0038-9153/90/6503-0151$02.00/0
STAIN TECHNOLOGY

Vol. 65, No. 3
Printed in U.S.A.

VISUAL ENHANCEMENT OF MYELINATED TISSUES IN THE CENTRAL NERVOUS SYSTEM OF THE RAT USING SUDAN BLACK B.

KAREN R. OLSON AND RICHARD K. TRAUB, *Neurotoxicology Branch, Pathophysiology Division, U.S. Army Medical Research Institute of Chemical Defense, APG, Maryland 21010-5425*

We investigated the quantitative distribution of radiolabelled compounds in the central nervous system of the rat using autoradiographic techniques (Traub 1985). To complement our autoradiographic images, we searched for a suitable fiber stain delineating myelinated structures using fresh frozen tissue. Surprisingly, the majority of staining procedures were exclusive to fixed tissues (Lillie 1977, Lillie and Fullmer 1976). These techniques applied to fresh frozen brain tissue produced poor differentiation of myelinated and nonmyelinated areas and were generally unsatisfactory. Therefore, we performed a series of studies using lipid soluble dyes such as oil red O, Luxol fast blue, hematoxylin and Nile blue in an effort to enhance differentiation of myelin. We found Sudan black B to be superior for our purpose. The procedure we developed to define myelinated structures is described below.

Materials and methods. Fresh frozen rat brain sections cut at 20 μm were thaw mounted on acid cleaned glass slides and air dried overnight. A saturated solution of Sudan stain was prepared by mixing 2 g of Sudan black B (C.I. 26150) with 100 ml of tetrahydrofuran (THF). Plastic staining dishes were used because this solution severely stains glassware with prolonged use. (Note: This procedure yields the best results when slides are hand held and stained one at a time.)

1. Immerse tissue portion of slide in Sudan stain for approximately 8 sec without movement. Optimization of immersion time may be required depending on tissue type and section thickness. It is suggested that practice slides be tested to determine times which yield sufficiently stained structures.
2. Move slide through 50% ethyl alcohol rapidly (approximately 1 sec).
3. Plunge slide into 90 C (±5 C) tap water for 10 sec moving the slide back and forth through the water. Change water after every 50 slides to prevent accumulation of undissolved stain.
4. Repeat step 3 with room temperature water.
5. Before coverslipping with Kaiser glycerol gelatin (Lillie and Fullmer 1976, p. 119), wipe the slide clean of excess stain around the section with a damp, lint-free cloth. Ring the coverslip with clear nail polish.

Results and discussion. Staining results are shown in Fig. 1. THF was chosen as the solvent because the Sudan dye and tissue lipids are soluble in it and because it has a low boiling point (65 C). Since THF is a solvent, this procedure was performed in a draft chamber. The 50% ethyl alcohol removes the excess stain from the surface of the section and is the first step in differentiation.

FIG. 1. A 20 μm thick fresh frozen rat brain section, thaw mounted on acid cleaned slide, air dried and subsequently stained with Sudan black B. Darkly stained areas are myelinated structures. Sudan black B also stains basement membranes and probably contributes to the staining of the choroid plexus as seen above. × 8.

The alcohol does not penetrate lipid structures as rapidly as THF, thus preserving differential lipid staining.

The second part of the process, hot tap water, flushes the THF, causing the dye to remain in myelin-rich areas. This step is essential for complete differentiation. Room temperature tap water brings the temperature of the tissue down quickly and prevents dye diffusion into nonlipid areas. It also maintains integrity of the tissue by preventing distortion due to heat. It is possible to differentiate further by a quick dip in 100% ethyl alcohol, followed by a rinse with room temperature tap water. The dye is removed quickly in this manner. Perform this differentiation step with care. Once the dye is removed by alcohol, the tissue will be impervious to stain because the alcohol also removes lipids.

