vation imply an increase in the repair mechanism directly proportional to increased radiation dose but not to photoreactivating light, as this is constant in every case. The increased rate of photorepair in cysts irradiated by higher doses of ultraviolet may be explained by one of the following statements concerning the enzyme-mediated mechanism: (i) k_1 increases; (ii) k_3 increases; or (iii) k_2 decreases.

Since k_1 depends on the nature of photoreactivating enzyme and repairable ultraviolet-damaged DNA, it will increase only if the activity of one or both of these is enhanced; k_2 will decrease and k_3 will increase only if the photoreactivating enzyme-DNA complex is altered by ultraviolet irradiation.

The above explanation implies (i) an ultraviolet-induced activation of normally inactive photoreactivating enzyme in the cyst with a greater activity than that of photoreactivating enzyme of the vegetative cells, (ii) an ultraviolet-induced change in the activity of the photoreactivating enzyme of cysts but not in that of the vegetative cells, (iii) an ultraviolet-induced increase in the activity (facilitated repair) of ultraviolet-damaged DNA in the cyst, or (iv) the presence of a DNA in the cyst with unique photoreactivation characteristics (*6*). Because the experimental times involved are probably not sufficient to allow even minimum protein synthesis, it is unreasonable to assume a *de novo* synthesis of a more active photoreactivating enzyme in the cyst. An increase in the activity of native photoreactivating enzyme in the cyst or an activation of normally inactive photoreactivating enzyme as a function of ultraviolet radiation is feasible. An increase in the activity of repairable ultraviolet-damaged DNA is also feasible (*7*).

We conclude that the cysts of *Azotobacter vinelandii* 12837 can be photoreactivated and that they possess a photoreactivating enzyme which is activated by ultraviolet or that the DNA which is damaged by ultraviolet is easier to repair in the cysts than in the vegetative cells of the same organism.

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Ionic Mobility in Muscle Cells

Abstract. *The diffusivities of ionic potassium, sodium, sulfate, and adenosine triphosphate inside a muscle cell are reduced by a factor of 2, relative to diffusivities in aqueous solution. The diffusion coefficients of nonelectrolytes are reduced by the same factor, showing that the diffusion of the ions is retarded by physical, rather than chemical, interactions. In contrast, the diffusivity of the calcium ion, which is taken up by the sarcoplasmic reticulum, is reduced fiftyfold.*

We report the first direct measurements of the intracellular mobilities of ions important to the function of skeletal muscle. Sorbitol and sucrose, which are not metabolized or known to interact with intracellular components, were used to evaluate the retardation of diffusion by physical factors. The main result is that the diffusivities of K^+, Na^+, SO_4^{2-}, and ATP^{3-} within the cell are reduced by the same extent as those of the nonelectrolytes, which shows that the transport properties of these ions are not affected by specific chemical interactions.

As expected, Ca^{2+} is an exception. Its diffusion coefficient in muscle is about 50 times lower than in aqueous solutions as it binds strongly to, and is accumulated within, the sarcoplasmic reticulum (*1*). These results are opposed to the view that a large fraction of intracellular Na^+ and K^+ is reversibly immobilized on a polyelectrolyte matrix in the cytoplasm, a concept suggested by some recent observations: (i) the NMR signal of a large portion of intracellular Na^+ is broadened beyond detection, and the Na^+ that replaces a portion of intracellular K^+ becomes similarly "invisible" to NMR (*2*); (ii) the activity coefficient of Na^+ in muscle cells appears to be low (*3*); and (iii) estimates of the diffusion coefficient of various ions and nonelectrolytes in cytoplasm, derived from flux measurements in whole muscles, come out several orders of magnitude lower than the respective diffusivities in aqueous solutions (*4*). It would appear, therefore, that the latter (i–iii) observations should be interpreted with considerable caution.

