A method for processing and sectioning whole laryngeal specimens

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Certain malignant tumours confined to the supraglottic larynx can be removed with a partial laryngectomy procedure that conserves the patient's voice. A requisite for the selection of these patients is the accurate preoperative assessment of the location of the tumour and the extent of its spread. Present diagnostic measures, including direct and indirect laryngoscopy and laryngograms, are limited in their description of the tumour and largely define its mucosal extent only. Applicable information regarding the behaviour of these tumours can be gained by sectioning of whole laryngeal specimens, and, when done in a stepwise fashion, such sectioning provides a crucial third dimension to the extent of tumour infiltration. Careful preparation and study of these sections provide data on the critical relationships of the tumour with the various laryngeal anatomic structures.

One of the deterrents to such studies is the difficulty of processing, sectioning, and studying the sections from whole larynges. Adequate decalcification (accomplished in reasonable time), embedding, and cutting large specimens are frequently-encountered problems. The purpose of this paper is to describe our method of preparing such specimens.

Previous Studies

The studies of LeRoux-Robert (1936), Kernan (1950), Hajek (1891, 1932), Pressman (1956), and Tucker (1961) have contributed immensely to the understanding of the behaviour of laryngeal carcinoma, including the theories of compartmentalization and submucosal spread. These studies have been based on some form of whole laryngeal sectioning. Tucker (1961) described a technique of preparing the laryngeal specimens utilizing celloidin-embedding technique. We have modified this technique so that the total processing time is significantly shortened (from approximately 20 to 14 weeks). This conservation of total preparation time is considered important.

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Our Method

Materials

(1) Formaldehyde-fixed larynges are photographed in order to record detail of the external and internal structures, after which the structures are trimmed of attached muscle and connective tissue. Care must be taken not to cut into the tumour or its margin of invasion.

(2) The specimen is immersed in a solution of 1 per cent nitric acid in 10 per cent formaldehyde. This solution is changed weekly and serves to decalcify the specimen. Complete decalcification is determined by lack of opacification as noted on roentgenograms of the specimen. Alternatively, calcium may be detected in the solution by the use of a simple precipitation test. Total time for this step is 21 days. In order to avoid skin contact with the nitric acid, rubber gloves are worn when the specimen is handled.

(3) The specimen is wrapped in loose gauze and is immersed in a solution of 5 per cent sodium sulphate for 1 day and in fresh solution for a second day. This step adequately neutralizes the nitric acid.

(4) The specimen is bathed in running cold tap water for 2 days.

(5) Dehydration is accomplished by immersing the specimen for 24 hours in each of eight increasing strengths (per cent) of ethanol: 50, 80, 95, 95, 100, 100, 100 with ether (1:1 ratio), and 100 with ether (1:1 ratio).

(6) Infiltration with celloidin* is also done in stages: the specimen is submerged 3 weeks in 7 per cent solution (Fig. 1) and 1 week in 14 per cent solution.

(7) Finally, each specimen is placed for 5 weeks in a Stender dish containing fresh 14 per cent celloidin (Fig. 2).

Thus, the fixation, dehydration, and embedding are completed in 14 weeks.

Sectioning.—A Jung microtome is used. The celloidin block is immersed in 70 per cent alcohol and is oriented with the anterior laryngeal surface upward (Fig. 3). Specimens are sectioned at intervals of 750 μ; three sections of 24 μ each are required at each level. Only one section is stained; the remaining two are held in reserve.

Staining.—Routine hematoxylin and eosin stains are most useful. Sections are placed in Ehrlich's hematoxylin solution for 15 hours, rinsed in water for a day, and then counterstained with eosin.

Mounting.—After being rinsed in xylene, sections are placed on 2- by 3-inch glass slides. Coverglasses (no. 1) are positioned over Permount and weighted down for 2 days. The sections are then ready for study.

* Parlodion, Mallinckrodt Chemical Works, St. Louis, MO.

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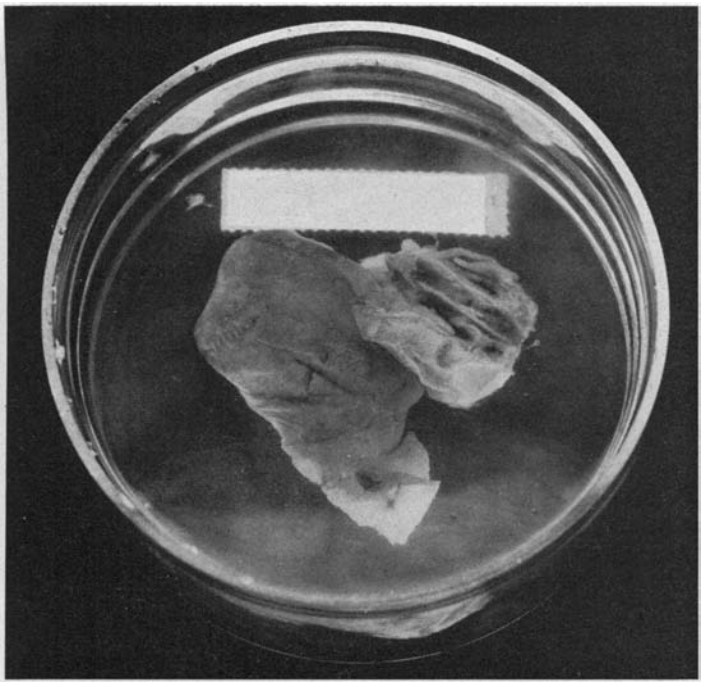


