

Preservation of Sudan Red Staining of Brain after Plastination Process

Punnarat Vibulchan* Jantima Intarapanya Ornsiri Cheunsuang

Abstract

Specimen preservation is an essential factor in gross anatomical study of the brain and spinal cord. Forced impregnation of the specimen using polymer, or plastination, is to date one of the best specimen preparation procedures. This study aimed to study the possibility of staining whole brain specimen using Sudan Red III dye prior to plastination. Dog brains were divided into 3 groups each stained with the dye for 1, 2 and 3 months, respectively. The stained specimens were then dissected to reveal the staining of deep structures before undergoing the plastination procedure. After the experiment we found that Sudan Red clearly stained the fiber tracts making it differentiable from the gray matter. However, after the strong acetone dehydration steps during the plastination procedure, most of the dye were thinned down in some specimen whereas in a few it was enough to differentiate the gray from white matter although not as clearly as prior to the procedure. Hence, Sudan Red III may not be a reliable dye of choice when followed by plastination.

Keywords: gray matter, plastination, Sudan Red III, white matter

Department of Anatomy, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand.

***Corresponding author: E-mail:** punnarat.v@chula.ac.th

บทคัดย่อ

การคงสภาพของสีย้อมชูดานเรด ในสมองภายหลังผ่านกระบวนการกำซาบ

ปณณรัตน์ วิบูลย์จันทร์* จันทิมา อินทรปัญญา อรสิริ ชื่นทรวง

การเก็บรักษาตัวอย่างมีความจำเป็นเพื่อการศึกษาทางกายวิภาคศาสตร์ของสมองและไขสันหลัง การนำตัวอย่างมาทำการกำซาบด้วยสารจำพวกโพลีเมอร์หรือการกำซาบด้วยพลาสติก เป็นวิธีการเก็บรักษาตัวอย่างให้มีสภาพเดิมที่ดีที่สุดในปัจจุบัน การศึกษาในครั้งนี้มีวัตถุประสงค์เพื่อศึกษาความเป็นไปได้ของการย้อมสีสมองที่ยังไม่ได้ผ่านการชำแหละด้วยสีย้อมชูดานเรด ก่อนทำการเก็บรักษาตัวอย่างสมองด้วยวิธีกำซาบด้วยสารพลาสติกที่อุณหภูมิห้อง การศึกษาทำโดยแบ่งสมองสุนัขเป็น 3 กลุ่ม ย้อมสีด้วยชูดานเรดทรี เป็นเวลา 1 เดือน 2 เดือน และ 3 เดือนตามลำดับ ก่อนทำการชำแหละสมองแล้วนำไปผ่านกระบวนการกำซาบด้วยพลาสติก จากการทดลองพบว่าการย้อมสีด้วยชูดานเรดทรี ทำให้แยกความแตกต่างของเนื้อสีขาวออกจากเนื้อสีเทาได้ชัดเจน แต่ไม่เหมาะกับการกำซาบด้วยพลาสติก เนื่องจากสีชูดานเรดทรี จะถูกละลายในอะซิโตนในขั้นตอนดึ่งน้ำออกจากเนื้อเยื่อ ทำให้การย้อมติดสีจางลง

คำสำคัญ: เนื้อสีเทา การกำซาบด้วยสารพลาสติก ชูดานเรดทรี เนื้อสีขาว

ภาควิชากายวิภาคศาสตร์ คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ปทุมวัน กรุงเทพฯ 10330

*ผู้รับผิดชอบบทความ E-mail: punnarat.v@chula.ac.th

Introduction

One of the major obstacles in organising gross anatomy class is specimen preparation. At present the use of laboratory animals for dissecting classes is restricted due to lack of supply and animal welfare issue. Therefore, the need for alternatives has never been greater. Plastination technique is the procedure in which the formalin-fixed specimen is dehydrated and the water molecule is replaced by polymer by forced impregnation. The plastinated specimen retains normal structure but will not decay. This method has been widely used in anatomy teaching due to the fact that the specimen is preserved permanently, thus can be used multiple times (von Hagens et al., 1987), and is arguably the optimum anatomical preservation technique to date. Also, the specimen can be dissected to reveal the internal structures prior to the plastination procedure so that they can be studied afterwards.

