With penetration of cresyl blue there is practically no difference between these 2 extractions. But with the 0.07% dye penetrating from azure B and methylene blue solution at pH 9.2, there is only 0.6 pH increase in the sap mixture instead of 1 pH as in the case of sap. In the case of methylene blue, this difference is not due to the additional penetration of methylene blue salt into the protoplasm, because the dye in the sap mixture gives the same absorption curve as the dye in the sap, with the primary absorption maximum at 653 to 655 mμ (characteristic of a mixture of azure B and methylene blue, with the preponderance of the former).

It is concluded that cresyl blue penetrates chiefly as free base, and that azure B also penetrates chiefly as free base from azure B solution and from methylene blue solution at pH 9.2.

In these experiments contamination of the sap by the stained cell wall was avoided as previously described.

Impurity in the free base of dye when added to the sap *in vitro* does not alter the pH value.

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Studies on the Penetration of Dyes With the Glass Electrode. III. Penetration into Valonia of Cresyl Blue and Azure B.

MARIAN IRWIN.

From the Laboratories of The Rockefeller Institute for Medical Research.

(1) The pH value of the sap extracted from the vacuoles of small living cells of *Valonia macrophysa* is found to be pH 5.4. The pH value remains unaltered for several hours, *i. e.*, much longer than the period required for the extraction of sap and subsequent measurement with the glass electrode.

(2) 0.01% cresyl blue penetrating (in about 20 minutes) from the sea water at pH 9.5, raises the pH value of the sap 0.5 pH. This increase is 0.2 pH less than the rise brought about by the addition of 0.01% free base of cresyl blue to sap *in vitro*.

(3) 0.03% cresyl blue penetrating (in about 1 hour) raises the pH value about 0.9 pH, which is about 0.4 pH less than the rise brought about by the addition of 0.03% cresyl blue free base to sap *in vitro*.

(4) If cells are kept 8 hours longer in this cresyl blue solution, they appear to be still in good condition, so that it is likely that the cells described under (2) and (3) are not at all injured.

(5) No alteration in the pH value of the sap occurs when cells

are placed even for 8 hours in sea water at pH 9.5 containing no dye.

(6) Azure B penetrating the sap also increases the pH value, and this increase is again less than the increase brought about by addition of azure B free base *in vitro*. Owing to a slower penetration and greater toxicity of azure B as compared to cresyl blue, these results on azure B are rather unsatisfactory but experiments are being continued.

(7) The penetration of dye from methylene blue solution at pH 9.5 is still slower than from the azure B solution, so that sufficiently accurate results are not obtainable before the cells are injured but experiments are being continued.

It is concluded that cresyl blue and azure B free base penetrate the vacuoles of living cells of *Valonia macrophysa*.

The fact that the penetration of dye causes the pH value of the sap to increase less than when the same amount of free base of the dye is added *in vitro*, may in all probability be due partly to the production of acid by the cell rather than to the penetration of dye salt, just as was proved to be the case with the sap mixture of *Nitella*. It is partly due to the impurity in the free base of dye which slightly raises the pH value when added to the sap *in vitro*.

Owing to the fact that the sap of *Valonia* is not so well buffered as that of *Nitella* and also to the fact that the electrode is affected by the 0.6 M inorganic salt in the sap of *Valonia*, these results with *Valonia* are not so convincing as those with *Nitella*.

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Analysis of Ether-Air Mixtures by Thermal Conductivity Method.

F. H. HOWARD AND E. N. GOODMAN. (Introduced by E. L. Scott.)

From the Department of Physiology, College of Physicians and Surgeons.

For the purpose of rapid determination of ether concentration in ether-air mixtures we have found the thermal conductivity method of gas analysis satisfactory. Since the heat conductivity of ether vapor is only about one half as great as is that of air, the admixture of ether vapor with air decreases the loss of heat from the wire and causes its temperature to rise. The consequent increase of electrical resistance of the wire is readily determined and, if the apparatus is calibrated, the change of resistance is a measure of the ether concentration.

The apparatus used in this investigation consisted of a rectangu-