The Study of Sectional Anatomy of Sheep and Human Brain by Various Staining Methods.

ABSTRACT

Five different staining methods were done on the sheep brain compared and the best method was employed to stain the human brain, Alstons staining method is found to be the best among the five staining method, which shows excellent differentiation of grey and white matter. Alston's method has low shrinkage percentage and the time taken for this procedure is around 8 hrs 30 min. When compared with the unstained section with the stained section show pronounced grey matter. Learning neuroanatomy with wet brain section is difficult in unstained brain specimens, with the induction of staining method in the nueroanatomical structures will be helpful in differentiating the grey and white matter of brain. With the coronal and axial sections in this study various macroscopic neuroanatomical structures can be studied easily.

Key words: Plastination, Nueroanatomy, Alstons stain, Mulligan Stain.

INTRODUCTION

Studying of neuroanatomy in non stained specimens is hard to differentiate between the grey and white matters, staining the neuro specimens are easy to understand it. Sheep brain specimen slices were stained with five different Staining methods and compared. The best method showing excellent white and grey matter was adapted to stain human brain slices. The technique designed for plastination was a simple cost effective, without involving any complex equipments and laboratory facilities (Cooper., 1990). The human brain plastinate prepared by this method is durable, convenient to handle, helpful tool in teaching neuroanatomy and radiological neuroanatomy as it is useful in the clinical correlations of modern diagnostic images of CT and MRI.

Sheet plastination is the process of preserving body tissue sections as sheets. It utilizes the basic plastination technique. The technique produces thin semi transparent to translucent slices which display anatomy with its normal anatomical relations. Normal sheet plastination of human brain shows less distinct differentiation between white and grey matter when compared to stained human brain specimen which shows excellent white and grey matter differentiation. In the

present study staining was followed by plastination procedure (Alfred Riepertinger., 1988). There is a lack of comparative research with statistically meaningful findings about the effects of using plastination in anatomy education. Positive responses from pupils were typically noticed (Chytas et al., 2019). Plastination also becomes a popular resource for displays outside of the educational and scientific fields as more and more shows incorporate plastinated specimens globally (Sora et al., 2019).

Plastinated specimen should be used more frequently, according to 100% of the students surveyed. The use of plastinated specimen in the dissection room along with wet cadaver dissection was thought to be helpful by the students when learning anatomy, especially when combined with their use in small-group tutorials (Latorre R et al., 2016).

There are usually certain gaps in a technique's development; in this case, ethical questions, religious conflicts, and even sex plastinates are quite troubling and shouldn't be disregarded (Neha & Vinoth., 2019).

MATERIALS AND METHOD

MATERIALS:

Fresh sheep whole brain - 10 in number was procured from the slaughter house. After obtaining Institutional Animal Ethical Board clearance. Fixed human whole brain specimen - 4 in number was obtained from the Anatomy Department of Saveetha medical college.

Fixative material used is Kaiserling solution(10% formalin + Potassium nitrate + Potassium acetate). Materials used for slicing were Brain knife, Dry ice, Gloves, Mouth mask. Materials used for staining were Mulligan's stain, Le Masurier's stain, Robert's stain, Modified Braak's stain - alcian blue & Alston's stain. Materials used for Plastination were Acetone, Methylene chloride & Epoxy resin. Materials used for casting chamber preparation were Glass slab, OHP sheet, Binder clips/Fold back clips, Latex tube & Thin metal wire.

METHODS

10 Fresh sheep brain, procured from slaughter house were selected. Fixation is necessary to yield the brain firm enough for slicing. Fresh sheep brain specimens were fixed by perfusion method with kaiserling solution and stored for 2 months. The fixed sheep brain specimens were frozen at 10°c with dry ice for 2 weeks. The 10 sheep brain specimens were divided into 5 groups, such that 2 specimens for each group.

In each group, one sheep brain specimen was coronally sliced with thickness of 6 -8 mm using a brain knife. The dust was removed by flushing with a stream of running water. The coronal slices were placed in plastic (acetone resistant) trays from frontal to occipital pole and numbered. Five different staining methods were carried out to stain different groups of sheep brain slices.

