Note on the Use of Bromophenol Blue for the Histochemical Recognition of Protein

By JOHN R. BAKER

(*From the Cytological Laboratory, Department of Zoology, University Museum, Oxford*)

SUMMARY

There is not sufficient evidence to prove that bromophenol blue is a reliable histochemical reagent for the recognition of protein. It is a powerful acid dye, capable of making direct links with basic groups in tissue-constituents (and perhaps also with certain acidic groups, through mercury).

BROMOPHENOL blue has recently come into general use as a histochemical reagent for the recognition of protein. It is the purpose of this note to inquire whether a microscopical object that is coloured by this dye necessarily contains protein.

Bromophenol blue is an acid phthalein dye, commonly used as a pH indicator. It was used by Durrum (1950) for colouring proteins in paper-electrophoresis. Durrum dissolved it at 0·1% in a saturated solution of mercuric chloride in 95% ethanol. The purpose of the protein coagulants (mercuric chloride and ethanol) was to immobilize the proteins on the paper. Durrum made no claim that the dye provided a specific test for proteins, but nothing else in human blood-serum was coloured by it, under the conditions of his experiments. The various proteins of serum were similarly coloured by it and were distinguished by the speeds at which they moved in electrophoresis.

Kunkel and Tiselius (1951) used bromophenol blue in a similar way. They sometimes dissolved the dye in an aqueous solution of mercuric chloride, acidified by acetic acid. They brought forward no evidence that the dye is specific for proteins, and indeed they noted that both dextran and paper can in certain circumstances be coloured by it. Cremer and Tiselius (1950) gave a special method for removing the colour from paper while leaving it in protein.

Geschwind and Li (1952) showed that certain amino-acids and peptides on paper chromatograms give a blue coloration with bromophenol blue. The results of their experiments did not depend primarily on the ability or inability of the dye to colour these substances, but rather on the capacity of mercuric chloride to fix the dyed substance on the paper, and thus prevent its removal by solution during the final rinsing in water. This would not necessarily be relevant in histochemical technique, for the amino-acid or peptide might have been rendered insoluble by the fixative used, and subsequently coloured by the dye.

[Quarterly Journal of Microscopical Science, Vol. 99, part 4, pp. 459–460, Dec. 1958.]

Mazia, Brewer, and Alfert (1953) showed that the tissues of animals, fixed in routine fixatives and sectioned, could be strongly dyed by Durrum's solution. They also showed that starch and glycogen had no affinity for the dye, and DNA and cholesterol very little. These results were to be expected, since bromophenol blue is an acid dye.

The affinities of bromophenol blue, as reported above, do not qualify the dye to act as a reliable reagent for the histochemical recognition of protein. Such qualification could only result from the test of a wide variety of lipids, carbohydrates, and other tissue-constituents.

In the present state of knowledge it seems safest to regard bromophenol blue as a powerful acid dye, capable of making direct links with basic groups in tissue-constituents, and perhaps also (as Mazia and his colleagues suggest) with certain acidic groups, through mercury. Interesting results can be obtained by using the dye with and without mercury, on the same tissue; but no certainty can be reached as to the chemical composition of the substances coloured, unless reliable evidence is available from other sources.

Azocarmine B has been used to visualize proteins in paper-electrophoresis (Turba and Enenkel, 1950), but this does not mean that azocarmine is a suitable substance for the histochemical recognition of protein.

REFERENCES

CREMER, H. D., and TISELIUS, A., 1950. Biochem. Z., **320**, 273.
DURRUM, E. L., 1950. J. Amer. chem. Soc., **72**, 2943.
GESCHWIND, I. R., and LI, C. H., 1952. Ibid., **74**, 834.
KUNKEL, H. G., and TISELIUS, A., 1951. J. gen. Physiol., **35**, 89.
MAZIA, D., BREWER, P. A., and ALFERT, M., 1953. Biol. Bull., **104**, 57.
TURBA, F., and ENENKEL, H. J., 1950. Naturwiss., **37**, 93.