THE STAINING OF BRAIN SLICES BY IMPREGNATION

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INTRODUCTION

Sheet plastination of brain tissue, using the standard P-35 technique with 4mm sections, results in outstanding differentiation between gray and white matter. Gray matter is rendered brown, while white matter remains close to its natural color. On occasion, however, modification of this effect maybe desirable to permit a different perspective. In this paper a method will be outlined for the quick, convenient staining of brain tissue slices for sheet plastination.

MATERIALS AND METHODS

This procedure was developed using 2 mm coronal sections of formalin-fixed human brain. It has not been used on 4 mm sections and may require some modification of stain concentration for this application.

Sectioning and Rinsing: Sections of fixed human brain, 2 mm thick, are prepared using a commercial meat slicer, modified in such a way that the blade is irrigated with a jet of water. These slices are then separated by filter paper discs and stored in 10% formalin until used. Sections to be processed are removed from storage, blotted dry and placed in a container that will serve for all subsequent steps. They are then rinsed in running tap water overnight.

First Dehydration: The next morning, the rinsed sections are blotted dry and placed in a generous aliquot (at least 10X the combined volume of the specimens) of 100% acetone which has been precooled to -20C. These sections, in acetone, are then placed in the freezer at -20C and allowed to dehydrate for approximately 24 hours.

Preparing the Stain: A stock solution of astra blue is prepared in the manner described by Ulfig, 1990. This consists of dissolving 0.1 gm astra blue[1] in 1 litre of distilled water and adding 1 ml of concentrated hydrochloric acid. This stock solution is then diluted to prepare a working solution of sufficient volume to cover the specimens in their impregnation chamber. The dilution determines the intensity of tissue coloration. Brain tissue exhibits unusual avidity for this stain in a slightly acid medium and individual specimens differ in uptake.

It is therefore recommended that several dilutions be tried. This should start with at least 1 unit of stock solution to ten or twenty of 0.1% Hcl diluent. Higher dilutions are quite feasible. Delicately stained sections are most useful.

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Impregnation Staining

Dehydrated specimens are drained of acetone and covered with the working solution of the stain. They are then placed in an appropriate chamber at room temperature and subjected to strong vacuum. This evaporates the acetone and draws the stain solution into the tissue. Bubbling caused by vaporizing acetone will usually stop within an hour or so (with 2 mm sections) and impregnation can be terminated.

Second Dehydration: At this point, overstained sections can be destained by rinsing in dilute 0.1% Hcl. Ideally, the gray matter will be green and the white matter a translucent blue. This metachromatic effect may be due to the nucleic acid content of cell bodies of the cortex and nuclei. When the color is satisfactory the sections are rinsed briefly, first in distilled water, then in room-temperature acetone. They are then covered with a 10X aliquot of acetone (which has been precooled to -20C) and placed in the freezer (at -IOC) overnight. The following day, the acetone is poured off and replaced with the same amount of fresh, precooled acetone. The specimens are returned to the freezer for another 24 hours.

Plastination: After dehydration the sections can be impregnated with polyester resin in the usual manner and mounted in sheets (Weber, 1994). Neither the second dehydration nor the plastination should affect the color.

Use of Impregnation Staining: As mentioned above, the procedure outlined here was developed for 2 mm sections of fixed human brain. There is no reason however, why this method could not be adapted to thicker tissue samples, or even to whole brains (or other organs). An entire organ could be stained and then sectioned or even stained, plastinated and sectioned.

REFERENCES

Ulfig, N., 1990. Staining of human fetal and adult brain slices combined with subsequent plastination. J Int Soc Plastination 4:33-38.

Weber, W, 1994. Sheet plastination of brain slices. J Int Soc Plastination 8/1:23.

AN ECONOMICAL APPROACH TO SHEET PLASTINATION

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In the past many plastinators have shown little interest in setting up a facility for sheet plastination because of the initial costs involved. Unless there is a great demand for sectional anatomy specimens, it is not economically feasible to buy the