Mulligan's solution preparation: 40 gm crystalline phenol, 5gm cupric sulphate & 1.25 ml 0.1 N HCL mixed with 1 litre of water.

MULLIGAN'S STAINING METHOD: GROUP I: 2 sheep brain specimen slices were used for this method. The Mulligan's solution was heated to 60° -65° C and the slices were immersed into the solution for 4 minutes. The slices were removed from the Mulligan's solution and immersed in ice water for 10 seconds. Slices were taken out from ice water and kept in 0.4% tannic acid solution at room temperature for 1 minute. The slices were taken out and kept in running water for 1 minute. The washed slices were taken out and then immersed in 0.08% ferric ammonium sulfate at room temperature. Finally, the slices are kept in running tap water for 8 hours.

LE MASURIER'S STAINING METHOD: GROUP II: 2 sheep brain specimen slices were used for this method. The Mulligan's solution was heated to 60° -65° C and the slices were immersed into the solution for 2 minutes. Remove the slices from the Mulligan's solution and immerse them in ice water for 1 minute. Slices were taken out from ice water and immersed in 1% Ferric chloride solution at room temperature for 1 minute. The slices were taken out and kept in running water for 5 minutes. The washed brain slices are taken out and then immersed in 1% potassium Ferro cyanide at room temperature for 3 minutes. Finally, the slices are kept in running tap water for 8 hours.

ROBERT'S STAINING METHOD: GROUP III: 2 sheep brain specimen slices were used. Heat the Mulligan's solution to 60°-65° C and immerse the slices into the solution for 6 minutes. Then the slices were removed from Mulligan's solution and kept in running water for 5 minutes.

The washed brain slices are taken out and then immersed in 2% Potassium Ferro cyanide at room temperature for a minute. Finally, the slices are kept in running tap water for 8 hours.

MODIFIED BRAAK'S STAINING METHOD: GROUP IV: 2 sheep brain specimen slices were used for this method. Slices are placed in a plastic tray containing per formic acid. (10 ml 30% H202 and 90 ml 100% formic acid) and shaked at room temperature for I hour. The slices were taken out and kept in running water for 1 hour. The washed brain slices are taken out and immersed in a plastic tray containing alcian blue solution (0.1 gm alcian blue and 1 ml 37% HCL in 1 litre of water) and shaked at room temperature. Finally, the slices are kept in running tap water for 8 hours.

ALSTON'S STAINING METHOD: GROUP V: 2 sheep brain specimen slices were used for this method. The slices were immersed in Mulligan's solution at room temperature for 20 minutes. Remove the slices

from the Mulligan's solution and immerse them for 20 seconds in xylene at room temperature. Slices were taken out from ice water and immersed in 1% Ferric chloride Solution at room temperature for 1 minute. The slices were taken out and kept in running water for 5 minutes. The washed brain slices are taken out and then immersed in 1% potassium Ferro cyanide at room temperature for 3 minutes. Finally, the slices are kept in running tap water for 8 hours.

DEHYDRATION: The stained brain slices were dehydrated in 100% acetone at -25°C for 2 days, with 3 daily changes of fresh acetone (Henry R.W, 2001).The acetone: brain volume ratio was 10:1 to avoid shrinkage during this procedure the specimen sections are precooled. The slices were transferred to acetone at room temperature for defattening for 1 week (Henry R.W et al, 2001). Extra defattening with methylene chloride improves the transparency of the specimen.

CASTING AND IMPREGNATION: Impregnation was done with some modifications. Vacuum chamber is not used for the impregnation procedure. The dehydrated slices were removed from the final acetone bath and immersed into the epoxy resin mixture for 4 hours at room temperature. The resin: hardener ratio is 4: 1.The acetone evaporated onto the surface of the resin as large air bubbles during this procedure. The space created by the evaporated acetone was replaced by the epoxy resin material.

CASTING CHAMBER PREPARATION: The casting chamber was prepared with 2 plates of 5mm tempered glass, OHP sheets, flexible latex tubing and several binder clips. They were taken out from the resin mixture and placed into the chambers. The chambers will be filled with epoxy resin material, with resin, hardener ratio 2:1, through a funnel made up of OHP sheet. Once the casting chambers were filled, large bubbles will be removed with a thin metal wire. The chambers are placed upright and position of the slices was adjusted if needed. The chambers were kept undisturbed for 3 days at room temperature for further impregnation. No curing is done in this method.

