Carcinoma of the Larynx in South Africa

R. T. Gregor
CARCINOMA OF THE LARYNX IN SOUTH AFRICA: HISTOPATHOLOGICAL EXAMINATION BY MACROSCOPICAL TRANSVERSE SECTIONING AND CORRELATION WITH COMPUTED TOMOGRAPHY FEATURES- WITH SPECIAL REFERENCE TO FRAMEWORK INVASION.

Reinhold Theophilus Gregor

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ABSTRACT

Invasion of the laryngeal framework by cancer is a significant feature to look for. It implies a tumour that has spread beyond the bounds of the organ of origin, and may affect the outcome of the disease. The diagnosis of this feature may be difficult clinically and Computed Tomography (CT) scanning has proved useful in this aspect. Framework invasion almost invariably takes place in ossified or calcified cartilage, and the reason for this has never before been adequately explained.

The finding of increased density on some CT scans where the tumour was invading the framework stimulated this study into the mechanisms of this type of spread. One hundred and fifty-eight consecutive laryngeal specimens were examined by a serial sectioning method to elucidate this and some other problems. As the South African population represents different ethnic groups, the study of laryngeal cancer here gave the opportunity of comparing the findings of this study, not only with those from overseas, but also between the ethnic groups. Several laryngeal specimens were examined for alkaline phosphatase (ALP) in the tissues; two specimens were examined for collagenase. A method of tetracycline labelling was used to measure the amount of osteoblastic activity in a further two specimens. The histological and some clinical data from 154 laryngeal specimens were computerized in SAS data programs, and several statistical analyses regarding these factors were performed.

There were no striking ethnic differences in laryngeal cancer in Black and White South Africans, except that supraglottic lesions were relatively more common in Blacks, and the latter ethnic group tended to seek medical advice later in the disease process. In all ethnic groups the clinical appreciation of the extent of the disease was poor. Pre-epiglottic space (PES) invasion was common in supraglottic tumours.
and had a strong association with deeper spread of tumour. Supraglottic
tumours also spread readily to the paraglottic spaces (PGS), casting doubt
on the efficacy of partial supraglottic laryngectomy in these patients.
The most frequent route of tumour out of the larynx was via the
cricothyroid membrane (CTM). Evidence of the origin of tumours in the
ventriculo-saccular complex was found, and this is thought to represent
a subgroup in the major laryngeal sites.

Framework invasion occurred mainly at the glottic level and exclusively
in ossified or calcified cartilage. This type of invasion was associated
with osteoblastic activity which appeared to be at least partially medi-
ated by tumour-produced ALP. Osteoclastic activity took place
hand-in-hand with the former process, and at this stage tumour remained
outside the perichondrium. Tetracycline labelling confirmed active bone
deposition in these areas, and appeared to explain the finding of in-
creased ossification seen on CT scans where early invasion was taking
place.
I declare that this thesis is my own, unaided work. It is being submitted for the degree of Doctor of Philosophy in Medicine in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University. Certain assistance has been given by several people, all acknowledged in the preface.

REINHOLD THEOPHILUS GREGOR

31st day of July, 1986.
DEDICATION

To Kelly and Fiona, who often went to bed without seeing Daddy; and to Chris, their mother, who understood.
My interest in sectioning the larynx began during my time as lecturer in the Professorial unit at the Royal National Throat, Nose & Ear hospital, London. I wish to thank my former chief, Professor D F N Harrison, Professor of Laryngology and Otology, University of London, for this initial stimulus. I also want to thank my colleagues in the first studies published here; Professor Leslie Michaels, Professor of Pathology, Institute of Laryngology & Otology, and Drs Glynn Lloyd and Peter Phalps, Consultant Radiologists, The Royal National Throat, Nose & Ear Hospital, London, for their contributions. These initial published studies are bound into the appendix section of this thesis.

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THE PROBLEMS

The incidence of carcinoma of the larynx in the South African Urban Black has been given as 3.73 per 100,000 of population.\(^1\) In Sweden, the values are 2.8 per 100,000 population for males, and 0.3 for females.\(^2\) and represents 1% of all cancer. The vast majority of laryngeal and hypopharyngeal cancers are squamous cell carcinomas, and other types of laryngeal tumours are rare. There have been suggestions that the incidence of laryngeal cancer is rising in western countries, paralleling the rise in incidence of bronchial carcinoma.\(^2\)

This study has been undertaken in an attempt to elucidate several problem areas.

1. **How the pathology of carcinoma of the larynx and hypopharynx** in South Africa compares with the findings of studies conducted elsewhere, in terms of the **site-distribution and spread** of cancer, and whether or not there are **ethnic differences** within these features.

2. **What the factors are which govern the local and regional spread** of laryngeal cancer.

3. **What the determining factors and mechanisms of framework invasion** by laryngeal cancer are, and
4. The value of **Computed Tomography (CT)** in laryngeal cancer, particularly in terms of framework invasion.

In the literature review which follows, the format of these 4 problem areas will be followed:

Chapter one covers the pathology of laryngeal cancer, detailing the results of previous studies using the serial sectioning method of examining the laryngeal specimens. A section on anatomy is included where it is felt that the anatomical features are relevant to the spread of cancer in the larynx. A short section on dysplastic and premalignant lesions is also included because of the importance of these lesions in deciding the site origin of certain tumours. Chapter two examines the factors determining the spread of laryngeal cancer, Chapter three focuses on framework invasion, and Chapter four on the place of CT in laryngeal cancer and particularly on framework invasion.
1.0 CHAPTER ONE: PATHOLOGY OF CARCINOMA OF THE LARYNX AND HYPOPHARYNX

1.1 ANATOMY

The anatomy of the larynx is intricate, and herein lies the main reason for a specialized method in the examination of pathological specimens. The study of the growth and spread of laryngeal cancer depends upon a sound knowledge of this intricate anatomy. The management, both surgical and otherwise, of laryngeal cancer, depends upon these factors.

The larynx is situated in the midline compartment of the neck, deep to the infrahyoid "strap" muscles. There are two layers of these muscles; the outer, sternohyoid, and the deeper sternothyroid, and thyrohyoid.

The cavity of the larynx is divided into supraglottic, glottic and subglottic areas. The site classification of carcinoma used in this thesis conforms to that of the UICC (Union Internationale Contre le Cancer, Geneva, 1982). The glottis consists of the true vocal cords with their anterior and posterior commissures. Above the vocal cords are the ventricles. The glottis extends to the apex of the ventricle (laterally) and to the conus elasticus below, for the purposes of site classification for cancer (figure 1).

The supraglottic region, or vestibule, consists of the ventricles, false vocal cords, laryngeal surface of the epiglottis and aryepiglottic folds as well as the expanse of mucosa covering the arytenoids, and extending from the false cords to the aryepiglottic folds.
Figure 1. View of the larynx from behind: Note that the mucosa has been removed on the right half.

The subglottic region begins at the level of the cornus elasticus and extends to the inferior border of the cricoid cartilage. (Figure 1) The subglottis is well seen on a coronal plane view, and is therefore well demonstrated by laryngography and laryngeal tomography. The symmetry of its shape is important in coronal and transverse planes in detecting carcinomatous invasion. The subglottis consists of an upper, mobile half and a lower fixed half. The upper half consists of mucosa covering the cornus elasticus, which lies over the thyroarytenoideus muscle. During
phonation this upper half appears as an arch. The lower half consists of mucosa covering the inner aspect of the cricoid cartilage. (figure 3)

The mucosa of the glottic and supraglottic areas consists of stratified squamous epithelium. The mucosa of the ventricles, saccules and subglottic regions is pseudostratified ciliated (respiratory) epithelium. The supraglottic and subglottic regions are rich in submucosal mucous and minor salivary glands, the glottic region is not.

1.1.1 THE LARYNGEAL CARTILAGES.

The framework of the larynx consists of nine cartilages, three single and three paired. The large single thyroid cartilage is "shield shaped", open posteriorly and angulated in front (figure 2). The angulation is more acute and the protrusion of the thyroid prominence in the midline is greater in males than in females. The cricoid cartilage is a single "signet ring" shaped cartilage that is thicker and stronger than the thyroid cartilage. The posterior portion, or lamina, measures 2-3 cm from above downwards, being considerably broader than the anterior arch, which measures 3-7 mm. The cricoid cartilage is extremely important for the stability of the larynx, for which it forms a type of platform. The arytenoids, which move the vocal cords, articulate with and are supported by the rostrum of the cricoid lamina. The intrinsic muscles of the larynx arise on the cricoid and insert on the arytenoids, causing them to rotate. The cricoid cartilage is the only part of the laryngeal framework which forms a complete ring. For this reason the mucosa covering the inside of the cricoid is particularly susceptible to pressure effects from tight endotracheal tubes.
The paired arytenoid cartilages are pyramidal in shape, each having three surfaces, a base and an apex. The base of each arytenoid articulates with the cricoid. The lateral and posterior crico-arytenoid muscles insert on the muscular process which is situated on the lateral angle of the arytenoid. The anterior angle is elongated into the vocal process, which receives the insertion of the vocal ligament. The arytenoid thus comprises the posterior one third of the vocal cord. The epiglottis is a thin leaf-shaped fibrocartilage situated in the anterior midline behind the angle of the thyroid cartilage, projecting upward behind the base of the tongue. The upper free end is broad and rounded. The narrow base, called the petiole, is attached to the midline of the thyroid cartilage.
by the thyro-epiglottic ligament. This attachment, which forms the lower limit of the pre-epiglottic space, is situated immediately above the anterior commissure. This fact has pathological significance, in terms of cancer spread and will be dealt with in more detail below. About half of the epiglottis projects above the hyoid bone and is called the suprathyroid portion. This portion is covered with mucosa on both sides, and has a lingual and a laryngeal (posterior) surface. The lingual surface is not part of the larynx in terms of UICC classification, but lies in the oropharynx. The infrahyoid portion of the epiglottis, has no free anterior surface, and forms the posterior wall of the pre-epiglottic space (figure 2). The epiglottic cartilage contains many pits, or lacunae, which are filled with mucous glands and thus provide an avenue for the spread of cancer. The paired corniculate and cuneiform cartilages are essentially vestigial structures situated within the ary-epiglottic folds at the tips of the arytenoids.

**Nerves and Vessels**

These are described in detail with the possible influences this anatomy may have on the spread of cancer in chapter 2, page 40.

**1.1.2 LARYNGEAL MEMBRANES, LIGAMENTS AND COMPARTMENTS.**

The cartilages mentioned above are bound together by the intrinsic ligaments of the larynx (figure 2). These intrinsic ligaments are portions of a broad sheath of fibrous tissue containing many elastic fibres, which is well developed in some areas and almost absent in others. The better developed lower portion, or conus elasticus is separated from the less
well developed upper portion, or quadrangular membrane, by the ventricles.

Studies of laryngeal specimens by serial sectioning have demonstrated the ligamentous, cartilaginous and mucosal relationships within the larynx. These connective tissue structures form barriers that divide the larynx into various compartments which influence the spread of cancer within the larynx.

1.1.2.1 Laryngeal Connective Tissue Structures.

The conus elasticus, or crico-vocal membrane, is composed of mainly yellow elastic tissue. The membrane extends from the cricoid cartilage below to the vocal cords above. In the vocal cords, it condenses to form the vocal ligaments which run from the vocal processes of the arytenoids to the thyroid cartilage in the midline. The vocal ligaments are attached to the thyroid cartilage by the anterior commissure tendon. This area is of interest because there is no intervening parichondrium at this attachment, and hence it may be an avenue of cartilage invasion by cancer. This feature will be discussed in more detail below.

The elastic tissue is almost absent in the ventricles, but above the layer resumes in the form of the quadrangular membranes, which attach anteriorly to the edges of the epiglottis, and extend posteriorly to the aryepiglottic ligaments (which form the substance of the aryepiglottic folds) and which attach to the medial surfaces of the arytenoids, and to the ventricular ligaments inferiorly (figure 2).

CHAPTER ONE: Pathology of carcinoma of the larynx and hypopharynx
1.1.2.2 The laryngeal spaces.

- Reinke's space. This is a potential space below the mucosa of the cords extending on their free edges and superior surfaces. This arises because the mucosa is attached rather loosely to the vocal ligaments. Its clinical significance lies in the fact that the space can become oedematous, (Reinke's Oedema) but more significantly that it may represent a barrier to cancer, due to a lack of communication with the lymphatics of the rest of the larynx. Other authors hold that the spread of cancer in the larynx is influenced by the topography of the mucous glands. The glottis normally has no mucous glands beneath the squamous epithelium lining the free vocal cord edge.

- Paraglottic space (PGS) The paraglottic space can best be summarized clinically as those portions of the larynx which contain all the paired structures of the larynx not normally visible to the examiner. The space is bounded by the thyroid cartilage laterally, the quadrangular membrane and conus elasticus medially and the anterior reflection of the pyriform fossa posteriorly. Inferolaterally the paraglottic space is continuous with the cartilaginous defect between the thyroid and cricoid cartilages. The paraglottic spaces embrace the ventricles and saccules (see figure 3). The importance of this space is in the spread of tumours from any mucosa which is adjacent to it. It is most commonly seen in cancers which arise from the rima glottidis and is also seen in hypopharyngeal tumours.

- The Pre-epiglottic Space (PES) This space has been called the space of Boyer, although according to Tucker, Boyer described a prethyrohyoid bursa. The pre-epiglottic space is bounded superiorly by the hyoepiglottic ligament, anteriorly by the thyrohyoid membrane.
and thyroid cartilage and posteriorly by the epiglottic cartilage and thyroepiglottic ligament. The space is filled with fat and areolar tissue. The space is continuous with the upper ends of the paraglottic spaces, although it has been said that cancer rarely spreads this way. Other authors feel that the pre-epiglottic space does not only lie in front of the epiglottis but extends in a horseshoe fashion around the epiglottis, but does not include the thyroarytenoid and vocalis muscles, as the paraglottic space does. They suggest that it should be called the "peri-epiglottic" space. The pre-epiglottic space appears to have a septum and does not extend through to the hyoid bone, and it has been suggested that this bone may not have to be resected in supraglottic- and total laryngectomy. 

- **Subglottic "space"** this has been called the subglottic "area". It is a potential space filled with the fibro-elastic submucosal tissue between the mucosa and the conus elasticus. For tumours to spread to this area the conus elasticus must first be breached.

- **Supraglottic space** The area between the supraglottic mucosa and quadrangular ligament. This space does not communicate with the right and left sides, and extends to the false cord, but does not extend into the ventricle and vocal cord. Pressman showed by dye injection studies that there is wide dissemination of the dye into the adjacent hypopharynx when it is injected into the aryepiglottic fold.

1.1.2.3 Barriers and compartments

The barriers and compartments described above have been popularized by Tucker (figure 3). We have seen that tumours of the vocal cords tend
to remain localised for long periods of time. The "bursa" or Reinke's space has been implicated in this, and this has been corroborated by Pressman's injection studies which tend to show that this area is lymphatically divorced from the rest of the larynx. There is however disagreement about the anatomy and significance of the pre-epiglottic space, as has already been mentioned above. There has been a strong school that believes that supraglottic tumours do, for the most part, remain supraglottic and do not spread to the paraglottic spaces and vocal cords.\textsuperscript{6} \textsuperscript{12} \textsuperscript{11} \textsuperscript{15} \textsuperscript{17} It has also been said that there is a difference in the behaviour of supraglottic tumours arising from the laryngeal surface of the epiglottis and those arising more laterally.\textsuperscript{12} The lateral lesions are said to invade the paraglottic space more readily and therefore are less amenable to conservation laryngeal surgery.

Bocca et al.,\textsuperscript{15} in a study of 160 laryngectomy specimens for supraglottic cancer, observed that "even in the most advanced stages of evolution primary vestibular growths never invaded the floor of the ventricle or vocal cords." This was attributed to the fact that the supraglottic portion of the larynx develops from the buccopharyngeal anlage whereas the glottic and subglottic portions develop from the tracheobronchial anlage. This has been confirmed by others\textsuperscript{16} and by clinical studies of supraglottic versus total laryngectomy.\textsuperscript{18} Supraglottic tumours rarely invade the thyroid cartilage, except when they become "transglottic".\textsuperscript{16} \textsuperscript{19}
1.1.3 THE ANTERIOR COMMISSURE

Tucker\textsuperscript{12} has pointed out that above the attachment of the anterior commissure tendon the anterior reflection of the ventricular commissural mucosa lies very close to the internal perichondrium of the thyroid ala. Above this cleft created by fusion of the ventricles, the "petiole" (see page 6) of the epiglottis inserts obliquely in the midline. This area would appear to be a site for cancer spread subglottically (to the cricothyroid membrane), supraglottically (via the pre-epiglottic space) or for cartilage invasion. For this reason radiotherapy has been condemned in the treatment of these lesions.\textsuperscript{10} In serially sectioned
larynges\textsuperscript{21} the anterior commissure tendon has been shown to extend from the area of the thyroid notch to the level of insertion of the vocal ligaments. At this level there is no perichondrium which might resist tumour spread. However Olofsson et al\textsuperscript{12} found radiotherapy to be effective in controlling anterior commissure carcinomas.

### 1.1.4 Anatomy of the Larynx Related to Transverse Sectioning.

This is described in Chapter 7, pp 133-136.

### 1.2 Embryology of the Larynx

Various authors,\textsuperscript{16} \textsuperscript{17} believe that the embryological development of the larynx influences the spread of laryngeal cancer. The larynx develops differently in its upper and lower halves.\textsuperscript{24} The lower portion develops around the cranial end of the trachea, whereas the supraglottic portion arises out of the pharyngeal floor, in the region of the laryngeal orifice. This fact has been used as an explanation for the hypothesis that supraglottic tumours remain in that position even when they are large.\textsuperscript{15}

The epiglottis is peculiar to mammals. Embryos of 5mm show a rounded prominence that elevates midventrally from the bases of the 3rd and 4th arches. By the 40mm stage, it has assumed a recognizable shape, and by birth the cartilage has developed.
The primitive glottis is a slit which develops in the floor of the pharynx and opens into the trachea. On either side of this slit develops rounded swellings of 4th and 5th arch origin, called the arytenoid swellings. These swellings grow caudal, and have the effects of making the slit-like orifice T-shaped. (7th intra-uterine week) During the 8th week the vocal folds (cords), cartilages and muscles develop from mesenchyme of the 4th and 5th arches.

1.3 HISTORY

The history of laryngology\(^3\) is relevant to understanding the development of the conventional sites in the larynx in which the origin of laryngeal cancer is described today.

The decade from 1850 to 1860 marked a turning point in the understanding of laryngeal pathology. The advent of laryngoscopy permitted examination of the larynx in the living subject, and the publication of Virchow's histopathology in 1858\(^*\) enabled accurate distinction of various laryngeal abnormalities.

Prior to this time there was much confusion concerning laryngeal disease. The earliest reference to laryngeal cancer is by Aretaeus, circa A.D. 100. Galen circa A.D. 200 described a malignant ulceration of the throat and apparently understood the nature and seriousness of laryngeal cancer. However this knowledge was lost for a time as there are no known refer-

\(^*\) Virchow, RLK; Die cellular Pathologie in Ihren Begrundung auf Phisiologische und pathologische Gewebelhre. A. Hirschwald, Berlin, 1858. Quoted by Silver\(^1\)

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ences to laryngeal cancer in the western literature of the middle ages. In 1732 Morgagni clearly described autopsy findings in two cases of laryngeal carcinoma.

The terminology used prior to the 18th century clearly indicates the general confusion which reigned during this time; granulomatous diseases and cancer were not distinguished. "Angina", "phthisis" and "Larynco-phthisis carcinomatosa" were used loosely. Following the turning point in the mid 19th century brought about by the advent of laryngoscopy and histopathology, understanding of laryngeal pathology blossomed. Isambert and Krishaber attempted to classify laryngeal tumours as intrinsic, extrinsic and subglottic. These classifications remained for 50 years until the modern topographical classification and staging systems replaced them.

Although between 1743 and 1854 various individuals claimed to have invented some rather ingenious contraptions for demonstrating the larynx, the credit for the modern technique of indirect laryngoscopy is usually ascribed to Manuel Garcia. During a holiday in Paris in 1854, this expatriate singing teacher, of Spanish descent, used a dental mirror and reflected sunlight to examine his own vocal cords. Van Turck of Vienna and Czermak of Budapest popularised the method of indirect laryngoscopy amongst the medical profession. It was the latter of these two who first used lamp-light for illumination. Direct laryngoscopy was developed after 1895, and particularly by Chevalier Jackson, who with others, laid the foundation of modern endoscopy.
1.4 DYSPLASTIC AND PREMALIGNANT LESIONS

The transformation of normal epithelium to dysplasia and carcinoma-in-situ with its association with invasive carcinoma, is relevant to the concept of deciding upon the site of origin of tumours within the larynx - particularly in the Ventriculo-saccular complex - a site which has been described as rare for the origin of squamous carcinoma.\(^2\) More than 90% of laryngeal malignancy is squamous carcinoma. There is an entire spectrum of laryngeal epithelial aberrations which are pre-malignant and may progress to frank carcinoma. Abnormalities of squamous epithelium include hyperplasia, keratosis and atypia. These can occur individually or in any combination. The epithelium showing such changes may be flat, or papillary.\(^25\)\(^26\)\(^27\) Malignant conditions range from Carcinoma-in-situ to micro-invasive squamous carcinoma, verrucous carcinoma and infiltrating squamous carcinoma.\(^28\)\(^29\)\(^30\)\(^31\)\(^32\)

1.4.1 KERATOSIS

Keratosis may be benign or atypical. The normal non-keratinizing squamous epithelium in the larynx may undergo keratinization which may vary from benign keratosis to frank carcinoma. In between these two are keratoses with various degrees of atypia.\(^27\) Keratosis is due to the formation of kerato-hyaline granules in the cytoplasm of the superficial epithelial cells. When the cytoplasm is totally replaced and the nucleus disappears, keratin is left. If the nucleus is left the condition is called parakeratosis. Keratinization within the prickle cell layer is called dyskeratosis.
1.4.2 CARCINOMA-IN-SITU

In this condition the squamous epithelium contains cells of malignant morphological pattern (loss of stratification and differentiation, altered polarity of cellular orientation and cellular atypism) that do not invade the basement membrane. The entire thickness of the epithelium is replaced by these cells in which no differentiation or maturation takes place.\(^2\) Fisher\(^2\) has proposed that the "bowenoid" type of carcinoma-in-situ in which only the basal layer of cells appears neoplastic and the prickle and squamous layers remain unchanged, be accepted. Carcinoma-in-situ is considered a malignant and not a pre-malignant condition. The distinction between this, and severe epithelial atypia or dysplasia may be difficult for the pathologist to make.

Carcinoma-in-situ contains all the generally accepted cytological criteria for malignancy, except invasion. However, there has been some question about the reversibility of this condition.\(^2\) These authors found that 16% of smokers had carcinoma-in-situ at autopsy, whilst in former smokers none had this condition. The values for atypia were 100% for smokers and 25% for former smokers. (Similar figures applied to former smokers and non-smokers.) The proportions of atypical cells were also related to the amount of smoking by the individual.

Most cases of carcinoma-in-situ of the larynx originate on the anterior ends of the vocal cords. There is no clinical appearance which strongly suggests the diagnosis. Many lesions are indurated thickenings with or without a whitish plaque; "Leukoplakia". Carcinoma-in-situ is nearly always in continuity with areas of squamous metaplasia. Intraepithelial changes may be present in the supra- and subglottic regions. There appears
to be a histogenic interdependence between the in situ cancer and atypical hyperplasia.

