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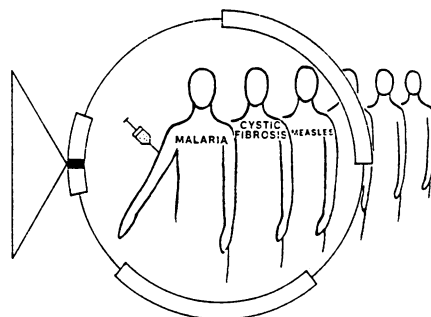
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MOLECULAR PATHOLOGY

Edited by J. Kay & M.J. Morgan



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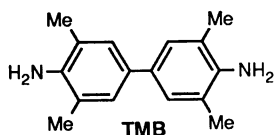
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Tetramethylbenzidine

A reported noncarcinogenic analog of benzidine



For many years benzidine was used as a sensitive and specific reagent for the detection of blood.¹ However, in 1974 the Occupational Safety and Health Administration banned its manufacture and use in the United States because of its extreme carcinogenicity.²

One of the early hypotheses for the carcinogenicity of aromatic amines involved *ortho* hydroxylation.³ Thus, it seemed that 3,3',5,5'-tetramethylbenzidine (TMB) (in which *ortho* hydroxylation is impossible) might be an effective and safe substitute for benzidine. Indeed, it was found to be nontumorigenic by subcutaneous injection into rats.⁴

The *Salmonella*/microsome test (Ames test)⁵ showed TMB to be nonmutagenic,^{6,7} suggesting noncarcinogenicity. Recently, TMB was shown to be nonmutagenic when compared to other benzidine compounds in a modified Ames assay.⁸

Garner *et al.*⁹ evaluated the use of TMB as a presumptive test for blood in forensic work. In various concentrations of glacial acetic acid, TMB reacted with blood in the presence of H₂O₂ to form a colored product. Comparative studies with benzidine showed TMB to be equally sensitive in blood detection.⁹ Piejko and Boemer¹⁰ have used TMB in a mixture spread on polyester film strips to determine glucose concentration in whole blood.

Standefer and Vanderjagt¹¹ found that methods employing TMB for plasma hemoglobin assay compared well with those using benzidine in accuracy, precision and sensitivity. Iron porphyrins, which can exhibit a peroxidase-like action, were detected at very low levels on paper chromatograms with TMB as the visualizing agent.¹² The peroxidase activity of the heme protein cytochrome P-450 was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis with TMB staining.¹³ In this method, TMB-H₂O₂ stain is superior to benzidine-H₂O₂. The blue-stained bands are distinct and the color is stable.¹³ Peroxidase activity was also detected by the presence of immunoprecipitates in Ouchterlony double-diffusion plates.¹³

In the area of neurohistochemistry, TMB proved more

sensitive than 3,3'-diaminobenzidine in demonstrating retrograde and anterograde axonal transport of horseradish peroxidase (HRP) in rat brain by light microscopy.¹⁴ TMB also gave results comparable to those obtained with benzidine dihydrochloride in the demonstration of retrograde transport of HRP.¹⁵

Current literature is replete with references to analytical applications of TMB. Recent applications include the use of TMB in a substrate to provide direct evidence of peroxidase activity in *Salmonella typhimurium*,¹⁶ of horseradish peroxidase on virally infected plant root extracts,¹⁷ and to measure the activity of peroxidase in an HRP-IgE complex.¹⁸ TMB is gaining recognition as a chromogen in enzyme immunoassay systems.¹⁸⁻²⁰

We also offer TMB dihydrochloride which, in contrast to the water-insoluble free base, dissolves slowly in water or in citrate buffer.²¹ TMB dihydrochloride appears to be suitable for the quantitative determination of hemoglobin in solutions, and for the identification and localization of myeloperoxidase in cells.²¹

TMB and TMB dihydrochloride have earned acceptance as reliable and safe substitutes for benzidine and its carcinogenic derivatives.

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