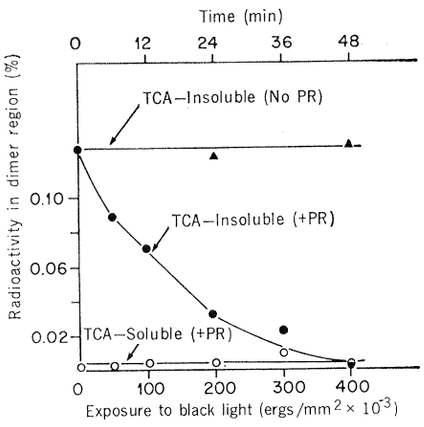

Fig. 1. The thymine-containing dimers in the TCA-insoluble (●) and TCA-soluble (○) fractions of paramecia irradiated with ultraviolet light after several exposures to photoreactivating light (PR). As a control, irradiated animals were kept in the dark after ultraviolet irradiation for the same periods of time as were used for photoreactivation. The dimers in the TCA-insoluble (▲) fractions were determined for the controls.

mm^{-2} sec^{-1}, as estimated from Jagger meter (*8*) readings. After this exposure, cell survival at 48 hours was 100 percent.

After irradiation the cells were divided into three groups. The first group was killed immediately by the addition of ice-cold, 5 percent trichloroacetic acid (TCA); the second was exposed to photoreactivating light (PR) from a General Electric BLB black-light bulb at a rate of 8000 ergs mm^{-2} sec^{-1}. A glass plate between the animals and the black-light bulb prevented transmission of wavelengths below about 3000 Å. The third group was placed in a lightproof chamber and killed at the same time as the corresponding sample exposed to PR, the cells being centrifuged from Dryl's solution and killed by the addition of TCA. Three ether extractions were used to remove the TCA from the TCA-soluble fractions. Calf thymus DNA was added to aid in chromatographic identification, and the samples were hydrolyzed for 30 minutes in 97 percent formic acid at 175°C. The hydrolysates were analyzed for radioactivity in thymine and thymine-containing dimers by two-dimensional paper chromatography, elution, and counting in a dioxane-based system (*4*) in a scintillation counter. The photoproducts were identified as dimers by their chromatographic mobility and by the kinetics and products of their monomerization at 2390 Å (*9*). It will be shown elsewhere (*10*) that the photoproducts measured were indeed dimers from *Paramecium* DNA, even though bacteria were used to label the paramecia.

Figure 1 shows the results of a typical experiment designed to determine the fate of dimers in cells irradiated with ultraviolet light that were subsequently exposed to PR or kept in the dark. In the dark the dimer content did not change; in the light, dimers disappeared from the TCA-insoluble fraction and did not appear in the TCA-soluble fraction—nor did they appear in the medium, because only 0.04 percent of the total radioactivity was released into the medium during the experiment. Even if all the material released from the cells were dimers, it would not be great enough to account for the disappearance of dimers in the light. These results strongly indicate that, in the presence of PR, dimers in *Paramecium* DNA are monomerized in vivo.

Mutational damage produced by similar doses of ultraviolet light are also reactivated (*11*) by exposures to photoreactivating light comparable to those used in the present experiments (*12*). However, evaluation of the biological role of pyrimidine dimers in mutagenesis due to exposure to ultraviolet light is not yet possible, because the dose-response relation for induction and photoreactivation of mutation in *Paramecium* by ultraviolet light has not been completely worked out. Nevertheless, the destruction of dimers in vivo by PR suggests that biological photoreactivation of mutation in *Paramecium* results from the monomerization of pyrimidine dimers, and that the induction of the mutations by ultraviolet light results from the formation of pyrimidine dimers.

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

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 μfarad/cm^2 in nerve (*1*) and 4 to 8 μfarad/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 μfarad/cm^2 and

the tubular membrane a capacitance of 4.1 μfarad/cm^2, and hence the total capacitance is 6.7 μfarad/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 m*M* 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 reimmersion 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 μ) in which the tubules were disrupted the membrane capacitance was 2.25 $\pm$ 0.14 μfarad/cm^2 (mean and standard error of the mean). The specific membrane resistance was 3708 $\pm$ 316 ohm cm^2, and the internal resistivity was 191 $\pm$ 51 ohm cm.

In the fibers without tubules the membrane resistance should have been much higher than 3708 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 μ) while they were still in the glycerol solution. The average membrane capacitance was

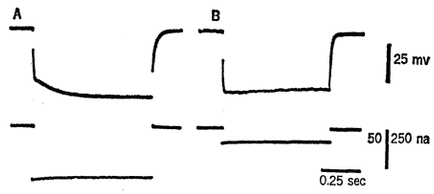

Fig. 1. The effect of hyperpolarizing currents on the membrane potential of muscle fibers. (A) In Ringer solution containing 400 m*M* glycerol (tubules intact); (B) in Ringer solution following treatment (tubules disrupted). Current calibration in A is 50 na and in B is 250 na.

6.13 $\pm$ 0.78 μfarad/cm^2, and the specific membrane resistance was 3858 $\pm$ 416 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 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 μfarad/cm^2) (*3*). Since the capacitance of our treated muscle fibers is even lower (2.25 μfarad/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 indistinguishable 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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