1.4.3 PRE-MALIGNANT LESIONS, AND THEIR PROGRESSION TO MALIGNANCY.

Traditionally, the term leukoplakia has carried with it a pre-malignant connotation. This is not warranted, since there are a number of white mucosal lesions which are either benign, or are only exceptionally associated with the subsequent appearance of malignancy. Similarly not all potentially malignant lesions appear as white patches. "The term leukoplakia therefore is entirely a clinical descriptive term, should not be used by pathologists, and should not carry with it any implication as to biological behaviour." However, the appearance should not be dismissed too lightly because the underlying changes in the squamous epithelium in a white plaque lesion may vary from harmless hyperplasia to invasive carcinoma. The malignant potential of "keratoses" of the larynx is difficult to assess. Keratosis without atypia is a benign lesion and is reversible with little inclination to progress further to atypical change or carcinoma. While there is by no means an obligatory progression from keratoses with or without atypia to cancer, the risk is small, yet significant. McGavran et al found 11 out of 18 cases of keratosis with atypia to have recurrent symptomatic disease as opposed to 14 out of 66 without atypia. However only 2 of the first group and 1 of the second developed invasive squamous carcinoma. These authors were however at variance with some earlier ones who found higher incidences of "malignant transformation". The natural history of keratoses is possibly altered by the very biopsy that establishes the diagnosis.
1.5 LARYNGEAL PATHOLOGICAL EXAMINATION

In most centres the pathological examination of laryngeal specimens is poorly performed. It has been shown above that the larynx has a minute and exact anatomy - comprising a composite of different types of tissue; mucosa, glandular structures, cartilage, ossified and non-ossified; synovial joints, muscles, tendons and ligaments; compartments and barriers. Obviously then, any pathological examination of the organ must take this into account. The whole organ serial sectioning technique is such an attempt. This was first performed by Le Roux Robert in 1936\(^a\). Since 1936 the serial sectioning technique was used by Kernan\(^b\), using celloidin embedding, and Fletcher\(^c\), using paraffin embedding. Szlezak\(^d\), used a 'serial block' method with paraffin embedding. It was Tucker, however, who popularised the celloidin embedding technique\(^e\), which has been employed in other laboratories. The paraffin embedding technique was refined by Hyams of the AFIP (Armed Forces Institute of Pathology, unpublished data quoted in 1) Olofsson has published a major series of 139 larynges processed by this method.\(^2\)

Whole organ serial sectioning performed by these methods is extremely time-consuming, expensive, and requires highly specialised equipment and expertise. It is not a method which is within the capabilities of an average histopathology laboratory. This was one of the reasons for the development of the method used in our study.\(^3\) Another reason was that the method was intended originally to correlate with preoperative computed tomography scans\(^4\). This method will be described in detail in part II, chapter five. The serial sectioning method does enable the examining


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pathologist to comment in detail on the growth and spread of tumour in the larynx.

As recently as 1955, Ogura, in a major paper on laryngeal pathology described laryngeal tumours as "endolaryngeal", "intrinsic", "extrinsic" and "subglottic".\textsuperscript{17} In his study the laryngeal specimens were examined by slicing the organ into multiple blocks, first sagitally and then in various other planes. It is my experience that this method gives a poor impression of the extent of laryngeal tumour. The modern concepts of site classification will be discussed later in this chapter.

Ogura's\textsuperscript{17} description of cancer of the larynx as endolaryngeal (true and false cords and ventricles), which included glottic and supraglottic tumours, makes correlation with more modern series difficult. (See figure 4) He noted that cancer of the true cord usually arose on the anterior one third of the vocal cord. There was the unusually high number of 13/59 "subglottic tumours". (In Olofsson's series\textsuperscript{2} only 4 occurred in 110 laryngeal carcinomas.) By our modern classification it would appear that most of these would be classified "glottic" with subglottic extension. The classification of "extrinsic" similarly bedevils comparison, as this includes sites which we would now call Supraglottic (aryepiglottic and epiglottic) and hypopharyngeal (pyriform sinus). These tumours were found to erode the thyroid cartilage in only one case, but invasion of the tongue base was well recognised.

In a correlation of nodal metastases in his study, Ogura found that nodal metastases were more common in tumours which had cartilage invasion. Invasion of the PES was also found to correlate well with cervical node metastases.
Figure 4. The lumen of a right hemilarynx: This shows the modern recognised regions of the larynx.

1.5.1 THE SERIAL SECTIONING METHODS

Tucker emphasized the need for a method of examination of the larynx which was capable of a more precise determination of the relationship of the cancer to the structures beneath the mucosa.
"The nature of most pathological routines is such that the material is dissected before histological preparation, and therefore cannot be re-examined in its original state"

Tucker favoured the coronal plane for his sections. His first publication\(^9\) reports the serial sectioning of 4 cases. Each larynx yielded between 1100 and 1700 sections. This method was extremely labour-intensive as is obvious from the number of sections cut. In order to maintain the sequence - and appreciate the position of the sections - all sections were numbered, and saved. Only every 10th section was stained, still representing 110-170 stained sections to be examined.

Kirchner used the same technique as Tucker, but was able to process 100 larynges by 1969\(^8\) and 200 by 1977\(^9\). This was made possible in spite of the expense and laboriousness by a National Cancer Institute Grant. Most specimens were sectioned in the coronal plane as described by Tucker, but a few were sectioned horizontally and sagittally. In this series every 50th section was stained. The sites classified were glottic(31 cases), supraglottic(10), Epiglottic(10) "transglottic"(19), infraglottic(3) and pyriform sinus(24). Partial laryngectomies numbered 3.

In his later paper, Kirchner\(^9\) has adapted to the modern TNM classification (See later in this chapter) and has dispensed with separating epiglottic tumours from the rest of supraglottic tumours. This series of 200 specimens included 8 subglottics(4%) with a high incidence of framework invasion.

Kirchner concurred with Ogura\(^7\) that glottic fixation was usually due to thyro-arytenoid muscle invasion. Only 3 tumours appeared to arise "infra-glottically"(subglottic). This represents 3% in comparison to Ogura's more than 26%. Supraglottic tumours were found to be less invasive as far as cartilage is concerned - in the epiglottic tumours 50%
invaded epiglottic cartilage. The specimens were felt to demonstrate that supraglottic tumours tended to remain supraglottic.

1.5.1.1 Transglottic tumours

The term transglottic was coined by McGavran et al\textsuperscript{41} to describe a growth extending within the deep tissues above and below the ventricle. Although it transcends the generally accepted modern concept of supraglottic, glottic and subglottic, it is a useful term to describe spread. (Kirchner used it only for those cases that had vocal cord fixation.)

Transglottic tumours were characterised by invasion of the paraglottic space described by Tucker\textsuperscript{7}, and were thought to have originated in the ventricle. These tumours exhibited an aggressive growth pattern, infiltrating the laryngeal framework in most cases, and emerging from the confines of the larynx by direct extension between the thyroid and cricoid cartilages at the crico-thyroid membrane. (15/19 cases) In a review of transglottic carcinomas\textsuperscript{19}, Kirchner discusses the origin of these tumours, suggesting that they originate in the ventricle. Kirchner concluded that it cannot always be determined where these originate, but that at least some originated supraglottically, and some glottically. These lesions responded poorly to radiation, and were best managed by wide surgical excision. (60% demonstrated framework invasion).
1.5.1.2 Framework invasion

Pyriform sinus growths usually invaded the laryngeal framework. Early invasion of the paraglottic space and laryngeal muscles occurred with decreased cord movement. Invasion of the laryngeal framework was observed to occur nearly always in the ossified portions. The hyoid bone was found to be free of tumour even in the most extreme cases of PES invasion. Invasion of the thyroid ala was found to occur usually in its lower one third. Size was important in predicting framework invasion, with 2 cm being a cutoff point; those larger than this had a high incidence of framework invasion.

1.5.1.3 Olofsson and van Nostrand's series

This is one of the most conclusive reports of the serial sectioning studies. These authors reviewed 110 carcinomas of the larynx and 25 hypopharyngeal carcinomas. They defined the glottic area as the area between the lateral angle of the ventricle and the conus elasticus (including the vocal ligament). Above this was the supraglottic and below this the subglottic area. Of the 110 larynx carcinomas, 73 were glottic and 25 supraglottic, 4 were subglottic and 8 defied classification and were called "multiregional". Twenty four of all these tumours could have been considered "transglottic" by other authors. (22%)

Ventriculo-saccular Tumours

Olofsson and van Nostrand believed tumours arising from the ventricles to be very rare, in support of this their findings indicated that there
was a very low incidence of squamous metaplasia in the pseudostratified columnar ciliated epithelium of this region. In no cases did they find dysplasia of this region. However, Michaels and Hassman\textsuperscript{a2} using the technique developed in London\textsuperscript{19} and used in the study for this thesis, found 11 cases of "ventriculo-saccular" carcinoma in 76 cases of carcinoma of the larynx. (14.5%) These tumours were characterised by concentric tumour growth in the paraglottic space, absence of invading processes of tumour and absence of cartilage invasion. None of these patients developed lymph nodal metastases. Clinically these tumours usually present as a mucosal covered mass in the supraglottis and may defy diagnosis by the unwary. Computed tomography scanning is useful for demonstrating the paraglottic space invasion. Micheau\textsuperscript{a3} found 22 cases of ventricular tumours out of 120 cases of carcinoma of the larynx (19\%). However, these tumours behaved entirely differently to those in Michael's and Hassman's patients. Eight out of 23 invaded cartilage and 16 out of 23 destroyed the conus elasticus. Another 22 cases were said to have developed "in a laryngocoele ". The significance of these findings will be discussed in more detail later in the chapter.

**Glottic carcinomas\textsuperscript{2}**

Most of these arose from the free margins of the vocal cords as found by Ugurá and Kirchner. Thirteen out of 73 primary glottic tumours were confined to the glottic region. Subglottic extension occurred in 24/73 cases, and to subglottic and supraglottic regions in 31 cases. Five tumours spread to the supraglottis alone and the opposite hemilarynx was involved in 35 cases. The most common site of thyroid cartilage invasion and breakthrough was in the anterior midline (21/26). The cricothyroid membrane was also a frequent site of breakthrough.
Supraglottic Carcinomas

There were 25 supraglottic tumours; 6 extended to the glottic region and 6 extended to the vallecula and 6 extended to the pyriform sinus. Ten invaded the epiglottic cartilage and PES. Of the epiglottic lesions (15) only one invaded the thyroid cartilage. Most supraglottic carcinomas extended upwards rather than downwards, and had "pushing margins".

Subglottic carcinomas

Only 4 carcinomas arose in this area. Three of the 4 were bilateral and invaded the laryngeal cartilages. The tumours extended outside the larynx in 3 cases.

Multiregional carcinomas

Eight of the 110 laryngeal carcinomas were so large as to defy classification. These could all be considered "transglottic" and 6 probably arose in the glottic region. All of these invaded the laryngeal framework, and spread beyond the larynx. (As in Kirchner's cases over 2cm in size).

Pre-epiglottic Space invasion

The fenestrations in the epiglottic cartilage were found to be frequent sites of invasion, as described by Ogura. Glottic tumours invaded the PES via the base of the epiglottis. In Micheau's series this occurred in 10% of cases of glottic tumours.

Vascular and peri-neural invasion occurred in 27 and 20 cases respectively. Fixation of the vocal cord was usually due to thyro-arytenoid
muscle invasion. (28/28) The crico-arytenoid joint was invaded less often (17/28).

Lymph nodal metastases were higher in supraglottic tumours and in those carcinomas with spread outside the larynx. The Delphian (pre-laryngeal) node was involved in 6 cases, all of which had spread to the subglottis.

Olofsson and van Nostrand² came to several conclusions, which can be summarized as follows:

1. Laryngeal tumours are often more deeply invasive than surface inspection clinically or pathologically might suggest.

2. The conus elasticus is an effective barrier to tumour spread, and was considered the boundary between the glottic and subglottic regions.

3. There were some weak points in the laryngeal framework; the anterior commissure and the crico-thyroid membrane were the most frequent sites where glottic tumours spread out of the laryngeal confines; elsewhere along the cord muscle and perichondrium intervene. The crico-thyroid spaces laterally are also routes of extralaryngeal spread.

4. Transglottic tumours are aggressive in behaviour and framework invasion was common.

5. Laryngeal framework invasion occurs at the ossified portions of the cartilage in most cases.

6. Supraglottic tumours tend to have "pushing margins" and often do not invade the glottic area, some however do become transglottic and invasive.
7. The pre-epiglottic space (PES) is frequently invaded in the supraglottic group.

8. Subglottic tumours are often advanced when diagnosed and invade the conus elasticus and the framework commonly.

9. Direct invasion of the thyroid gland may occur in glottic, subglottic or transglottic tumours and therefore the isthmus and ipsilateral lobe should be removed in these cases when performing laryngectomy.

10. Supraglottic tumours have the highest incidence of metastatic lymph nodes. The Delphian node may be involved in tumours of the subglottis or with subglottic involvement by glottic tumours. However in this series it only occurred in 8 out of 60 potential cases. This low incidence of Delphian node involvement has been found by other authors as well.\textsuperscript{41}

11. Fixation of the vocal cord was usually due to thyro-arytenoid muscle invasion. More than half of the glottic tumours with a fixed cord (T3) had extension outside the laryngeal framework and this was poorly appreciated clinically pre-operatively.

12. Hypopharyngeal carcinomas (N=29) were less differentiated than laryngeal carcinomas. Extensive submucosal spread was a feature of these. Pyriform sinus carcinoma was the most common and often involved laryngeal structures causing vocal fixation. Lymph nodal metastases were more frequent in hypopharyngeal than in laryngeal carcinomas.
1.5.2 OTHER STUDIES

Harrison\textsuperscript{36} reviewed the results of 50 excised specimens of hypopharyngeal carcinoma. There were 14 from the pyriform fossa, 2 from the posterior pharyngeal wall, and 34 postcricoid carcinomas. The pyriform fossa tumours showed a high degree of submucosal spread, the average being 10mm. This has been confirmed in Black patients in South Africa by Davidge-Pitts and Mannel,\textsuperscript{44} who found up to 30mm of submucosal spread in these tumours. Harrison also found an extremely high incidence of lymph nodal metastases in these patients.

The two cases of posterior pharyngeal wall malignancies showed widespread invasion of muscle and prevertebral tissues.

Postcricoid lesions were at first treated by pharyngolaryngectomy\textsuperscript{37} but most of these (21) had recurrence due to positive margins inferiorly or paratracheal lymph nodes left behind. In 13, laryngopharyngoesophagectomy was performed with recurrences in only 2. Harrison\textsuperscript{36} emphasized the frequency of oesophageal, tracheal and paratracheal node involvement. (More adequate removal was achieved by manubrial resection.)

Micheau et al.\textsuperscript{42}, examined 120 larynges by a radial sectioning technique. These authors felt this to be superior to coronal sectioning. Eight "codified" planes were chosen, 2 sagittal, 2 coronal and the other 4 the radial planes between the sagittal and coronal plane. They found framework invasion in 58\% of cases, 94\% of these were in the ossified portions of the cartilage.

This series comprised 26 supraglottic tumours; Of these 18 were epiglottic tumours which in only one case invaded the vallecula and base of tongue. Pre-epiglottic space (PES) invasion took place nearly always by de-
struction of the thyro-epiglottic ligament ("foot of the epiglottis"). Not one of these epiglottic tumours spread to the glottis. Six of the supraglottic tumours were more extensive and invaded the epiglottis, false cords and paraglottic space, involving the ventricles and cords in one third of cases.

Twenty-three cases of "cancer of the ventricles" were found. Of these, 11/23 invaded the PES via the base of the epiglottis. These tumours frequently invaded the glottic areas as well, and a high percentage invaded the subglottis, either submucosally or via the paraglottic space (16/23). Thyroid cartilage was invaded in 16/23 cases. Micheau also found 22 tumours which were said to be "cancer developing in a laryngocoele". Microscopically the tumour was found to "develop along the ciliated epithelium of the laryngocoele."

These cases would appear to compare with those of Michaels and Hassman\(^2\), except that the behaviour appears to be more aggressive. Micheau's cases showed cartilage invasion in 50\% of cases, and PES invasion in 19/22. It may be that these patients represented a more advanced stage of this disease. The difficulty in diagnosis has already been mentioned. It is clear that there is disagreement in the literature as to the question of carcinoma developing in the ventriculo-saccular complex.

Micheau found 36 cases of glottic carcinoma, 72\% of which showed invasion of the arytenoid. Olofsson\(^2\) \(^3\), found a much lower rate of about 42\%. Subglottic tumours were rare, in agreement with Kirchner\(^4\) \(^5\), and Olofsson\(^4\) with only 4 cases being found.
"If 700 people, 150 of whom have spent the whole week here, finish up by not being able to define the vocal cord, that's something that we're going to have to keep very much to ourselves."

Professor DFN Harrison, Professor of Laryngology and Otology, London.
(At the Centennial Conference of Laryngeal Cancer, Toronto, May 1974.)

The difficulty in reporting cancer in the larynx has been the pre-occupation of laryngologists for over 100 years. In 1879 Krishaber* divided laryngeal tumours into intrinsic and extrinsic. Krishaber rightly considered the latter tumours to be more malignant, and this terminology remained in general use for the next 50 years.

In 1886, Sir Felix Semon introduced the term "endolaryngeal" for all tumours within the larynx. The term subglottic was mentioned by Morrell McKenzie in 1880, but it was not until 1930 that St Clair Thompson recommended that the classification of subglottic cancer be differentiated from intrinsic, because of its different behaviour.†

Gradually, therefore, a new more specific division of the intrinsic region of the larynx into supraglottic, glottic and subglottic areas gained universal acceptance. "Extrinsic" tumours are now considered to be hypopharyngeal. (Pyriform fossa, posterior hypopharynx and postcricoid


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areas.) This classification has formed the basis for the TNM classification of cancer of the larynx accepted by the UICC (Union Internationale Contre le Cancer, Geneva) in 1962 for the purposes of cancer staging and reporting end results.

Prior to 1972, the supraglottis was defined by the UICC as including the posterior aspect of the infrahyoid epiglottis, the arytenoids, the false cords and the ventricles. The so-called "marginal zone" of the larynx which comprised the suprahypoid part of the epiglottis and the ary-epiglottic folds was excluded from the supraglottis. Since 1972, and confirmed in 1978 and 1982 the supraglottis includes the tip of the epiglottis and the ary-epiglottic folds. The anterior aspect of the suprahypoid epiglottis is, however, still considered to be part of the oropharynx by the UICC.

The American Joint Committee for Cancer Staging and End Results Reporting (AJCCS) was organised on January 9th, 1959 with the purpose of developing a system of clinical staging of cancer by site acceptable to the American medical profession. The AJCCS attempts to develop classifications which are compatible with those published by the UICC. There are, however, several differences now in these two classifications, as last published in 1982. The AJCCS considers the lingual surface of the suprahypoid epiglottis part of the larynx and not the oropharynx. There are also significant differences in the bilateral lesions, and in the reporting of positive lymph nodal metastases.

Deficiencies

The TNM system of classification, now widely accepted as the standard method of reporting laryngeal cancer, not only ignores the biological behaviour of the tumour - all patients being erroneously considered as having the same disease - but possesses important intrinsic deficiencies.
which need defining.\textsuperscript{46} One of the problems is that there is not even total agreement in the basic anatomical boundaries. Harrison\textsuperscript{46} states that:

"The first real attempt to reach an acceptable definition of the mucosal boundaries of the glottic region, the cornerstone for any laryngeal classification, was made in workshop number one at the Centennial Conference on Laryngeal Cancer (CCLC), held in Toronto, May 1974. It was agreed that this was that portion of the vocal cord which approximates its neighbour during phonation and which is normally covered with squamous epithelium. It extends from the anterior commissure to the tip of the vocal process of the arytenoid, having a vertical height in the male of approximately 5mm at its midpoint, but only 2mm anteriorly."

The UICC classification considers the laryngeal ventricles to be part of the supraglottis, although the junction between the squamous epithelium covering the vocal cord and the respiratory mucosa of the ventricle is the histological boundary line.\textsuperscript{46} Considerable disagreement centered around this aspect during the panel discussion during workshop 1 at the CCLC.\textsuperscript{46} Ölofsson and van Nostrand\textsuperscript{2} considered the upper limit of the vocal cord to be the lateral angle of the ventricle (nearest to the thyroid lamina). (Figure 3.)

It would seem from the conclusions of this panel discussion that the most universally acceptable definition of the upper margin of the vocal cord is the superior arcuate line, where the mucosa changes, and which corresponds with the limit of the visible surface of the cord when looking from above, clinically. In order to look more laterally, the false cord must be moved by an instrument, which then brings the ventricle into view. However the new AJCCS classification uses a horizontal plane passing through the lateral apices of the ventricles. (in agreement with Ölofsson and van Nostrand).
All classifications are compromises, and in this thesis the UICC classification has been used. However, the term "transglottic" has also been used but where possible the site of origin of the tumour is indicated. Both the UICC and the AJCCS classifications are reproduced below:

THE CLASSIFICATION OF THE UICC

Supraglottis

Epilarynx (including marginal zone):

i) Posterior surface of the suprahoid epiglottis (including the tip)
ii) Aryepiglottic fold.

Supraglottis excluding the epilarynx.

i) Infrahoid epiglottis.
ii) Ventricular bands (false cords).

Glottis

i) Vocal cords.

ii) Anterior commissure.

Subglottis

TNM PRE-TREATMENT CLINICAL CLASSIFICATION

T - Primary tumour.

Supraglottis

Tis  Pre-invasive carcinoma (carcinoma in situ.)

T0  no evidence of primary tumour.

T1  Tumour confined to the region with normal mobility.

T1a  tumour confined to the laryngeal surface of the epiglottis
     or to an aryepiglottic fold or
     to a ventricular cavity or to a ventricular band.
T1b Tumour involving the epiglottis and extending to the ventricular cavities or bands.
T2 Tumour with extension to the glottis without fixation.
T3 Tumour confined to the larynx with fixation and/or other evidence of deep infiltration.
T4 Tumour with direct extension beyond the larynx.
Tx The minimum requirements to assess the primary tumour can not be met.

Glottis
Tis Pre-invasive carcinoma. (Carcinoma-in-situ)
T0 No evidence of primary tumour.
T1 Tumour confined to the region with normal mobility.
T1a Tumour confined to one cord.
T1b Tumour involving both cords.
T2 Tumour confined to the larynx with extension to either the supraglottic or the subglottic regions with normal or impaired mobility.
T3 Tumour confined to the larynx with fixation of one or both cords.
T4 Tumour with direct extension beyond the larynx.
TX The minimum requirements to assess the primary tumour can not be met.

Subglottis
Tis pre-invasive carcinoma (Carcinoma-in-situ)
T0 No evidence of primary tumour.
T1 Tumour confined to the region
T1a Tumour confined to one side of the region.
T1b Tumour with extension to both sides of the region.
T2 Tumour confined to the larynx with extension to one or both cords with normal or impaired mobility.
T3 Tumour confined to the larynx with fixation of one or both cords.
cords.

T4 Tumour with destruction of cartilage and/or with direct extension beyond the larynx.

Tx The minimum requirements to assess the primary tumour can not be met.

Regional lymph nodes.

N0 no evidence of regional node involvement.
N1 Evidence of involvement of movable homolateral regional lymph nodes.
N2 Evidence of involvement of movable contralateral or bilateral regional lymph nodes.
N3 Evidence of involvement of fixed regional lymph nodes.
NX the minimum requirements to assess the regional lymph nodes can not be met.

Distant metastases

M0 no evidence of distant metastases
M1 evidence of distant metastases
MX The minimum requirements to assess the presence of distant metastases can not be met.

