4827

Studies on Permeability of Living Cells. XII. Further Studies on Penetration of Oxidation-Reduction Indicators.

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In a previous paper[1] it was shown that there was a definite relation between the penetration of oxidation-reduction indicators into cells of *Valonia macrophysa* and their position in the oxidation-reduction scale. Those dyes which were at the electro-positive end of the scale penetrated very readily, while those which were farther down towards the electro-negative end did not penetrate. The former were represented by the indophenols, which were reduced in the sap; and the thiazines, which were not reduced. The latter included the indigo-sulphonates, which did not penetrate.

The question was then raised whether the position of the dye on the oxidation-reduction scale was the determining factor in its penetration. Fortunately Rapkine, Struyk and Wurmser[3] have determined the potentials of some vital dyes on the E_h scale, and found that Janus green and neutral red are more electro-negative than any of Clark's dyes; in fact the neutral red system lies very near to the H electrode. These 2 dyes were, therefore, used in further penetration studies.

About one dozen small plants of *Valonia ventricosa* which had been kept in the laboratory for some months and seemed to all outward appearances to be normal living cells, were placed in solutions of sea water containing 0.001% Janus green and buffered with Clark's buffers at various pH values from 5.8 to 9.0. The total M concentration of buffer was 0.009. The temperature was 25° C. The sap was examined at various intervals up to 12 hours and in no case was the dye found in the sap in detectable amounts either in the oxidized or the reduced state.

Other plants were placed for one hour in a solution of 0.001% Grübler's neutral red dissolved in sea water containing buffers as previously described at pH 5.8. A spectrophotometric analysis of the neutral red dissolved in sea water gave an absorption maximum at wave length of 523 mμ, which corresponds to that found by For-

[1] Brooks, M. M., *Am. J. Physiol.*, 1926, lxxvi, 360.

[2] Clark, W. M., Cohen, Barnett, and Gibbs, H. D., *Pub. Health Rpts.*, 1925, xl, 1131.

[3] Rapkine, L., Struyk, A. P., and Wurmser, R., *Compt. Rend. Soc. Biol.*, 1929, c, 1020.

manek and Grandmougin.[5] There was also a secondary maximum at about 465 mμ. Spectrophotometric analysis of the pink sap which was extracted from 5 cells kept under these conditions for one hour, gave the same absorption maxima as the dye itself. The method of extraction is described in another paper.[1] That the cells were not irreversibly injured is shown by the fact that they lived for months afterwards in the laboratory and appeared normal.

These results confirm unpublished data on the penetration of these dyes into sap of freshly collected plants of *Valonia macrophysa* which the writer obtained in 1925 while in residence at Bermuda.

These experiments show, therefore, that the position of the dye system on the oxidation-reduction indicator scale as described by Clark, is the factor determining whether or not a dye is reduced by the cell, but that it is not the exclusive factor which determines the penetration of these dyes into living cells.

4828

Actions of Sodium Bismuthate.*

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The bismuth compounds in current use contain the bismuth as cation, or in basic form, and do not dependably penetrate the brain and appear in the cerebrospinal fluid. Compounds containing the bismuth as anion, or in acid form, have not been tried previously, but, on theoretical grounds, might be expected to penetrate the central nervous system more readily than those containing bismuth as cation, and thus be more valuable in neurosyphilis. Accordingly, we have explored the possibilities of sodium bismuthate and obtained results worthy of record at this time in order to indicate the desirability of further study of anionic bismuth.

[4] Clark, W. M., "The determination of H ions." Williams and Wilkens, Baltimore, 1928.

[5] Formanek, J., and Grandmougin, E., *Untersuchungen und Nachweis organischen Farbstoffe auf Spektroskopischen Wege.* (Berlin), Julius Springer, 1908.

* This research was supported in part by a grant from the Committee on Research in Syphilis.