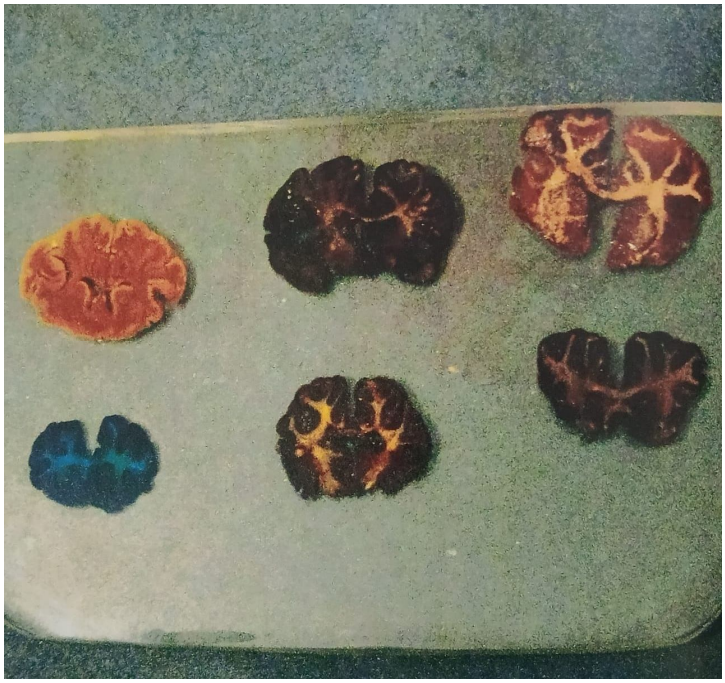

Figure 1 Plastinated sheep brain with five different stains.

The casting chambers were dismantled and the glass slabs are separated from the OHP sheet and plastinate. Then the OHP sheet was removed from the plastinate slowly and gently from one corner to the other.

Five different staining methods were carried out to stain different groups of sheep brain slices (Figure: 1). Out of these, the best method was selected to stain the human brain.

RESULT AND DISCUSSION

Table 1 Comparison of various staining method on their grey & white matter differentiation, working time & shrinkage.

METHOD	GREY & WHITE MATTER DIFFERENTIATION	WORKING TIME (HRS)	SHRINKAGE %
GROUP 1 Mulligans	------	8hrs 10min	1.5
GROUP 2 Le Masuries	+	8hrs 20min	1
GROUP 3 Robets	++	8hrs 10 min	1
GROUP 4 Modified Braaks	+++	58 hrs	2
GROUP 5 Alstons	++++	8 hrs 40 min	1

MULLIGAN'S STAINING METHOD:

Shrinkage is observed before and after staining. The shrinkage percentage after staining was 1.5%. The grey and white matter differentiation was not clear. The time taken for the entire staining period was 8 hours and 10 minutes (Mulligan., 1931)

LE MASURIER'S STAINING METHOD:

In this method the shrinkage difference observed before and after staining was 1% almost like Mulligan's method. The grey and white matter differentiation is seen well than the Mulligan's method. But still it needs clarity. It may be due to the failure of phenol in the Mulligan's solution to react with the lipid in white matter of brain to form a jelly coat over the surface of the white matter to prevent its staining. The time taken for entire staining period was 8 hours and 21minutes. This method is better than the Mulligan's in grey and white matter differentiation and

cost wise. In relation to working time and shrinkage percentage it is almost similar to Mulligan's method. (Lalita suriyaprapadilok et al., 1997)

ROBERT'S STAINING METHOD

The shrinkage difference observed before and after staining was 1% like the Le Masurier's method and slightly less than the Mulligan's method. The grey and white matter differentiation is better than the Le Masurier's method. (++). Working time for this method was 8.20 minutes. This is almost similar to Le Masurier's method.

