Preparation of Semithin Serial Sections of Epon Embedded Material

Sections of Epon embedded material cut at a thickness of 0.5–1 μm have been increasingly used for light microscopy. Since they yield a greater definition of tissue structures than the 3–5 μm thick paraffin sections routinely used, they have proved to be of great help in clarifying many problems in classical histology.

Studying the origin and renewal of epithelial cells of the small intestine, using 1 μm thick sections, we encountered the need for serial sections for mapping and counting crypt cells (MERZEL and LEBLOND[1]).

In previous attempts at serial sectioning Epon material, ribbons of serial sections were transferred to a strip of coverslip glass fitted into the bottom of the boat on a glass knife. In this way about 30 serial 0.5 μm sections were obtained (WARSHAWSKY[2]). However, in addition to the many technical difficulties of this procedure, we needed about 100, sometimes over 200, serial sections; furthermore we had to radioautograph them which was impratical using coverslips. Therefore, a technique was devised which enabled us to obtain any number of sections and to mount them on regular microscope glass slides.

The blocks were trimmed to a rectangular shape with one of the upper angles cut off. Successive 1 μm sections were then cut with a diamond knife.

The sections were kept in the proper order either by preparing ribbons, when feasible, or by having only a few, at the most 5 sections at a time floating in the trough. After spreading them with fumes of trichloroethylene released from a glass rod near their surface, they were transferred, one by one, maintaining their order, to a large drop of distilled water on a microscope slide. To transfer the sections, a piece of human hair mounted with wax or adhesive tape on the tip of a small wooden rod was used. The hair was placed beneath the floating section in the trough and by a quick movement upwards, the section was picked up, folding itself over the hair (Figure 1), and conveyed to the slide. By touching the surface of water on the slide it was released and refloated (Figure 2). Towards the side where the label will be the drop of water was drawn into a straight line with a hypodermic needle. Using the hair, the section was then pushed against this straight line in such a way as to have its upper edge (the one that had one of its corners cut off) stuck to the glass (Figure 3). In this manner the sections were kept from moving and in a proper order while others were added. Visualization of the sections was facilitated by working over a black surface. Once 10 or more sections were aligned, the slide was gently tilted, as shown in Figure 4, to make the water run down slowly; in this way, the whole surface of the section became stuck to the glass. If a section did not have its upper edge well attached to the glass, it tended to run down with the water. However, by using the hair, while the slide was still wet, the section could be properly realigned. The last step was to wipe off the excess water below the line of sections.

To prevent the sections from coming off during the staining procedure, the slides were dried overnight in an oven at 60 °C.

The results of the present technique were shown to be highly reproducible. We were able to obtain any number of serial sections, and once mastered, it was possible to produce about 200 sections in 4 h.

The use of a diamond knife was required when more than 20 sections were needed. Common glass knives became dull after cutting 10–20 sections, depending upon the size of the block. Changing the position of the cutting edge or the knife itself usually resulted in loss of orientation of the structures.

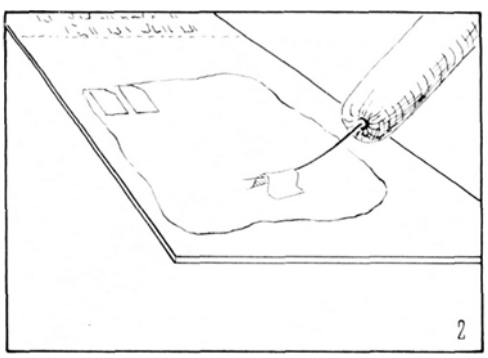

Fig. 2. By touching the surface of the water on the slide, the section is refloated. Note 2 sections already aligned on the straight line of water. The work is done over a black surface.

[1] J. MERZEL and C. P. LEBLOND, Am. J. Anat. *124*, 281 (1969).
[2] H. WARSHAWSKY, Anat. Rec. (Proc.) *160*, 446 (1968).

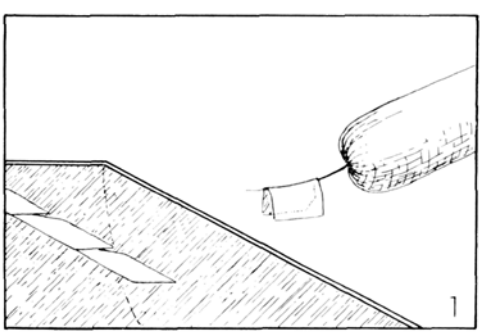

Fig. 1. A piece of human hair mounted on a wooden rod is placed underneath a floating section to pick it up. The section folds over the hair and in this way it is conveyed to the slide.

