tubular membrane a capacitance of  $4.1 \mu\text{farad}/\text{cm}^2$ , and hence the total capacitance is  $6.7 \mu\text{farad}/\text{cm}^2$ . A method has been described (5) which reportedly destroys selectively the morphology of the transverse tubular system in frog skeletal muscle. We have measured the membrane capacitance of muscle fibers after such treatment. The value obtained agrees closely with that attributed to the surface membrane from impedance measurements (3).

To destroy the transverse tubules we placed sartorius muscles (*Rana pipiens*) for 1 hour in a Ringer solution to which 400 mM glycerol had been added (5). The muscles were then transferred to a normal Ringer solution. Destruction of the transverse tubular system occurs not during the 1 hour in the glycerol solution but only after re-immersion of the muscle in Ringer solution (5). All solutions contained curare ( $10^{-5}$  g/ml) to preclude possible neuromuscular effects and tetrodotoxin ( $10^{-7}$  g/ml) to block action potentials (6). Two intracellular microelectrodes were used to make a conventional cable analysis of surface muscle fibers (2).

Resting potentials were used as an index of the integrity of the surface membranes of treated muscle fibers. The membrane potential of some fibers declined progressively, but many maintained membrane potentials of 70 to 90 mv for up to 3 hours, and we used these. In 18 fibers (mean diameter  $59 \mu$ ) in which the tubules were disrupted the membrane capacitance was  $2.25 \pm 0.14 \mu\text{farad}/\text{cm}^2$  (mean and standard error of the mean). The specific membrane resistance was  $3708 \pm 316 \text{ ohm cm}^2$ , and the internal resistivity was  $191 \pm 51 \text{ ohm cm}$ .

In the fibers without tubules the membrane resistance should have been much higher than  $3708 \text{ ohm cm}^2$  if the surface and tubular membranes had the same resistive properties. However, it has been suggested that the chloride conductance, which makes up two thirds of the total membrane conductance, is located principally in the exposed surface membrane (7). That the membrane resistance of muscle fibers with disrupted tubules is essentially normal lends support to this suggestion.

To test the possibility that glycerol might directly affect membrane characteristics we analyzed nine muscle fibers (average diameter  $54 \mu$ ) while they were still in the glycerol solution. The average membrane capacitance was

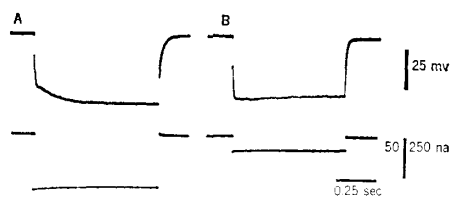


Fig. 1. The effect of hyperpolarizing currents on the membrane potential of muscle fibers. (A) In Ringer solution containing 400 mM glycerol (tubules intact); (B) in Ringer solution following treatment (tubules disrupted). Current calibration in A is 50 nA and in B is 250 nA.

$6.13 \pm 0.78 \mu\text{farad}/\text{cm}^2$ , and the specific membrane resistance was  $3858 \pm 416 \text{ ohm cm}^2$ . These figures are in close agreement with those previously cited for normal fibers in Ringer solution (2). The internal resistivity was  $133 \pm 16 \text{ ohm cm}$ ; this value suggests that the internal ionic concentration is raised as might be expected if glycerol remains extracellular and thus osmotically dehydrates the fibers (8).

Therefore, the low capacitance of treated fibers cannot be attributed to a direct effect of glycerol on the membrane since the abrupt fall in membrane capacitance occurs not while the muscle is in glycerol but only when it is returned to Ringer solution. This is in accord with the results of Howell and Jenden (5), obtained by electron microscopy, which have been confirmed in our laboratory (9); that is, the transverse tubular system appears intact during the exposure to glycerol but becomes grossly disorganized shortly after the muscle is placed in Ringer solution. Thus, the decrease in membrane capacitance which was found is clearly related to the destruction of the transverse tubular system.

In muscle fibers without any transverse tubules the membrane capacitance should be approximately that previously attributed to the surface membrane ( $2.6 \mu\text{farad}/\text{cm}^2$ ) (3). Since the capacitance of our treated muscle fibers is even lower ( $2.25 \mu\text{farad}/\text{cm}^2$ ), our results seem to indicate that no transverse tubules remain. However, the small difference between these capacitances is not decisive evidence that there are no tubules left in treated fibers since the number derived from impedance measurements may be a significant overestimate of the capacitance of the surface membrane. This overestimate might occur if the impedance of the membrane of the tubules near the outer surface of the fiber is indis-

tinguishable at high frequencies from that of the surface membrane.

An interesting observation was that long hyperpolarizing pulses did not produce the progressive increase in potential (creep) which is evident in normal muscle fibers (2, 10). Long hyperpolarizing current pulses caused a voltage change which reached a plateau in the first 100 msec and showed no further increase (Fig. 1B). In several experiments we tried to increase any residual creep by substituting impermeable anions for chloride. Under normal conditions this procedure greatly increases the creep. In our treated preparations no creep was observed even in solutions free of chloride. The absence of creep was not caused by a direct chemical effect of the glycerol since muscle fibers in glycerol solution [which have been shown to have an intact transverse tubular system (4)] displayed the normal behavior (Fig. 1A).

Our figure for the capacitance of muscle fibers with disrupted tubules, in conjunction with the results of Falk and Fatt (3), suggests that most, if not all, of the tubules are destroyed by the treatment. The membrane which remains appears to be intact since it can still maintain normal resting potentials and generate action potentials. This preparation can therefore be used to study the properties of the surface membrane of muscle fibers.

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#### References and Notes

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9. Surface fibers of treated muscles were fixed in glutaraldehyde, then in  $\text{OsO}_4$ , embedded in Araldite, and sectioned for electron microscopy. The transverse tubular system was disorganized; the contractile system was normal in appearance. We thank Brenda Eisenberg for performing these experiments. Supported by NIH grant 9M06753.
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11. We thank Dr. Paul Horowitz for his generous support during these experiments; supported in part by NIH grant GM08803 and by NSF grant GB5023.

26 September 1967