

This Week's Citation Classic

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Adams C W M. A p-dimethylaminobenzaldehyde-nitrite method for the histochemical demonstration of tryptophan and related compounds. *J. Clin Pathol.* **10**:56-62, 1957.
[Bernhard Baron Inst. Pathol., London Hosp., London University, England]

This paper described a new method for the histochemical detection of tryptophan. Staining depends on a reaction between tryptophan and p-dimethylaminobenzaldehyde (DMAB) and a subsequent stage of nitrosation with nitrous acid. Tryptophan-containing proteins in tissue-sections are stained in an intense blue colour. [The SC[®] indicates that this paper has been cited over 250 times since 1961.]

C.W.M. Adams
Department of Pathology
Guy's Hospital Medical School
London University
London SE1 9RT
England

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"This paper was written at the beginning of a career in pathology when I was a Freedom Research Fellow at Bernhard Baron Institute at London Hospital. It was stimulated by the need for better histochemical methods for identifying tissue proteins. I am happy to remember that this enthusiasm was shared with John Sloper and Ken Swettenham at the London Hospital and with George Glenner and Ralph Lillie at the NIH.¹ Other colleagues took a friendly but sometimes less committed attitude to this work, particularly when the fumes of strong acids penetrated their offices! In the mid-1950s exhaust ventilation was not as effective as now and largely depended upon a strong draft and an open window!

"The method itself is remarkably simple and takes only two minutes to complete. The reagent is DMAB dissolved in concentrated hydrochloric acid. It was adapted from spot tests used in organic chemistry, coupled with the substitution of nitric acid by nitrous acid in the second stage. It is this last partly serendipitous

addition that results in the intense blue colour and makes the method of practical use. The reaction is between tryptophan and the aldehyde dissolved in a strong mineral acid. Similar but weaker results can be obtained with glyceraldehyde and even formaldehyde, hence the need to avoid prolonged fixation with formalin or other aldehydes. Unfortunately, it is still not known how nitrosation or diazotization enhances the pigment properties of the tryptophan-aldehyde adduct. However, since the tryptophan molecule is an integral part of the final pigment complex, the specificity of the method is virtually absolute.

"In pathology, this DMAB-nitrite method proved to be particularly useful for demonstrating amyloid, fibrin, immuno- and other globulins, intestinal and salivary zymogen granules, and the structural proteins of the myelin sheath in the PNS. By contrast, connective tissues contain relatively little tryptophan and are unstained. At the time, the method added a useful amino acid stain to back up those for tyrosine, cystine, and arginine.² The relative staining intensity for these various amino acids provided a rough 'fingerprint' to distinguish different proteins in tissue-sections. However, this application has been largely superseded by the well-known immunohistochemical methods using a specific antibody to a particular protein, which is then detected by a fluorescein- or peroxidase-labelled antiimmunoglobulin antibody.²

"The possible reason for the interest that has been shown in the DMAB method is that it is remarkably quick and easy to perform. Moreover, the method is quite reliable and the reaction rapidly reaches completion. Nevertheless, I must admit to be more than a little surprised at this interest. However, it could also be that the method has come to be used as a sprayreagent in chromatography: it would certainly be appropriate for this purpose."

1. **Glenner G G.** The histochemical demonstration of indole derivatives by the rosindole reaction of E. Fischer. *J. Histochem Cytochem* **5**:297-304, 1957.
2. **Pearse A G E.** *Histochemistry: theoretical and applied* London:Churchill.1968.vol 1. p. 106-247.