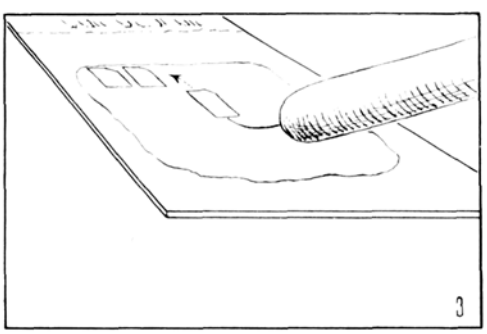

Fig. 3. With the tip of the hair, the section is pushed against the straight line of water.

Fig. 4. Once 10 sections are aligned, the slide is tilted, forcing the water to run down slowly. If any section tends to run with the water, it can be repositioned by pushing it with the hair while there is still water on the slide.

Although most of the time, only 1 row of 10 sections per slide was used, it was easy to obtain more, say up to 5 rows of 10 sections, hence 50 sections per slide.

This method for mounting sections and removing the excess water from the slides appears to be a very useful one, even in mounting routine. 0.5–1 µm Epon sections for radioautography, since deposits around the section, which results from the drying of water, were avoided. These deposits are usually responsible for an excessive background fog in the emulsion[3].

Resumen. Describese una tecnica para la obtención de cualquier número de cortes seriados de 0,5–1 µm de espessura, de material incluso en Epon, usandose una cuchilla de diamante. Ademas de su uso para seriación de cortes, la tecnica muestrase útil para la montage de cortes para radioautografias.

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A New Method for the Cytochemical Demonstration of *p*-Diphenol: O_2 Oxidoreductase (Laccase)

Phenol oxidases are widespread among plants and animals. In micro-organisms these enzymes are especially frequent in fungi capable of degrading lignin[1]. In this particular case, their most important purpose is the detoxification of heartwood toxins[2]. A direct participation in lignin decomposition could not be proved[3]. Melanin synthesis in fungi is also catalyzed by these enzymes[4]. The phenol oxidases are divided into *p*-diphenol:O_2 oxidoreductase (laccase; E.C. 1.10.3.2) and *o*-diphenol:O_2 oxidoreductase (tyrosinase; E.C. 1.10.3.1). However, these 2 enzymes cannot be clearly distinguished from each other because their substrate specificity is comparatively broad and overlaps. Laccase oxidizes *o*- and *p*-diphenols and *p*-phenylenediamine, but not monophenols, whereas tyrosinase catalyzes the oxidative degradation of mono- and *o*-diphenols, but not that of *p*-diphenols and *p*-phenylenediamine[5].

The procedures that have been developed for the cytochemical demonstration of phenol oxidases are comparatively nonspecific and do not permit a separate demonstration of laccase and tyrosinase. In this case the incubation medium consists of a buffered solution of L-dioxyphenyl-alanine (DOPA)[6]. The essential disadvantages of this technique are a nonenzymatic oxidation of the substrate and the water solubility of different intermediates[7]. In addition, L-tyrosine has been used as substrate[8].

In the course of investigations of the cytochemical detection of enzymes in fungi[9], an attempt was made to detect phenol oxidase activity with the procedure of LAIDLAW and BLACKBERG[10]. Even after prolonged incubation, no staining could be observed. Therefore, a better technique for the intracellular demonstration of phenol oxidases in fungal cells had to be developed.

SCHENK et al.[11] described a method for the stabilization and subsequent detection of the quinones that resulted from the oxidation of the oxicoumarins unbelliferone and aesculetin. The quinones are coupled with BESTHORN's[12] hydrazone(3-methyl-benzthiazolon(2)-hydrazon-hydrochloride) to an azo dye[13]. In food chemistry this procedure is used for the evaluation of phenol oxidase activity[14]. The resulting azo dye is not water-soluble and could therefore be of a certain importance for the development of a cytochemical procedure.

The experiments were performed with the fungi Aspergillus fumigatus (laccase producing according to[15]), Aureobasidium pullulans (laccase producing according to[16]) and Neurospora sitophila (tyrosinase producing according to[17]). Cultures on cover glasses in malt extract solution (1.7% malt extract, 0.3% peptone; Oxoid) were