$(1 - k_2 f_2)$, and $w_3 = y\,(1 - k_3 f_3)$, we showed earlier that the regions allowing stable polymorphism are increasingly larger for overdominance models and higher values of s (for example, see Fig. 3) (k_i, constants to make w_i, selective values, functions of the frequencies f_i). Computer simulation of models involving overdominance in b_{ij} values also gave significantly wider regions for heavy inbreeding. However, in nature the underdominance models discussed above cannot be assumed a priori to be any less frequent than the very widely hypothesized overdominance situations. The role of frequency-dependency merits further detailed analyses in terms of both genetic and ecological aspects of variation in natural populations. Some recent developments in *Drosophila* research (*10*) indeed clearly attest this viewpoint.

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13 June 1969

Azotobacter Cysts: Reactivation by White Light after Inactivation by Ultraviolet Radiation

Abstract. *Cysts of* Azotobacter vinelandii *12837 inactivated by ultraviolet radiation can be reactivated by white light. This photoreactivation mechanism is not seen in the vegetative cells of the same organism.*

Harm and Rupert (*1*) propose that photoreactivation in bacteria exposed to ultraviolet radiation is brought about by a photochemical reaction involving a photoreactivating enzyme. They state that the reaction is characterized by the Michaelis–Menton equation for enzyme mediated reactions:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

where E is the photoreactivating enzyme; S is repairable ultraviolet-damaged DNA; and P is repaired DNA. Photoreactivation in many different organisms results in a dose-reduction phenomenon. Harm *et al.* (*2*) state that this dose-reduction parameter is determined by k_3, which is a light-dependent photolysis of the photoreactivating enzyme–DNA complex resulting in repaired DNA. This implies that in the absence of the photoreactivating enzyme (*3*) or light, repairable ultraviolet-damaged DNA will not be repaired, and the cell will be damaged. There are three assumptions in this theory: (i) the concentration of photoreactivating enzyme is not limiting within the doses of ultraviolet used; (ii) the activity of photoreactivating enzyme does not vary; and (iii) the reaction rate constants do not change. If these three assumptions are correct, the effect of photoreactivation is that of dose reduction as commonly seen.

Azotobacter lends itself well to certain types of experiments (*4*). These organisms form cysts which are not totally inactive metabolically; that is, they will oxidize substrates but they probably do not synthesize proteins (*5*). We assumed that these cysts would undergo photoreactivation as proposed (*1*) only if they were metabolically active. Photoreactvation of the *Azotobacter* cysts has not been reported. Washed vegetative cells of *A. vinelandii* 12837 grown in Burk's medium with 1 percent glucose for 18 hours in cultures at 30°C were placed in petri plates to a layer depth of less than 1 mm, exposed to a flux of light of approximately 2537-Å wavelength and approximately 100 erg mm^{-2} sec^{-1}, and kept constantly in motion to insure uniform exposure. Photoreactivation was obtained by subjecting the irradiated bacteria, in test tubes, to the light emitted by a 500-watt Sylvania Photoflood lamp located approximately 10 cm from the tubes in a running-water bath at 12° to 15°C. Controls were subjected to identical manipulations, except that the tubes were wrapped in aluminum foil. Survival rates were obtained by plate counts on Burk's medium after the cultures were incubated for 48 to 72 hours at 30°C.

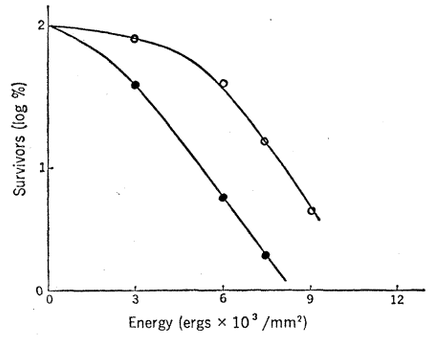

Fig. 1. Ultraviolet inactivation (solid circles) and photoreactivation (open circles) of vegetative cells of *Azotobacter vinelandii* 12837.

Photoreactivation of the vegetative cells follows a simple dose-reduction mechanism and our assumptions are tenable (Fig. 1). The coefficients of regression of the rectilinear portions of these lines are -0.03 (-0.0302) for the death curve and -0.03 (-0.0300) for the photoreactivation curve.

The organism was induced to encyst by culturing it on plates of Burk's medium containing 0.2 percent *n*-butanol in lieu of glucose. The cysts were treated in the same manner as were the vegetative cells. Cysts of *A. vinelandii* 12837 could be photoreactivated (Fig. 2) in the same manner as the vegetative cells of the same organism could. Further analysis of photoreactivation of the cysts showed a higher rate of photoreactivation at higher ultraviolet doses. The inactivation curve has a slope of -0.05 (-0.0481), and the photoreactivation curve has a slope of -0.02 (-0.0198). Photoreactivation of cysts of this organism does not represent the classical dose-reduction phenomenon but rather an ultraviolet-enhanced photoreactivation. The kinetics of cyst photoreacti-

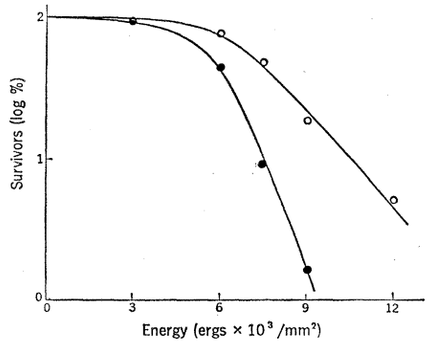

Fig. 2. Ultraviolet inactivation (solid circles) and photoreactivation (open circles) of cysts of *Azotobacter vinelandii* 12837.

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,

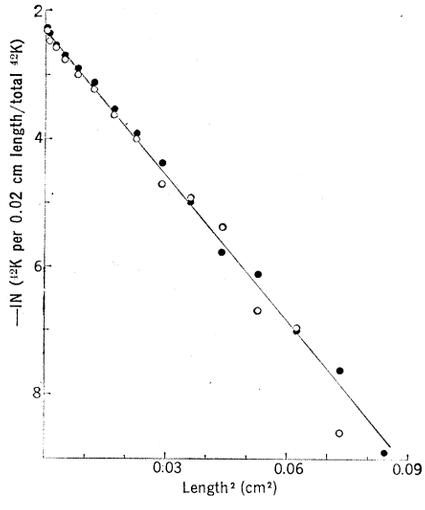

Fig. 1. Longitudinal distribution of $^{42}K^+$ at 0.02 cm intervals after diffusion for 320 seconds at 20°C. The data are plotted as a logarithmic transform of the diffusion equation for an infinite slab from an infinitely thin initial distribution (*9*):

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

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.