various glands on the uterus of the cat could likewise be inhibited by epinephrin. It is therefore interesting to note that invertebrate smooth muscle cells which appear to be under the control of the sympathetic nervous system exhibit actions similar to the above.

It was found that epinephrin is about twenty times as effective as ephedrine in antagonising the action of the extracts of the parathyroid and the anterior lobe of the pituitary gland; twice as effective in antagonising the action of the extract of the posterior lobe of the pituitary gland. Ephedrine is almost ten times more effective than epinephrin in antagonising barium chloride. (This work will be published in full elsewhere.)

[1] Roth, G. B., *Arch. Internat. Pharm. et Therap.*, 1923, xxvii, 333; PROC. SOC. EXP. BIOL. AND MED., 1922-23, xx, 43.

[2] Fawcett, G. G., Beebe, S. P., et al., *Am. J. Physiol.*, 1915, xxxvii, 453; xxxix, 154.

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Influence of Salts and Acids on Penetration of Brilliant Cresyl Blue into the Vacuole.

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The theory[1] underlying the experiments described in the present paper is that the basic dye, brilliant cresyl blue, exists in two forms which we may call DB, a free base (predominant at higher pH values), and DS, a salt (predominant at lower pH values). It is assumed that the living cell of *Nitella** is permeable primarily to DB, and only very slightly to DS. Thus when we speak of the entrance of the dye into *Nitella* we refer to penetration of DB and not of DS. At equilibrium DB in the vacuole is equal to or proportional to DB in the external solution, and the amount of the total dye (DB and DS) in the sap depends on the extent to which DB changes to DS in the sap.

* The *Nitella flexilis* used is a fresh water plant, with multinuclear cells about four or more inches in length, having an outer cellulose wall, a very thin protoplasmic layer, and a relatively large vacuole filled with sap at about pH 5.5, containing about 0.1 M halides, protein, potassium, and other substances.

The rate of penetration of DB into the vacuole may depend on conditions in (1) the external solution, (2) the protoplasm, (3) the vacuole. It is assumed[1] that under varying conditions the seat of the controlling factor may change. One of the important problems in permeability is to locate this controlling factor. An attempt to throw light on this question is made in the present paper by exposing living cells to certain salts and acids and determining the rate of penetration of dye into the vacuole, when the cells are subsequently placed in a solution of dye.

1. *Effect of Chlorides with Monovalent Cations on the Cell.*

Cells were placed in 0.03 M NaCl solution[2] for 10 minutes, after which they were removed, wiped, and washed in distilled water (pH 5.4) for 5 seconds, then again wiped, and placed in 0.00007 M dye solution[3] at pH 7.7 (M/150 borate buffer solution). The rate of penetration of dye after 2 minutes was found to be about 75 per cent less than in the control cells which were transferred directly from distilled water to the same dye solution. This decrease is not due to the direct effect of NaCl on the dye, since, when the cells are placed in the 0.00007 M dye solution at pH 7.7, containing 0.03 M NaCl, the rate of penetration of dye is slightly higher than that of the control cells placed in dye solution containing no NaCl. Nor is this decrease due to the adhering of NaCl to the cell wall in such fashion that it cannot be washed or wiped away, because no decrease is found to take place if we dip the cells in NaCl solution for a few seconds, instead of for 10 minutes. No change in the concentration of halides is found to take place in the sap after the cells have been exposed for 10 minutes to NaCl solution.

These results indicate that the decrease in the rate of penetration of dye results from the effect of NaCl on the protoplasm (either at the surface or interior). The same result is obtained when KCl and LiCl are used.

2. *Effect of Chlorides with Bivalent and Trivalent Cations.*

The cells were exposed as before to solutions of chlorides with bivalent cations, $MgCl_2$ and $CaCl_2$, and with a trivalent cation, $LaCl_3$. In contrast to the chlorides with monovalent cations, there was with these salts no decrease in the rate of penetration of dye when cells were previously exposed to the same series of

concentrations of salt solutions for 10 minutes whereas there was an increase (about 10 per cent) in the rate of penetration of dye when the cells were placed directly in the 0.00007 M dye solution at pH 7.7 containing 0.003 M salt.

The effect of these salts on the dye, increasing penetration, is not sufficient to mask entirely the possible effect of the salt on the protoplasm, decreasing penetration, in case the latter is equal to that of the chlorides with monovalent cations, so that there is a difference in the behavior of the chlorides with monovalent cations on the one hand, and with bivalent and trivalent cations on the other, toward the protoplasm.

3. *Effect of Salts with Different Anions.*

By using the same experimental procedure as before we find that $NaNO_3$ and Na_2SO_4 give the same result as the chlorides with monovalent cations, while $MgSO_4$ and $La(NO_3)_3$ behave like $MgCl_2$ and $LaCl_3$.

These experiments show that specific effects of these salts either on the protoplasm or on the dye are due chiefly to the cations.

