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A new stabilizing agent for the tetramethyl  
benzidine (TMB) reaction product in the  
histochemical detection of horseradish peroxidase  
(HRP)

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In this paper, an alternative procedure for the histochemical detection of HRP using ammonium heptamolybdate (AHM) as a stabilizing agent and tetramethyl benzidine (TMB) as a chromogen is reported. This procedure avoids the two main problems that occur in previous methods using sodium nitroferrocyanide (SNF) as the stabilizer, namely, the appearance of needle-shaped crystals at non-specific anatomical sites, and intensive tissue shrinkage. A comparative study of both, the TMB-AHM and TMB-SNF methods, was performed in the analysis of cerebral cortex afferent connections of the lizard *Podarcis hispanica*. This study demonstrates that the two methods are of similar sensitivity. The TMB-AHM reaction can be carried out at physiological pH (from 6 to 8), thus, avoiding tissue contraction. The reaction product is of an intense blue-green colour and, as with the TMB-SNF method, shows granulation. The appearance of non-specific precipitates is completely avoided when the incubation medium is maintained at a pH in excess of 5.

#### Introduction

One of the most extensively used techniques in the tracing of neural connections is the injection of horseradish peroxidase (HRP). Since its discovery (Kristensson and Olsson, 1971; La Vail and La Vail, 1974) a large number of neurohistochemical techniques, using different chromogens and/or experimental procedures, have been developed in order to increase the sensitivity: diaminobenzidine (DAB) (La Vail and La Vail, 1974; Streit and Reubi, 1977; Adams, 1977); DAB-glucose oxidase (DAB-GOD) (Itoh et al., 1979), O-DIA (De Olmos, 1977); benzidine dihydrochloride

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Els 3 articles més citats, publicats als anys 1984-85 i que complien els requisits del premi Ramon Turró, van ser:

<p><b>Olucha F, Martinez-Garcia F, Lopez-Garcia C (1985).</b></p> <p>A new stabilizing agent for the tetramethyl benzidine (TMB) reaction-product in the histochemical detection of horseradish-peroxidase (HRP)</p> <p>Journal of Neuroscience Methods 13; 131-138</p> <p>Facultat de Ciències Biològiques, Universitat de València</p>	<p><b>271 cites</b></p>
<p><b>Artigas F, Sarrias MJ, Martinez E, Gelpi E (1985)</b></p> <p>Serotonin in body fluids. Characterization of human plasmatic and cerebrospinal fluid pools by means of a new HPLC method</p> <p>Life Sciences 37; 441-447</p> <p>Institut de Química Bioorgànica, CSIC</p>	<p><b>89 cites</b></p>
<p><b>Armario A, Restrepo C, Castellanos JM, Balasch J (1985)</b></p> <p>Dissociation between adrenocorticotropin and corticosterone responses to restraint after previous chronic exposure to stress</p> <p>Life Sciences (1985) 36; 2085-2092</p> <p>Facultat de Ciències , Universitat Autònoma de Barcelona</p>	<p><b>86 cites</b></p>

L'entrega del 1r Premi Ramon Turró al Dr. Carlos López-García es va fer durant el VIII Simposi de Neurobiologia Experimental de la Societat Catalana de Biologia (19-20 octubre 2010).



El Dr. Carlos López-García, de la Universitat de València, rep el 1r Premi Ramon Turró de mans del Sr. Sergio Dantí, net de Ramon Turró.



El Dr. Carlos López-García, de la Universitat de València, imparteix la conferència d'acceptació del 1r Premi Ramon Turró.



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