TNM Post Surgical Histopathological Classification

The TNM classifications are the same as above, with the prefix "p".

THE TNM AND STAGING SYSTEMS OF THE AJCCS

These systems are summarized and correlated in figure 5.
Figure 5: diagram correlating the AJCCS staging and TNM systems: The TNM system of the AJCCS is similar but not identical to the TNM system of the UICC. See text for explanation.
2.0 CHAPTER TWO: FACTORS DETERMINING THE SPREAD OF LARYNGEAL CANCER

2.1 COMPARTMENTS

As has been seen in Chapter 1, the anatomy of the larynx does include compartmentalization. The concept of compartments and barriers was popularized by Tucker. These anatomical features are reviewed below with respect to the growth and spread of laryngeal cancer.

The compartments of the larynx were first studied by Pressman who used a dye injection technique. This study showed that the subglottic submucosal compartments were separated from one another in the midline. These findings were supported by Dayal et al. The paraglottic spaces were described by Tucker who used coronal serial sectioning of adult and embryo larynges to demonstrate them. The compartments and barriers were described in detail in Chapter 1. However, how effective the so-called barriers are to limiting the growth of cancer was not clearly understood by Tucker.

More recently Lam and Wong using a similar technique to our own studied normal and pathological larynges to determine the importance of the pre-epiglottic spaces to spread of carcinoma of the larynx. This study supported those mentioned above in that a fibrous sheet of tissue was found separating the pre-epiglottic space (PES) and paraglottic spaces. This fibrous membrane extends from the edge of the epiglottic cartilage posteriorly to the edge of the thyroid notch anteriorly. The PES is therefore triangular with the apex pointing inferiorly. The roof is the hyo-epiglottic ligament. Behind is the epiglottic cartilage, and
in front the thyroid cartilage and the thyro-hyoid membrane. The para-glottic spaces (PGS)\(^{12}\) were separated from the PES by the membranous partition, and extend down as far as the conus elasticus; they are limited posteriorly by the pyriform fossa mucosa, laterally by the thyroid cartilage and they embrace the saccules, ventricles and thyroarytenoid muscles.

Lam and Wong\(^{43}\) found that tumour reaches the PES either via the fenestrations of the epiglottic cartilage, or by the destruction of the epiglottic cartilage, or by destruction of the thyro-epiglottic ligament. This was in agreement with previous authors.\(^{2, 17, 42}\)

The fibro-elastic membranous partition between the PES and PGS did in some cases contain tumour, so that when the PGS was invaded the PES was not necessarily invaded and \textit{vice-versa}. However, when the tumours became large enough these barriers were breached. Spread of tumour in the PES is limited, at least temporarily, by the boundaries of the space; superiorly by the hyoepiglottic ligament, anteriorly by the thyrohyoid ligament and the thyroid cartilage and inferiorly by the thyroepiglottic ligament. This is why a supraglottic tumour may remain localized and not invade the glottis; however if the para-glottic space is invaded the glottis is easily invaded. This has confirmed Tucker’s original observation\(^{12}\) and has shown that the statement that all supraglottic tumours remain localized is incorrect.\(^{15, 16, 17}\)

Subglottic tumours penetrated the cricothyroid membrane early due to its proximity. This has been confirmed by other authors.\(^{2, 91}\) Laterally and posteriorly the cricothyroid membrane is deficient (it is sometimes more correctly called the cricothyroid ligament); the reason for this is that the cricothyroid membrane fuses with the conus elasticus leaving a gap laterally between the conus elasticus and the lower edge of the thyroid

\textit{CHAPTER TWO: Factors determining the spread of laryngeal cancer}
This cricothyroid space is a common area for the escape of tumour from the confines of the larynx.

When tumours which had invaded the FGS spread posteriorly, they invaded the mucosa of the pyriform fossa (18/35) or the posterior aspect of the cricoid cartilage (13/35). In Lam's series 63% were transglottic and 17% supraglottic. The most common sites for extralaryngeal spread were through the thyroid cartilage and cricothyroid membrane. Thyrohyoid membrane penetration was only seen in transglottic tumours, and in all cases was also associated with thyroid cartilage penetration.

2.1.1 VASCULAR SUPPLY TO THE LARYNX

The vascular supply to the larynx and its effects on laryngeal cancer has been studied by Sokjer and Pearson. Below is a brief description of this and comment as to the possible influences on cancer spread are in bold.

The larynx receives its blood supply from the superior and inferior laryngeal arteries. The superior laryngeal artery usually originates from the superior thyroid artery and enters the larynx through the thyrohyoid membrane (although it may pass through a foramen on the postero-superior thyroid ala). The inferior laryngeal artery, a branch of the inferior thyroid artery, accompanies the recurrent laryngeal nerve, and enters the larynx behind the cricothyroid joint. Both these vessels ramify extensively in the plane between the thyroid cartilage and the intrinsic laryngeal musculature.
The Anterior Commissure Area

This area is related to the pre-laryngeal area; there is a ramification and anastomosis of vessels related to the angle of the thyroid cartilage and the isthmus of the thyroid gland. This arterial arcade is deep to the sternothyroid muscles and below their attachment to the oblique line of the thyroid ala. The superior thyroid artery follows and supplies the upper margin of each thyroid lobe, and anastomoses with its opposite member on the upper edge of the thyroid isthmus in front of the second tracheal ring. The cricothyroid artery arises at the level of the sternothyroid insertion and contributes to this anterior pre-laryngeal arterial arcade. There are connections with the anterior divisions of the descending branch of the superior laryngeal artery via the cricothyroid triangles. This represents an anastomosis of the endolaryngeal and extralaryngeal vessels; or can be thought of as an anterior vascular pedicle.

Several vessels arise from the cricothyroid arcade; a fine midline arteriole passes through the cricothyroid ligament ("membrane") and reaches vertically upwards to the anterior commissure, and into the anterior commissure tendon\(^{21}\) and supplies the thyroarytenoid muscles. Companion veins drain the anterior commissure directly to the subglottic mucosa and out under the inferior margin of the thyroid cartilage to the soft tissues immediately in front of the cricothyroid ligament.\(^{33}\) Branches of the cricothyroid arcade supply the perichondrium of the thyroid cartilage both inside and outside in this region. Small inferiorly directed vessels supply the subglottic mucosa and cricoid perichondrium.

The anterior commissure has been widely noted to be a problem area for cancer involvement. Many authors have reported the fact that cancer spreads quickly subglottically and then outside the larynx, via the
crico-thyroid membrane, once the anterior commissure is involved.\textsuperscript{6} \textsuperscript{7} \textsuperscript{13} \textsuperscript{24} \textsuperscript{21} \textsuperscript{22} The vascular anatomy may well be partly responsible for this.

The Supraglottic larynx

Epiglottis and Pre-epiglottic Space

The epiglottis is supplied by the Superior Laryngeal Artery via its ascending branch and by the ventral branch in the petiole area.\textsuperscript{53} The lingual surface of the epiglottis is also supplied by branches of the lingual artery, which create an arcade below the hyoid bone. These branches also supply the upper PES, and by penetrating the epiglottis also supply the central upper area of the laryngeal surface mucosa. The lower reaches of the PES are supplied by branches of the ventral branch of the superior laryngeal artery.

If carcinoma followed the periarterial preformed pathways, it would explain early invasion of the PES from a laryngeal surface epiglottic tumour; a feature which has been noted by numerous authors.\textsuperscript{2} \textsuperscript{8} \textsuperscript{9} \textsuperscript{43} The branches from the lingual artery would explain spread to the vallecula and base of tongue, a feature also well noted.\textsuperscript{17}

Ventricles and Paraglottic Space (PGS)

The PGS and ventricle are supplied by the ventral branch of the superior laryngeal artery. Branches are also given to the petiole, the lower PES and saccule. Below there are anastomoses with the anterior division of the descending branch over the thyroarytenoid muscle.

The large perivascular spaces that run vertically and obliquely in this region lie lateral to the intrinsic laryngeal musculature and conus elasticus. The nearest mucosal surfaces to this vascular pedicle are those
in the ventricle and pyriform fossa. Cancer reaching the paraglottic space would be expected to spread vertically with little surface evidence. This type of behaviour might be expected in ventricular tumours.

False cords and Aryepiglottic folds

These are supplied by the medial branch of the superior laryngeal artery. It also supplies the cuneiform, corniculate and arytenoid perichondria.

**Glottic and Subglottic Larynx**

This area is supplied from the cricothyroid arcade and branches of the anterior division of the descending branch of the superior laryngeal artery. These vessels give rise to the linear vasa recta of the true vocal cord (which run the length of the cord.)

The tendency of cancer to spread along the length of the cord toward the anterior commissure and extend into the subglottic region, from where spread may occur to the cricothyroid membrane, the lower edge of the thyroid cartilage and the isthmus of the thyroid is suggested by this arterial pattern and has been extensively observed.

**The postcricoid region**

Upon entering the larynx behind the cricothyroid joint, the inferior laryngeal artery divides into numerous branches which ramify throughout the postcricoid submucosa superficial to the posterior cricoarytenoid muscle. There is an anastomosis with the dorsal branch of the superior laryngeal artery on the posterior aspect of the interarytenoid muscle. Several other vessels anastomose in this area.
Carcinoma arising in the postcricoid region lies in immediate contact with peri-vascular pathways through which bilateral local extension can occur. There is no muscle to be penetrated first, since the vascular anastomoses are submucosal.\textsuperscript{53} This correlates well with Harrison's experience with post cricoid carcinomas in which local extension to the oesophagus and trachea, and lymph nodal metastasis to the paratracheal nodes was common.\textsuperscript{36}

In summary, there appears to be a definite correlation between the vascular patterns within the larynx (there is a close relationship between arteries, veins and lymphatics\textsuperscript{53}) and the observed spread of cancer as studied by various serial sectioning techniques. The actual mechanism of cancer spread has not been elucidated since it may be that tumour uses the loose connective tissue surrounding the vascular pedicles as a path "of least resistance". Lymphatic spread to the neck would correlate with the high incidence of lymph nodal metastases in many instances; such as involvement of the pre-epiglottic space\textsuperscript{17}, subglottis\textsuperscript{51}, pyriform fossa and postcricoid areas.\textsuperscript{36}

Invasion of structures does not appear to be entirely related to blood supply; it has been well described that ossified cartilage is easily invaded while avascular cartilage is not.\textsuperscript{41} In explanation it has been suggested that this is because of the better blood supply of ossified cartilage. However, the perichondrium is well supplied with arterioles, and perichondrium has been seen to be a barrier to tumour spread. It is clear that there are mechanisms at work in tumour spread other than purely mechanical ones, and it is this aspect (to be reviewed later in this chapter) that has formed an important part of the investigative work of this study.
2.1.2 CANCER SPREAD RELATED TO LARYNGEAL MUCOUS GLANDS

The theory of Bridger and Nasser\textsuperscript{54, 55} has it that the topography of mucous glands within the laryngeal mucosa largely determines the spread of laryngeal tumours in the larynx. These authors demonstrated by serial sectioning techniques that invasion along the mucous gland pathways does occur. This fact appears to correlate with some of the observations already emphasized.

Epiglottic tumours: Carcinomas arising from the tip of the epiglottis do tend to remain localized at first. The fenestra in the cartilage have been mentioned often already - these contain mucous glands - and are far more numerous lower down. These facts are felt by the authors to support the theory in that tumours of the infrathyroid epiglottis quickly invade the pre-epiglottic space.

Aryepiglottic folds: The lack of mucous glands in the upper vestibule and aryepiglottic folds would appear to correlate with a limitation of submucosal spread in this area, but again an early involvement of the PES.

False cords and ventricle: The false cords contain numerous mucous glands and cancer may spread within an extensive tubulo-alveolar system forwards and superiorly into the "medial compartment" of the pre-epiglottic space. Tumours from the roof of the ventricle are said to behave similarly. Such a tumour may also spread into the saccula. The mucous glands in the ventricle, posterior to the saccula, extend upwards and laterally towards the thyroid ala. Bridger and Nassar's findings in relation to saccular tumours correlate well with the findings of Michaels and Hassman\textsuperscript{42}

Glottic tumours: The oft repeated observation of the early localisation of glottic tumours - said to be related to Reinke's space and the paucity
of lymphatics - can also be ascribed to the lack of mucous glands in the mucosa. As a tumour spreads to the floor of the ventricle, where the mucosa changes, and mucous glands are found, it may spread into the thyroarytenoid muscle. Subglottic spread also results in the tumour reaching an area of mucous glands, with deep penetration of the tumour. Particularly in the anterior commissure area there are numerous tubulo-alveolar glands in its subglottic aspect. Tumours following these glands find an easy passage into the fibrous structures at this point: the conus elasticus, cricothyroid ligament and anterior commissure tendon. It is also Bridger and Nasser's contention that the spread of posterior commissure tumours (although rare) to the postcricoïd mucosa is related to the rich supply of this area with mucous glands. The theory is felt to explain why tumours of the ventricle or the superior vocal cord with spread into the ventricle become "transglottic", extensively invading the paraglottic area via the numerous branching mucous glands, of which the saccula is only one. This theory would appear to support the belief that transglottic tumours originate from the ventricle.

2.2 COMMENT

The factors which influence the growth and spread of cancer within the larynx are multiple. Physical factors such as fibrous tissue barriers, particularly the conus elasticus and perichondrium contain tumour, at least at first. Tubulo-alveolar mucous glands may be invaded by tumour, and are likely to influence its local mucosal spread. Bridger and Nasser's findings are interesting, and it is probable that this mechanism may operate in conjunction with the ones already mentioned, so as to influence the spectrum of spread of laryngeal cancer. The structure of the vocal cord with Reinke's space and the vascular and lymphatic supply certainly
influence the spread of early lesions and it is probable that blood vessels provide a pathway for tumours to spread.
It has been well described before that ossified cartilage is much more susceptible to cancer invasion than non-ossified cartilage. The reason for this is not yet fully explained, but it has been suggested that cartilage releases a substance or substances which inhibits tumour, or inhibits tumour substances which enhance invasion.

Olszewski has related the invasion of ossified cartilage to vascularization. Twenty larynges were studied by silicone rubber blood vessel injection. This enabled the author to study the vascularization of tumour vessels within the cancerous larynges. There was evidence of "the direction of invasion of a neoplasm developing in accordance with the direction of the penetration of the vessels into the focus of ossification from the ... perichondrium." This evidence, in addition to the discovery of a tumour angiogenic factor (TAF), suggests that there may be a relationship between the ossification of cartilage, the production of tumour 'hormones', and the invasiveness of laryngeal tumours.

3.1 OSSIFICATION OF CARTILAGE

The ossification of the laryngeal cartilages has been studied by Kirchner, Keen and Wainwright and Harrison and Denny. Ossification usually occurs in the 3rd decade of life and not before the age of 21, however the correlation with age is poor. The changes are similar to ordinary endochondral ossification, and are preceded by calcification of
the cartilage. There appears to be no substantial sex difference according to some but Keen and Wainwright found that ossification proceeds at a slower rate in females.

Ossification of the thyroid cartilage invariably begins at the posterior border near the root of the inferior horn; spreads along the inferior border, and reaches the midline, where there is usually a separate centre of ossification. It then extends upwards along the posterior border towards the superior horn (cornu) which was only ossified in 20% of Harrison and Denny's series. Ossification proceeds upward in the thyroid laminae eventually involving the superior border and usually leaving two unossified islands of cartilage in each lamina. These islands may suggest erosion of cartilage and therefore tumour invasion radiologically.

Cricoid ossification occurs about the same time as the thyroid and begins at the upper border of the posterior lamina, just anterior to the crico-arytenoid joint. From these two points ossification spreads into the lamina, and forwards into the arch, the anterior extent of which is usually the last to ossify. Ossification of the arytenoids starts in the muscular processes and spread forwards, but rarely reaches the apex or vocal process.

The pattern of ossification in the larynx has been thought to correspond to the insertion of the main intrinsic and extrinsic laryngeal muscles. Possibly these sites represent areas of increased tension leading to ossification. Harrison and Denny, having studied various mammalian larynges felt this was a tenable explanation for the progression of bony changes.
3.2 INVASION BY CANCER

The pattern of framework invasion has been studied by Kirchner\(^7\) in 185 larynges.(64 glottic/subglottic, 56 transglottic and 65 supraglottic.) Invasion of the laryngeal framework occurred almost invariably in the lower one third of the thyroid cartilage and the upper edge of the cricoid ring in the early stages. The perichondrium was thought to be a barrier to the spread of cancer, because when cancer infiltrated one point, and expanded the cartilage, most of the perichondrium remained intact. This has been seen by other authors as well.\(^{43} 51 64\)

Transglottic lesions showed the highest incidence of framework invasion (50%) as has been previously reported by the same author\(^9\) and others,\(^1\) although Harrison reports that in his series\(^5\) no patients with a transglottic tumour had penetration of the external perichondrium without tumour escape via the (lateral) crico-thyroid gap (space) or membrane. None of the pure supraglottic tumours in Kirchner's series showed cartilage invasion.

3.3 CLINICAL SIGNIFICANCE

The fact that cartilage invasion per se, without invasion of extralaryngeal structures, influences the survival of the patient has been reported by Kirchner\(^7\) but disputed by Harrison.\(^8\) Kirchner found that invasion of the framework reduced the 3 year survival for glottic carcinomas from 72% to 38%. As pointed out by Harrison, the reason for this most probably lies in the high rate of lymph node metastases with cartilage invasion, a finding also reported by Ogura\(^17\), but not confirmed.
by McGavran et al. However, framework invasion remains an important finding even though its greater morbidity is probably because of an increase in the incidence of lymph nodal metastases.

3.4 SITES AND ROUTES OF FRAMEWORK INVASION

Yeager and Archer have studied the sites of cartilage invasion, and have confirmed that they are the same as found by Kirchner and Harrison and that these are also the areas of ossification. In addition these areas are those related to the attachment of laryngeal musculature, a fact that has been related to their ossification because they are the areas of most stress. Yeager and Archer have pointed out, however, that these are also areas which are deficient in perichondrium, because of the attachment of fibrous collagen bundles ("Sharpey's fibres"). Perichondrium has been shown to present a barrier to cancer, but in the following areas the perichondrium is deficient:

1. The area of the anterior commissure tendon (ligament).

2. The junction of the anterior one fourth and posterior three fourths of the lower thyroid lamina

3. The medial aspect of the posterior border of the thyroid lamina.

4. The cricoarytenoid joint.

5. The areas of attachment of the cricothyroid and cricotracheal membranes.
Cancer cells have been seen to separate the collagen bundles (which pass through the perichondrium) finding a linear passage to the cartilage. This feature was pointed out by Broyles in 1943 as far as the anterior commissure tendon is concerned. Archer and Yeager feel that the observation of cancer invading ossified cartilage more easily is related to their findings regarding the collagen bundles, they believe that the ossification is related to the attachment of fibres and muscles and lines of stress and that the correlation between tumour invasion and ossification is incidental.

3.5 HISTOLOGICAL FEATURES OF FRAMEWORK INVASION

This has been extensively studied by Carter et al.

Carter and Tanner found no histological pattern which appeared to predispose to framework invasion, in agreement with other authors. Pittam and Carter found in agreement with Kirchner that cartilage invasion represented a group of patients with a higher mortality.

These authors found that the ossification of the laryngeal cartilages faithfully reproduced organized bone, with bone trabeculae lined with osteoblasts and haemopoietic marrow. Osteoclasts were inconspicuous. In cases having framework invasion the invasive process was concentrated in regions of metaplastic ossification. Direct invasion of unchanged cartilage was unusual, and was always overshadowed by deeper infiltration into nearby foci of metastatic ossification.

The morphological changes associated with the carcinomatous invasion into metaplastic bone were consistent in all cases studied. Most commonly
there was an increase in osteoclasts which accumulated near the edge of spreading tumour cells. The bone was found to be progressively destroyed by characteristic "scalloping" defects produced by the osteoclastic activity. Direct mingling of tumour cells and osteoclasts on the bone surface was rarely seen. Osteoblasts, the predominant type of cell in uninvaded bone, were always outnumbered in the invaded regions.

In some cases with extensive destruction of the laryngeal framework, the osteoclastic activity appeared to wane and direct contact between the absorbing bone and tumour cells occurred. These authors did not feel that radiotherapy or infection were responsible for these changes. 59

In a study of bone invasion in other tumours of the head and neck, Carter et al found similar changes. 65 However in some cases there was a combination of osteoclastic and osteoblastic activity in advance of the invading carcinoma. Although the osteoclasts outnumbered the osteoblasts, they usually evoked a measure of osteoid and new-bone formation.

3.6 BIOCHEMICAL FACTORS

These findings have indicated that bone invasion is a complicated process. Most of the bone destruction is achieved by host cells which are apparently stimulated by the presence of the tumour.

In another study, Bennett, Carter et al 68 have demonstrated a prostaglandin-like substance which is produced by carcinomas of the head and neck. The cellular origin of these prostaglandins, and other tumour-associated osteoclast-activating products, is unclear; however, it is thought by these authors that host cells such as macrophages may
play a part in their production. The mechanisms responsible for osteoblastic stimulation, apparently linked to the increased osteoclastic activity, are obscure. 65

Similar mechanisms have been described in skeletal metastases. Galasko 69 has shown in autopsy specimens and in animal experiments that bone is resorbed by osteoclasts, and the tumour secretes a diffusible osteoclast activating factor. In many cases an associated osteoblastic reaction and new bone production was taking place independently. There was usually a fibrous reactive stroma in the space between the osteoclasts and the tumour mass. The osteoclasts disappeared when the tumour had grown sufficiently large to envelope residual trabeculae of bone, but bone destruction continued. There appeared to be two mechanisms; one in which a humoral substance 70 was involved and another in which the tumour cells seemed to mediate late bone destruction by a mechanism not involving osteoclasts.

In another study Galasko 71 showed that Prostaglandin E 2 could be extracted from tumours with bone invasion, and could be abolished by treating the experimental animals with Indomethacin. In the indomethacin-treated animals bone destruction, and osteoclastic activity was markedly diminished.

In previous studies Galasko 71 showed that skeletal metastases are associated with new bone formation and destruction which occur simultaneously. The new bone formation and destruction have some relationship, as for example in a fracture. Once the destruction is gross, and malignant cells surround the residual bone, no new bone is formed.

Other authors 72, have found that during the process of resorption, bone releases a factor that is strongly chemotactic for tumour cells. This factor is released by a variety of agents which induce resorption of bone.

CHAPTER THREE: Framework invasion
Collagenase activity has been measured in various head and neck carcinomas. These authors cultured cells from biopsies from 14 patients with head and neck cancer; 8 were cases of cancer of the larynx. Oral cavity tumours showed greater activity than laryngeal carcinomas while both sites were more active than uninvolved mucosa from the same patients. At 18 months after the diagnosis, four of the six patients with most active collagenase activity were dead of cancer and one patient was alive with persistent cancer. These authors concluded that high collagenase activity may be a factor in the clinical aggressiveness of epidermoid carcinomas of the head and neck.

The explanation for the fact that cartilage appears to be resistant to tumour invasion, may be related to the discovery of the inhibition of tumour angiogenesis mediated by cartilage. Brem and Folkman have shown that cartilage inhibits capillary proliferation induced by tumour. Using the rabbit cornea as an assay, neonatal scapular cartilage inhibited capillary proliferation induced by tumour. When the cartilage was heat inactivated, this effect was abolished. These results suggested that there was a diffusible material from normal cartilage which inhibited capillary proliferation by tumour.