4. *Effect of Buffer Mixtures and Acids on the Cell.*

What happens if we place cells in a buffer solution which contains sodium or potassium salts at the same pH value as the control? When cells are placed in a phosphate buffer solution at pH 7.7 for 10 minutes and then transfrered to the dye solution the rate of penetration of dye is about 30 per cent lower than the rate of the control (cells directly transferred from tap water at pH 7.7 to the same dye solution). With borate buffer solution at pH 7.7 there is no change from that of the control. Since the pH value is the same in all cases, this decrease in the rate with the cells exposed to the phosphate buffer mixture cannot be due to the effect of H or OH ions on the cell, but is due to some other cause.

It is not due to a specific effect of potassium because the same result is obtained whether we use the phosphate buffer mixture made up with Na_2HPO_4 containing KH_2PO_4 or NaH_2PO_4.

It is not due to the effect of the phosphate buffer mixture on the dye because the rate of penetration is the same whether the cells are placed for 1 minute in the dye solution at pH 7.7 made up with borate or phosphate buffer mixture.

It may be due to phosphoric acid, which enters the protoplasmic surface as undissociated molecules and dissociates after entrance, thus, lowering the pH value of the protoplasm. This lowering of the pH value would reduce the amount of DB in the protoplasm as compared to the control, thus resulting in a decrease in the rate of penetration of DB from the protoplasm into the vacuole.

When cells are exposed for 10 minutes to solutions at pH 4 (1) of hydrochloric acid, (2) of boric acid, (3) of phosphoric acid, and then placed in the dye solution, the rate of penetration of dye as compared with the control is the same with (1) and (2), but it is about 20 per cent lower with (3). It would appear from (1) that H ions do not enter the protoplasm, while (2) indicates that if undissociated molecules of boric acid enter and dissociate it is too weak an acid to affect the pH value of the protoplasm, whereas (3) indicates that phosphoric acid enters and dissociates sufficiently to cause a decrease in the pH value thus decreasing the rate of penetration of dye into the vacuole or acts on the surface.

It may very well be that the effect of the phosphate buffer solution on the protoplasm is twofold, (1) due to phosphoric acid,[4] (2) due to the Na and K salts, in view of the fact that Na and K aschlorides have considerable effect on the cell.

The fact that sodium borate has no effect cannot be due entirely to its low concentration since the concentration of Na is about 0.01 M at pH 9, at which concentration NaCl has a marked inhibiting effect, and yet there is no effect of the sodium borate. Whether this is due to the masking of the decreasing effect of Na by an opposite action of borate ions or due to the effect of high pH value it is at present undecided.

5. *Effect of Acetic Acid on the Cell.*

These results may help to explain the reason why a decrease[5] in the rate of penetration of dye takes place when cells are exposed to an acetate buffer solution in which the pH value of the sap is lowered, though according to the theory[1] already outlined we might expect an increase in the rate of dye penetration to take place. If we suppose that the increase of penetration due to lowering of pH value of the sap is masked by the decreasing effect of (1) sodium acetate on the protoplasm, and

(2) of acetic acid either on the protoplasm or on the "film of liquid" between the protoplasmic surface and the cell wall, then we might expect a decrease in the rate of penetration, on the basis of the theory[1] mentioned above.

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[1] Irwin, M., *J. Gen. Physiol.*, 1926-27, x, 75.

[2] Different concentrations of NaCl solution were used (from 0.05 M to 0.0006 M). It was found that there was no decrease at 0.0006 M salt solution. The decrease caused by an exposure of cells to 0.03 M salt solution cannot be due to injury, though the cells can live in this solution only a few hours, since the same type of decrease may be obtained in the salt solution, at 0.005 M for example, in which the cells can live for several days.

[3] Solutions are not stirred unless otherwise stated.

[4] Another way of interpreting this decrease is as follows: The pH value of the very thin film of liquid between the protoplasmic surface and the cell wall is lowered as a result of diffusion of phosphoric acid from the protoplasm into this film when cells are transferred from the phosphate buffer solution to the dye solution, but at pH values of the phosphate buffer solution above pH 7 the decrease in the rate of penetration of dye cannot be attributed to this, since the pH value of the solution to which the cells are previously exposed is higher than the pH value of the dye solution.

[5] The writer has found that in some cases there is an increase in the rate of penetration of dye instead of a decrease. Such a difference may be due to the difference in the condition of the protoplasm. Irwin, M., *J. Gen. Physiol.*, 1925-26, ix, 561.

This decrease in the rate of penetration of dye is not caused by the lowering of the pH value of the external solution outside the cell wall as a result of diffusion of acetic acid, since stirring the solution does not change the relative decrease in the rate of penetration of dye (on comparing the rate of the test experiment with that of the control experiment when in both cases the solutions are stirred).

When the living cells of *Valonia macrophysa* are exposed for 1 hour to sea water at pH 5.9 containing (1) acetic acid in which the pH value of the sap is lowered, (2) hydrochloric acid, in which the pH value remains unchanged, and subsequently transferred to the sea water containing 0.00007 M dye, after 20 minutes the rate of penetration is found to be much lower than that in the case of the control experiment (cells directly transferred from sea water to the same dye solution). The rate of penetration of dye is found to decrease (1) when cells are previously exposed to sea water at pH 8.1 containing 0.003 M NH_4Cl until the pH value of the sap is increased, and then transferred to the sea water containing dye, (2) when cells are placed in sea water at pH 8.1 containing dye and 0.003 M NH_4Cl.