MODIFIED BRAAK'S METHOD

The shrinkage before and after staining was 2%. The grey matter differentiation was seen clearly (+++) and Better than the Robert's method. The working time for the preparation of staining the slice was 58 Hours as seen in the Table: 1 , shrinkage and working time is higher.

ALSTON'S STAINING METHOD

The shrinkage before and after staining was 1% as. the grey and white matter differentiation is seen very clearly (++++) than the other methods. The working time for the staining is 8 hours and 38 minutes (Table: 1). In this method, grey and white matter contrast is superior (++++). The shrinkage percentage was similar to that of Le Masurier's and Robert's method and less than the Mulligan's and Modified Braak's method. The time consumption for staining one slice was slightly more than the Mulligan's, Robert's and Le Masurier's Methods and very much less than the modified Braak's method.

HUMAN BRAIN PLASTINATION CARRIED OUT WITH ALSTON'S STAINING METHOD

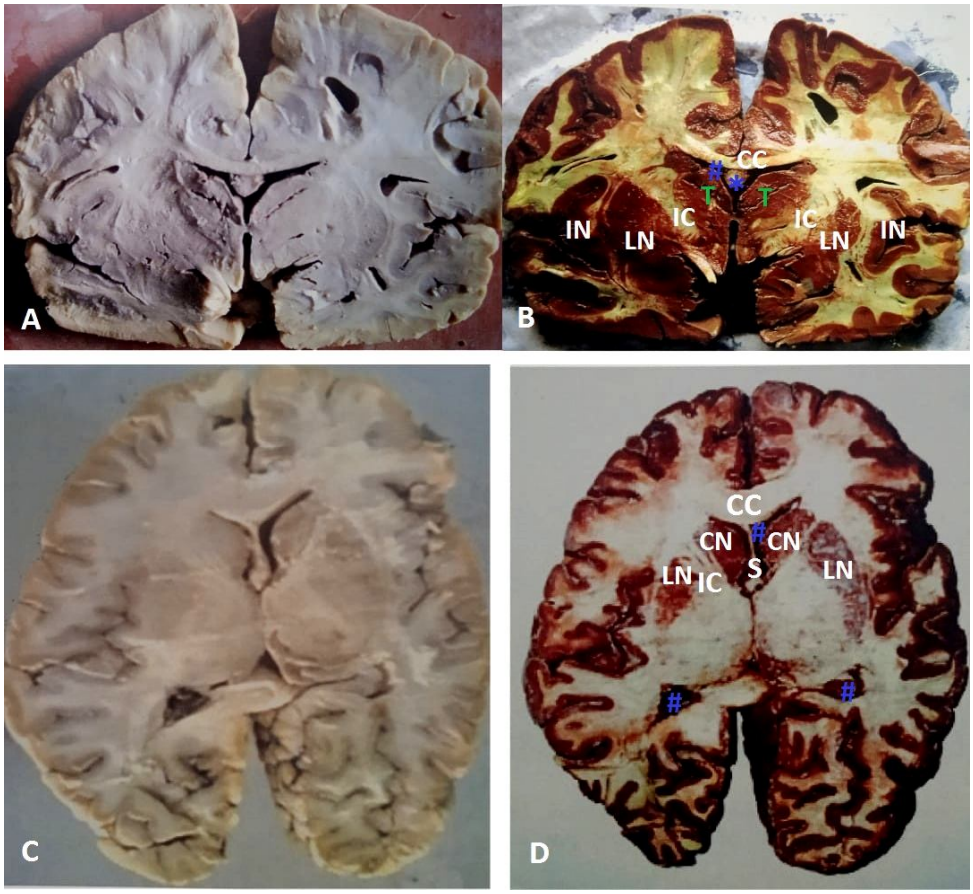

Figure 2, Human Brain (A) Unstained coronal section (B) Stained coronal section (C) Unstained axial section (D) Stained axial section. (CC- Corpus Collosum, T - Thalamus, IC - Internal Capsule, LN - Lentiform Nucleus, S - Septum Pellisudum, CN – Caudate Nucleus, # - Ventricles, * - Fornix & IN – Insular Lobe)

Since Alston's method was concluded as the superior staining method it was carried out to stain the coronal and axial slices of human brain

OBSERVATION FOR CORONAL SECTIONS OF HUMAN BRAIN SPECIMENS:

The fixed human brain was coronally sliced with 6mm- 8mm thickness and was stained by Alston's Method. The stained human brain slice shows excellent grey and white matter contrast.

