IODO-EOSIN AS A TEST FOR FREE ALKALI IN DRIED-UP PLANT TISSUES

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The free dye-acid of iodo-eosin has been employed by Professor Ehrlich as an extremely delicate reagent for free alkali in the red-blood corpuscles of man.¹

The writer of the present note subsequently found that the same reaction proved also a useful test for the feeblest free alkalis in dried-up plant tissues.²

The dye used is iodo-eosin, the potassium salt of tetraiodo-fluoresceïn. Iodo-eosin as a salt dissolves in dilute alkalis with red colour, but is insoluble in ether or any other organic solvent. The free dye-acid, however, obtained as a yellow precipitate from the alkaline solution of iodo-eosin by adding hydrochloric acid in excess, dissolves easily in ether or in any other organic solvents, but is insoluble in water.

It is for this reason that the reaction can be made use of for testing free alkali in a perfectly dry tissue.

Suppose we have a free hand transverse section of a dry twig of a common forest-tree, for example, a spruce fir, and put the section into a solution of the dye-acid of iodo-eosin that has been dissolved in ether, leave it there for some minutes, then wash out the section thoroughly with ether. The preparation brought into xylene should now be examined under a low power of the microscope, when some of the histologically differentiated elements of the tissue will be stained distinctly red. In our section, for example, the cambium, the resin canals and the secreting cells lining the cavity appear plainly red-stained.

It is easy to see that these red-stained elements of the tissue must be those that contain free alkali, and, further, that their red colour is due to the red alkali salts formed.

We are justified in supposing that the free alkali located in these tissues is acting as a dye-base as soon as the dye-acid of iodo-eosin is

1. Ehrlich-Lazarus, *Die Anaemie*, Vienna, 1898.

2. A. C. Hof, *Botanisches Centralblatt*, LXXXIII, 1900.

added, yielding immediately the characteristic red dye-salt. If there is no water present the red colour remains in the tissue fixed at the exact place where it has been formed. Thus we get an exact idea of the distribution of free alkali in dry tissues.

METHODS

Preparation of the dye-acid of iodo-eosin.—Dissolve 1 gramme iodo-eosin, the commercial dye, in 1 per cent. potassium hydroxide, add hydrochloric acid in excess. The dye-acid precipitates at once. Filter and wash the precipitate with hot water thoroughly till the filtrate is absolutely free from hydrochloric acid. Dry the precipitate of dye-acid and dissolve it in 100 c.cm. ether.

Staining of sections.—Bring the sections, either sectioned free hand or in a microtome, into the above solution of dye-acid, leave them in it for some minutes, wash out the sections carefully with ether, transfer to xylene, and seal preparation in Canada-balsam. For preserving the sections distinctly stained it is absolutely essential that the balsam used is of perfectly neutral reaction. The ordinary balsam bought from dealers in microscope supplies often reduces the dye-salts immediately. By this reduction of the dye the colourless compound—the leuco-base or leuco-compound—is formed.³

I venture to hope that the foregoing reaction, simple, effective and feasible as it is in the case of almost any drug, may be of some use to students of pharmacognosy.

3. P. G. Unna, *Centralblatt für Bakteriologie und Parasitenkunde*, Vol. III, 1888.