The two main anatomical components found in the central nervous system are nuclei and the axonal tracts. The nucleus is the area where neuronal cell bodies that have the same function are grouped together, and send their axons in a bundle or "tract" to their targets. The nuclei in the CNS, where cell bodies are abundant, appear darker, hence are referred to as the gray matter whereas the fiber tracts, paler in appearance due to the myelin around the axon, are called the white matter. However, this difference is not clearly apparent to an untrained eye. In gross neuroanatomical laboratory classes, attempts have been made to ease this obstacle by way of staining. Unfortunately several dyes used for neuronal staining cannot withstand the harsh

procedures, such as prolonged dehydration in acetone, which are necessary for plastination. To date there are a few reports of staining brain section prior to plastination (Ulfig and Wuttke, 1990; Suriyaprapadilok and Withyachumnarnkul, 1997; Baeres and Moller, 2001), but not whole brain staining. The commercial dyes, Sudan Red III and Sudan Red IV, have been reported to have superior results in staining fixed whole brain specimen compared to other dyes (Bayliss and Adams, 1972; Jordanov and Zaprianova, 1972; Olson and Traub, 1990) due to the specific attachment of the dye to lipid in the myelin. However, the use of these dyes in combination with plastination has never been reported. This study aimed to ascertain the possibility of staining whole brain specimen using Sudan Red III dye prior to plastination procedure at room temperature.

Materials and Methods

Specimen preparation: Nine dog brains collected from the laboratory of Department of Anatomy, Faculty of Veterinary Science, Chulalongkorn University were fixed in 10% buffer formalin for 1 month at room temperature.

Staining procedure with Sudan Red III: Sudan Red III was prepared by dissolving 100 g of the dye in 1 l of 70% ethanol : 100% acetone (1:1). The fixed specimens were divided into 3 groups (group 1-3) then immersed in the dye solution at 28°C for 1, 2 and 3 months, respectively. The dye penetration was checked regularly by cutting at 5 mm from the end of the spinal cord that was attached to the brain to

determine whether the cord was stained throughout. After the staining the brain specimens were then washed and dissected to reveal the inner structures (for neuroanatomical study).

Forced impregnation with polymer

The protocol used for plastination of whole brain in this study was as first described by von Hagen (1987) and modified by Raoof (2001). Dehydration was done prior to plastination procedure using 70%, 90% and absolute ethanol (2x24 hours) respectively, followed by absolute acetone 2x24 hours. The dehydrated brains were immediately submerged in a mixture of S10: Acetone (2:1) for 9 days followed by immersion in a mixture of S10: S3 (100:1) for 30 days. After that the impregnated brains were placed on a mesh in a drying chamber saturated with S6 for 3 days.

Results and Discussions

Sudan Red III staining

Sudan Red III dye attached firmly to myelinated nerve fibers of the specimen rendering distinguishable white matter which were red in colour whereas the gray matter appeared much paler. The dye penetrated throughout the whole brain, therefore even deep structure such as corpus callosum, internal and external capsules were clearly stained (Fig 1). No apparent difference in staining pattern or intensity between the three groups were observed. All areas were clearly and equally stained and the white and gray matters were easily separated (Fig 1 & 2).

Preservation of Sudan Red III after plastination

During the long acetone dehydration step before plastination procedure, Sudan Red appeared to fade considerably. Theoretically, Sudan dyes are lipid soluble and insoluble in polar solvents such as water or alcohol. Upon close inspection after plastination the Sudan Red staining within the specimens either faded or changed to yellowish brown. There was inconsistency in intensity of the staining amongst the specimens. Despite fading, the staining was enough to determine the white and gray matter (Fig 3), thus the stained specimens were nonetheless useful for neuroanatomical study.

In this present study we investigated a commercial dye, Sudan Red III in whole brain staining followed by forced impregnation with polymer (plastination). Due to strong chemicals used in the plastination procedures, many dyes that have been tested were not able to sustain within the specimen. Suriyaprapadilok and Withyachumnarnkul (1997) tested several dyes in sectioned brain specimens and reported that some dyes were preserved but dispersed into adjacent structures after plastination.

