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Salts Affecting Penetration of Brilliant Cresyl Blue into Nitella at Different pH Values.

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I. When cells are exposed to 0.05 M sodium chloride,[1] and then placed in a solution of brilliant cresyl blue[2] at pH 7.5, there is a decrease[3] in the rate of penetration of dye into the vacuole,[4] as compared with the control.[5] There is no such decrease[3] when sodium chloride is replaced by magnesium or calcium chloride. The same results are obtained at pH 7.9.

II. When cells are transferred directly from tap water to the dye solution at pH 7.5, containing 0.05 M (*a*) sodium chloride, (*b*) magnesium chloride, or calcium chloride, there is a slight increase[3] in the rate of penetration with (*a*), while there is practically no increase[3] with (*b*). When the experiments are repeated at pH 7.9, there is no appreciable change in the rate from that of the control,[5] when the dye solution contains sodium chloride, while there is a slight decrease when the dye solution contains magnesium or calcium chloride.

This decrease is not due to an increase in the concentration of sodium in the buffer resulting from the addition of more borax, because there is no such decrease when the equivalent amount of sodium is introduced by adding sodium chloride to the dye solution containing either 0.05 M magnesium or calcium chloride, at pH 7.5. This decrease may therefore be attributed to the increase either in hydroxyl ions[6] or in borate ions. This decrease,[7] furthermore, does not seem to be due to the direct effect of such anions on the protoplasm in the presence of a salt with bivalent cation, because there is no such decrease when cells are exposed to 0.05 M calcium, or magnesium chloride made up with borate buffer mixture at pH 7.9, and then placed in the dye solution containing neither of these chlorides, at pH 7.5. If there were such an effect on the protoplasm it would in all probability persist after transferring the cells to the dye, as indicated by other experiments. It may, therefore, be attributed to a change in the dye brought about by the anions (hydroxyl[6] or borate or both) in presence of any of the salts with bivalent cations, so that (1) the dye has an inhibiting effect on the protoplasm, or (2) it becomes less soluble in the cell, or (3) it becomes less diffusible.

III. When cells are placed in the dye solution containing 0.05 M sodium chloride at pH 8.2, there is a decrease in the rate of penetration of dye, which is less than the decrease with the dye solution containing magnesium or calcium chloride at pH 7.9, though the dye solution contains the same proportion of borax to boric acid in both cases. This decrease may be interpreted in the same way as in section II.

IV. When cells are previously exposed to 0.05 M (*a*) sodium chloride, or (*b*) magnesium or calcium chloride, and then placed in the dye solution at pH 7.9, containing 0.05 M magnesium or calcium chloride, there is a decrease in the rate of penetration of dye as compared with the control,[5] but the extent of this decrease is slightly less than when cells are placed in the same dye solution without previous exposure to salt. Furthermore, the decrease in the rate of penetration is less in the case of cells previously exposed to sodium chloride solution and then placed in dye solution at pH 7.9, containing no salt other than buffer salts.

V. When cells are previously exposed to 0.05 M (*a*) sodium chloride, (*b*) magnesium chloride, (*c*) calcium chloride, and then placed in the dye solution containing 0.05 M sodium chloride at pH 8.2, the rate of penetration of dye is practically the same as that of the control,[5] with (*a*), and shows possibly a slight increase with (*b*), and a very slight decrease with (*c*).

These experiments indicate that the method of adding salts to the dye introduces variables (such as changes in the dye and in its effect on the protoplasm) which make the interpretation difficult. To study the effect of salts on the protoplasm it seems better, as previously suggested by the writer,[3] to expose the cells to the action of salts and then place them in dye made up with as little salt as possible.

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[1] All salts are dissolved in distilled water unless otherwise stated. Living cells are exposed to the salt solutions for 10 minutes, during which time no irreversible injury is brought about.

[2] In all cases, 0.00014 M dye solution is made up in M/150 borate buffer mixture (borax plus boric acid). The pH values are determined by means of the hydrogen electrode. Cells are placed in the dye solution for one-half minute.

[3] This result confirms the result given in the writer's previous papers, PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 54, and *J. Gen. Physiol.*, 1926-27, x, 271.

[4] The concentration of dye in the vacuole is determined colorimetrically.

[5] Experiments where the cells are transferred directly from tap water to the dye solution containing no salt (except buffer salts) represent the control in all cases.

[6] The action of hydroxyl ions at such pH values on the dye has been found to increase the relative amount of the more readily diffusible form of dye, free

base, so that if they have an inhibiting effect, in presence of such chloride, their action on the dye must be different in presence of such salts.

[7] Addition of different concentrations of sodium chloride to the dye solution at pH 7.9 containing 0.05 M calcium chloride, diminishes progressively the extent of this decrease.

[8] The conflicting results obtained by various investigators seem to be due to the use of this method.

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On the Experimental Transmission of Arsenic to the Aqueous Humor.

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(Introduced by S. C. Harvey.)

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This investigation is a study of the arsenic content of the aqueous humor after the intravenous injection of neo-arsphenamine in dogs.

Eleven dogs were used. Fifty milligrams of neo-arsphenamine per kilogram of body weight was injected into the jugular vein of each dog. The eyes were anesthetized with cocaine. As much aqueous humor as possible was removed from each eye with a fine bore needle at varying intervals of time after injection. The fluids of the two eyes were pooled in every case. After digestion[1] with acid, a modified Gutzeit method[2] was used in the determination of the amount of arsenic in the samples.

The details and results are shown in the accompanying table.

TABLE I.

Dog No.	Injection of 50 mg. of neo-arsphenamine per kilogram.							
	Before inject.	After Injection						
	0.5 hr.	1 hr.	2 hr.	4 hr.	8 hr.	24 hr.	48 hr.	96 hr.
1	0	8	20	15		2	0.6	
2	0	7	16	13		2	1	
3		T	24	15		2		
4		T	21	16		1	1	
5			T	9		1.5	0.8	
6				T	2.5	1	0	
7						1	2	0.5
8							1	0
9		T		5		1.5	T	
10		T			2.	T	T	
11		T				T	T	

T represents trace. Results in micromilligrams or arsenic trioxide.