Our method is based on the analysis of the distribution of radioactivity in a length of single fiber at various times after point application of a labeled substance. A segment (3 to 6 mm long) of a fiber from the semitendinosus muscle of the frog *Rana pipiens* was isolated in mineral oil, and the surface membrane was removed (*5*). The ends of the segment were mounted in stage micromanipulators, the preparation was raised so that it was completely surrounded by oil, and the sarcomere length was set close to 2.4 μm (*6*). Opposing micropipettes, one filled with a radioactive solute and the other with 140 m*M* KCl,

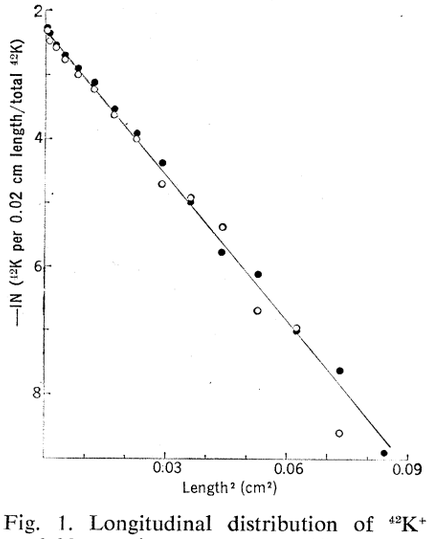

Fig. 1. Longitudinal distribution of $^{42}K^+$ at 0.02 cm intervals after diffusion for 320 seconds at 20°C. The data are plotted as a logarithmic transform of the diffusion equation for an infinite slab from an infinitely thin initial distribution (*9*):

$$\ln\left(\frac{\text{counts at distance } x}{\text{total counts}}\right) = -\frac{x^2}{4Dt} - \ln 2(\pi Dt)^{1/2}.$$

The diffusion coefficient, D, was calculated from the slope of the line. The two kinds of circles (open, closed) represent the two sides of the radioactivity distribution, the center of which is between the two points nearest the origin.

were placed near the center of the segment. The isotopes used were ^{22}Na, ^{42}K, ^{45}Ca, ^{32}S in sulfate ion, and ^{14}C in uniformly labeled ATP, sucrose, and sorbitol. Tracer quantities of the solutes were injected into the fiber (*7*) by applying a short voltage pulse across the micropipettes. The time for diffusion ranged from 1 to 45 minutes. After diffusion had taken place, the fiber was dehydrated quickly in acetone, stained, embedded in paraffin, and cut into 25 μm transverse sections (*8*). The longitudinal distribution of the radioactivity was determined with a Geiger counter. The overall accuracy of the method was checked by using the same procedure to measure diffusion coefficients in cylinders of 2 percent agarose 400 μm in diameter. The values obtained in this way for Na^+ and sucrose were within 5 percent of the reported values (*9*).

Except for Ca^{2+}, the distribution of radioactivity in the muscle fibers could be accurately fitted by the equation for unidimensional diffusion of a single component from an infinitely thin plane (*10*) (Fig. 1). The diffusion coefficients (D) were found to be independent of the diffusion time. In the case of Ca^{2+}, the tracer generally moved as a single component ($D = 1.4 \times 10^{-7}$ cm^2 sec^{-1}) but, in a few preparations, for reasons not understood, a slower fraction was also present.

Figure 2 summarizes the data. The reduction of the diffusion coefficient of sorbitol and sucrose by a factor of 2 shows that presumably inert solutes are subject to physical interactions which lower their mobility within the cell (*11*). This is not surprising in view of the large content of soluble protein in cells and the highly structured nature of muscle fibers.

The reduction of the diffusion coefficients of Na^+, K^+, SO_4^{2-}, and ATP^{3-} by the same factor as sorbitol and sucrose suggests that these ions have the same diffusion path as the nonelectrolytes and that retardation in both cases is due to physical factors rather than specific chemical reactions. Two likely mechanisms for the retarded diffusion are obstructions by structures within the cell (a tortuosity factor) and increased viscosity of the cytoplasm. Thus the upper limit for the viscosity of the cytoplasmic solution is about twice that of water.