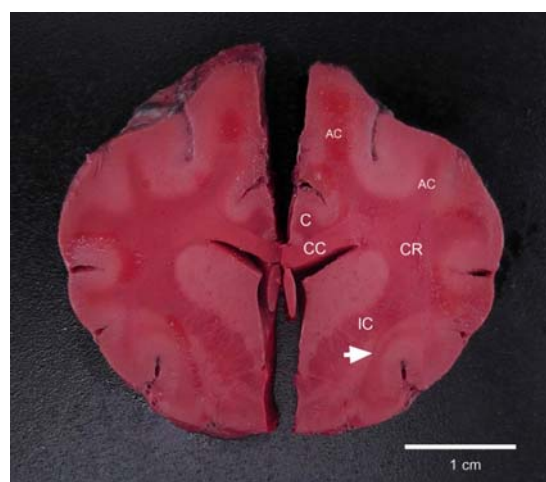


Figure 1 Crossed section of the brain specimen at the level of cruciate sulcus of the cerebrum showing Sudan Red III staining in the white matter of fiber tracts; corpus callosum (CC), cingulum (C), arcuate fibers (AC), internal capsule (IC), external capsule (arrow)



Figure 2 Sudan Red III staining was apparent in the arbor vitae of the cerebellum, which is the white matter.

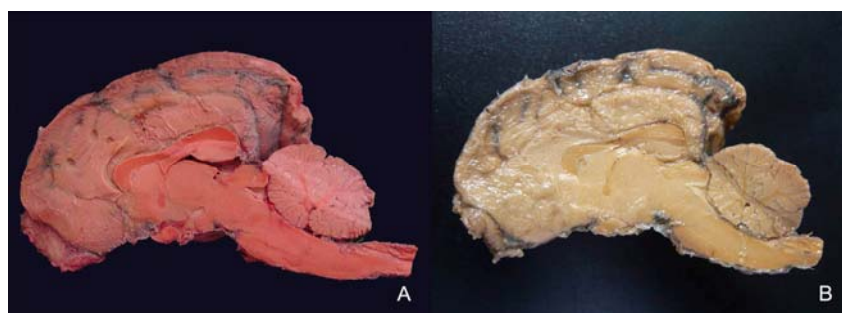


Figure 3 Hemisected dog brain (A) after staining with Sudan Red III one month, and (B) the same specimen after plastination procedure. The colour turned from bright red to yellowish brown

The principle of such staining depends upon the efficacy of Sudan stains for lipids which were major components of the myelin. Therefore, the white matter in the brain is clearly stained. It attached firmly and therefore has been commonly used in neuronal tract studies. Due to these properties of Sudan Red, it was selected in this study. The staining of the white matter was prominent throughout the whole brain as expected. However, the dye was thinned down during acetone dehydration leaving little staining in some specimens. Strong dehydration is one of the crucial steps in plastination procedure. Specimens should be free of water in order that the polymer molecules can infiltrate thoroughly resulting in effective preservation of specimens. Hence, it is not recommended to shorten or omit this step which might help sustaining the staining. Further investigation into additional steps to fix the dye in the specimen prior to dehydration may be useful to enhance the consistency of staining.

Acknowledgements

This research was financially supported by the Chulalongkorn University-Veterinary Science Research Fund (RG 1/2554).

References

- Baeres, F.M. and Moller, M. 2001. Plastination of dissected brain specimens and Mulligan-stained sections of the human brain. *Eur J Morphol.* 39: 307-311.
- Bayliss, O.B. and Adams, C.W. 1972. Bromine-Sudan Black: A general stain for lipids including free cholesterol. *Histochem J.* 4: 505-515.
- Jordanow, J. and Zaprianova, E. 1972. A technique combining Baker's acid hematein and Hadjioloff's hydrotropic Sudan 3 stains for identification of choline-containing phospholipids within the same tissue section. *Acta Histochem.* 42: 360-366.
- Olson, K.R. and Traub, R.K. 1990. Visual enhancement of myelinated tissues in the central nervous system of the rat using Sudan black B. *Stain Technol.* 65: 151-153.
- Raoof, A. 2001. Using a Room-Temperature Plastination Technique in Assessing Prenatal Changes in the Human Spinal Cord. *J Internat Soc Platinat.* 16: 5-8.
- Suriyaprapadilok, L. and Withyachumnarnkul, B. 1997. Plastination of stained sections of the human brain: Comparison between different staining methods. *J Internat Soc Platinat.* 12: 27-32.
- Ulfig, N. and Wuttke, M. 1990. Platinatation of stained sections of the human brain. *Anat Anz.* 170: 309-312.
- von Hagens, G., Tiedemann, K. and Kriz, W. 1987. The current potential of plastination. *Anat Embryol. (Berl)* 175: 411-421.