3.7 COMMENT

Framework invasion would appear to be more complex than simply a question of blood supply. Tumours have been found to produce prostaglandin-like substances which activate osteoclasts to erode bone. Cartilage has been found to inhibit tumour angiogenesis. It appears likely that there is a combination of osteoblastic and osteoclastic activity taking place when bone invasion takes place. This may account for some
of the Computed Tomography appearances which have been found in cancer of the larynx. The agents responsible for an increase in osteoblastic activity are, however, still obscure. The CT changes are to be discussed in the next chapter.
4.0 CHAPTER FOUR: COMPUTED TOMOGRAPHY OF LARYNGEAL CARCINOMA

Computed Tomography (CT) was first introduced in England in 1971. The original EMI unit was installed in the Atkinson Morley Hospital in London and was not intended for examining the body below the base of the skull. As higher resolution scanners were made the method was extended to the base of the skull and paranasal sinuses. The introduction of the high-resolution body scanner by EMI in 1978 allowed CT to be applied to the temporal bone and middle ear, and as scanning times improved, to the neck structures.

Early publications on the use of CT for the larynx found that for the first time the laryngeal cartilages could be adequately assessed, and suggested that CT should make a significant contribution to more accurate planning of radiotherapy portals. Comparative evaluations of CT and the conventional methods of laryngeal examination followed, e.g. laryngography and cinelaryngoscopy and direct laryngoscopy. The advantages of non-invasiveness and the ability to image for the first time the deep infiltration of cancer, rather than relying on the mucosal surface appearances were acclaimed. It was even suggested that, while complimenting direct laryngoscopy, CT had made all previous forms of radiological evaluation obsolete. CT was also found to be cost effective and to have decreased the radiation dose delivered by conventional radiology.

The larynx, when removed at operation, gives the unique opportunity for whole organ study, and comparison with pre-operative CT scans. This study was undertaken by myself and colleagues at the Royal National Throat, Nose & Ear Hospital, London in 1979.
A method of examining the larynx in the Histopathology laboratory which was rapid, cost effective and equivalent in accuracy to the methods of whole organ serial sectioning, had to be developed. At the time of rapidly improving technology in CT scanners, there was no point in using a method of laryngeal examination which took 6 months to complete!

Other studies were published describing the normal anatomy of the larynx on CT, and a correlation of the CT of laryngeal specimens scanned in air and water, in comparison with sections of larynges cut using a bandsaw. No histological correlation was made.

The study performed by us in London was published in 1981 and comprised the examination of 17 patients - correlating the clinical findings on direct laryngoscopy and on full ENT examination, conventional tomography and CT scanning with the pathological findings post-operatively in the total laryngeal specimen, including histology. This paper is included in the appendix of this thesis. The method of laryngeal examination will be discussed in Chapter 6, but the original published report is also included in the appendix.

4.1 THE VALUE OF CT IN LARYNGEAL CANCER.

The original scanners were slow in scanning and took about 27 seconds to perform one high-resolution scan. Modern scanners can perform more than one scan per second. There is therefore no merit in dwelling on the technical difficulties originally encountered because of movement of the patient or movement within the larynx. The scan is done in the axial plane, but modern scanners can produce coronal reconstructions, which mimic the picture seen on conventional tomography. This feature has made
this modality unnecessary, whereas previously tomography was found to be complimentary to CT because of the image being at $90^\circ$ to the axial plane. \(^5\)

The imaging produced by CT is vastly superior to any other modality currently generally available. Not only does the framework show well, particularly when calcified or ossified, but the pre-epiglottic and paraglottic spaces are displayed. The mucosal surfaces are accurately delineated since air produces a contrast in attenuation value to the soft tissues, so that the relative thickness of the tissues between the framework and the lumen can be assessed. Any asymmetrical thickening of the paraglottic space is interpreted as abnormal. The anterior commissure where mucosa lies directly in contact with the thyroid cartilage is well displayed, so that any thickening is abnormal. In the same way the subglottis can be accurately assessed because any disturbance in its even, oval shape, or thickening of the mucosa as seen over the cricoid cartilage is abnormal. The problem with CT is that it does not differentiate between tumour and mucosal oedema or fibrosis in the absence of framework invasion.

4.2 THE LARYNGEAL FRAMEWORK ON CT

The pattern of the laryngeal cartilages in the living patient has been studied using conventional radiography. \(^8\) Basically the patterns of ossification were the same as found by Keen and Wainwright\(^5\) and Harrison and Danny\(^6\) who performed examinations on laryngeal specimens.

Of interest is the fact that the calcification and ossification which follows is symmetrical in terms of right and left sides. \(^5\) These authors
performed their work mainly on black South African patients, so it has
great bearing on the work of this study. However there were no essential
differences between their results and those of the European studies.
Ossification of the thyroid cartilage is essentially as described in
Chapter 3. Keen and Wainwright and Hately et al\textsuperscript{81} found that ossification
progressed at a slower rate in females, and that the front parts of the
laminae and the midline area remain cartilaginous to a more advanced age.
Similarly radiological changes of the cricoid mimic the description given
by the previous authors\textsuperscript{61} with ossification beginning in the superior
border of the lamina (in front of the crico-arytenoid joint) and spreading
forwards and downwards, the anterior extent of the arch being the last
to ossify.

The arytenoid cartilages start to calcify and ossify in the muscular
processes, then into the base, with spread upwards into the body. The
ossification never reaches the apex or the vocal process. Hately et
al\textsuperscript{81} found ossification of the arytenoid to have a higher incidence in
females, but Keen and Wainwright found the opposite.\textsuperscript{61}

CT has allowed a much better assessment of the laryngeal framework,
whether or not the cartilage has ossified; however cartilaginous areas,
particularly in the thyroid cartilage may mimic tumour invasion. Several
studies have concentrated on the assessment of the laryngeal
cartilages.\textsuperscript{64 82 83 84 85 86 87 88 89 90}

A striking feature noted in the arytenoid and cricoid cartilages in our
original series\textsuperscript{88}, was an increase in density on CT which had a high
correlation with early tumour invasion in these larynges. This appearance
had not been previously reported. This feature was at first thought by
us to be an artefact of some kind, but examination of the histology slides
and X-rays of the tissue slices confirmed that there was increased density
in the bone, and that in most cases there was early invasion of cancer,
or very close microscopic proximity by cancer. One or Archer and Yeager's publications demonstrated the same feature, but they made no comment on this phenomenon.² Two of the figures in this paper show the appearance affecting the right arytenoid in one of their patients.

In a follow-up study on the original observation made⁶⁶, Lloyd, Michaels and Phelps⁵ studied 8 patients who showed invasion of cartilage on subsequent histology. In all there were 14 sites of cartilage invasion which could retrospectively be diagnosed on CT in 11 instances. Four of these showed erosion of cartilage, and 5 showed osteosclerosis. The arytenoid was most often affected by the characteristic change and in 5 cases showed strong, moderate or minor degrees of increased density on the side affected by tumour in comparison to its fellow on the normal side. Most of these patients had had previous radiotherapy, and these authors concluded that the changes of increased bone formation were related to previous radiation and/or perichondritis.

### 4.3 OTHER CT FEATURES

Some authors found the use of phonation scans, which showed vocal cord fixation or mobility, useful.⁶⁷⁶⁸⁶⁹ These examinations have been made possible by very fast scanning times in the latest generation scanners. Symmetry of movement of the arytenoids is important to find. When this is absent it may indicate a fixed (T3) vocal cord. These authors found a good correlation between a fixed cord as shown by phonation scans, and thyroid cartilage invasion. They also found this examination useful for evaluating the ary-epiglottic folds and pyriform fossae. During phonation the pyriform fossae distend, and the ary-epiglottic folds become more prominent, and more easily evaluated for tumour involvement.
A lucent line, between the thyroid cartilage and the thyro-arytenoid muscle was described by Mancuso et al\textsuperscript{7}, and observed by us.\textsuperscript{6} This line was found to correspond with areolar tissue containing blood vessels on microscopical section. This line was not found by us to be invariably present, but when present, asymmetrical obliteration was significant for paraglottic invasion but not cartilage invasion. This line has also been seen by others\textsuperscript{4, 8} but disputed by some authors.\textsuperscript{17}

Yeager and Archer identified certain criteria to help with the diagnosis of possible cartilage invasion.\textsuperscript{6, 8, 10} These studies correlated the tumour's maximum dimension with the level in the larynx in relation to the arytenoid cartilage. Tumours below the level of the apex of the arytenoid had an 86% association with cartilage invasion, while those above this level only had an 8% incidence of cartilage invasion.

CT has been found useful in distinguishing supraglottic from pyriform fossa tumours.\textsuperscript{11} This may be a difficult distinction to make clinically. These authors found that pyriform fossa tumours can be recognised on CT because of their position, direction of spread and higher incidence of cartilage invasion. The supraglottic tumours grow in a circumferential pattern, invading the PES extensively and becoming bilateral at a relatively early stage. The pyriform fossa lesions were situated posterolaterally, invaded the paraglottic space from behind, insinuating themselves between the cricoid lamina and posterior edge of the thyroid ala. They tended to remain on one side only and extended into the lateral neck tissues, often rotating the larynx to the opposite side. It is of interest to note that these authors excluded transglottic lesions from this series of 25 cases. Transglottic tumours would be particularly difficult to distinguish from pyriform fossa tumours. CT has also been able to show involved lymph nodes in the neck which were not clinically palpable.\textsuperscript{8, 11}
There is little doubt that CT has become the preferred imaging technique for carcinoma of the larynx. Laryngography and even conventional tomography has become largely obsolete with the newer generation scanners. For the first time the deep infiltration by laryngeal cancers has been demonstrable pre-operatively. In the same way as the serial sectioning techniques have shown that the spread of laryngeal cancer is often deeper than was thought clinically, so this has been reflected in the CT scans. The one area in which CT scans have been disappointing has been in the detection of framework invasion. Clinicians had hoped that the scans would be able to detect early invasion, thus making the decision of the mode of management easier. In a recently completed thesis on CT and laryngeal cancer, Gerritson has reached very similar conclusions. This author has used the method of laryngeal examination published by us and has used the same method of comparing these to CT scans.

Although there has been general disappointment with the demonstration of early framework invasion, there have been several features from the CT scans which have helped our understanding of the invasion of cartilage, both ossified and unossified. The work of Archer and Yeager has been valuable in the identification of the sites which are most commonly invaded, and the histological correlation of these areas with the attachment of collagen bundles through the perichondrium. The position of the tumour and the level of it's maximum dimension related to the arytenoid has reflected the findings of many large pathologic studies showing that it is the transglottic, subglottic and advanced glottic tumours that show most of the cartilage invasion.
The finding of increased density on CT and evidence of osteosclerosis on histology originally reported by us has not received any attention in the world literature, although the same findings have been noted in the illustrations of at least two other reports. Many authors have reported that ossified cartilage bears the brunt of invasion. The fact that tumour angiogenesis factor, which aids the tumour in establishing a host blood supply, and osteoclast stimulating factors have been found, suggests that the tumour is able to create for itself an environment which is suitable for invasion. Galasko's and Carter's work on bone invasion, the finding of a prostaglandin-like substance and the observation that osteoclastic and osteoblastic activities seem to be taking place together, suggests to me that early tumour invasion may elicit an osteoblastic response at some stage during the invasion process. If cartilage is inhibitory to the TAF, then the production of calcification and ossification may actually aid the tumour in its framework invasion. It is this hypothesis which, it is hoped, will be shown to represent a factor in the spread of cancer within, and through the laryngeal framework.
PART TWO: METHOD
5.0 CHAPTER FIVE

5.1 METHOD OF COLLECTION, FIXATION AND DECALCIFICATION.

The majority of the laryngeal specimens were collected from the operating rooms and fixed in 10% buffered formal saline for at least 48 hours. In the early part of the series these larynges were then processed further by slicing. (See below.) Later it was found that better slices were made if the whole larynx was decalcified before slicing. In the latter part of the series, where tissue was required for biochemical assay, the larynges were collected fresh from the patient and immediately deep-frozen. The frozen larynges were then sliced in the same manner. Following the collection of tissue specimens for biochemical assay, the tissue slices were fixed in 10% buffered formal saline.

Decalcification was accomplished by the following solution:

\[
\begin{align*}
\text{Sodium citrate} & \quad 120g \\
\text{Formic acid (90%)} & \quad 350 \text{ ml} \\
\text{Distilled water} & \quad 608 \text{ ml}
\end{align*}
\]

The sodium citrate is dissolved in the distilled water and the formic acid added. It was found that the average larynx including the hyoid bone was decalcified in four days. At first it was my practice to dissect off the hyoid bone⁴⁴, and then to cut the undecalciﬁed larynx as described below; but later it was found that decalcification of the whole specimen improved the quality of the tissue slices, and did not impair the staining capa-
bilities of the histological sections. There is also a distinct advantage in not dissecting off the hyoid bone in the case of tumours which involve the pre-epiglottic space (PES). In the case of larynges which were cut either fixed and undecalcified, or unfixed and frozen, the tissue slices were fixed and then decalcified in the formic acid solution. In this case 2 days was usually enough. Nitric acid was abandoned as a decalcifying medium because of the poor histological sections resulting from this method.

5.2 TISSUE SLICING AND HISTOLOGICAL PREPARATION

The larynges were sliced transversely in a slicing machine. The machine used was an Excell/Boston 10 inch gravity slicing machine (supplied by Staines Group Catering equipment Ltd., 15-19 Brewer str., London W1R 9FL.) which was supplied with special grinding equipment to keep the blade sharp. Slicing was carried out transversely starting with the epiglottis. The machine was set for cutting slices at 4mm thickness by turning the wheel regulating the distance between the circular blade and safety plate. (Figure 6.)
Figure 6. The Excell/Boston 10 inch gravity slicing machine: Above: The machine showing the circular blade and sliding tray. The supplied holder on right was not used. Below: The technique of slicing a laryngeal specimen.

The specimens were held in the right hand wedged firmly against the vertical plate on the movable tray so that the posterior surface was down-
wards, and so that the epiglottis was exposed for the first slice. (Figure 6.) Sequential sections were cut by sliding the movable tray sharply against the moving circular blade with the left hand. (figure 6.) The cut slices were then carefully orientated with the superior surfaces uppermost. A sequence of slices produced in this way was easily reconstructed to determine their relationship to the whole larynx. (Figure 7.) The slices were then laid out in a special perspex tray with numbered compartments and a macroscopical report was made on each larynx. The report focused on the site and degree of spread of the tumour. An accurate description of the 3-dimensional extent of the lesion was achieved by this method. The frozen specimens were fairly easily cut in the same way, without having to remove the hyoid bone. The best quality tissue slices were, however, obtained after first decalcifying the whole specimen.

The tissue slices were then each photographed using a Chinon CE-5 SLR camera (Chinon Industries, Inc. Tokyo, Japan) fitted with a Pentax 50mm F4 Macro lens. (Asahi Optical Co., Ltd, Japan.) The camera was mounted on a copy stand with a tungsten light source. In all cases Ilford 125 ASA monochrome film was used to produce black and white print photographs. In some cases colour slide Kodak Ektachrome 160 ASA Tungsten film was used. Colour prints were obtained from the colour slides so produced.
Figure 7. A sliced laryngeal specimen, left sided supraglottic tumour: Above: The reconstructed larynx, below: The slices laid out, arranged with the superior surfaces uppermost.

The camera was fitted with a digital Data-imprinting unit (DP-520) also made by Chinon, so that each negative or slide was imprinted with the
name, hospital number, hospital, and histology number. (figure 8). When the black and white prints were produced each therefore bore the patient’s data. Using a sharp scalpel, representative tissue blocks were then cut from the tissue slices, and these blocks were then carefully mapped out on the photographs, using a felt-tip pen. (Figure 8.) The prepared and stained histological sections could therefore be orientated by matching them to the photographs relating to the tissue slices from which they were taken. In this way a 3-dimensional picture was built up for each specimen, relating not only to the macroscopical appearances, but also to the histological findings.

The tissue blocks were inserted into standard histology laboratory (Labotec) tissue capsules and processed in the labotec automatic tissue processor. The largest size tissue block which could fit in the capsules was 30mm×25mm which was usually large enough. In some cases the blocks taken were larger than this, and, in these cases the blocks were bisected and placed in two capsules. Each block was labelled according to the number of the tissue slice from which it was taken. When there was more than one block per slice, they were subscripted "a" and "b" and so on. The blocks were then embedded in paraffin wax, and sections cut at 4 microns using a standard microtome. The sections were stained in haematoxylin and eosin in the usual way, and in some cases, Masson's stain.

5.3 BIOCHEMICAL ASSAY

Specimens were taken for biochemical assay from the frozen specimens. After cutting the specimens and photographing the slices, tissue was taken and again deep-frozen immediately. Specimens were taken in the following
way; tumour tissue, control tissue (usually from the opposite side of the larynx which was not involved with tumour) and extrinsic muscle from the outside of the larynx.

Figure 8. Correlation of histology slides with photographs of the tissue slices: The glass slide has been superimposed over the photograph to show the correlation of the section to the whole tissue slice. Above; an example of a left sided pyriform fossa tumour invading the left paraglottic space. (Massons stain.) Arytenoid = "A". Below; a right sided ventriculo-saccular tumour; the tumour is clearly seen coming off the right saccule (S). (H&E.)
5.3.1 BIOCHEMICAL ASSAY FOR ALKALINE PHOSPHATASE.

Tissue extraction

Each tissue sample was cut into fine pieces and homogenised with 5 volumes 20mM Tris-HCl (pH=7.0) in a Potter-Elvehjem homogeniser and then in an Ultra-Turrax homogeniser. This crude homogenate was centrifuged at 200g for 5 minutes in a Hettich Universal 28 centrifuge and aliquots of the supernatant used in the assay below.

Enzymatic assay

The assay method employed was a modification of that of Bowers and McComb. Into a cuvette were added 2.7ml of 0.84M ammonium buffer, pH 10.2; 0.2ml 60mM p-nitrophenol phosphate in 1.5mM MgCl₂ as final concentrations in the reaction mixture. The absorbance was read at 404nm on a Perkin-Elmer Spectrophotometer and the results were given in terms of Units/g, where 1 Unit is defined as the amount of enzyme which converts 1μmol of substrate to product per minute under the conditions specified.

Electrophoretic separation of alkaline phosphatase isoenzymes

The procedure for the separation of alkaline phosphatase isoenzymes uses a continuous Tris-borate buffer on a 5% acrylamide gel. Tissue extraction: One gram of tissue was homogenised with 5.0ml of 0.25M sucrose (1 in 6) at 0°C and centrifuged for 10 minutes at 15 000g in a Beckmann J21-C centrifuge. From the crude homogenate, 4.0ml of the supernatant was stirred in an ice bath and 0.8ml butanol was slowly added over a period of 15 minutes and stirring continued for a further 30 minutes. The mixture was then centrifuged at 38 000g in a Beckmann 18-70M Ultracentrifuge for 30 minutes at 0°C, and the bottom layer taken out for electrophoresis.
The buffer and gel solutions were as follows:

Gel buffer- 45,5g Tris were dissolved in 900ml distilled water to which was added 1.2ml NNN'N'-tetramethylethlenediamene (Temed). The mixture was brought to pH 9,5 with boric acid and the whole made up to a final volume of 1,0l.

Running buffer- this buffer was prepared as described above but with the omission of Temed. This buffer was diluted one in four with distilled water for use in both electrode vessels.

Running gel monomer- 19,0g acrylamide were added to 1,0g BIS as this was dissolved in 100,0ml distilled water. Persulphate - 0,2g ammonium persulphate were dissolved in 100,0 ml water. All of the above solutions were kept in the dark at 4°C and were stable for a period of 4 weeks except for the persulphate which was prepared fresh at the beginning of each electrophoretic run. The gel solution was prepared by first warming to room temperature one volume of monomer solution, two volumes of persulphate and one volume of Tris-borate-Temed buffer. These solutions were then mixed and placed directly into the cassettes of either the electrophoretic systems used. Polymerisation was complete in 30-45 minutes and gels used on the same day.

In all experiments a control serum (50,0ml) was run together with variable amounts (10,0-60,0µl) of tissue extract in order to determine an optimal volume (eventually found to be 50,0µl) which would produce sharp, discrete enzyme bands. Initially these gels were run on the Pharmacia Gel Electrophoresis Apparatus GE 2/4 and subsequently on the LKB 2001 Vertical Electrophoresis System. Each system was run at a constant current of 10,0mA per gel for 150 minutes at 10°C.
Collagenase assay was performed on two of the specimens. The tissue was collected in the same way as for the alkaline phosphatase assay. The method of collagenase assay used was one employing chromogenic substrate. The chromogen is PZ-Pro-Leu-Gly-Pro-D-Arg which is cleaved by collagenase at the Leu-Gly binding site.

To 0.5ml prepared tissue extract, 1.0ml of 0.5M Tris-HCl buffer pH 7.8 containing 0.1M CaCl2 was added. Since the substrate was only available in crystalline form, 10.0mg PZ-Pro-Leu-Gly-Pro-D-Arg were dissolved in 0.2ml methanol and the whole made up to 10.0ml with 0.1M Tris-HCl buffer pH7.8 in order to give a substrate concentration of 1mg/ml. To the tissue extract and buffer solution, 1.0ml of the soluble substrate was added.

The reaction mixture was incubated for one hour at 25°C with gentle agitation, following which the reaction was stopped with 1.0ml 10% citric acid. This was followed by the addition of 5.0ml ethyl acetate and the whole mixture shaken intermittently in a vortex mixer for 15 minutes. Finally, the top layer was removed (3.0ml) and read at 320 nm in a LKB Ultraspec 4050.

Since a standard curve was required, collagenase from Clostridium Histolyticum was used. The concentrations of collagenase used in the standard curve ranged from 0.25μ-2.5μ.
5.4 EXAMINATION OF HISTOLOGICAL SECTIONS

The histological sections were examined and compared to the photographs. In this way the histological appearances were correlated with the macroscopic patterns of spread of the tumour. When further histological staining or sections were required, this was possible by finding the relevant tissue slices, which were stored in formalin. The photographs were used to identify the correct slice. The tissue blocks were also filed, and so additional sections were readily available from this source. The advantage of this method is that all the tissue is still available for further study, after the larynx has been completely sectioned.

The data from each specimen was entered on to a data card which contained some clinical information as well. The clinical information was obtained in most cases from the hospital notes, but many of the patients were my own and in such cases the information was entered directly from my own observations.

Using the data cards, 22 variables were entered into a computer program. The University mainframe IBM computer was used, employing the SAS data analysis software. A program was written with the help of the computer centre staff to enable an interactive, full-screen (FSP) data entry and display facility. The variables were grouped into 3 types; information about the patient, information about the tumour, and clinical information:

PATIENT INFORMATION

1. Sex - entered as M or F

2. Age entered in years
3. Hospital

4. Hospital number

5. Histology number

6. Ethnic group

**TUMOUR INFORMATION:**

7. Site of tumour - entered as either: Supraglottic, glottic, subglottic or hypopharyngeal.

8. TNM - The pathological TNM classification, entered as pT1a, pT1b, pT2, pT3 or pT4.

9. Differentiation of the tumour - entered as "poor", "moderate", "well" or "anaplastic".

10. Cartilage invasion (framework invasion) was entered in a code depending which of the cartilages were histologically invaded. (see figure 17, part three - results pp. 105,106)

11. Tongue invasion - entered as "yes" or "no"

12. Muscle - strap muscle invasion entered as "yes" or "no"

13. Cricothyroid membrane penetration - entered as "yes" or "no"

14. Pre-epiglottic space (PES) invasion - "yes" or "no"
15. Paraglottic space (PGS) invasion - "yes" or "no"

16. Transglottic spread - spread of tumour across the ventricle - "yes" or "no"

17. Ventriculo-saccular origin of the tumour - where there was histological evidence of this, entered "yes" otherwise "no"

18. Thyroid gland invasion was entered as "yes" or "no"

CLINICAL INFORMATION:

19. Neck dissection: when a neck dissection had been performed and the nodes were histologically positive, "POS", if they were negative; "NEG" was entered. If no neck dissection, "NO" was entered. If a patient subsequently developed nodes, this was treated as "POS".

