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BRIEF COMMUNICATION

A New, Simple Myelin Stain

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KAATZ, K. W., T. J. BAZZETT AND R. L. ALBIN. *A new, simple myelin stain.* BRAIN RES BULL **29**(5) 697–698, 1992.— A new and convenient myelin stain is described. Paraformaldehyde fixed tissue is serially immersed in a nitroblue tetrazolium solution and then in a diaminobenzidine solution. The result is distinct blue staining of myelinated fiber tracts. This technique has advantages over presently used myelin stains.

Myelin Stain Central nervous system

MYELIN stains are commonly employed in the study of the central nervous system. In the course of recent histochemical studies, we inadvertantly discovered a new technique of staining myelinated fiber tracts that is simpler to use than prior techniques.

METHOD

Male Sprague–Dawley rats were deeply anesthetized with ketamine/xylazine and transcardially perfused with 100 ml 0.1 *M* phosphate buffer (PB, pH 7.4) followed by 500 ml 4% paraformaldehyde-PB. Brains were extracted from the calvarium, immersed overnight in fixative at 4°C, and cryoprotected in 20% sucrose-PB. Forty micron frozen sections were cut on a sliding microtome and collected in PB.

Solution A

Solution A consists of 12 mg monosodium malate and 10 mg nitroblue tetrazolium per 10 ml 0.1 *M* Tris-HCl/0.9% saline (TBS, pH 8.0) containing 0.8% Triton X-100 (v/v). Warm TBS-Triton to 37°C prior to adding other reagents. This should be made up fresh on day of staining.

Solution B

Solution B consists of 50 mg diaminobenzidine and 4 g sucrose per 100 ml 0.1 *M* phosphate buffer (PB, pH 7.4). Make up fresh on day of staining and warm to 37°C.

Free-floating sections are washed 2 × 1 min in 0.1 *M* TBS and immersed in solution A for 30 min in a 37°C waterbath while undergoing agitation. Rinse sections with 2 × 1 min washes in TBS and immerse in solution B. Sections are incubated for 1 h at 37°C in a waterbath while undergoing gentle agitation. The reaction is terminated with 2 × 1 min washes in PB. Sections are mounted onto gelatin coated slides and air dried overnight. Drying overnight seems to intensify staining. Coverslips are affixed with DPX.

RESULTS

As shown in the figure, the staining procedure visualizes myelinated fiber tracts. The resulting stain is blue in color and there is very little staining of grey matter.

DISCUSSION

This stain has several advantages over prior techniques. Most commonly used myelin stains, such as Weigert methods, Luxol fast blue stains, or phosphotungistic acid techniques, require several steps, a variety of reagents, and are time consuming (1,2). Alternative techniques based on osmication are complicated by the need to use highly toxic OsO_4 and since staining takes place with vapor, the staining may be difficult to control (1). This stain uses inexpensive reagents and is easy to perform.

This technique may have additional advantages. We have combined it with NADPH-diaphorase histochemistry and cytochrome oxidase histochemistry to stain simultaneously my-

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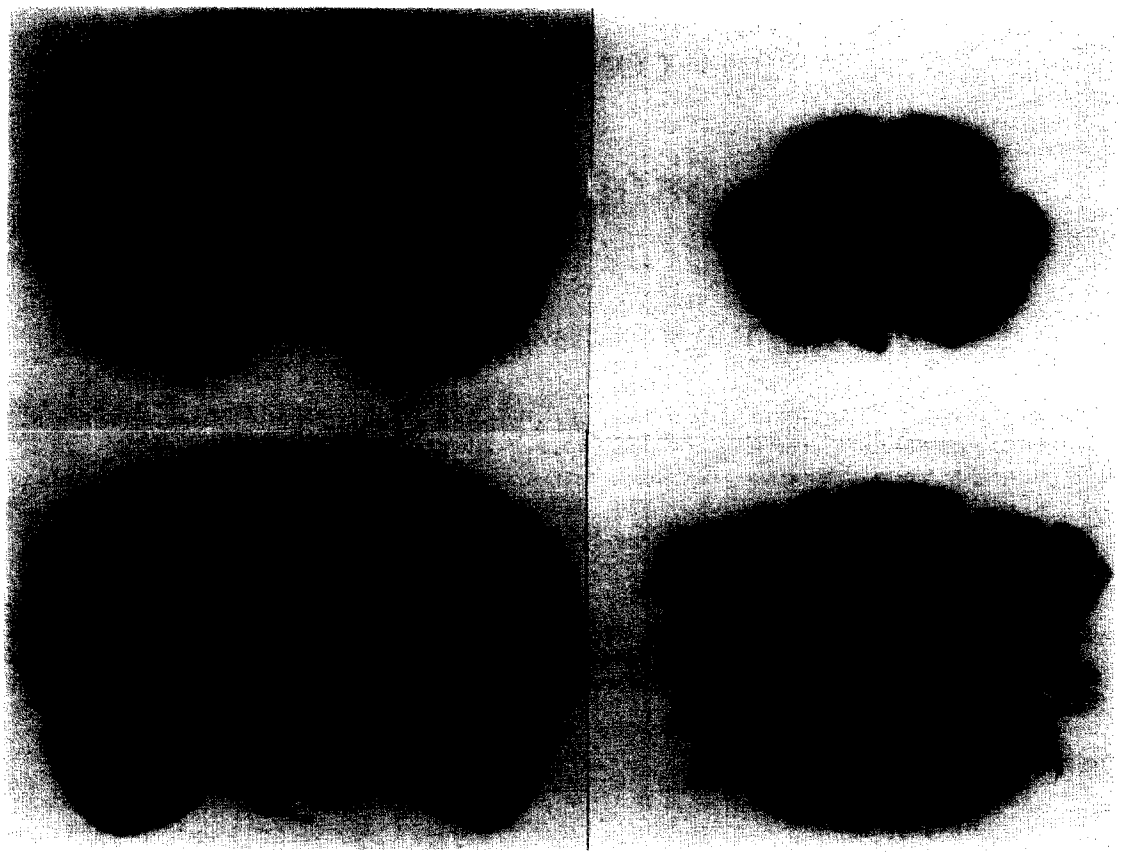

FIG. 1. Staining at several different levels of rat brain. Myelinated fiber tracts are well stained with little staining of grey matter.

elinated fiber tracts and neurons containing these enzymes. This stain may be compatible with other stains or histochemical techniques.

ACKNOWLEDGEMENTS

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REFERENCES

1. Clark, G.; Coulson, R. E.; Nordquist, R. E. Animal histology. In: Clark, G., ed. Staining procedures. Baltimore: Williams & Wilkins; 1973:89–102.

2. Luna, L. G. Manual of histologic staining methods of the Armed Forces Institute of Pathology. New York: McGraw-Hill; 1968.