These two preliminary studies indicate the possibility of quantitative revision of our vitamin B tables, through use of the Peters' or the Williams' preparations. The study of Williams' preparation in bird tests has shown, however, that while antineuritic for these animals and capable of checking weight decline, it is not able to restore them to normal weight. Autoclaved yeast also fails to provide the restorative for birds though effective in rats. Finally yeast or whole grains prove both antineuritic and restorative of weight for pigeons. As Williams and Waterman have pointed out, these factors appear to indicate that yeast and whole grains contain a B factor which is not antineuritic, not present in autoclaved yeast and necessary to bird weight control. Our tests on Peters' fraction are not yet sufficiently advanced to state whether his fraction is like Williams' or different, but in the only two observations reported by Peters it failed to prevent weight decline in pigeons.

The doubts raised by the above tests as to whether vitamin B is dual or still more multiple in nature, are obvious, and suggest further study of the vitamin B fractionation before attempting extensive revision of the existing tables. Tools are, however, now available for assaying at least two of these factors with the rat as test animal.

[1] Smith and Hendrick, Public Health Report, U. S. P. H., 1926, xli, 201.

[2] Goldberger and coworkers, Public Health Reports, U. S. P. H., 1926, xli, 297, and U. S. P. H., 1926, xli, 1025.

[3] Salmon, *J. Biol Chem.*, 1927, lxxiii, 483; Hauge and Carrick *J. Biol. Chem.*, 1926, lxix, 403.

[4] Chick and Roscoe, *Biochem. J.*, 1927, xxi, 698.

[5] Kinnersley and Peters, *Biochem. J.*, 1925, xix, 8-20, and personal communication of later date.

[6] Williams and Waterman, PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, 63.

[7] Osborne and Mendel, *J. Biol. Chem.*, 1919, xxxvii, 187.

[8] Eddy, Kohman and Carlsson, *J. Indust. and Eng. Chem.*, 1925, xvii, 69.

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Multiple Partition Coefficients of Penetration.

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Overton's lipoid theory[1] which states that only dyes soluble in lipoid can penetrate into living cells, is inadequate in certain re-

spects.[2] This is due in part to the fact that he considered only one partition coefficient, that of the dye between the external solution and the lipoid layer surrounding the living cell. Since it is chiefly cytoplasmic inclusions, granules, fat globules, vacuoles, etc., which are stained rather than the cytoplasm proper, it is necessary to consider several partition coefficients, such as: (1) That of the dye between the non-aqueous layer surrounding the cytoplasm and the external solution. (2) That of the dye between the same non-aqueous layer and the aqueous cytoplasm. (3) That of the dye between the aqueous cytoplasm and the inner non-aqueous inclusions (granules, or fat globules) or the non-aqueous layer surrounding the vacuole. (4) That of the dye between the inner non-aqueous layer of the vacuole and the aqueous vacuolar sap.

Experiments from this point of view have been made on *Nitella flexilis* and *Valonia macrophysa*. The results are presented here with *Valonia*, the cells of which consist of an outer cell wall, an inner protoplasmic layer surrounding a relatively large central vacuole filled with sap, at about pH 5.8 and containing about 0.6 M halides.

We may assume[3] that the dye diffuses through the protoplasm into the vacuole in succession through an outer non-aqueous layer, a middle aqueous layer, an inner non-aqueous layer (surrounding the vacuole), and then into the vacuolar sap.

By applying these ideas experimentally we can predict the behavior of the dyes in penetrating *Valonia*. The dye in sea water at pH 9.5 or at pH 5.5 is shaken with chloroform so as to represent the two non-aqueous layers. Then the chloroform is removed and shaken with freshly collected *Valonia* sap. We should then expect that the dye, which penetrates the vacuole most readily, is that which is most readily absorbed by chloroform from sea water, and is most readily given up by chloroform to the sap. This is found to be the case. The penetration of dye into the vacuole of living cells was measured by placing the living cells in about 0.03% dye dissolved in sea water at pH 9.5 and at pH 5.5 for 1 to 3 hours. The amount of dye found in the vacuole was determined in the manner already described.*

The experiments show the following results: (1) Trypan blue, trypan red, and Bordeaux red are not absorbed by chloroform from sea water, and they do not readily* enter the vacuole (*viz.*, no dye

* The phrase "does not readily enter" is used even in the case when no dye enters during the experiment because it might be found to enter with a much longer exposure.

penetrates in 3 hours). (2) Acid fuchsin and basic fuchsin are very slightly absorbed by chloroform from sea water, and they are readily absorbed from the chloroform by the sap. They do not readily enter the vacuole (only a trace of basic fuchsin is found from pH 9.5 after 3 hours). (3) Crystal violet and methyl violet are readily absorbed by chloroform from sea water and are not readily given up to the sap. They do not readily enter the vacuole. (4a) From methylene blue dissolved in sea water at pH 9.5, azure B is chiefly absorbed by chloroform. Azure B is also chiefly absorbed by sap from this chloroform. It is chiefly azure B that penetrates the vacuole from methylene blue in sea water at pH 9.5. (b) Practically no thionin is absorbed by chloroform from sea water. A violet-pink dye is found in chloroform, and is absorbed by the sap. The dye which is found in the vacuole is also violet-pink, and not chiefly thionin which is violet. (c) From methyl green in sea water, chiefly methyl violet is absorbed by chloroform, but only methyl green is given up to the sap. Mostly methyl green and not methyl violet penetrates the vacuole. (5) Brilliant cresyl blue, azure B, toluidine blue, new methylene blue, methylene green, are more readily absorbed by chloroform from sea water at pH 9.5 than at pH 5.5. These dyes penetrate the vacuole more readily from sea water at pH 9.5 than at pH 5.5.

There are, of course, certain factors which must be considered. If a dye enters more readily in one form than in the other (*e. g.*, free base[5] as contrasted with the salt of a basic dye), the rate of penetration and degree of accumulation will depend in part on the ratio of one form of dye to the other.

The penetration, accumulation, and exit of the dye may also depend on compounds formed between the dye and the various substances in the cell.

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[1] Overton, E., *Jahrb. wissensch. Bot.*, 1900, xxxiv, 669.

[2] For reviews of the literature see Robertson, T. B., *J. Biol. Chem.*, 1908, iv, 1, and Höber, R., Physikalische Chemie der Zelle und der Gewebe, 6th ed., Leipzig, 1926.

[3] For a diagram of the cell see Irwin, M., *J. Gen. Physiol.*, 1926-27, ix, 75.

[4] Irwin, M., *J. Gen. Physiol.*, 1926-27, x, 927.

[5] Irwin, M., *J. Gen. Physiol.*, 1925-26, ix, 561.