20. Pre-op TNM: The clinical TNM classification; entered as T1a or T1b, T2, T3 and T4. The classification given by the clinicians pre-operatively was used.

21. Radiation - only pre-operative radiation was noted, as "YES" or "NO"

22. Follow-up - When reliable clinical follow-up was possible the patients were grouped as follows:

AN - alive without disease

AD - alive with disease

DD - dead of disease
DN - dead of other causes

All these criteria were decided upon after careful examination of the histological slides. By means of various functions of the SAS data program, it was possible to compare and contrast various relationships of these variables one to the other. Graphs and histograms were printed out by the computer after running the relevant programs. The illustrations were drawn from the computer printouts by the Central Graphics division of the University.

The most valuable aspect of the data analysis available on this program was the Chi-squared test. This allowed the statistical analysis of the relationships of various variables, and the simultaneous calculations of the Chi-square ($x^2$) and the "probability" (p) value. Thus it was possible to determine genuine statistical relationships. The total number of relationships between the 22 variables is almost infinite, but the decision of which relationships to evaluate was essentially educated guess-work, based on clinical and pathological experience.

5.5 TETRACYCLINE LABELLING

In the case of two patients, tetracycline was given preoperatively. Three hundred mg. of Ledermycin was given twice a day for 2 days, followed by a 12 day free period, followed by 300 mg b.d. for 4 days.

The larynges of these patients were treated in the same way as described above. After sectioning the larynges in the slicing machine (without decalcification) the sections were prepared in the following manner.
The tissue slices were processed by immersion in 70% alcohol for one day. This was followed by 80% alcohol until the framework turned whitish (about 11 days) and then absolute alcohol for 2 days. This was followed by toluene for two days and then methylnmethacrylate I for one day and methylnmethacrylate II for one day. The slices were then placed in the embedding medium in a 32°C waterbath until set.

The embedding medium was made up in the following way: Benzoylperoxide powder 25g is placed into a 1000 ml graduated container. To this is added 150 ml of dibutylphthalat and 500 ml of methylnmethacrylate to make up 750 ml. This is placed in a stirrer until the powder is dissolved. The liquid is stoppered and kept in the fridge.

The sections were then cut on a Reichert-Jung sledge microtome Model K. (G. Reichert Optische Werke Aktiengesellschaft, Hernalser Hauptstrasse 219, Vienna, Austria.) The sections were then examined under ultra-violet light.

The Clacification rate (CR) was measured by obtaining an average distance between the labelled lamellae (figure 27) using the following formula:

\[ CR = (p \times 2.5) \mu + (d \times n) \mu m/day \]

When \( p \) is the number of points read on the microscope graticule multiplied by a factor of 2.5 to correct to \( \mu m \); and \( d \) the number of days over which the two doses of tetracycline were given, and \( n \) the number of readings.

The percentage of trabeculae which were labelled was measured using a grid-graticule. This was expressed as a percentage by noting the number grid-crossings by labelled trabeculae and dividing by the total number of grid-crossings and multiplying by 100:
\[(LS + OS) \times 100\]

Where LS (Labelled Surface) represents the number of labelled
grid-crossings, and OS (Osteoid surface) represents the total number of
trabecular grid-crossings.

Bone formation rate (BFR) = calcification rate (CR) \times \text{fraction labelled trabeculae}. The units are expressed as $\mu m^3/\mu m^2$/day.
PART THREE: RESULTS
6.1 PATHOLOGY, STATISTICS AND ETHNIC FACTORS.

One hundred and fifty-four laryngeal specimens were examined and included in the series. These were obtained from the Johannesburg, Hillbrow and Baragwanath Hospitals. Patients in either J.G. Strijdom or Coronation Hospitals are normally referred to one of the former 3 for this type of surgery. An additional 4 specimens were examined too late to be included in the statistical analysis but are reported for the alkaline phosphatase values, and tetracycline labelling.

Ethnic Composition of Sample

There were 87 White patients (56%), 60 Black patients (39%) and 7 Coloured patients (5%) who underwent laryngectomy and had their laryngeal specimens submitted between November 1981 and August, 1985; i.e. over a period of 46 months. Please note: in the statistical analyses which follow, with the exception of figure 11, the Coloured ethnic group has been omitted, because of the small sample number. The number of these patients is so small in relation to the total number, that no statistical conclusions could be drawn on this ethnic group.

Age Distribution

The ages of the patients ranged from 33 to 81, with 60 years being the mean age. The mean age was 60 for males and 62 for females. (See figures 9 and 10.)
Figure 9. Frequency bar chart: This shows the age distribution in males and females.

Figure 10. Frequency bar chart: This shows the overall age distribution.
For Black patients the age distribution showed no significant difference from the whole series. (See figure 11.) There was no particular site-predilection in relationship to age.

Figure 11. Frequency bar chart: This shows the age distribution overall and w.r.t. ethnicity

**Sex Distribution**

Twenty-nine (19%) of the patients were female, and 125 (81%) were male. Most of the females were amongst the white patients (82.7%). Only 3 Black females were encountered. There was no difference in the differentiation of the tumours in relation to sex. The females were more or less equally distributed between glottic and supraglottic sites, but there was only one female patient with a hypopharyngeal lesion. (See Figure 12)
Figure 12. Frequency bar chart: This chart shows the relationship between ethnicity, sex & site. (Coloured patients have been omitted because of small numbers).

Differentiation

The differentiation of the tumours was assessed histologically by taking into account the propensity to keratin formation, and the regularity of the prickle-cell layer, as well as cellular characteristics. The most dedifferentiated area of the tumour was used as the definitive area for the purposes of grading. The tumours were graded as being well differentiated, moderately well differentiated, poorly differentiated and anaplastic. Only 3 tumours were adjudged to be anaplastic (1.9%), 41 poorly differentiated (26.6%), 52 moderate (33.7%) and 58 well differentiated (37.5%).
There was no statistical difference in prevalences of differentiation
groups in the ethnic groups, except that all anaplastic tumours occurred
in the Black patients, but this was probably not statistically signifi-
cant. There was no relation between differentiation and sex.

Site distribution

The site distribution can be seen in Table I.

<table>
<thead>
<tr>
<th>Site</th>
<th>No</th>
<th>%</th>
<th>Black %</th>
<th>White %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glottic</td>
<td>46</td>
<td>29.9</td>
<td>18.3</td>
<td>37.9</td>
</tr>
<tr>
<td>Supraglottic</td>
<td>81</td>
<td>52.6</td>
<td>55.0</td>
<td>52.9</td>
</tr>
<tr>
<td>Subglottic</td>
<td>2</td>
<td>1.3</td>
<td>0</td>
<td>2.3</td>
</tr>
<tr>
<td>Hypopharyngeal</td>
<td>19</td>
<td>12.3</td>
<td>16.7</td>
<td>6.9</td>
</tr>
<tr>
<td>Multiregional</td>
<td>6</td>
<td>3.9</td>
<td>10.0</td>
<td>0</td>
</tr>
</tbody>
</table>

TABLE I: Site and ethnic group; Coloured patients omitted.

Six patients had lesions so extensive that they defied classification,
and were therefore classified as "multiregional" as was done by Oleffson
and van Nostrand.² Black patients had significantly less than the expected
number of glottic tumours. (This was statistically significant; p=0.02.)
The Black patients also had almost 40% more hypopharyngeal tumours than
the expected number (p=0.02.) There was however no relative difference
in the prevalence of supraglottic tumours in the different ethnic groups.
When the uncommon sites and hypopharyngeal lesions are removed for sta-
tistical purposes, as well as the coloured ethnic group (only 7 patients),
a more realistic pattern emerges as to the relative prevalences of glottic
and supraglottic tumours in the white and black ethnic groups:

CHAPTER SIX
<table>
<thead>
<tr>
<th>SITE</th>
<th>BLACK</th>
<th>WHITE</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLOTTIC</td>
<td>25%</td>
<td>42.3%</td>
</tr>
<tr>
<td>SUPRA</td>
<td>75%</td>
<td>57.7%</td>
</tr>
</tbody>
</table>

TABLE II: Relative prevalences of the glottic and supraglottic sites in black and white patients, excluding other sites and ethnic groups.

However the only statistically significant feature remains the low number of glottic tumours in the Black ethnic group.

TNM Classification

Table III shows the pathological T (pT) classification, overall and in relation to the main ethnic groups.

<table>
<thead>
<tr>
<th>pT Class</th>
<th>pT1a</th>
<th>pT1b</th>
<th>pT2</th>
<th>pT3</th>
<th>pT4</th>
</tr>
</thead>
<tbody>
<tr>
<td>number</td>
<td>5</td>
<td>2</td>
<td>19</td>
<td>28</td>
<td>100</td>
</tr>
<tr>
<td>overall %</td>
<td>3,2</td>
<td>1,2</td>
<td>12,3</td>
<td>18,1</td>
<td>64,9</td>
</tr>
<tr>
<td>Black %</td>
<td>1,7</td>
<td>0</td>
<td>3,3</td>
<td>26,7</td>
<td>68,3</td>
</tr>
<tr>
<td>White %</td>
<td>4,7</td>
<td>2,3</td>
<td>18,8</td>
<td>9,4</td>
<td>64,7</td>
</tr>
</tbody>
</table>

TABLE III pT-CLASS and ETHNIC GROUP (excluding Coloured ethnic group)

T2 tumours were more common in the white patients, whilst T3 tumours were more common in the Blacks, but T4 tumours were almost equal to the expected values in the ethnic groups. (p=0.0212). In the Black patient sample, 95% were pT3 & pT4, as opposed to 74,1% amongst white patients.

A correlation of pre-op TNM and Post-op TNM classifications reveals that the correlation is good for T1a and T1b lesions. Of the lesions classified as T2 pre-operatively, 40% were upgraded to pT4 pathologically. The T3 lesions had 65% upgraded to pT4. All the pre-operatively diagnosed T4 lesions were correctly assessed, and none were pathologically downgraded.
This shows a very significant underestimation of the tumour spread in T2 and T3 lesions. The reasons for the upgrading will be assessed below, and discussed in detail in Chapter 7.

6.2 FACTORS WHICH GOVERN LOCAL AND REGIONAL SPREAD OF TUMOUR

Spread of tumour in the larynx was assessed in terms of the following parameters which were all introduced as variables in the computer program. The mainframe University IBM computer was used, employing the SAS program for data analysis.

- Differentiation
- FES invasion
- Tongue invasion
- Strap muscle invasion
- Thyroid gland invasion
- Neck node status
- Pre-operative radiation
- Cricothyroid membrane penetration
- PCS invasion
• Transglottic spread

• Ventriculo-saccular tumour

• Framework (cartilage) invasion

Differentiation

There was no significant association between differentiation and site or T-classification. Statistical analyses were done to see if differentiation influenced the ability of the tumour to spread by framework (cartilage) invasion, strap muscle invasion, and tongue invasion. In none of these was there any association between differentiation and spread of tumour in these areas. This analysis was also performed for transglottic spread of tumour; and again there was no association between these lesions and differentiation.

In this series 140 patients were studied in terms of their lymph node status. The neck nodes were graded as histologically positive or negative if a neck dissection had been performed. When no neck dissection had been performed, either at the time of primary surgery or subsequently, the nodes were graded as "NO". For statistical purposes, these were also regarded as "negative". Table IV shows the results:

<table>
<thead>
<tr>
<th>DIFF</th>
<th>Anaplastic</th>
<th>Poor</th>
<th>Moderate</th>
<th>Well</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>0,0%</td>
<td>48,6%</td>
<td>50,0%</td>
<td>65,4%</td>
</tr>
<tr>
<td>Positive</td>
<td>100%</td>
<td>51,4%</td>
<td>50,0%</td>
<td>34,6%</td>
</tr>
</tbody>
</table>

N=133

TABLE IV: NECK NODE STATUS AND DIFFERENTIATION

The only statistically significant associations are those for anaplastic tumours (which had 100% positive nodes) and well differentiated lesions which had only 35% positive nodes. (p=0.07). However, this is only mar-
Finally statistically significant, bearing in mind that there were only 3 anaplastic lesions.

PES invasion

In this series PES invasion has been interpreted as spread outside the larynx. There is controversy about this in the literature, and while the AJCCS (See Chapter 1 page 34) exclude the PES from the borders of the larynx, it specifically mentions PES invasion under the categories and classifies this type of spread "T3". The UICC however, does not specifically mention the PES either under anatomy or under the categories, but also implies that the PES is outside the borders of the larynx. The justification for my use of the T4 category for PES invasion will be presented under discussion. (Part Four).

PES invasion was entered into the computer program as positive or negative and could therefore be statistically correlated with all the other variables. This exercise was performed with site, tongue invasion, strap muscle invasion, cartilage invasion and lymph nodal metastasis.

In the case of cartilage invasion and positive neck nodes, there was no increase in relation to the presence of tumour spread into the PES.

As far as site was concerned, it was suspected that PES invasion would be most common in supraglottic tumours because of proximity. This suspicion was borne out by the facts. See table V below.

<table>
<thead>
<tr>
<th>Site:</th>
<th>Glottic</th>
<th>Hypopharynx</th>
<th>Multiregional</th>
<th>Supraglottic</th>
</tr>
</thead>
<tbody>
<tr>
<td>%age</td>
<td>11.3</td>
<td>2.5</td>
<td>2.5</td>
<td>82.3</td>
</tr>
</tbody>
</table>

TABLE V PES INVASION AND SITE

CHAPTER SIX
This shows that 82.3% of PES invasion originated in the supraglottis. The mechanism of invasion was by direct spread either through the epiglottic cartilage (where the tumour usually passed initially through the fenestra in this structure which contain the tubulo-alveolar mucous glands) or passed lateral to the cartilage through the quadrangular membrane. (Figure 13).

Of all the supraglottic tumours, 61.2% had PES spread, so that in many cases tumours were upgraded to pT4. A statistical analysis shows that 15 T2 tumours, and 32 T3 tumours were found pathologically to have PES invasion, and were therefore categorised pT4. (This represents 48.4% of all T2 tumours, and 41.0% of all T3 tumours.) It is obvious from this that the clinical appreciation of PES invasion is poor.

The correlation of PES invasion and strap muscle invasion was striking. Of the patients with strap muscle invasion, 73.9% had PES invasion as well. This was statistically highly significant (p=0.0004). Conversely, 42.5% of larynges with PES invasion, had cancer spread to strap muscles. This association was also highly significant (Cell Chi-square=4.3). The correlation of PES invasion with tongue invasion was also impressive; nearly one fourth (23.7%) of larynges with spread to the PES also had spread to the base of tongue. This means that, of all the cases with PES invasion, nearly 25% had tongue invasion, and nearly 50% had strap muscle invasion, justifying the T4 category. Of further interest is that, a correlation of tongue invasion and strap muscle invasion shows that there was only an overlap in 3 cases between these two. A further extrapolation of this shows that, of 80 cases with PES spread, 50 cases had either tongue or strap muscle spread, a percentage of 62.5%.
Figure 13. PES invasion: Examples of PES invasion. Above, the epiglottic cartilage has been almost completely destroyed, below, the tumour has passed to the right of the epiglottic cartilage, via the lateral compartment of PES.
PES spread and Transglottic spread

A correlation of these two types of spread shows that of 64 transglottic lesions, 70.3% had PES spread as well. Conversely, of 79 cases with PES spread, 57.0% had transglottic spread. When it is borne in mind that that 81.2 of lesions with PES spread originate in the supraglottis, it is obvious that spread to the glottis and below is common in supraglottic tumours.

Tongue and strap muscle invasion

The significance of these two degrees of spread in relation to PES spread has already been presented. It remains to consider these in relation to other types of spread, and its significance in relation to nodal metastases. The overall figure of positive nodes for supraglottic tumours was 46.9%. While 58.8% of patients with tongue invasion had involved nodes proven pathologically after neck dissection, 23.53% had neck dissections with uninvolved nodes. Patients with strap muscle invasion had a rate of positive nodes of 53.8%. Although these figures are both higher than the rates for supraglottic lesions as a whole, the difference is not statistically significant.

Tongue invasion did not occur in any cases without PES invasion. Strap muscle invasion occurred with PES invasion, cartilage invasion, and cricothyroid membrane penetration. Table VI refers to these figures:

<table>
<thead>
<tr>
<th>site of spread</th>
<th>cricothyroid</th>
<th>cartilage</th>
<th>PES</th>
</tr>
</thead>
<tbody>
<tr>
<td>% muscle invasion</td>
<td>68.7</td>
<td>48.7</td>
<td>42.5</td>
</tr>
</tbody>
</table>

TABLE VI: ROUTES OF STRAP MUSCLE INVASION

As this table shows, the commonest mode of spread to the strap muscles is via cricothyroid membrane penetration. (This was highly significant;
$x^2 = 9.6, \ p=0.0001$.) However strap muscle spread of tumour did not relate to the site of origin per se.

**Thyroid gland Invasion**

It has been common practice to resect the thyroid lobe on the side of the tumour during laryngectomy. How commonly is the thyroid gland involved by direct spread? In this series the thyroid gland was invaded histologically in 3 cases (5.2%). One was in a glottic tumour, 2 in supraglottic, and 4 in pyriform fossa tumours. The only strong association was with the latter site, where this type of spread occurred in 21.6% of cases.

**Cricothyroid membrane penetration**

The strong association of this type of spread and strap muscle invasion has been mentioned. A correlation of site and this type of spread reveals that 50% of cases occurred in glottic tumours. Surprisingly 37.5 occurred in supraglottic tumours. The high association with transglottic lesions (74% of larynges having cricothyroid membrane penetration occurred in transglottic tumours) indicates that supraglottic tumours with transglottic spread are responsible for this unexpectedly high figure. Patients exhibiting cricothyroid membrane penetration did not have a statistically significant higher rate of malignant neck nodes, although a few cases did demonstrate spread to the Delphian node.

Cricothyroid membrane penetration was almost invariably associated with subglottic spread of a glottic tumour, or subglottic spread in a transglottic tumour. One case occurred in a hypopharyngeal lesion. The strong association of anterior commissure involvement and cricothyroid membrane invasion was almost invariable; as was the finding of subglottic spread at this point. See figure 14.
Transglottic spread and FGS invasion

Transglottic spread, that is, tumour having crossed the ventricle, occurred in a total of 64 patients. Seventy-one percent originated in supraglottic tumours, and 29% in glottic tumours. The 6 multi-regional tumours, although they all had transglottic spread were omitted from this estimation, but are included in the 64. Transglottic spread depends upon
spread of tumour into the paraglottic space (PGS). This occurred in 119 patients;

48.5% of these occurred in supraglottic tumours, in fact supraglottic tumours invaded the PGS in 72.5% of the cases. This strong association shows that, in this series, the statement that supraglottic tumours infrequently involve the glottis, is false.

What are the consequences of transglottic spread? The high association with cricothyroid membrane penetration has already been mentioned. The association with cartilage invasion is also high; 75% of cases having cartilage invasion as well. (This association has a cell Chi-square of 7.7, p=0.001.) See figure 19, page 110)

A correlation of transglottic lesions with ethnic group has borne out the clinical impression that these lesions are more common in Black patients. There were significantly more of these lesions amongst these patients; 57.6% of Black patients, and 29.9% of white patients had transglottic spread. (p=0.0027). In view of the fact that there was not a statistically significant difference in the overall pT-classification for the ethnic groups, this phenomenon is not due only to a more advanced presenting lesion. There was no significant association between transglottic spread and differentiation of the tumour. Although 48% of patients with transglottic spread had positive lymph nodes, this was not a significantly higher figure than for lesions without transglottic spread.

Ventriculo-saccular tumours

Tumours of this origin are not always easy to identify, since the features may be lost when the tumour is advanced. Nevertheless, 22 cases from this series showed clear evidence of coming from the ventricular saccular complex. These lesions commonly spread deep to intact epithelium,
burrowing into the false cord area. Tumour is often not evident when looking from above. The tumour enlarges by concentric expansion eventually involving the whole saccular epithelium and ulcerating through to the false cord mucosa. Ten of the 22 cases had spread transglottically. Several of these larynges clearly showed the pseudostratified columnar epithelium of the ventricle or saccule undergoing squamous metaplasia, with areas of carcinoma-in-situ, and invasive tumour in other areas. Figure 15 shows sections taken through the saccule in case number T27. Pseudostratified columnar epithelium has undergone squamous metaplasia with malignant change. There are also areas of carcinoma in situ. Figure 16 shows sections through the saccule of case number T58. Change from pseudostratified columnar epithelium to squamous epithelium, and invasive squamous carcinoma has taken place.

This is presented as evidence that squamous tumours may originate in the ventricle and saccule. The ventriculo-saccular tumours did not occur within any specialised sub-group in terms of ethnic group, differentiation or T-classification. Nine of the 22 cases had positive nodes, and 5 patients had cartilage invasion (less than the overall average.)
Figure 15. Ventriculo-saccular carcinoma - case T27: Low power view (above) and high power view (middle) showing saccular mucosa which has undergone squamous metaplasia and invasive carcinoma. Below: an area of carcinoma in situ. (H&E - Haematoxylin and Eosin)
The status of the neck lymph nodes was recorded as "positive" if the nodes were histologically involved and "negative" if a neck dissection had been performed.
performed, but the nodes were histologically uninvolved. If a subsequent neck dissection became necessary and the nodes were involved, the status was also recorded as "positive". If, however, no neck dissection was performed, the nodes were recorded as "No". For statistical purposes, this has been taken as synonymous with "negative".

In this series 140 cases could be studied with regard to their neck-node status. Of these, 76 (54.2%) were negative, and 64 (45%) were positive. Statistical correlations were performed for Site, T-classification (pre- and post-operative), Strap muscle invasion, Tongue invasion, Cricothyroid membrane penetration, Pre-operative Radiation, Transglottic spread, Cartilage invasion and PGS and PES invasion.

The only variables of the above which had any correlation with neck nodes at all were SITE and T-CLASS.

SITE

Site was the overall most important determining factor regarding neck nodes. These results are shown in Table VII, below:

<table>
<thead>
<tr>
<th>Site</th>
<th>hypopharynx</th>
<th>supraglottic</th>
<th>glottic</th>
</tr>
</thead>
<tbody>
<tr>
<td>% pos nodes</td>
<td>86.7</td>
<td>47.2</td>
<td>33.3</td>
</tr>
</tbody>
</table>

TABLE VII POSITIVE NECK NODES AND SITE

The most significant of these was the hypopharyngeal site; 86.7% of these having positive nodes ($x^2 = 5.5$). The low incidence of positive nodes in glottic lesions was also statistically significant.
T-CLASSIFICATION

T-class was much less important with the exception of T1, which had a very low incidence of positive nodes. Table VIII shows these results:

<table>
<thead>
<tr>
<th>T-class</th>
<th>T1a</th>
<th>T1b</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>% positive</td>
<td>0</td>
<td>33.3</td>
<td>40.7</td>
<td>48.6</td>
<td>52.9</td>
</tr>
<tr>
<td>Total No.</td>
<td>5</td>
<td>3</td>
<td>27</td>
<td>70</td>
<td>34</td>
</tr>
</tbody>
</table>

n=139

TABLE VIII—NECK NODES VERSUS PRE-OP T-CLASS

Although these figures are not statistically significant because of small numbers and a sparse distribution, it does show a convincing clinical trend which is probably valid.

