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Accumulation of dye in Nitella as related to dissociation.

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In previous papers[1] the writer has described the accumulation of brilliant cresyl blue in the sap of *Nitella* and has discussed certain theoretical explanations. The present paper deals with the possibility of explaining accumulation on the basis of dissociation as recently discussed by Osterhout.[2] For this purpose we may assume that the dye enters in the form of dye hydrate (DOH) but that ions are unable to pass in or out. At equilibrium DOH has the same concentration inside the cell as in the outside solution, but if the pH value is lower in the sap there will be more dissociation, and consequently the total concentration of the dye (ions plus undissociated molecules) will be greater than in the outside solution. The difference in concentration can be calculated if the dissociation constant of the dye and the pH values inside and outside are known. When the previous papers were published, such calculations were attempted but at that time the experiments were not sufficiently complete for this purpose. Since then the writer has been able to secure the necessary data.

Living cells of *Nitella* were placed at 25 ± 0.5° C. in 0.00002 M dye solutions at different pH values, from pH 6.4 to pH 9.2 (M/150 phosphates or borates). At definite intervals the cells

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[1] **Irwin, M., *J. Gen. Physiol.*, 1925-26, viii, 147.**

[2] **Osterhout, W. J. V., *J. Gen. Physiol.*, 1925-26, viii, 131.**

were removed from the solutions. The end[3] of each cell was then cut and the sap was squeezed out upon a glass slide. The sap was then drawn up into a capillary tube, the color of which was matched with that of the capillary tube containing the standard dye solution.

It was found that the greater the pH value of the outside solution, the higher was the rate of penetration, and the greater the concentration of the dye in the cell sap at equilibrium. A maximum was reached at pH 9, where further increase in pH value of the external dye solution brought about no increase in the rate of accumulation.

At pH 6.4, 6.6, and 6.9 the process reached an equilibrium, but at higher pH values the cells died before the equilibrium was attained. When the rates taken at a very early stage of the time curve are plotted as ordinates, with the pH values of the external dye solutions as abscissæ, an S-shaped curve is obtained which resembles the curve obtained when the amount of dye absorbed by chloroform is plotted as ordinates against the pH values as abscissæ. In both cases the curve reaches a maximum at about pH 9, beyond which further increase in the pH value brings about no increase in the amount of dye taken up by chloroform or in the rate of penetration into *Nitella*. This is regarded as indicating that the percentage of undissociated molecules has reached its maximum value. This maximum is therefor taken as 100 per cent in each case and the amount of DOH at different pH values is calculated on this basis. When these values are plotted against pH values, the curves agree, though at lower pH values the curve obtained with *Nitella* is a little below the curve for chloroform. This discrepancy may be due either to experimental error or to conditions in the cell. The theoretical curve calculated from the dissociation constant of the dye agrees fairly well with these two curves. This may indicate that the rate of accumulation is dependent on the concentration of DOH in the external solution.

If we assume that equilibrium is reached when DOH inside is equal to DOH outside, the total concentration of dye at equilibrium will depend on the concentration of DOH in the outside solution and on the degree of dissociation of the dye in the sap (*i. e.*, on the concentration of D^+ ions). Calculations made upon this basis agree well with the observed facts.

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[3] For details of technique, see Irwin, M., *J. Gen. Physiol.*, 1925-26, viii, 147.

It may be added that it is possible, by making certain assumptions, as in the case of CO_2 discussed by Osterhout and Dorcas[4] to account for the relation between the inside and outside concentrations of dye on the basis of Donnan equilibrium. But in that case we might expect the rate of penetration to increase as the percent of dissociation increases, which is contrary to observation.

The high temperature coefficient (about 4.8) indicates that the dye does not pass in by simple diffusion but combines chemically with some constituent of the protoplasm on its way into the vacuole: or if it passes in by simple diffusion the process is complicated by other factors.

It is evident that the accumulation of dye may be explained on the basis of dissociation as set forth in the present paper or on other grounds as described in previous papers. Which of these explanations is correct may remain for the present an open question.

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Successive changes in the electrocardiogram following acute coronary artery occlusion.

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By the use of the portable electrocardiograph[1] it has been possible to secure a series of electrocardiograms in four patients soon after the onset of symptoms diagnostic of coronary artery occlusion. As none of these patients died, there was no opportunity to confirm the diagnosis by a pathological examination of the coronary arteries, but the diagnosis is justified by considerable experience in similar cases in which one or two characteristic electrocardiograms were obtained, which also presented typical symptoms and associated phenomena, and in which necropsy con-

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[4] Osterhout, W. J. V., and Dorcas, M. J., *J. Gen. Physiol.*, 1925-26, ix, 255.

[1] Mann, H., PROC. SOC. EXP. BIOL. AND MED., 1925, xxiii, 19.