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Effect of Potassium Chloride on Rate of Penetration of Dyes.

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A previous paper[1] has shown that KCl decreases the rate of penetration of dyes into the sap of *Nitella*. (1) The results with a cell model, consisting of a non-aqueous substance placed between cresyl blue solution and the artificial sap, show that when chloroform is used the rate is not decreased when 0.1 M KCl is added to the sap, but when aniline is employed it is decreased somewhat. (2) The same difference is observed when chloroform and aniline are shaken with 0.1 M KCl and then placed between the 2 aqueous phases when the sap contains no KCl. (3) When *o*-anisidine in chloroform is employed as the membrane, cresyl blue can be made to penetrate into the sap rapidly whether the sap contains KCl or not, provided the external pH value is considerably higher than the internal, but KCl has some inhibitory effect. An acid dye like phenol red is capable of penetrating the sap rapidly whether the sap contains KCl or not.

In dealing with the rate of penetration of substances into the sap in the model it is essential to consider several properties of the constituents of the non-aqueous layer such as chemical constitution, acid or basic character, dissociation constant, and solubility in water.

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Importance of the Internal Phase Boundary in Penetration of Dye into the Vacuole.

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A cell model[1] was used consisting of chloroform placed between the dye solution and the artificial sap. R_o = the rate of penetration of dye from the dye solution into the chloroform, and R_s = the rate of penetration of dye from the chloroform into the sap. These are obtained by determining the concentrations of dye in the aqueous phases during one hour. The absorption coefficients of the dye are

[1] Irwin, M., PROC. SOC. EXP. BIOL. AND MED., 1926, **24**, 54.

[1] For description see Irwin, M., PROC. SOC. EXP. BIOL. AND MED., 1928, **26**, 135.

determined by shaking chloroform with the aqueous solutions. These are C_o = conc. of dye in the chloroform/conc. of dye in the dye solution, and C_s = conc. of dye in the chloroform/conc. of dye in the sap.

(1) The absorption coefficients of crystal violet do not differ very much between pH 5.5 and pH 9, and the rate of penetration of dye into the sap does not vary to any extent in this range. This is because the dye exists chiefly in one form.

(2) The absorption coefficient of methyl violet varies roughly from 4 at pH 5.5 to 20 at pH 9. This is due to the fact that methyl violet is a mixture. (*a*) When $C_o = C_s = 4$ the rates R_o and R_s are comparatively rapid. (*b*) When $C_o = 20$ and $C_s = 4$ there is an increase in R_o and R_s, but this is very small compared to the increase in C_o. (*c*) When $C_o = 4$ and $C_s = 20$, the change in R_o may be considered negligible while the decrease in R_s is roughly proportional to the increase in C_s as compared to the results in (*a*). (*d*) When $C_o = C_s = 20$, R_o is slightly higher than in (*a*), but the increase is very small in comparison with that in C_o, while the decrease in R_s is roughly proportional to the increase in C_s.

(3) The absorption coefficient of methyl violet increases approximately from 4 to 371 when 0.1 M KCl is added to the dye solution at pH 5.5. The rate of penetration of dye into the sap is greatly reduced when the sap contains 0.1 M KCl, but the presence of the same amount of the salt in the dye solution does not affect the rate to the same extent. The rate of penetration of dye into the chloroform from the dye solution is not appreciably affected by the presence of salt in the dye solution or in the sap. These results confirm those on crystal violet.[2]

In the cell model the rate of penetration of dye into the sap appears to be influenced more by the alteration in the absorption coefficient at the internal phase boundary than at the external phase boundary.

In these experiments, volume relations, thickness of the chloroform layer, distance between the phase boundaries, and rate of stirring play important parts. The fact that R_o is not greatly affected by the alteration in C_o may be partly due to the volume relations. Experiments are in progress to determine this point.

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