DIFFERENTIATION

The effect of differentiation on the neck node status has already been presented above. (see table IV p 91)

ETHNIC GROUPS

There was no difference at all between the ethnic groups with regard to neck node status.

FOLLOW UP

As this study was not a clinical one, per se, reliable clinical follow up could only be obtained in 51 patients. The patients were categorised as follows: AN=alive, no disease; AD=alive with disease; DN=dead of other causes; DD=dead of disease. For statistical purposes, those with persistent cancer were grouped together, and those apparently free of cancer were grouped together.
Of the patients with negative neck nodes, 78.3% were free of disease, and 21.7% had persistent cancer after treatment. Conversely, of the patients with positive nodes, 57.1% had persistent disease, and 42.9% were free of disease after treatment. The follow up ranged in time from 4 years to 6 months.

Pre-operative radiation

Pre-operative radiation was administered in 38 cases (27.3%) and 101 cases did not receive any pre-operative radiation (72.7%). In 15 cases information was not available.

The presence or absence of pre-operative radiation had no effect whatsoever on the following variables; cartilage invasion, pathological (pT-) class, strap muscle invasion or transglottic spread. Of great interest, however, was the apparent effect of radiation on the differentiation of the tumour. Of the 38 patients who received pre-operative radiation, only 6 (15.8%) had well-differentiated tumours, 47.4% had moderately differentiated tumours, and 34.2% had poorly differentiated tumours. See table IX, below.

| Diff | poor | mod | well | number=101%
|------|------|-----|------|----------------
| no radiation | 22   | 33  | 45   | %
| * | 21.8% | 32.7% | 44.5% | Cell Chi-square
| 0.5 | 0.4 | 1.7 |
| * radiation | 13   | 18  | 6    | number=38%
| * | 34.2% | 47.4% | 15.8% | Cell Chi-square
| 1.2 | 1.2 | 4.5 |

TABLE IX - RADIATION AND DIFFERENTIATION

As can be seen from this table, the low number of differentiated tumours in the group who received pre-operative radiation is highly significant (cell chi-square = 4.5; p=0.018.) The large number of well differentiated...
tumours in the group who did not receive radiation, is also statistically significant.

As presented above, the presence or absence of radiation pre-operatively had no effect on the prevalence of positive or negative neck nodes. In a correlation of radiation and follow up, however, an interesting feature did emerge; in the group that had radiation, a lower than expected number of cases had persistent disease, 23.5% as opposed to 50% in the no radiation group.

6.3 FRAMEWORK INVASION

All histological sections were carefully examined for any signs of invasion of the laryngeal cartilages. In this series most larynges showed at least early calcification or ossification of the laryngeal cartilages; hence the reference to "framework" invasion. Cartilage per se was almost never invaded; however the mechanisms will be presented in more detail below.

The larynges showing framework invasion and those which did not were almost exactly equally distributed in numbers; 76 (49.4%) showed no invasion and 78 (50.6%) had some degree of invasion. The pattern of invasion was reported in the following way: ARYCR= invasion of arytenoid and cricoid; ARY= invasion arytenoid alone; THYHYOID= invasion of thyroid and hyoid; THYCRI= invasion of the thyroid and cricoid; THYARYCR= invasion of thyroid, arytenoid and cricoid; THYARY= invasion of thyroid and arytenoid; and THY= invasion of thyroid alone. (See figure 17.)

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As can be seen from this the commonest cartilage to be invaded was the thyroid, which was invaded in 37.7% of the whole series of 154 cases. Thyroid was also the commonest cartilage to be invaded alone, 16.2% of the whole series. The second commonest combination was thyroid, arytenoid and cricoid invasion, 10.4% of the whole series, followed by arytenoid alone. The hyoid bone was invaded in 4 cases, 2.6%, and was always associated with thyroid involvement as well.

When the thyroid was involved alone this was either the anterior superior or inferior lamina in the midline. When the thyroid was involved with the arytenoid and/or cricoid, it was the posterior inferior lamina which was invaded by tumour in the posterior crico-thyroid space from tumour in the paraglottic space.
Framework invasion and Tumour Site

Eight glottic tumours invaded the arytenoid alone, as well as 5 supraglottics (all transglottic.) Arytenoid invasion alone was not considered to be suitable for a pT4 classification, so all these cases were classified pT3.

Arytenoid and cricoid, crico-arytenoid joint and/or thyroid cartilages were involved in 15 glottic tumours, and 11 supraglottic tumours (with transglottic spread) and in 9 pyriform fossa tumours that invaded the paraglottic space.

In 16 patients with supraglottic tumours, the thyroid cartilage alone was invaded - this was the upper anterior (midline) portion, and was associated with PES invasion. An additional 4 also invaded the hyoid bone, making up 11.1% of supraglottic tumours with framework invasion. Figure 18 is a map of the pattern of framework invasion in this series.
Figure 18. Map of framework invasion: Each dot represents one case of framework invasion, when the dot lies between two of the structures, it indicates invasion of both.

No one site showed a particular tendency for framework invasion. All specimens with cartilage invasion were classified pT4, except those that involved only the arytenoid.
Framework invasion and Ethnic group

Black patients had more than the expected number of larynges with framework invasion; 63.3% of Black patients and 42.5% of white patients showed this degree of spread.

Framework invasion and Neck Nodes

There was no effect of invasion of the laryngeal framework on neck node status.

Framework Invasion and Strap Muscle Invasion

As presented above, a very significant number of patients, 48.7%, with framework invasion had spread of tumour through the cartilage and into pre-laryngeal strap muscles. Also there was a very strong relationship with transglottic lesions, 75% of these having framework invasion.

Within the bounds of the limited number of reliable follow-up records, framework invasion did not appear to relate to survival or to local control of disease.

Framework invasion in transglottic lesions

As presented above, transglottic lesions were the subgroup with the largest proportion of framework invasion. Of transglottic tumours 75% invaded the laryngeal framework at some point. Conversely, transglottic lesions accounted for 62.4% of all tumours which showed this type of invasion. Figure 19 below shows the proportion of transglottic lesions in the group of patients with framework invasion.
Figure 19. Frequency bar chart: This shows the proportion of transglottic lesions in the group with framework (cartilage) invasion.

Mechanisms of Invasion

Histology

Framework invasion varied from massive through and through invasion with a large amount of destruction of cartilaginous/bony tissue, to micro-invasion. The mechanism of invasion is easier to appreciate in the cases with early invasion as opposed to those with massive destruction.
Almost invariably the ossified cartilage is involved in the destructive process. In some cases, it appears histologically that the cartilage is being eroded, but even in these cases I have doubt that cartilage per se is ever invaded. This will be discussed in detail in the next section.

Focal ossification occurred extensively in the specimens studied. The areas involved generally conformed to the pattern described by Harrison and Denny⁶¹ and Keen and Wainwright.⁶¹ In these areas the normal bone structure was reproduced with striking accuracy; with bone trabeculae enclosing spaces containing fatty and haemopoietic marrow. See figure 20. These spaces usually do not show much activity, but in the presence of tumour nearby an intense stromal reaction is seen - with numerous osteoblasts and osteoclasts.

This effect is often seen even before the tumour has penetrated the perichondrium (Figure 21). These findings have confirmed the findings of Carter et al., where extensive osteoclastic activity is seen eroding away bone trabeculae well in advance of the edge of the tumour. Figure 21 shows the tumour some distance away and separated by periosteum from the hyoid bone which is being eroded. A very reactive stroma is seen, containing mixed inflammatory cells and collagen fibres. The high power view shows numerous osteoclasts (O) actively eroding the bony trabeculae (T). In other cases, relatively few osteoclasts are seen, and predominantly osteoblastic activity is seen (Figure 22). As the process continues, eventually only the fragments of bone are left, surrounded by the reactive zone of tissue (figure 23); later the tumour penetrates the perichondrium and enters the area, to develop into overt tumour penetration of the framework.
Figure 20. Focal ossification of the thyroid cartilage in T66: Note the tumour (T) behind intact perichondrium (P) in the upper (low power) view and the intense reactive tissue between the bony trabeculae (T). (below.) (H&E; low power (LP) above, X160 below)
Figure 21. Hyoid bone in T64: Above (low power) tumour in the PES, middle X160, below X400, H&E. See text for description.
Figure 22. Micro-invasion of the cricoid in T30: Tumour (T) is outside the perichondrium (P). There is predominantly osteoblastic activity. (H&E, LP above, HP below)
In those larynges in which relatively little bony metaplasia has occurred, a process of micro-invasion is seen which appears to give a clue to the mechanism of invasion. Figure 24 exemplifies this mechanism. Here the
tumour is seen as before behind intact perichondrium. In the higher power view (X160/middle) a small area of micro-erosion of the cartilage (C) is seen; this area shows some stromal tissue penetration of the cartilage (S), but at the interface of these two tissues, bone lamellae (L) have formed. In this immediate vicinity chondrocytes have changed to osteocytes. Clearly osteoblastic activity is taking place. In the X400 magnification (below) osteoclasts (O) are seen eroding the apparently recently laid down bone lamellae of metaplastic bone. These appearances suggest that the mechanism of cartilage invasion involves an osteoblastic phase, followed by an osteoclastic phase. That is, cartilage is first changed to bone, which is then eroded by osteoclastic activity by the "host", presumably due to some tumour humoral factors, which eventually allows the invasion by tumour cells.
Figure 24. Invasion associated with early ossification in T52: See text for explanation. (H&E, LP, X160, X400; above-downwards).
Another mechanism of cancer invasion which was more direct was also noted. This confirmed the work of Yeager and Archer. Tumour cells are seen to find a route to the anterior thyroid cartilage via the collagen bundles in the conus elasticus (vocal ligament) and thus reach the area of the framework where there is no perichondrium. (The anterior commissura tendon area.) See figures 25 & 26; this is one example of direct penetration of cartilage apparently without any metaplastic bone formation/resorption.

Figure 25. Macroscopic view of T67: Left, H&E; right, Masson's stain. Outlined area enlarged in next figure.
Figure 26. Invasion of cartilage at anterior commissure in T67: Tumour cells are seen between collagen bundles, (C); above H&E; below, Masson's stain. Cartilage=0. (Both low power)
Biochemical Investigation

The results of the biochemical estimation of alkaline phosphatase (ALP) are given below in Table X.

<table>
<thead>
<tr>
<th>Histology findings</th>
<th>Larynx number</th>
<th>Tumour u/gram</th>
<th>Control u/gram</th>
<th>Ex. mus u/gram</th>
</tr>
</thead>
<tbody>
<tr>
<td>invasion++</td>
<td>T65</td>
<td>1.04</td>
<td>0.146</td>
<td>0.016</td>
</tr>
<tr>
<td>inv. hyoid</td>
<td>T64</td>
<td>1.28</td>
<td>0.136</td>
<td>0.045</td>
</tr>
<tr>
<td>invas. thy.</td>
<td>T66</td>
<td>0.48</td>
<td>0.133</td>
<td>0.013</td>
</tr>
<tr>
<td>invas. thy.</td>
<td>T67</td>
<td>0.32</td>
<td>0.034</td>
<td>0.12*</td>
</tr>
<tr>
<td>no invasion</td>
<td>T69</td>
<td>0.08</td>
<td>0.048</td>
<td>0.014</td>
</tr>
<tr>
<td>no invasion</td>
<td>T70</td>
<td>0.388</td>
<td>0.043</td>
<td>0.011</td>
</tr>
<tr>
<td>CAJ invasion</td>
<td>T72</td>
<td>0.3</td>
<td>0.106</td>
<td>0.06</td>
</tr>
<tr>
<td>Invasion++</td>
<td>T75</td>
<td>0.56</td>
<td>0.091</td>
<td>-</td>
</tr>
<tr>
<td>invas. ary</td>
<td>T76**</td>
<td>0.67</td>
<td>0.355</td>
<td>-</td>
</tr>
</tbody>
</table>

TABLE X: ALKALINE PHOSPHATASE ASSAY IN LARYNGEAL CARCINOMA. ex.mus.=extrinsic muscle, CAJ=crico-arytenoid joint. Ary=arytenoid. Thy=thyroid cartilage. **had tetracycline labelling.

In all specimens examined the alkaline phosphatase was highest in the tumour tissue, intermediate in the control tissue and lowest in the extrinsic muscle. In the first specimen examined, the method of sampling was different, and the results have therefore not been compared with those above; however they do conform to the pattern. Tumour was 1.04 u/gram, tissue containing tumour and adjacent tissue; 0.92 u/gram, and control tissue (opposite side of larynx), 0.008 u/gram. In almost all cases the level of alkaline phosphatase was extremely low in the extrinsic muscle.

*This was the exception T67 - in this case there was found to be tumour invasion of the extrinsic strap muscles and the thyroid gland on histology. The difference in the ALP levels between the tumour and control tissues was statistically significant. (p=0.005)
From these results it would appear that the tumour may be producing the alkaline phosphatase which is diffusing away from the tumour into the normal adjoining tissue. There appears to be a correlation between the alkaline phosphatase levels and the degree of framework invasion in these cases. (Table X)

Isoenzyme studies
The results of electrophoresis performed on several of the alkaline phosphatase samples obtained from the larynx tissue specimens show what appears to be a bone form and not a variant form of alkaline phosphatase.

Collagenase studies
Collagenase activity was measured in two of the laryngeal specimens. In both of these, collagenase activity was 4 to 5 times higher in the tumour tissue than in the control tissue.

6.4 TETRACYCLINE LABELLING

Tetracycline labelling was carried out in two patients to show the activity of ossification in the framework of their larynges. Both larynges showed extensive labelling. (Figure 27.) The label showed a double line in keeping with the interval during which no tetracycline was given. The bone formation rate (BFR) was estimated for specimen T76. Normal levels are given as: Males= 0.56 standard deviation=0.22; females= 0.48 standard deviation=0.19. The left arytenoid (uninvolved with tumour) = 0.43 $\mu$m$^3$/µm$^2$/day. The right arytenoid (involved with tumour)= 0.63 $\mu$m$^3$/µm$^2$/day. The thyroid cartilage = 0.57 $\mu$m$^3$/µm$^2$/day.

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The right arytenoid (figure 27) showed scalloping of the recently laid down lamellae, indicative of active bone deposition and resorption taking place together.
Figure 27. Tetracycline labelling of the right arytenoid of larynx

T76: Above; the tetracycline label (l) demonstrated by viewing under ultraviolet light. Below; the same area seen under polarised light. In both cases the scalloping due to osteoclastic activity is seen (s).
Unfortunately, because of logistical constraints, CT scans were only obtained in 9 cases included in this study. This was due to the severe shortage of CT time available in the Provincial Hospital System, and the fact that the Baragwanath Hospital scanner was of a generation which did not produce body scans of sufficient quality for research purposes on larynges. The Hillbrow Hospital scanner, on which all the scans were done was only installed in late 1984. All the patients scanned were from my own unit, and received their scans on the 4th generation Siemens Somaton DR3 at the Hillbrow Hospital. All the patients were assessed pre-operatively and operated upon by myself or my registrars under my direction.

In general the findings of these scans confirmed and amplified the previous findings of Gregor, Lloyd and Michaels and other workers in this field. The findings will be discussed in the next section, but the following figures and their descriptions will serve to describe the results of this small series.
Figure 28. CT and tissue slice of T27: This scan, correlated with the tissue slice pictured below shows: The outline of the mucosal surface delineated by the air-filled hypopharynx; demonstrating the aryepiglottic folds, (A) and the pyriform fossae (P). The hyoid bone and PES can be seen; the scan shows infiltration of the PES by a decrease in lucency (T). Note the right aryepiglottic fold thickened by tumour. This tumour arose from the right ventriculo-saccular complex.
Figure 29. CT scan and tissue slice of T58: Ventriculo-saccular tumour, transglottic, no cartilage invasion, early calcification/ossification (see figure 14). The scan shows infiltration of the right PGS and calcification in the cricoid/arytenoid complex.
Figure 30. CT scan and tissue slice from T66: Scan shows asymmetry of the airway, invasion of the left PGS (PG) and the PES (PE). The left pyriform fossa (PY) is compressed but the right pyriform fossa (FY) shows well.
Figure 31. CT scan and tissue slice from T69: Level 9: Shows infiltration of the right paraglottic space, note the apparent increase in density of the cricoid/arytenoid complex. (X)
Figure 32. CT scan and tissue slice from T69: Level 10: This scan, taken at bone window levels, shows increased density on the side of the tumour. (X)
Figure 33. CT scan and tissue slice from T59A: T4 Glottic tumour. Invasion of the arytenoid and cricoid cartilages. Scan shows extensive destruction in the cricoid/arytenoid area by a ulcerating tumour with contrast-enhancing edge.
Figure 34. CT and tissue slice from T68: T4 Supraglottic, ventriculo-saccular tumour; no cartilage invasion. Scan shows infiltration of the PGS (P6) and PES (PE). Note both saccules on the tissue slice, and the tumour comming off the right saccule (S).
Figure 35. CT and tissue slice from T65: T4 Glottic tumour invasion of cricoid and arytenoid with much destruction of framework. Scan shows massive destruction of right sided cricoid and arytenoid; infiltration of the right PGS, and an irregular air/tissue interface on that side.
7.0 Chapter Seven: Discussion

7.1 The Method

The method of examination of laryngeal specimens used in this study, which had been reported in 1981,\(^3\) was further perfected, and was found to be a rapid, cost-effective and highly satisfactory method. The method can be used by any standard histopathology laboratory, the only additional requirement being the slicing machine, which is relatively inexpensive, and obtainable from any catering suppliers. It was possible to process a larynx in 7-10 days including producing a report. This contrasts with the serial sectioning methods\(^6\) \(^8\) \(^9\) \(^11\) \(^12\) \(^13\) \(^15\) \(^16\) \(^17\) \(^18\) which require extremely expensive specialized equipment and expertise; with the length of period required for producing a report being several months.\(^9\) One of the criticisms of our method is that it is only possible to section the larynges transversely; it is true to say that there are cases in which coronal or sagittal sections demonstrate certain features more graphically. However, it is my contention that, in general, transverse sections are superior in demonstrating the anatomical features. Figure 36 shows the features of laryngeal anatomy as seen in transverse sections. The sections from above downwards are representative of levels indicated to right and left of the sections in the models of a whole larynx. The following features may be noted on these sections: (See figure 36)

Level 1

Supraglottic area. This section is taken from a level above the thyroid notch. Outside the thyroid cartilage can be seen the strap muscles (S). Inside is the lumen (I) and the pre-epiglottic space (PES). Lateral to this, and divided from it by a sheet of fibrous tissue,\(^49\) are the paraglottic spaces (PGS). Posteriorly, are seen the ary-epiglottic folds.
(a) with the cuneiform sesamoid cartilages. Blood vessels (b) can be seen running in the long axis of the larynx in the PGS.

LEVEL 2

Supraglottic area. This section is taken slightly lower down, at the level of the false vocal cords (f). The saccules are seen in the PGS (sac), and posteriorly are seen the corniculate sesamoid cartilages (c) and the upper end of the posterior commissure. Again the blood vessels in the PGS (b) are seen running longitudinally.

LEVEL 3

Glottic level. This section is taken from the upper vocal cord level. Note the thyro-arytenoid muscles (t) in the PGS running from the arytenoid (ar) to the thyroid cartilage. In the edge of the vocal cord can be seen the crico-vocal membrane (conus elasticus)(ce) running from the vocal processes of the arytenoids to the anterior commissure (v). Between the thyro-arytenoid muscles and the thyroid cartilage laminae, is a layer of areolar tissue, in which run the blood vessels of the larynx (b).

LEVEL 4

Lower glottic level. This section is taken from the area below the free edge of the vocal cords. The cricoid lamina is seen posteriorly (cr) with the crico-arytenoid joints (caj) laterally.

LEVEL 5

Subglottic level. In this section the cricoid cartilage (cr) is shown as a complete ring. The crico-thyroid joints (ctj) with the lower cornua
of the thyroid cartilage are seen. The lateral crico-arytenoid (lc) and posterior crico-arytenoid muscles (pc) are demonstrated.
Figura 36. The anatomy of the larynx as seen in transverse sections: See text for explanation
In general terms the findings of this study demonstrated many factors in common with previous studies.\textsuperscript{2-6 7 9 17 19 31 38} The age and sex distribution was similar to earlier studies. There was no age difference in the ethnic groups. The sex distribution in Black patients showed very few females, but it must be remembered that this series represents a select group; in that it included only the patients who were treated by laryngectomy. This same reservation should be borne in mind with all the statistics related to epidemiology. Only 24 larynges were received from Baragwanath hospital, yet during 43 of the 46 months of the study, 119 new cases of carcinoma of the larynx were diagnosed at that hospital. During the same time 55 new cases were diagnosed at Hillbrow hospital. The average number of new cases, that is the incidence of carcinoma of the larynx in the Black population of the Witwatersrand, is therefore calculated at 48.6 per annum, \((\text{174}/\text{43}) \times 12 = 48.6\). This series of 60 patients therefore only represents about 32\% of the total number of patients. These figures are probably fairly accurate for Black patients, as the majority would be expected to receive treatment at one of the these two hospitals. In the period 1956-1975 Isaacson et al\textsuperscript{1} found the incidence of laryngeal cancer in the Black population of the Witwatersrand to be 18.6 per annum. This represents an increase of 2.6 fold in the incidence in 10 years; even allowing for the population increase the incidence appears to be increasing. Similar figures were not available for the Johannesburg Hospital, and would probably be less accurate in terms of incidence, since more of these patients would be treated in the private sector.

One feature in which this study differs from most of those from Europe and North America, is in site distribution. Our patients included very few subglottic tumours (1.3\%) which is in agreement with most more recent
series, but at variance with Ogura. Glottic lesions were far less prevalent in this series (29.9%) than those from North America and Britain. Olofsson and van Nostrand found 54%, and Lederman found 70%. Prevalences of glottic carcinoma similar to our figure (29.9%) have been reported from Finland (32%), whilst Mediterranean studies have found intermediate figures of 50-60%.

When our patients are divided into Black and White groups (Coloured patients being omitted because of small numbers), it can be seen that the Black patients had only 25% glottic tumours and 75% supraglottic tumours and White patients 42.3% glottic and 57.7% supraglottic. Many findings therefore are that there is a reversal in the prevalences of glottic and supraglottic tumours in our region as opposed to North America and Western Europe, and that the feature is more marked amongst the Black patients. The figures for our White patients are similar to those for Eastern Europe and the Mediterranean regions.

The TNM classification of the patients in this study showed that the Black patients tended to present with advanced lesions. However the proportions of T4 tumours were almost the same for Black and White patients; whilst T3 were more common in Black patients, T2 were more common in White patients. These figures show that, although there is the tendency noted above, there is no marked difference in the TNM staging in the ethnic groups.

Of more interest, however, is the statistical correlation between the pre-op TNM and the pathological (pTNM) classifications. This revealed that 40% of T2 and 65% of T3 were in reality T4-rated tumours. This shows that there is a very significant underestimation of the tumour in the clinical grading of these patients. This may have bearing on the treatment program and the ultimate outcome of the disease. Usually the reasons for upgrading to T4 were framework invasion, PES and tongue invasion,
strap muscle invasion and cricothyroid membrane penetration. These mechanisms are discussed below.

