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The Form of Dye Penetrating the Cell as Determined by the Glass Electrode.

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Measurements with the glass electrode show that cresyl blue in the form of salt* decreases the pH value of distilled water and in the form of free base* increases it. The free base also increases the pH value of the sap freshly extracted from the vacuoles of living cells. This difference in the behavior of the dye salt and the free base enables us to determine whether free base of cresyl blue penetrates into the vacuoles of living cells of *Nitella.*

The pH value of the sap of *Nitella* (obtained from Maryland) was measured by means of the glass electrode immediately after extraction at about 25°C. The duration of each measurement was about 5 minutes. The pH value of the sap of the control cells was pH 5.77. Using indicator methods previously employed[1] with brom cresol purple (appearing yellowish when dissolved in distilled water) the sap from the control cells was only 0.1 pH lower. Each measurement was made immediately after extraction and took about 5 minutes. As the dye penetrates, the pH value of the sap is gradually increased to the extent of 0.5 pH or more.

The same concentration of brilliant cresyl blue produces approximately the same increase in pH value whether it penetrates the vacuole of a normal cell or is added in the form of free base *in vitro* to freshly extracted sap.

The work is being continued.

The measurements in distilled water were made by Dr. A. E.

* Cresyl blue (prewar Grübler) is purchased in form of salt. The free base, free from impurities, may be obtained by shaking the aqueous solution with chloroform.

[1] Irwin, M., *J. Gen. Physiol.*, 1925-26, ix, 240.

Mirsky[2] while those in the sap were made by Dr. M. Dole.[3] I wish to thank them for their kindness and courtesy.

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The Isoelectric Point of the Dick Toxin.

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In the course of experiments having for their purpose purification of Dick toxin it became desirable to ascertain the electrical charge carried by the toxic material. This point seemed worthy of investigation not only because of our own immediate needs for such information, but also because the electrical charge borne by a toxin is an important factor in filtration, adsorption, flocculation, etc.

The method described by Krueger, Ritter and Smith,[1] based upon electrophoresis of the test substance into agar and subsequent resuspension in some appropriate menstruum, was employed. Toxin* of high titre was adjusted to the required pH value with N/10 NaOH or HCl, placed in the two cells of the apparatus and exposed to a current of 70-85 v. and 5-12 milliamps. for 20 hours. At the end of this time the agar cylinders from anode and cathode were removed and triturated with 10 cc. normal saline solution for 1 hour. The extracts so obtained were then made up in 1/100 dilutions with normal saline and injected in quantities of 0.1 cc. into the skin of individuals known to be Dick positive. Each test patient also received a control injection, *i. e.,* 0.1 cc. of a 1:10,000 dilution of toxin previously adjusted to the pH at which the test was run, maintained at this C_{H^+} for the same length of time as the toxin in the apparatus and neutralized before injection. This injection served to detect inactivation of the toxin by acid or alkali and also furnished us with a criterion by which to read the other skin reactions. We found it necessary to employ in the cataphoresis cells a gel containing not more than 0.4% purified agar. Percentages higher than this did not permit the toxin to migrate.

[2] Mirsky, A. E., and Anson, M. L., *J. Biol. Chem.,* 1929, lxxxi, 581.

[3] MacInnes, D. A., and Dole, M., *J. Gen. Physiol.,* 1928-29, xii, 805.

[1] Krueger, A. P., Ritter, R. C., and Smith, S. P., *J. Exp. Med.,* 1929, (in press).

* Dr. Gladys Dick very kindly supplied us with large quantities of toxin having a titre of 12,500 S.T.D.'s per cc.