FIG. 1.
First stage of celloidin infiltration. Trimmed larynx is submerged in a 7 per cent solution.

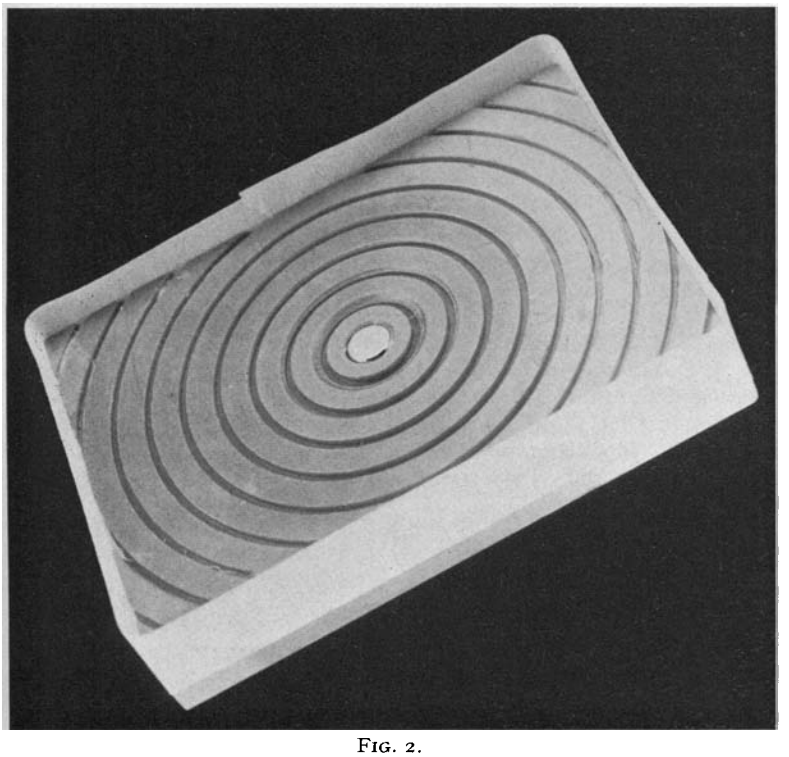


FIG. 2.
This 'dish' is used for final stage of celloidin embedding.

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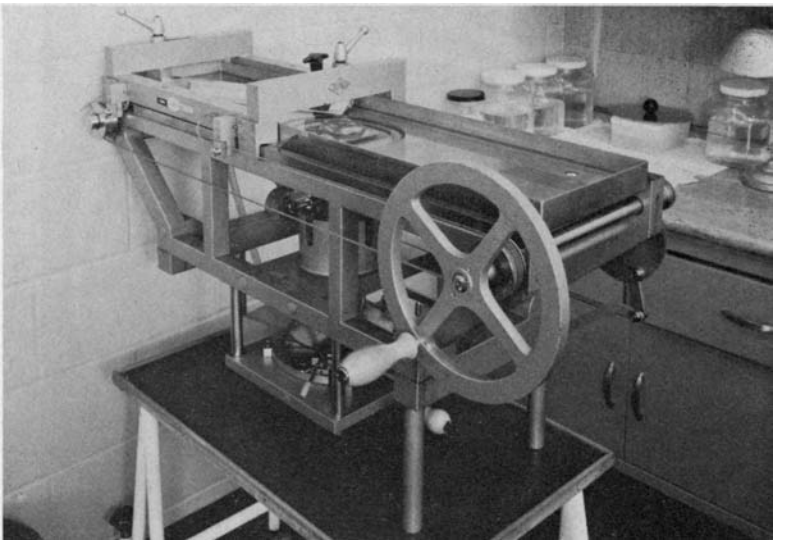


FIG. 3.
Celloidin-blocked larynx in position to be sectioned.

Discussion

This method has proved satisfactory for the study of whole laryngeal sections. The shortened preparation time is desirable, and the 24 μ sections are adequate for observing the growth and spread characteristics of carcinoma and the resulting tissue reaction. If thinner sections are necessary, embedding in paraffin is preferable to the method described (Ekem, 1972).

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