7.3 FACTORS WHICH GOVERN LOCAL AND REGIONAL SPREAD OF TUMOUR

7.3.1 DIFFERENTIATION

In this series, the differentiation of the tumour had no statistically significant effect on the local or regional spread. The only exceptions were the 3 anaplastic lesions which all had positive nodes. This was in agreement with McGavran et al. However, this number is extremely small, and no valid conclusions can be drawn from them. (See also "Spread to regional lymph nodes", p141)

7.3.2 PES INVASION

Traditionally invasion of the PES has been considered to represent a T3 classification. (See Chapter one, page 34.) In this study PES invasion has been designated T4. See also Chapter 6, pp 91-93. PES invasion was frequently involved with tumour spread to tongue or strap muscle (23.7% and 42.5%, respectively.) Spread to these areas was very commonly responsible for upgrading tumours to T4. A very strong case must therefore be made for PES invasion to constitute a T4 grading. However, the clin-
ical appreciation of early PES invasion is poor, and, as the TNM classification is mainly a clinical one, this may create problems in making this diagnosis. Computed tomography (CT) scanning is an effective method of assessing this mode of spread, and should be used more often when there is a possibility of spread into the PES.

I believe these facts justify my use of the T4 category for PES invasion. In only 37.5% of the cases in this series was PES invasion NOT associated with tongue or strap muscle invasion. Furthermore, PES invasion is frequently associated with spread into the paraglottic spaces (PGS). In this series this occurred in 57% of cases, to produce transglottic tumours. The findings of this study is that the two spaces (PES and PGS) are contiguous, and that there is no significant barrier between the two. This is in agreement with Mcguire and Deyal\textsuperscript{13} and at variance with many other authors.\textsuperscript{6,8,12,15,16,17} These findings cast suspicion on the use of the supraglottic (horizontal partial) laryngectomy as an effective cancer procedure on most of our patients. Certainly very few of the cases in this series could have been controlled by such an operation. It is not suggested, however, that this procedure does not have a place for early (T1,T2) supraglottic lesions, as an alternative to radiation therapy.

7.3.3 CRICOTHYROID MEMBRANE (CTM) PENETRATION

The usual route of tongue invasion was via PES invasion, however the commonest route for strap muscle invasion was via the crico-thyroid membrane (CTM), (68.7%). This type of spread occurred with subglottic spread of a glottic tumour (50% of cases of CTM penetration); or transglottic spread of a supraglottic tumour (37.5% of cases of CTM penetration) Anterior commissure involvement was usually associated with subglottic
spread, and CTM penetration. This has confirmed the reports of earlier studies.\textsuperscript{12, 20, 21} Although a few cases demonstrated spread to the Delphian node, there was not a statistically higher rate of positive neck nodes in these cases. This tends to confirm the findings of others.\textsuperscript{2, 31}

7.3.4 TRANSGLOTTIC SPREAD

Sixty-four (47\%) of the patients of this series had transglottic spread in comparison with 22\% of Olofsson and van Nostrand's\textsuperscript{2} cases. Most of these (71\%) originated in the supraglottis; 29\% came from glottic tumours. In 6 cases the tumours were so extensive that their origin remained obscure. Transglottic tumours had a high rate of CTM penetration and framework invasion (75\%). The findings have confirmed the work of Kirchner.\textsuperscript{16}

Transglottic spread appeared to be more common amongst the Black patients (57.6\%) than amongst the White patients (29.9\%); and this was statistically significant. This is all the more remarkable when the fact that supraglottic tumours are more common in the Black ethnic group is taken into account. It further amplifies the findings of how readily supraglottic tumours in our series spread to the PGS.

7.3.5 VENTRICULO-SACCULAR TUMOURS

In chapter one it was shown that the origin of tumours in the ventricular-saccular complex was controversial. (Chapter 1 pages 24 &
30.) In this study, 22 cases showed clear evidence of comming from the ventricles and saccules which were considered to be one region: The ventriculo-saccular complex. In these patients areas of squamous metaplasia and dysplasia with carcinoma-in-situ were seen. In many cases the tumour cells could clearly be seen comming off these areas. (Figures 15 & 16.) This has confirmed the findings of Michaels and Hassman.\textsuperscript{42} These authors found these lesions to constitute 14,5\% of their series; whilst our figure is 14,3\%. Our cases also tended to confirm Michaels and Hassman's findings in that they appeared less aggressive than the average. This was not in agreement with Micheau.\textsuperscript{43} It may be that these tumours are more common than these figures suggest, but the diagnosis is difficult to make when the tumour is advanced. In both this series, and Michaels and Hassman's\textsuperscript{42} the cases were only classified so when there was clear evidence of origin in the ventriculo-saccular complex. Many of the transglottic lesions may originate in this area. My findings confirmed the fact that these tumours are difficult to diagnose in their early stages, since they remain mucosal-covered at first. The patient may present only with hoarseness and a mucosal-covered swelling in the false cord area. The importance of examination of the inside of the ventricles during laryngoscopy is emphasized by these findings. (See Chapter 6, pages 98-101)

7.3.6 SPREAD TO REGIONAL LYMPH NODES

The rate of involvement of the regional lymph nodes was related to only two factors, viz. SITE and T-classification. Hypopharyngeal lesions had an extremely high rate of positive nodes, i.e. 66,7\%. This was in agreement with Harrison.\textsuperscript{36} Supraglottic lesions had nearly 50\% positive lymph nodes (47,2\%) and glottic only one third (33,3\%). This tended to
confirm McGavran's figures, although in my series there was no significant difference in the rate of positive nodes between supraglottic lesions and transglottic lesions. McGavran, however, classified these sites separately so that the supraglottic group did not contain any with transglottic spread. "Transglottic" is not an accepted site classification in either the UICC or the AJC. (See chapter one, pp23, 34) The rate of positive lymph nodes rose with increasing T-classification, but these figures were not statistically significant. (Table VIII.) Differentiation also had a tendency to affect the lymph node status; the more undifferentiated the tumour, the higher the rate of positive nodes, but this was also not statistically significant. (Table IV.) Although the number of cases in which reliable clinical follow-up was available was small (51), positive nodes appeared to have a direct relationship to the outcome of the disease; of those patients with negative nodes, nearly 80% were free of disease at between 4 years and six months follow-up. Conversely, of those with positive nodes, only just over 40% were free of disease at this time.

7.3.7 PRE-OPERATIVE RADIATION

Information on pre-operative radiation was available in 139 of the patients. A very interesting finding was that radiation appeared to induce a "shift to the left" in the differentiation of the tumours (see table IX, page 104). However this finding has to be regarded with caution, as the pre- and post-operative histology was not correlated to see whether or not there a change in the differentiation between the pre-operative biopsy and the histology post-operatively. Even if this were done, representative sections are not always obtained at biopsy since in one tumour there may be areas of different differentiation; so that a discrepancy
in the reported differentiation between the pre-operative biopsy and definitive specimen may occur. Also there may have been a degree of selection in the patients chosen for radiation therapy. In my own unit differentiation did not influence the treatment program and, to my knowledge there is no prevailing protocol in the other units in regard to differentiation in laryngeal tumours. The radiated patients also did not represent a significantly more advanced group on pT-classification. Another finding was that a statistically lower than expected number of patients had persistent disease in the radiated group.

It would be unwise to attempt to draw conclusions on these findings, but the observations remain interesting, and worthy of further study.

7.4 FRAMEWORK INVASION

About half of the specimens demonstrated invasion of the laryngeal framework. The commonest area to be involved was the glottic level; the thyroid cartilage being the usual structure involved. The thyroid was usually involved at the upper or lower midline portion at its angle. The arytenoid-cricoid complex was the next commonest site, with involvement of the posterior inferior lamina of the thyroid related to this area. This pattern basically confirms the findings of previous authors.\textsuperscript{57} \textsuperscript{58} \textsuperscript{59} \textsuperscript{60} Strap muscle was invaded in nearly half of the cases with framework invasion (48.7\%) and this is not in agreement with Harrison's\textsuperscript{58} findings, which suggest that framework invasion \textit{per se} is not sufficient grounds for the T4 grading. The exception to this is where only the arytenoid was invaded, and in this series I have classified these as T3.
Framework invasion was more common in Black patients (63.3%) and in transglottic lesions (75%).

**Mechanisms of Invasion**

Histological examination has confirmed that cartilage which has not undergone calcification or metaplastic bone formation is almost never invaded. These findings have also confirmed the work of Carter who found that early metaplastic bone invasion starts with the tumour separated from the framework by an intact perichondrium. Osteoclastic activity was observed producing scalloping of the bone lamellae. Bennett et al. found that the tumours produced a prostaglandín-like material which stimulated osteoclastic activity. The presence of osteoclastic activity has been of more interest to me as an early feature of framework invasion, particularly where metaplastic bone was not already present. In chapter 6 histological evidence has been presented which shows early osteoclastic activity associated with osteoclastic activity in the framework invasion process.

Biochemical assay performed by us has shown that the tumours appear to produce alkaline phosphatase. The activity of alkaline phosphatase was found to be higher in tumour tissue than in corresponding control tissues, and was lowest outside the larynx, unless the outer tissues were invaded. Electrophoretic studies showed no apparent difference between normal and cancerous tissue; the mobility of the major band of activity appeared to correspond to that of the bone form of alkaline phosphatase. Alkaline phosphatase activity seems to be associated with active deposition of bone and the enzyme may act by hydrolysing pyrophosphate, which is an inhibitor of bone mineralisation. There is a possibility that 2 enzymes with pyrophosphatase activity may exist, one identical to alkaline phosphatase (ALP), and one a separate protein. It has been suggested that ALP in general may play a central role in controlling DNA synthesis. The
enzyme is capable of dephosphorylating thymidylate which may impair DNA synthesis.

Tumour forms of serum alkaline phosphatase have been found in bronchogenic carcinoma.\textsuperscript{182} This isoenzyme is similar to the placental form of ALP, but quite different to the liver form; and has been found in breast, genitourinary and gynaecological cancers.\textsuperscript{101} Another isoenzyme has been found in hepatoma.\textsuperscript{101} No previous report has been found of a bone type of ALP produced by squamous carcinomas of the larynx.

Further evidence of intense osteoblastic activity was shown by the tetracycline labelling in two cases with framework invasion. The presence of active osteoblastic activity coupled with osteoclastic activity and scalloping of the recently laid down lamellae was shown. Estimations of the bone formation rate (BFR)\textsuperscript{15} showed increase in the arytenoid involved with tumour as opposed to the arytenoid on the opposite (uninvolved) side. Normal BFR rates are not available for the metaplastic bone of the laryngeal framework. The normal figures quoted in Chapter 6 (p121) are from Melsen and Mosekilde\textsuperscript{85} and are for large bones. It may be that the figures obtained are higher than normal for the larynx.

The evidence tends to suggest that as part of the mechanism of framework invasion, tumours produce various substances which stimulate "host" cells to alter the framework in such a way that erosion of the bony lamellae by osteoclastic activity is a preliminary step to penetration of the framework. An important step of this process appears to be one of intense osteoblastic activity, in which alkaline phosphatase, presumably produced by the tumour, may play a part. This process may explain the often observed fact that cartilage per se is not invaded. This may be because the cartilage is first mineralised or ossified under stimulation by humoral factors, some of which may be produced by the tumour, and is then
eroded mainly by means of osteoclastic activity mediated by a prostaglandin-like substance.

Another mechanism, confirming the work of Archer and Yeager," was identified. In this mechanism tumour gains entry to the framework by following the collagen bundles where fibrous structures attach to the framework. These areas are devoid of perichondrium.

It is clear that tissue invasion by tumours is a complex process, involving humoral factors produced by the tumour, "host" cells and probably many other unidentified factors. It would appear that the above findings may explain the findings of increased ossification, as seen on computed tomography, in the framework closely related to laryngeal tumours. This may be a marker of early tumour invasion, or of aggressiveness of a laryngeal lesion.

7.5 COMPUTED TOMOGRAPHY OF THE LARYNX.

As indicated in the last chapter, correlation with pre-operative CT scans was only possible in 9 cases. Also, the findings of this small series did not materially differ from those of our original paper and those of other workers in the field. (See Chapter 4.) The exception to this is the enquiry into the finding of an apparent increase in density of the framework adjacent to tumour sometimes seen in scans. (Figures 29, 31 & 32) The explanation for this finding now appears to be the osteoblastic changes described above.

The findings will be summarized in the next section; "Conclusions".
8.0 CHAPTER EIGHT- CONCLUSIONS

8.1 THE PATHOLOGY OF CARCINOMA OF THE LARYNX AND HYPOPHARYNX

1. Supraglottic carcinomas are relatively more prevalent than glottic carcinomas in the Black population studied compared with Europe and North America. The prevalence of supraglottic carcinoma in the white population of the Witwatersrand is similar to that reported for the Mediterranean regions.

2. There was no marked difference in the presenting T-classification between the Black and White ethnic groups, but Blacks tended to present later in the disease process.

3. In all ethnic groups there was a poor appreciation clinically of the extent of the tumour, with over one third of T2- and T3-rated cases being in reality T4 tumours.

4. The incidence of carcinoma of the larynx in the Black South African of the Witwatersrand has increased 2.6-fold in 10 years and this appears to represent an increasing incidence of laryngeal cancer as reported elsewhere.²

5. A method of examination of the larynx should comprise serial sectioning as part of its technique; the method described in this thesis is quick, cheap, and easily performed in any histopathology laboratory.
8.2 FACTORS WHICH GOVERN LOCAL AND REGIONAL SPREAD

1. PES (Pre-epiglottic space) invasion was common in supraglottic tumours, and associated in more than half of the cases with base of tongue and strap muscle invasion; and in more than half with transglottic spread.

2. The invasion of the PES therefore justifies a T4 classification.

3. The horizontal partial laryngectomy should be regarded with caution in the management of all but the earliest of supraglottic tumours.

4. CTM (Cricothyroid membrane) penetration was the commonest route of tumour to the strap muscles and occurred equally readily in subglottic spread from glottic and supraglottic tumours.

5. Transglottic spread occurred in half of the series; and was associated with CTM penetration and framework invasion. This occurred more commonly in the Black patients.

6. There was definite evidence of tumours arising in the ventriculo-saccular complex. These lesions may be difficult to diagnose because they remain submucosal and the ventricular cavity should be carefully examined during laryngoscopy in order to detect these lesions.
1. Framework invasion occurred mainly at the glottic level and exclusively involves calcified or ossified cartilage.

2. This type of spread was highest in the transglottic tumours and was associated with a high degree of penetration through to strap muscles, justifying the T4 classification.

3. Framework invasion was associated with osteoblastic activity as seen histologically and measured by tetracycline labelling in two cases.

4. In 10 patients studied, the tumour appeared to produce alkaline phosphatase (ALP) of a bone type; the levels of ALP were lower in control tissue from the larynx, and lowest outside the larynx, except where tumour had spread through to the extra-laryngeal tissues.

5. Osteoclastic activity with characteristic "scalloping" of the bone trabeculae occurred hand-in-hand with the above features; while tumour remained outside the perichondrium. Penetration of the perichondrium occurred as a late feature.

6. The results suggest the presence of a mechanism for invasion of the laryngeal framework which involves an osteoblastic phase; which is probably under at least the partial control of tumour-produced ALP; followed by an osteoclastic phase.

Chapter Eight- Conclusions 150
8.4 COMPUTED TOMOGRAPHY OF THE LARYNX

1. Computed tomography is presently the imaging method of choice for the larynx but in future will probably be enhanced by magnetic resonance imaging (MRI).\(^{123, 124}\)

2. The method is effective in showing the PES and PGS as well as the framework. Mucosal surfaces, particularly at the anterior commissure and subglottis are effectively displayed.

3. The presence of a localized increase in density of the framework, particularly the arytenoids, in close apposition to tumour is usually associated with early invasion of these structures by tumour. The mechanism appears to involve the production of ALP by the laryngeal tumour, resulting in active bone deposition. This feature may be a marker of tumour aggressiveness, and, since the osteoclastic phase is mediated by prostaglandin, this tendency could be delayed by the use of prostaglandin E\(_2\) antagonists such as indomethacin. This drug may therefore have a therapeutic indication in laryngeal cancer.
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Examination of the larynx in the histopathology laboratory

L Michaels and RT Gregor
Examination of the larynx in the histopathology laboratory

L Michaels and R T Gregor

From the Institute of Laryngology and Otology, University of London, 339 Gray's Inn Road, London WC1X 8EE, UK

Summary A method is described for examination of the larynx in the histopathology laboratory. Using a slicing machine, transverse slices of the whole larynx are obtained from which representative histological samples may be prepared. This method offers the advantages of a complete gross examination of the normal and pathological structures of the larynx supplemented by histological studies using any of the methods of paraffin embedding, frozen section, plastic embedding, or electron microscopy on any part of the larynx.

The histological examination of the larynx presents problems that are related to the complex anatomical configuration of that organ. To obtain an adequate picture of the extent of spread of tumour in the laryngectomy specimen multiple sections of the whole specimen are required. This has been achieved by the whole organ serial sectioning method. In this method the whole larynx specimen is cut serially after decalcification and embedding in celloidin1 or paraffin wax. The coronal plane is favoured for the serial sectioning of most laryngeal tumours; epiglottic tumours are sectioned serially in the sagittal plane.

Serial sectioning of the larynx is, however, far too time-consuming for use in most histopathology laboratories. The method necessitates prior decalcification of the whole organ, a process that requires longer exposure to acid than with smaller blocks of tissue, and therefore leads to inferior histological appearances. By this method also the opportunity for gross study of special areas is lost, and the application of modern histological methods, such as frozen sections, plastic embedding, and electron microscopy is not possible. Whole organ serial sectioning requires long periods of embedding which do not suit the clinical need for a reasonably quick laboratory assessment of the degree of tumour spread.

In a recent 'improved method' of laryngeal examination three large vertical blocks of tissue are taken by sagittal section through tumour and adjacent larynx. We have used this method extensively and found it to be unsatisfactory in certain respects. It does not allow an adequate gross study to be made of the tumour in the larynx, particularly in the case of large or posteriorly situated tumours. The close relationship of the thyroid aea, the cricoid lamina, and the arytenoids in this part of the larynx makes it difficult for well-aligned slices of tissue to be cut by vertical section in this area. The need for whole organ decalcification again results in histologically inferior results. We have tried cutting the strips of larynx for this method on a band-saw before decalcification with resultant improvement of histological appearances but with the separation of mucosa from cartilage due to the method of cutting. Even when decalcified as a whole, the larynx is still tough and elastic, and gross sectioning requires a very sharp knife which does not stay sharp for long. The large, vertically placed slabs of ossified cartilage in proximity to tumour and mucosa make satisfactory microtomy difficult as the softer tissues contract away from the harder ossified cartilage during processing. It is not easy to study intrinsic laryngeal muscles by this method.

The recent introduction of computerised tomography allows a series of horizontal radiographs of the larynx to be taken at 5 mm intervals. In order to correlate the appearances of such radiographs with pathological changes we have recently turned to horizontal slicing of larynges at similar intervals. We have found a slicing machine to be an ideal means of producing such sections. With this machine a complete gross picture of the tumour in situ in the larynx can be obtained, and very satisfactory histological studies may be carried out in the material so sliced.
Method

The larynx is fixed in 10% buffered formal saline for at least 48 hours. It is then opened by a vertical cut along the midline of the posterior surface, and the lesion is photographed (Fig. 1). After the gross appearances have been described the hyoid bone is carefully dissected off the larynx. If tumour is seen in the pre-epiglottic space, either grossly at this stage or microscopically at a later stage, the hyoid is sectioned transversely by sawing and sampled for histological examination.

![Fig. 1 Gross specimen of carcinoma of larynx. The larynx has been opened by a vertical cut along the midline of the posterior surface and kept open by a glass rod inserted at the lower end. Note the exophytic tumour extending from the epiglottis to the laryngeal ventricles on each side. The right lobe of the thyroid is attached to the specimen and can be seen projecting from the posterior edge of the right thyroid larynx.](image)

The larynx is then sliced transversely in a slicing machine. The machine used by us is an Excel/Boston 10 inch gravity slicing machine.* The machine is supplied with special grindstone equipment for sharpening the circular blade. Slicing is carried out transversely starting at the tip of the epiglottis. The machine is set for cutting slices of 4 mm thickness by turning the wheel regulating the distance between the spherical cutting blade and the safety plate. Four millimetres is the maximum thickness of a block of tissue that can be inserted into a tissue capsule for embedding. The larynx is held in the right hand at its inferior end and wedged firmly against the vertical plate on the movable tray so that its posterior surface is downward. The tip of the epiglottis is exposed for the first slice. The holder supplied with the instrument is not used. Slices are produced by sliding the movable tray sharply against the moving circular blade with the left hand (Fig. 2). When each slice is cut it is carefully orientated so that it represents a view of the specimen as previously seen from above. A sequence of slices is easily produced in this way, representing the whole larynx by sections which are usually smooth and even (Figs 3 and 4). Occasionally a section from a heavily ossified larynx may become wedged after cutting between the cutting blade and the back metal safety plate, but this may usually be removed intact by widening the space between the cutting blade and the safety plate. The slices of larynx are laid out and identified by a letter in sequence, and each one is photographed by a Polaroid CU5 Land Camera with a 3-inch lens and 1:1 frame using Polaroid Type 107c film. Each slice is examined, and representative blocks are taken for histological study using a sturdy scalpel with a fresh disposable blade. This is usually sufficient to cut through the 4 mm thick slices of ossified cartilage. The exact position of each block taken for microscopy is marked by drawing corresponding lines with a black felt pen on to the Polaroid photograph. The tissue blocks are carefully orientated by marking the reverse surface to that to be cut after paraffin embedding with India ink. The blocks taken for microscopy are decalcified, processed, and embedded in paraffin wax, and sections are cut at 4 microns and stained in the usual way. Each histological section

*Supplied by Staines Group (Cutting Equipment) Ltd, 15-19 Brewer Street, London W1R 3FL.
Fig. 3 A series of slices from a larynx with a small right glottic carcinoma. The slices are labelled A to E. The laminae of the thyroid cartilage form a V-shaped anterolateral boundary on each slice. The right lobe of the thyroid is present in each slice; it shows nodular colloid changes and in C a calcified nodule. 1, epiglottic cartilage; 2, dished laryngeal sacro-ana; 3, cuneiform cartilage; 4, corniculate cartilage; 5, arytenoid cartilages, partially ossified; 6, tumour; 7, cricoarytenoid joints.

is easily related to its origin in the original tissue slice by placing the section on to the corresponding Polaroid photograph (which is the same size as the original tissue slice) so that it fits into the shape made by the felt pen lines. Selected areas of tissue may also be subjected to frozen sectioning, plastic embedding, or processing for electron microscopy as required.

Discussion

By slicing the fixed larynx with a slicing machine, material for an accurate gross study of the larynx is provided quickly and easily (Fig. 5). The following normal structures may be identified in the tissue slices: epiglottis, laminae of the thyroid and cricoid
cartilages, corniculate and cuneiform cartilages, ventricular folds (false vocal cords), ventricles and sacculae, vocal folds (true vocal cords), inferior horns of the thyroid cartilage, cricarytenoid joints, cricothyroid joints, and arch of the cricoid cartilage. Any small structure that is not displayed on the surface of a block for microscopy may be included in a paraffin block and can be subsequently displayed in histological sections by cutting down on to the required area during microtomy. Portions of hypopharynx that are removed with the larynx can be studied in the horizontal sections and the method is particular suitable for hypopharyngeal carcinoma that has been treated by pharyngolaryngectomy.

In addition to the normal structures mentioned above, the intrinsic laryngeal muscles may be conveniently displayed and sampled for histological examination by this method. One of us (LM) has studied the laryngeal muscles in routine postmortem larynges and in postmortem cases of autonomic failure with multiple system atrophy (Shy-Drage syndrome) in which there was laryngeal muscle abductor muscle palsy. In this study the intrinsic laryngeal muscles were each separately dissected out and sampled for histology. More recently, postmortem laryngeal intrinsic muscles from a further case of the latter condition have been examined and sampled by slicing the larynx with a slicing machine.
The individual intrinsic muscles were easily recognized in the transverse sections so produced. The method proved especially valuable in providing serially cut slices of the atrophic posterior cricoarytenoid, a specific feature of autonomic failure with multiple system atrophy.

