A new differential staining method of semithin sections of polyester-embedded salivary glands

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The present communication reports a rapid and simple staining method in which Ponceau de Xylidine and Giemsa's solutions provide ample contrast of cellular elements in 1 µm thick sections of rat salivary glands prepared for electron microscopy.

Key-words: Sublingual gland; submandibular gland; granules, striated duct, staining method

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A number of staining methods have been employed for glutaraldehyde and osmium-fixed tissue embedded and sectioned in resins (10, 13, 14, 1, 6, 7, 2, 5, 3). All of them, however, use only a single basic stain so that the resultant monocromatic staining lacks histological contrasts, and fails to differentiate satisfactorily between nuclei, cytoplasmic and extracellular tissue components. Methods utilizing two or more different dyes have, to a limited extent, been employed in an attempt to overcome this problem. Inconveniences inherent in these methods include such factors as prolonged staining time, stain precipitation, removal of the embedding media and the oxidation of osmicated sections prior to staining (4, 9, 12). These disadvantages restrict their usefulness in routine processing. The ideal method for staining semithin sections of plastic-embedded tissue should be rapid, simple and reproducible. It should not require any removal of the embedding medium but provide adequate differentiation of cellular components as well as resistence to fading. During the course of ultrastructural studies of salivary glands, requiring frequent examination of semithin sections for orientation, we have developed a method which seems to meet these requirements.

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MATERIALS AND METHODS

Tissue section preparation

Fixation of rat salivary glands for electron microscopy was performed by vascular perfusion with dextran (Macrodex 6 %, Pharmacia® for 1 min), followed by 1.7 % glutaraldehyde in 0.1 M phosphate buffer, pH 7.3, for 10 min. The specimens were rinsed in 0.15 M phosphate buffer, pH 7.3 for 10 min and post-fixed in 1 % osmium tetraoxide at 3°C for 2 hr (8). The fixed specimens were dehydrated in increasing acetone concentrations and embedded in Vestopal-W® (11). Sections, 1–2 μm thick, were cut on a LKB Ultratome®, transfered to clean glass slides and allowed to dry on a hot plate for at least 5 min at 120°C prior to staining.

Dye solutions

A. Ponceau de Xylidine solution.
1 g Ponceau de Xylidine (Merck No. 1386®) is dissolved in 100 ml distilled water and 1 ml of glacial acetic acid added.

B. Giemsa's solution.
Azur-eosin-methylene solution according to Giemsa (Riedel-De Häen AG 655731 238 84®), is diluted 2:3 in phosphate buffer pH 6.8.

Staining procedure

1. A few drops of staining solution A are placed on the sections and allowed to remain for 10 min.

2. The slide is rinsed in running tap water for 2 min to remove all the excess of dye. The water is removed by blotting with filter paper and the slides allowed to dry at room temperature helped by a jet of compressed air.

3. A few drops of solution B are applied to the sections where the stain is allowed to remain for 6 min.

4. The staining agent is rinsed off the slide and the sections allowed to dry in the same manner as described in point 2.

5. Cover slips are mounted using the synthetic resin Entellan (Merck 368989®).

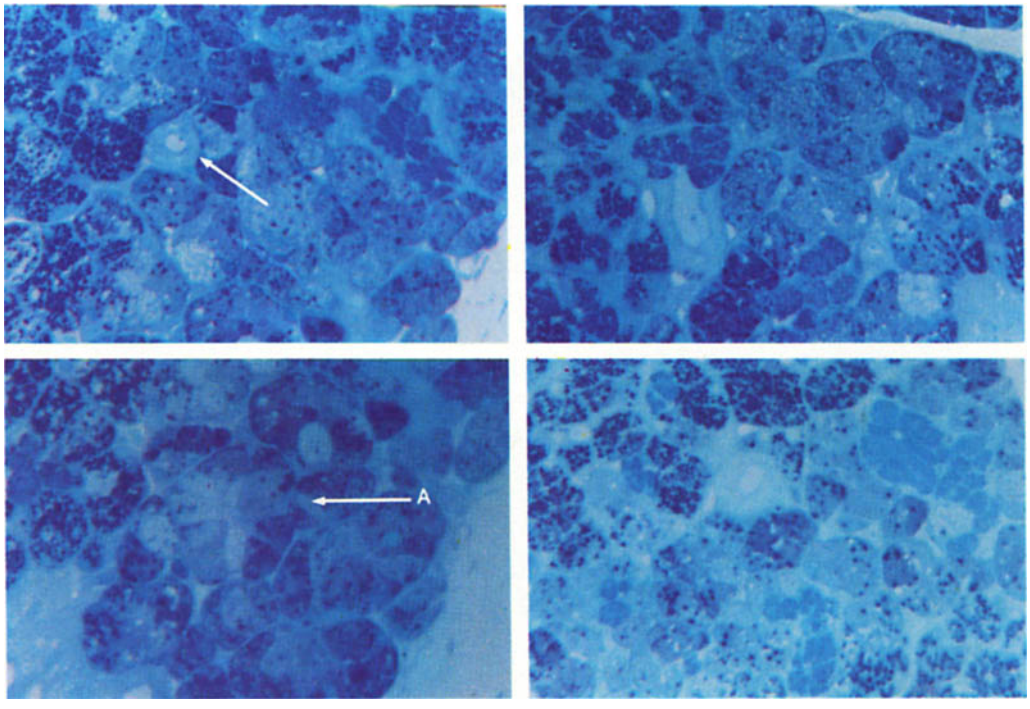
RESULTS AND DISCUSSION

The characteristic colors obtained with the present staining procedure applied on rat salivary glands are shown in Figs. 1–4. Cell nuclei, which are blue, are clearly differentiated from the cytoplasmic portion of the cells.

The sublingual glands consist of serous and mucous acini, both of which are filled with zymogen secretion granules (Fig. 1). These vary in color from unstained to deep violet blue both within single cells as well as between different cells and acini. Granules of serous cells tend to be more homogenously stained than those found in mucous cells. The serous granules are also more sharply demarcated, and not so densely packed as are the granules of mucous cells. Striated duct cells are stained homogenously light blue and are readily distinguished from the rest of the gland tissue. Submandibular gland mucous acini are more heterogenously stained than those of sublingual glands (Fig. 2). Within single acini the cells may vary considerably in color (Fig. 3).

Since orientation and trimming of tissue blocks often involves problems when using only one dye, the present method offers major advantages as a routine staining in localizing specific granules of interest to be prepared for electron microscopy.

The Ponceau de Xylidine-Giemsa method meets all requirements for an ideal staining procedure with the following two exeptions: 1. Stain precipitates form within 30 min, which necessitates the reagents to be mixed immediately before use. 2. The synthetic



Figs. 1 – 4. Polyester-embedded rat salivary glands stained with Ponceau de Xylidine and Giemsa as described. All illustrations are reproductions mede from Kodak Ektachrome 50L transparencies.
1. Sublingual gland. Striated duct.
2. Submandibular gland.
3. Submandibular gland. A, Acinus showing cells, that vary considerably in color.
4. Sublingual gland section that shows fading after two years storage.

resin Entellan was not found to be a completely satisfactory mountant since tissue components tended to fade if slides were kept for long periods (Fig. 4). Since resins undergo chemical alterations by time, unstained specimens stored for several years may require staining times which are different from those specimens which are recently polymerized. It may also be necessary to alter the staining time in order to correct for different thicknesses of the section.

The rapidity and ease with which this staining method is accomplished should make it well suited for routine use and photomicroscopy.

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