The stained human brain in the coronal section were compared with the unstained coronal section of human brain (Fig: 2 A). In the stained brain, the grey and white matter contrast were brought out clearly by brick red Colour and white colour respectively. The brick red colour indicates cortical & subcortical grey matter. The white coloured areas indicate the association,

commissural and projection fibers. The brick red colour indicates the cortical and sub cortical grey. In this staining method, the corpus callosum connecting the two cerebral hemispheres was seen clearly in white colour .Even the fornix can be made out easily. The ventricles are clearly visible between the two thalami. The inner grey matter Putamen and thalamus are seen clearly in brick red color (Norbet., 1990). The outer grey Matter which is in brick red colour is bordering the white matter. The entire white matter appears like branches of a tree. The Corticospinal tract can be traced out clearly from the internal capsule upto the brain Stem. The hippocampus of both the sides of the brain are stained in brick Red colour and is clearly visible after staining. The white and grey matter of the unstained coronal slice in (Fig: 2 A) are not clear when compared with the coronal slices stained by Alston's Method as shown in (Fig: 2 B)

The stained coronal section (Fig: 2 B) shows white matter like corpus callosum, internal capsule, external capsule ,extreme capsule and fornix in white color. The grey matters, head of caudate nucleus, thalamus and lentiform nucleus are stained with brick red color. Claustrum is also seen clearly. The stained coronal section of brain in Fig: 2 B, shows corpus callosum connecting the cerebral hemispheres. The caudate nucleus and lentiform Nucleus are clearly visible. Thalamus is situated on both sides of the ventricle. The Insula of cerebrum are stained in brick red color. The plastinated coronal section of human brain is shown in Fig: 2 B. The Plastination technique adopted is the same method followed for sheep brain Plastination (Baeres et al., 2001).

OBSERVATION FOR AXIAL SECTIONS OF HUMAN BRAIN SPECIMEN

The fixed human brain specimen was cut axially with 6mm to 8mm of thickness. The grey and white matter differentiation is not well appreciated in the unstained axial section of human brain specimen as seen in Fig: 2 C. The axial section of human brain was stained by Alston's technique. Thus it shows a very good contrast between the grey and white matter of the human brain slices as shown in Fig 2 D.

The sub cortical grey matter, the caudate nucleus, lentiform nucleus and the thalamus were seen in brick red colour. The superficial grey matter in brick red colour borders the inner white matter. Internal capsule were seen in white colour. Thus, the axial sections of human brain The white matter, corpus callosum, fornix, septum pellucidum and Show good contrast between grey and white matter after staining.

The cerebellum section stained by Alston's method excellently demarcates the dentate nucleus as seen in Fig: 3 A.