Dye may be observed to accumulate on the sections as small round clusters. This may be due to a supersaturated Sudan solution. Although some undissolved dye is acceptable, this may be a problem and can be corrected by adding a small amount of THF. It is important to mention that THF evaporates during normal use so the quantity of undissolved dye constantly increases. Reconstitute the solution with fresh THF as needed. Air currents produced by the swift movement from the stain to alcohol to hot water contribute to

the formation of dye crystals. Holding the slide with the section facing away from the air current as it is transferred will greatly reduce dye precipitation. It is also important to use hot tap water within 5° C of the stated temperature. If the water is too cool, THF will not vaporize from the tissue and poor differentiation will result. If the water is boiling, the tissue will be destroyed. Isopropyl alcohol can be substituted for ethanol, but methyl alcohol should not be used because it blisters the tissue.

This staining technique is satisfactory at low magnifications for defining fiber bundles and myelinated areas. However, at higher magnifications, the stain is inadequate. The resolution of these areas is poor, and tiny crystal accumulations restrict the crisp image of the tissue that is seen at the lower powers.

Counterstaining with (0.05%) neutral red for 30 sec gives good definition of cell nuclei in contrast to the fibered areas, and this dual staining becomes very useful for identifying areas of interest. It is important to stress that after counterstaining with neutral red, a wash with distilled water followed by mounting as discussed in step 5, gives the best results.

Slides stained with only Sudan black B keep well for more than five years and do not fade.

REFERENCES

Lillie, R. D. (Ed.) 1977. *H. J. Conn's Biological Stains*, 9th ed. Williams & Wilkins, Baltimore, p. 577.

Lillie, R. D. and Fullmer, H. M. 1976. *Histopathologic Technic and Practical Histochemistry*, 4th ed. McGraw-Hill, New York, p. 571.

Traub, K. 1985. *In vivo* distribution of ^{14}C radiolabeled soman ((3,3-dimethyl-2-butoxy)-methyl-phosphorylfluoride) in the central nervous system of the rat. Neurosci. Lett. *60*: 219–225.

UNCLASSPIED
SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) P89-057	5. MONITORING ORGANIZATION REPORT NUMBER(S) P89-057
6a. NAME OF PERFORMING ORGANIZATION US Army Medical Research Institute of Chemical Defense 6b. OFFICE SYMBOL (If applicable) SGRD-UV-YN	7a. NAME OF MONITORING ORGANIZATION US Army Medical Research Institute of Chemical Defense, SGRD-UV-RC
6c. ADDRESS (City, State, and ZIP Code) Aberdeen Proving Ground, MD 21010-5425	7b. ADDRESS (City, State, and ZIP Code) Aberdeen Proving Ground, MD 21010-5425
8a. NAME OF FUNDING/SPONSORING ORGANIZATION 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code)	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 61102A; PROJECT NO. 3M161102B; TASK NO. 511-AA; WORK UNIT ACCESSION NO.

11. TITLE (Include Security Classification)
Visual Enhancement of Myelinated Tissues in the Centeral Nervous System of the Rat Using Sudan Black B.

12. PERSONAL AUTHOR(S)
Karen R. Olson and Richard K. Traub

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Open Literature Publ.	FROM _____ TO _____		3

16. SUPPLEMENTARY NOTATION
Stain Technology 1990

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	
			fiberstain Sudan Black B Myelinated tissue

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

This report ~~We~~ investigated the quantitative distribution of radiolabelled compounds in the central nervous system of the rat using autoradiographic techniques ~~(Traub 1985)~~. To complement our autoradiographic images, we searched for a suitable fiber stain delineating myelinated structures using fresh frozen tissue. Surprisingly, the majority of staining procedures were exclusive to fixed tissues ~~(Lillie 1977, Lillie and Fullmer 1976)~~. These techniques applied to fresh frozen brain tissue produced poor differentiation of myelinated and nonmyelinated areas and were generally unsatisfactory. Therefore, we performed a series of studies using lipid soluble dyes such as oil red O, Luxol fast blue, hematoxylin and Nile blue in an effort to enhance differentiation of myelin. We found Sudan black B to be superior for our purpose. The procedure we developed to define myelinated structures is described below. (772)

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS	
22a. NAME OF RESPONSIBLE INDIVIDUAL Wade, John V., LTC	22b TELEPHONE (Include Area Code) 301-671-2553
	22c. OFFICE SYMBOL SGRD-UV-YN

DD Form 1473, JUN 86 *Previous editions are obsolete.* SECURITY CLASSIFICATION OF THIS PAGE

UNCLASSIFIED