The diffusion coefficient of Ca^{2+} in the fiber was at least 50 times less than that in water. This demonstrates that chemical interaction of an ion with fixed structures within the cell (that is, the sarcoplasmic reticulum) has a very much greater influence on mobility than the physical interaction discussed above.

In summary, K^+, Na^+, SO_4^{2-}, and ATP^{3-} are influenced by physical interactions in the cytoplasm of muscle so that their apparent diffusivities, relative to aqueous values, are all reduced by a factor of 2. Thus binding of these ions to immobile structures within the cell is not extensive compared with the total amounts present, and chemical interactions do not limit their mobility.

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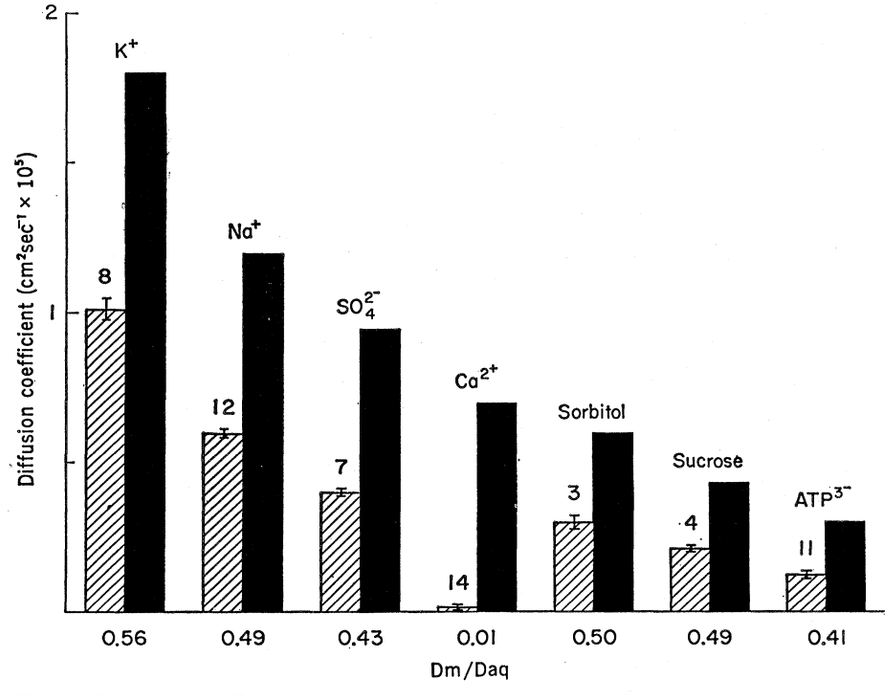

Fig. 2. Diffusion coefficients observed at 20°C in single, skinned frog muscle fibers (shaded bars) and in water (*9*) (black bars). Error limits, indicating the standard error of the mean, are given for muscle; the number of experiments is above each error bar. The ratio of the diffusivity in muscle to that in aqueous solution (Dm/Daq) is given beneath each pair of bars.

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8. Almost all the tracer was retained during these steps; at the dehydration step, a loss of 10 percent or less occurred in the case of Na^+ and K^+, but the loss was much less for the other compounds. Furthermore, the striation spacing was not changed by dehydration and embedding.
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11. This appears to be at variance with the report that the diffusivity of urea, glycerol and water in barnacle muscle is not reduced [W. H. Bunch and G. Kallsen, *Science* **164**, 1178 (1969)]. With our method, the intracellular diffusion coefficient of Na^+ in single fibers of *Balanis nubilis* was reduced by nearly a factor of 2 [$D = 0.07 \pm 0.03$ (standard error of the mean) $\times$ 10^{-5} cm^2 sec^{-1}; $N = 10$]. A possible reason for this discrepancy is that the diffusivity observed in the former study might have been enhanced by bulk transport of solute along the muscle-oil interface due to capillarity.

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