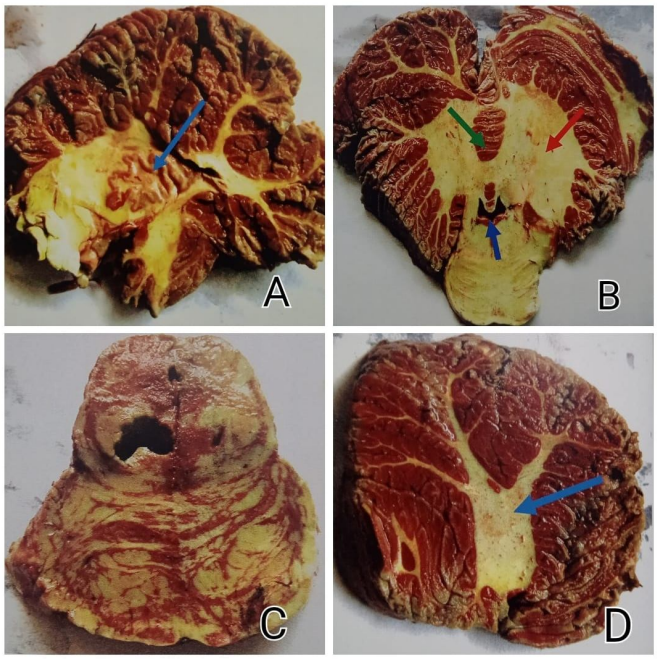

Figure 3 A. Deep nucleus of Cerebellum (blue arrow - Dentate nucleus), B. Stained Axial section of Brain stem and Cerebellum (green arrow – Vermis, red arrow – Cerebellar peduncle, blue arrow – IV ventricle), C. Stained axial section of brain stem (Various nucleus & cross over fibres) and D. Stained axial section of cerebellum arrow showing Arbor Vitae Cerebelli.

The inferior cerebellar peduncle, IV ventricle and the vermis part of the cerebellum are very well appreciated in Fig: 3 B. The stained axial section of brainstem with the cerebral aqueduct Is seen in fig: 3 C. Stained section of cerebellum (Fig: 3 D) shows the branching pattern of white matter with grey matter bordering it excellently shows the Arbor vitae Cerebelli.

CONCLUSION

In neuroanatomy, the low availability of human brain for practical classes coupled with their fragility adds importance to make a more specimen that can be easily handled and due to ever increasing costs, in this study the sheet plastination of sheep brain was simplified with low cost equipments and time saving procedures to produce stained brain sections which can be plastinated of much reduced cost.

Staining of human brain by Alston's method is superior to all the methods because the grey and white matter contrast, shrinkage and time consumption were superior in this method.

This kind of staining of brain in coronal and axial sections would help to understand neuroanatomy in a three dimensional way. Applying this technique in learning neuroanatomy would help the anatomist, neuroradiologist and neurosurgeons.

REFERENCE:

Alfred Riepertinger, Fixation of the human brain for plastination: special considerations. J Int Soc Plastination 2(1):8-12, 1988.

Angelie Jessica S, Mukilan R. A Study on Microscopic Vascular Anatomy by Luminal Cast Plastination of Renal Blood Vessels Using Epoxy Resin In Bulls Kidney.

Baeres F.M.M Plastination of Dissected Brain Specimens and Mulligan-stained sections of the human Brain. European Journal of Morphology, Volume 39, Number 5, December 2001, pp.307-311(5),

Chytas D, Piagkou M, Johnson EO, Tsakotos G, Mazarakis A, Babis GC, Nikolaou VS, Kaseta MK, Natsis K. Outcomes of the use of plastination in anatomy education: current evidence. Surg Radiol Anat. 2019 Oct;41(10):1181-1186.

Cooper, The technique and use of plastinated specimens in teaching and research: Gross anatomical sections of the head and neck.J IntSoc of Plastination 4(1):4,1990.

Henry R.W, History & principles of dehydration.J Int Soc Plastination 16:31 41, 45-50(2001).

Henry R.W,Brown MA, Henry CL, Reed RB, Dehydration: 6000 BC., 19th century, First quarter century of plastination and the New Millennium.J Int Soc plastination 16: 31 -41, 45-50(2001).

Lalita suriyaprapadilok, Boonsirm Withyachumnarnkul. Plastination of stained sections of the human brain: Comparison between different staining methods. J Int Soc Plastination Vol 12,No 1:27-32, 1997.

Latorre R, Bainbridge D, Tavernor A, López Albors O. Plastination in Anatomy Learning: An Experience at Cambridge University. J Vet Med Educ. 2016 Fall;43(3):226-34.

Mulligan,J.H .A method study of staining the brain for macroscopic study. J Anat London ., 65, 468 -472 July 1931.

Neha hassan patankar and Vinod m. Choudhari., Plastination an incipient way of learning anatomy-a review., World journal of pharmaceutical and medical research, 2019, 7(8), 164 – 168.

Norbert Ulfig. Staining of human Fetal and adult brain slices combined with subsequent plastination. J IntSoc Plastination, Vol4:33-38, 1990.

Sora, Constantin & Latorre, Rafael & Baptista, Carlos & Lopez Albors, Octavio. Plastination—A scientific method for teaching and research. Anatomia, Histologia, Embryologia. (2019). 48. 10.1111/ahe.12493.