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Can a Dye Base Penetrate into Living Cells from a Relatively Strongly Basic Dye Solution?

MARIAN IRWIN.

From the Laboratories of the Rockefeller Institute for Medical Research.

The color of azure B begins to alter from blue to violet at about pH 10 (the more the higher the pH) until at about pH 13 it turns red. These color changes are instantaneous and readily reversible on appropriately lowering the pH values; they are not progressive on standing for 3 hours. They are due to tautomeric change from the dissociated blue dye salt to the undissociated red dye base. The half-way point in the color change from blue to red occurs roughly in the neighborhood of pH 12. These results show that azure B is a relatively strong base existing chiefly in the form of salt in the pH range available for living cells of *Nitella* (roughly between pH 6 and pH 9).

On shaking the azure B solution at pH 9.4 and pH 8.5 with carbon tetrachloride the red dye base is extracted by the latter, the more the higher the pH value. The ratio of the apparent absorption coefficients[1] is roughly (K_{ap} at pH 9.4)÷(K_{ap} at pH 8.5)$=\frac{1.3}{0.15}=8.7$. This ratio agrees with the ratio (percent of dye base at pH 9.4)÷(percent of dye base at pH 8.5 $= 2.44 \div 0.31 = 8$, when the basic dissociation constant of the dye is $K_b = 10^{-3}$. Such a ratio at higher pH values might show whether the dissociation constant is $K_b = 10^{-3}$ or higher, but owing to the color change this determination is not possible by this method. These results show that azure B is strongly basic but the dye base has a high absorption coefficient between carbon tetrachloride and water so that it can be extracted on shaking the mixture in spite of its low percentage in the aqueous phase at these pH values.

The rate of penetration of azure B at these pH values into the vacuoles of artificial[1] cells (with carbon tetrachloride as the membrane) as well as into living cells of *Nitella* increases with rise in the pH values. The rate represents the reciprocal of time it took for the dye to reach a given concentration in the vacuolar sap. The ratio is (rate at pH 9.4)÷(rate at pH 8.5) = 3, for the artificial cell and for the living cell. This ratio is lower than the ratio obtained for the apparent absorption coefficients. These results show that the

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[1] Irwin, M., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 993.

rate is a function of the absorption coefficient but the function is not a linear one. The concentrations employed are dilute (below 0.0006 M) at which the concentrations are expected to be about equal to activities.

The azure B which penetrates the vacuole is capable of raising the pH value of the sap, and has the same absorption spectrum as the azure B outside.

The importance of these results is in showing that if the dye base has a high enough absorption coefficient and if it is capable of being changed into the dye salt by the sap, it will pass into the acid vacuolar sap rapidly in spite of the fact that the dye is strongly basic. These results support the author's theory of multiple absorption coefficients.[1]

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Influence of Carbon Dioxide upon Hydrogen Ion Concentration of Frog's Skeletal Muscle.

WALTER S. ROOT.*

From the Department of Physiology, College of Medicine, Syracuse University.

The anterior tibial muscles of *Rana pipiens* were removed, sprinkled with powdered quinhydrone, and mounted between a platinum plate electrode and an agar-KCl bridge connected with a saturated KCl-calomel half-cell.[1] The muscle and electrodes were inserted in a glass chamber through which known gas mixtures could be flushed. The potential differences were read in the usual way by means of a Type K potentiometer and galvanometer.

After the initial rapid acid shift, muscle macerated with quinhydrone slowly became more and more acid in 5% CO_2 in O_2. Intact muscle in 5% CO_2 in O_2 shifts rapidly acid in the first few minutes' exposure. This reaction is followed by a small alkaline drift, succeeded by a plateau which is maintained for 20 to 40 minutes. The secondary alkaline drift is attributed to the mobilization of base which is presumably a less rapid process than the inward diffusion of CO_2. The plateau usually terminated in an acid drift.

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[1] Benson, C. C., *J. Biol. Chem.*, 1928, **78**, 583.