Larynges with neoplastic growth can be satisfactorily studied by this method and an accurate picture built up based on gross and microscopic examination of the relationship of the tumour to normal structures. This is a far less laborious procedure than the serial section cutting method. The tissue blocks taken for histology need little decalcification time as they are small so that the stained sections show minimal harmful acid-produced effects. Mucosa seems to adhere to cartilage better by such transverse cutting. Blocks may be taken from the transverse slices for a wide variety of modern histological, histochemical, and electron microscopical procedures. A report may be issued on the specimen within eight days of the laryngectomy operation. The 4 mm slices used in this method provide a thickness close to the 5 mm section obtained with computerized tomography. We are at present using this method to help in the interpretation of the computerised tomography scans.

We are indebted to Professor DFN Harrison for suggesting the use of the slicing machine in the examination of the larynx and for his encouragement and advice in the development of the method described.

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Graedel GM, Michaels L, Bannister Sir Roger, Gibson W.

Pathology of the intrinsic laryngeal muscles. *Clinical Otolaryngology*; in press.

Requests for reprints to: Professor L Michaels, Department of Pathology and Bacteriology, The Institute of Laryngology and Otology, 330/332 Gray’s Inn Road, London WC1X 8EE, UK.
COMPUTED TOMOGRAPHY OF THE LARYNX: A CLINICAL AND PATHOLOGIC STUDY

R. T. GREGOR, FRCS, G. A. S. LLOYD, DM, FRCR,
and L. MICHAELS, MD, FRCPath, FRCP (G)

Since its introduction in England in 1971, computed tomography (CT) has had a profound effect on the neuroradiologic diagnosis of intracranial tumors. CT in ear, nose, and throat practice was first applied to the investigation of acoustic tumors, but with the introduction of coronal sectioning in conjunction with the body scanner, the method came into use for the investigation of paranasal sinus disease. The recent introduction of a high-resolution system has allowed CT to be applied to the temporal bone and middle ear. In addition to precise anatomic delineation, CT gives the measurement of tissue density by means of attenuation values. This information helps in the separation of fat from muscle, blood from other fluids, and vascular lesions from nonvascular lesions. Intravenous contrast enhancement makes it possible to obtain good visualization of the arteries and veins in the neck and upper mediastinum. This aids in differentiating the normal vessels from tumors or nodes, and better defines vascular tumors. Faster scanning techniques now allow the evaluation of organs in which there is inherent movement, such as the pharynx and larynx.

In the larynx, CT has been found to be useful in the more accurate planning of radiation therapy portals. Certain authors working in this field feel that conventional tomography and laryngography are now obsolete. Many advantages of CT are mentioned: the increased comfort for the patient, the noninvasiveness of the procedure, and the ability to assess the volume of tissue masses and to measure tissue density. CT also allows more accurate assessment of the paracordal, para arytenoidal, and pre-epiglottic spaces than is possible with conventional tomography. CT scans have revealed changes in the laryngeal cartilages not visible by any other means. In laryngeal injuries CT has been found to display clearly the cartilaginous injuries and displacement, soft tissue changes, and resulting encroachment on the airway. Even in severe injury, the larynx can be examined conveniently at the same time as the brain and facial structures. In blunt injury, contralateral cricoid fractures not suspected clinically have been well demonstrated.
Conventional tomography has been used successfully in the assessment of tumors of the larynx, with the exception of certain "blind" areas, such as the laryngeal surface of the epiglottis and anterior commissure. These areas are sometimes poorly seen in indirect and direct laryngoscopy. Furthermore, conventional tomography does not demonstrate the laryngeal cartilages well. Laryngography has its enthusiastic advocates, but the procedure is time-consuming, uncomfortable for the patient, and contraindicated in cases of airway obstruction and stridor.

MATERIALS AND METHODS
The larynx offers the opportunity of whole organ study after total laryngectomy. It is therefore possible to correlate gross and microscopic findings with CT scans made before laryngectomy. For this reason we initiated a prospective study of CT scanning and conventional tomography of all patients with malignant disease of the larynx seen at the Institute of Laryngology and Otology, London. Seventeen patients were assessed as shown in Table 1. Our conclusions were also based on experience with 9 patients who either were not treated by laryngectomy or were scanned before we began our study.

After routine clinical examination including full ENT evaluation and indirect laryngoscopy, all the patients were submitted to direct laryngoscopy under general anesthesia. Conventional tomograms were obtained and then scanned by an

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Site of tumor, staging</th>
<th>Age (years)</th>
<th>Treatment</th>
<th>Anatomy of tumor, histology</th>
<th>Conventional tomography</th>
<th>CT scan</th>
<th>Laryngography contra-indicated?</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>LVC T3N0</td>
<td>65</td>
<td>DXT 6,600 rad failed; total laryngectomy failed DXT</td>
<td>Ulcerating tumor of LVC extending onto false cord; ant. comm. to arytenoid; poorly diff. squamous Ca</td>
<td>Not done</td>
<td>Irregularity and thickening of paraglottic area (HR)</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>RVC T3N0</td>
<td>52</td>
<td>DXT (elsewhere; dosage unknown); total laryngectomy</td>
<td>Warthy growth from ventricle and RVC; erodic invasion; wall-ciff squamous Ca</td>
<td>RVC thickening; ventricle partly obliterated</td>
<td>Right paraglottic area infiltration; absence of &quot;Hanafee line&quot; (HFL)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Left piriform fossa T2N0</td>
<td>65</td>
<td>Laryngectomy, partial pharyngectomy, and functional neck dissection</td>
<td>Piriform fossa tumor extending onto AE fold</td>
<td>Obliteration of air filling in piriform fossa</td>
<td>Asymmetry of AE fold; piriform fossa obliteration (SP)</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>Epiglottis T3N0</td>
<td>49</td>
<td>Total laryngectomy</td>
<td>Extensive tumor of posterior surface; pre-epiglottic space and ant. comm. infiltration</td>
<td>Unhelpful</td>
<td>Excellent delineation of tumor anatomy (Fig. 2) (SR)</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>RVC T2N0</td>
<td>67</td>
<td>DXT 6,600 rad failed; total laryngectomy</td>
<td>Tumor from mouth of right sacculating ventricle; round ant. comm. to left ventricle</td>
<td>Right ventricle obliterated; left laryngocele (Fig. 4)</td>
<td>Ant. comm. involvement; widened irregular right paraglottic space (HR)</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>Left transglottic T4N0</td>
<td>54</td>
<td>Total laryngectomy</td>
<td>Extensive from left AE fold to 2 cm below LVC</td>
<td>Transglottic lesion well shown</td>
<td>Ant. comm., a arytenoid, and subglottic extent well shown (HR)</td>
<td>Yes</td>
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<tr>
<td>7</td>
<td>LVC T3N0</td>
<td>41</td>
<td>DXT 6,800 rad failed; total laryngectomy</td>
<td>Tumor of LVC extending to left AE fold and ventricle including left arytenoid; poorly diff. Ca</td>
<td>Ventricle obliterated; suggests (wrongly) subglottic extension</td>
<td>Widened left paraglottic space; suggests arytenoid involvement; no evidence of subglottic spread (SR)</td>
<td>No</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Patient no</th>
<th>Site of tumor, staging</th>
<th>Age (years)</th>
<th>Treatment</th>
<th>Anatomy of tumor, histology</th>
<th>Conventional tomography</th>
<th>CT scan</th>
<th>Laryngography contraindicated?</th>
</tr>
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<tbody>
<tr>
<td>8</td>
<td>RVG T3N0</td>
<td>53</td>
<td>DXT 5,000 rad failed; total laryngectomy</td>
<td>Ant. comm. to arytenoid; false cord to 5 mm below cord (right); squamous-cell Ca</td>
<td>Transglottic tumor</td>
<td>Poor scan because of movement; however, suggests (correctly) arytenoid involvement (SR)</td>
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<tr>
<td>9</td>
<td>LVG T3N0</td>
<td>74</td>
<td>DXT 5,000 rad failed; total laryngectomy</td>
<td>Ant. comm. and thyroarytenoid muscle invasion; no cartilage invasion</td>
<td>Shows obliteration of left ventricle</td>
<td>Thickening of left paracordal space; some loss of lucency (SR)</td>
<td>No</td>
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<tr>
<td>10</td>
<td>Left supraglottic T3N0</td>
<td>51</td>
<td>DXT 5,000 rad failed; total laryngectomy</td>
<td>From left AE fold, deeply ulcerating false cord, into LVG; no subglottic</td>
<td>Not very helpful</td>
<td>Excellent delineation of the ulcerated false cord; widened paraglottic (Fig. 9) (SR)</td>
<td>No</td>
</tr>
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<td>11</td>
<td>Left supraglottic T3N0</td>
<td>42</td>
<td>Total laryngectomy</td>
<td>Tumor arising from left saccule and false cord; extends up to but not through thyroid cartilage; poorly diff. squamous Ca</td>
<td>Left transglottic mass</td>
<td>Left supraglottic mass false cord level to subglottic; extends up to arytenoid and thyroid cartilage (HR and SR)</td>
<td>Yes</td>
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<td>12</td>
<td>Left supraglottic T3N0</td>
<td>67</td>
<td>Total laryngectomy; left block dissection</td>
<td>Supraglottic tumor of left side of epiglottis, extending into left piriiform fossa; pre-epiglottic and arytenoid invasion</td>
<td>Left supraglottic mass with minimal subglottic extension; piriiform fossa compression</td>
<td>Left-sided supraglottic mass; left AE fold compressing piriiform fossa; invasion of pre-epiglottic space and left arytenoid; no invasion of thyroid cartilage</td>
<td>Yes</td>
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<tr>
<td>13</td>
<td>LVG (pedunculated) T3N0</td>
<td>70</td>
<td>Total laryngectomy</td>
<td>Pedunculated tumor ant. vs LVG invading thyroarytenoid muscle</td>
<td>Pedunculated lesion, side uncertain (subglottic)</td>
<td>Clearly shows pedunculated lesion attached to ant. vs LVG</td>
<td>Yes</td>
</tr>
<tr>
<td>14</td>
<td>Left supraglottic T3N0</td>
<td>66</td>
<td>Total laryngectomy with neoglottic reconstruction</td>
<td>Tumor of LVG ext. into left and right ventricles and round ant comm.; moderately diff. squamous Ca surrounding left arytenoid</td>
<td>Left-sided transglottic lesion; early subglottic spread</td>
<td>Left-sided thickening of paraglottic space; ant. comm. and left arytenoid involvement</td>
<td>Yes</td>
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<tr>
<td>15</td>
<td>Bilateral glottic T3N0</td>
<td>70</td>
<td>Laryngofissure and cordectomy; recurrence; total laryngectomy with neoglottic reconstruction</td>
<td>Recurrence of tumor in area of left cordectomy; microinvasion of right cord</td>
<td>Left ventricle obliterated; swelling of true and false cords</td>
<td>Not contributory; shows split thyroid lamina from laryngofissure</td>
<td>No</td>
</tr>
<tr>
<td>16</td>
<td>Right transglottic T4N0</td>
<td>46</td>
<td>DXT 1,976 rad; total laryngectomy</td>
<td>Lesion of RVC and subglottis; ext. up to thyroid lamina; invading the lateral cricoarytenoid muscle</td>
<td>Subglottic mass on right</td>
<td>Right-sided supraglottic mass, extending into left arytenoid, suggesting (correctly) invasion</td>
<td>Yes</td>
</tr>
</tbody>
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(Continued)
EMI 5005 (London, England) series 2 body scanner. The patient lay supine on the scanning table with the head slightly extended; scanning was commenced at the level of the hyoid bone, and sequential scans of 5-mm slices were made in a caudal direction. We prefer to use high resolution because it produces better delineation of the cartilages of the larynx, but in several cases we used standard resolution because of the shorter exposure time of 27 seconds. However, even this time is too long to expect cessation of breathing, and so the patients were requested to breathe as quietly as possible. We have not found it advantageous to ask patients to protrude their tongues slightly, as advocated by Mancuso et al.9

In the histopathology laboratory, the larynges of those patients who received total laryngectomies were fixed in 10% formalin and the hyoid bones were dissected off the specimens. A series of 5-mm tissue slices were made by sectioning the larynx transversely on a slicing machine (Excell/Boston 10-inch gravity slicing machine, Staines Group Ltd, London England). This method, described in detail elsewhere,10 has proven to be so satisfactory that it has been adopted for routine use in our laboratory. The tissue slices were arranged in sequence, labeled, and photographed. When pieces of the slices were taken for embedding, they were marked on the photographs with a felt-tipped pen, thus allowing a spatial correlation of the histopathologic features. The information obtained was then correlated with the preoperative CT scans taken at corresponding levels.

The anatomy of the normal larynx, as seen in the transverse plane, was studied by sectioning several cadaver larynges and was found to correspond to the findings of other authors.11-13

Figure 1. (A) A CT scan of a normal larynx at the level of the hyoid bone, showing the crescentic epiglottis suspended in the air-filled oropharynx. The valleculae are delineated by the median and lateral glossoepiglottic folds. (B) CT scan taken at a similar level from patient 4 showing the filling in of the valleculae by an epiglottic tumor with infiltration of the pre-epiglottic space.
RESULTS

The Supraglottic Larynx. The epiglottis, valleculae, and base of the tongue. As shown in Figure 1, the suprahypoid epiglottis appears as a curved structure suspended in the air-filled oropharynx. The upper part of the hyoid bone can be seen, and the median and 2 lateral glossopiglottic folds delineate the 2 valleculae (Fig. 1A). When there is infiltration of the pre-epiglottic space, the valleculae are obliterated (Fig. 1B).

Figure 2 shows scans taken below the level shown in Figure 1B, with the corresponding laryngeal sections on the left. In Figure 2B the widened pre-epiglottic space correlates with tumor infiltration in the laryngeal section. The tumor is slightly asymmetrical and tends to compress the left piriform fossa. The tumor involves the anterior commissure below. The tumor was not demonstrated on conventional tomography, but was seen on the lateral x-ray. This patient was a difficult subject for indirect or direct laryngoscopy. The tumor proved to be carcinoid, a very rare occurrence in the larynx.

The piriform fossae, aryepiglottic folds, and laryngeal vestibule. The aryepiglottic folds are well shown in Figure 3, separating the piriform fossae from the laryngeal vestibule. The upper CT scan through the hyoid bone shows the asymmetry of the left aryepiglottic fold (Fig. 3B). Obliteration of the left piriform fossa is seen both on conventional tomogram (Fig. 3A) and on the CT scan (Fig. 3D). Tumors filling the laryngeal vestibule and encroaching on the airway are outlined in the air-filled space (see Fig. 5).

The Glottic Larynx. At this level the thyroid laminae, arytenoid cartilages, and cricoid lamina are seen (Fig. 4). The paraglottic space, that is, the space between the mucosa and the cartilage, is well delineated. A translucent line extending to the thyroid lamina has been described. We, however, have not found this line to be constantly present. When it is absent on one side, it contrasts with the normal side, indicating tumor infiltration of the thyroarytenoid muscle and possible early infiltration of the thyroid cartilage (Fig. 4).

Figure 2. Transverse sections of the larynx of patient 4 on the left and on the right, the CT scans from the same levels. A and B represent the level of the hyoid bone, and C and D represent the level of the vocal cords. The extensive epiglottic tumor has infiltrated the pre-epiglottic space (B), right down to the anterior commissure (D). The glottic chink is normally boat-shaped; note the calcified arytenoid cartilages (D).
Figure 3. (A) Conventional tomogram showing the obliteration of the left piriform fossa. (B) CT scan at the level of the hyoid bone, but below the velloculae. The left aryepiglottic fold is involved in the piriform fossa neoplasm. (C) Laryngeal slice at the level of the notch of the thyroid cartilage and (D) CT scan taken at the same level. The CT scan clearly shows the piriform fossa to be involved. (E) Laryngeal slice and (F) CT scan taken at the glottic level where there is no evidence of tumor. Note that the mucosa lies directly on the thyroid cartilage in the midline between the anterior attachment of the cords.
When the cartilage is macroscopically invaded, this can be seen on CT scans (Figure 5).

The anterior commissure. The anterior commissure is an important structure in the larynx, and in some cases it may be difficult to assess involvement. It is seen as a small free space consisting of mucosa lying directly on the cartilage in the midline between the anterior attachments of the cords to the thyroid cartilage (Fig. 3F). If the air space at this level does not extend right up to the junction of the thyroid laminae, this can be interpreted as involvement (Figs. 2D, 4B, 4D, and 6).

The laryngeal succules. These appendices of the laryngeal ventricles may appear as round, air-filled objects at the junction of the anterior and middle thirds of the false cords (Fig. 6). In the patient shown in Figure 6, there is considerable...
edema of supraglottic structures associated with radiation therapy. The tumor is seen on the right, projecting into the airway. Figure 61 shows the conventional tomogram and the presence of distended succules (laryngoceles).

The arytenoid cartilages, cricoarytenoid joints, and posterior commissure. The arytenoid cartilages are usually homogeneously calcified, roughly triangular structures lying above and anterolateral to the cricoid lamina (Fig. 2). We have found widening of the paraglottic space and irregularity of the air-tissue interface to be highly significant. When it extends to the posterior commissure, it suggests arytenoid and possible cricoarytenoid joint invasion (Fig. 7).

The Subglottic Larynx. The subglottic area is characterized by the oval shape of the airway, bounded posteriorly by the cricoid lamina and anteriorly by the lower rounded margin of the thyroid cartilage. As the thyroid cartilage gives way to the cricothyroid membrane, the arch of the cricoid completes the oval. The cricothyroid joints are clearly shown (Fig. 4E).

Subglottic extension is well shown by the asymmetry of the oval shape of the subglottis (Fig. 7). Figure 7 shows the larynx of a patient with an extensive transglottic lesion on the left side.

**DISCUSSION**

CT provides a noninvasive, quick, and effective radiologic investigation for the larynx, that is not uncomfortable for the patient. It can be done without risk in cases of respiratory obstruction and after suspected laryngeal injury. It gives an accurate assessment of laryngeal anatomy and involvement by tumor, particularly of the preepiglottic space, paracordal area, anterior commissure, and cricoarytenoid area. These are all areas not well assessed by conventional tomography. The presence of anterior or posterior commissure involvement is of paramount importance in precluding the possibility of conservative laryngeal surgery. This diagnosis can usually be made clinically or upon direct examination under general anesthesia, but may be problematic in patients who have short, thick necks, gagging, or prominent dentition. CT may prove valuable in such cases.

Because CT and conventional tomography present images at 2 different planes at right angles to each other—coronal and transverse—the two methods are totally complementary. CT is better than conventional tomography for showing

the laryngeal cartilages, epiglottis and preepiglottic space, paracordal infiltration, and anterior and posterior commissure involvement. However, conventional tomography is superior in showing the ventricles, the thickening of the false and true cords, and subglottic extension. In general, structures that run at right angles to the imaged plane are better shown than those that run obliquely or in the same plane.

Laryngography was not performed in any of the patients in this series, but would have been contraindicated in 10 of the 17 cases because of airway obstruction and stridor (Table 1).

The shape and symmetry of the airway has been shown to be of particular significance in the CT scans. Obliteration of piriform fossae occurred because of compression by a bulky supraglottic tumor, as well as by invasion. Tumors encroaching on the airway (Fig. 6), deeply ulcerating tumors (Fig. 8), or pedunculated tumors (Fig. 9), are accurately outlined. Irregularity of the air-tissue interface, particularly at the true cord level, is highly significant, and when accompanied by a broadening of the paraglottic space, suggests an infiltrating tumor. The anterior commissure should be sharp and extend right up to the thyroid cartilage. Any tissue swelling in this area is highly suspicious of anterior commissure involvement by tumor. Similarly, irregularity and widening of the paraglottic space posteriorly means that tumor infiltration extends as far as the arytenoid cartilage, and possibly the cricoarytenoid joint. In some patients, increased density has been seen in this area on the CT scan. This may be due to the tumor displacing the
Figure 6. Left: A series of laryngeal slices shown on the left with corresponding CT scans on the right from patient 5. A and B represent the level of the base of the epiglottis. C and D are at the level of the upper portion of the arytenoid cartilages. Note that the tumor arose from the area around the mouth of the right saccule, also obstructing the left saccule. The left saccule was distended, forming a laryngoceles. E and F represent the level of the lower arytenoid cartilages. Note in F the irregularity of the air-tissue interface on the right due to the tumor encroaching on the airway and the widening and increased density in the arytenoid area. G and H show the subglottic area. (I, right) The conventional tomogram showing the laryngoceles (arrows).

Figure 7. A series of laryngeal slices and corresponding CT scans from patient 6. A and B represent the level of the vocal cords; C and D represent the upper subglottic area; E and F the lower subglottic area. An extensive left-sided transglottic lesion is seen. Note the widening of the left paraglottic space, extending back over the arytenoid area. The arytenoids and cricoid lamina are seen in B. Subglottic extension is shown by the loss of the symmetry of the oval subglottis (F).
Figure 8. Laryngeal sections and CT scans from patient 10. A and B represent a level at the thyrohyoid membrane; C and D represent the level of the thyroid cartilage notch; E and F represent the false vocal cord level; G and H represent the lower false cord level.
Figure 8. I and J show a cut through the ventricles; and K and L represent the true vocal cord level. A deeply ulcerating lesion is seen extending from the base of the epiglottis and left aryepiglottic fold area, through the false cord and ventricle to the glottic level.
Note in J the widened left paraglottic space and the increased density in the arytenoid area.

Figure 9. Laryngeal section and CT scan at the vocal cord level showing pedunculated tumor of the left vocal cord for patient no. 13.
arytenoid cartilage upwards, thus increasing the attenuation value at a particular level, or may be the result of fixation of the arytenoid cartilage by the tumor (Fig. 8J). (We have recently studied this feature in more detail by submitting the laryngeal tissue slices to x-ray; these radiographs suggest an actual increase in ossification of the affected arytenoid and cricoid cartilages.)

Two of the patients in the series were monitored for radiation levels. The average skin dose per CT scan was recorded as 3.1 rem, compared with an average measurement for conventional tomography of only 0.16 rem per section. Thus, with the present apparatus, CT gives more than 19 times the radiation of linear conventional tomography performed on the Polytomie machine. These levels of radiation are not likely to be of importance in a patient with a laryngeal malignancy. A scanner with an under-the-couch tube mounting should reduce the CT dose to the thyroid to more acceptable levels for the investigation of nonmalignant conditions.

CONCLUSION

Routine CT has proved to be a valuable adjunct in the diagnosis and assessment of laryngeal tumors for surgery or radiation therapy, and can be valuable in the follow-up of the latter.

CT is complementary to conventional tomography of the larynx, which gives a longitudinal section in addition to the transverse sections provided by CT scan. We cannot agree with Ward et al. that conventional tomography no longer has a place in the investigation of the larynx. Subglottic extension is much easier to determine by conventional tomography, whereas CT is superior in demonstrating arytenoid and anterior commissure involvement and erosion of the thyroid cartilage.

We did not find it necessary to augment these studies by contrast laryngography. This technique was contraindicated in 10 patients because of airway obstruction and stridor, and is not considered a necessary procedure in the preoperative investigation of these patients when CT scanning is available.

Examination of the excised laryngeal specimen by means of transverse sectioning has been highly satisfactory, and findings in the tissue slices correlated well with the results of CT scans taken preoperatively.

REFERENCES

4. Goad LV, Camannings CW, Robuzzi DD, Reed GF, Chung CT. Use of computerized axial tomography of the head and neck region. Laryngoscope 57:1270–1276, 1977