LYMPH NODE METASTASIS IN ORAL CANCER

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by

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ABSTRACT

Lymph Node metastasis in Oral Cancer

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Oral cancer is an important and serious disease. Although it is a relatively rare disease in the U.K. (1,900 new cases per year), the incidence is increasing in females and younger age groups. The disease and its treatment may have a profound effect on the aesthetics and functions of the face and upper aerodigestive tract. The prognosis is poor (960 deaths per year), and failure to control the disease, locally at the primary site and/or regionally in metastatic cervical lymph nodes, is the usual cause of death. The extent of disease at presentation has a major influence on prognosis and treatment needs, but clinical assessment of the extent, in particular, the presence and extent of cervical metastasis, is frequently inaccurate.

The aims of my study were to investigate the incidence and extent of cervical node metastasis in patients presenting with oral cancer, and to determine the extent to which selected clinical, histological, and morphometric features of the primary tumour influence the development of lymphatic spread.

Detailed observations were made on the pattern of lymphatic spread in 80 surgical neck dissection specimens using a protocol designed to facilitate an accurate clinicopathological correlation. My results show that histological assessment of every lymph node that has been harvested from the gross specimen is essential, since metastatic deposits may be present in small nodes (while larger nodes within the same anatomical group are histologically free of tumour), and as emboli or micrometastases with only minimal replacement of normal architecture of the node. Extracapsular spread of metastatic carcinoma was diagnosed in 71% of positive dissections, and its extent ranged from microscopic embolisation/permeation of perinodal lymphatics to gross involvement of major vessels, muscles and skin.

Clinical assessment of the metastatic status was in agreement with the histological assessment in 66% of necks. Pathological findings suggest that some misdiagnoses are unavoidable, given the inherent limitations of current scanning facilities. Micrometastases or metastasis to small nodes accounted for nine of the 14 false-negative assessments, and non-metastatic nodal enlargement accounted for six of the 13 false-positive assessments.

The mean number of nodes harvested from the deep cervical chain in functional and supra-omohyoid dissections was only 65% and 46%, respectively, that achieved in radical dissections, raising questions concerning the efficacy of modified surgical procedures.

The relationship between selected clinical and histological features of the primary tumour and the histological metastatic status was investigated in a series of 45 tongue/floor-of-mouth cancers. There were significant differences in tumour surface-dimension, tumour thickness, the multifactorial malignancy grade, and the presence of perineural and vascular invasion in patients with and without metastasis. A logistic regression model (with total malignancy grade and vascular invasion as the two predictor variables) correctly classified 39 (87%) of the 45 cases.

Assessment of nuclear features by histological malignancy grading and stereological estimation of the volume-weighted mean nuclear volume by point-sampling of linear intercepts had no prognostic value in predicting metastasis.

Further studies should be directed at correlating the pattern of spread in the neck dissection with regional recurrent and systemic disease; and at the prognostic value of an assessment of the microvasculature in and around the primary tumour.

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Chapter 1.

INTRODUCTION.

Oral cancer is an important and serious disease. The incidence varies widely in different parts of the world (Parkin *et al.*, 1988), and changing patterns of exposure to certain risk factors account for the recent increased incidence in females and younger age groups in the Western World (Hindle and Nally, 1991). In the United Kingdom, there are approximately 1,900 new cases each year, and 960 deaths (Cancer Research Campaign Atlas of Cancer Incidence in England and Wales, 1991). Hence, oral cancer is a highly lethal neoplasm. In addition, it causes great morbidity, with patients having to cope with both the aesthetic and functional changes resulting from the disease and its treatment (Barnes and Johnson, 1986a).

The extent of the disease at presentation has a major influence on prognosis (Binnie and Rankin, 1988). In addition to local spread, carcinoma of the oral mucosa is characterised by metastatic spread via the lymphatics, to the cervical lymph nodes (Willis, 1973a). Distant blood-borne metastases usually only occur much later in the course of the disease, and are rarely the primary cause of death (Kotwall *et al.*, 1987). The true incidence of lymph node metastasis in oral cancer is unknown since clinical assessment of the cervical nodes is frequently inaccurate and many patients are treated by radiotherapy alone (Richard *et al.*, 1987). However, the presence of occult micrometastases accounts for late regional disease which develops in 20-50% of patients whose initial treatment was confined to the primary intra-oral tumour (Cunningham *et al.*, 1986).

The incidence of cervical metastasis is related to the site and the size of the primary tumour, and to the histological degree of differentiation (Arthur and Farr, 1972). More recent reports suggest

tumour thickness (Spiro *et al.*, 1986; Mohit-Tabatabai *et al.*, 1986; Urist *et al.*, 1987) and the histological pattern of invasion (Crissman *et al.*, 1984; Frierson and Cooper, 1986; Okamoto *et al.*, 1988) are also important risk factors, but the relative importance of these different histological features is unknown at present. In particular, more discriminating methods are needed to identify from among individuals with small-to-moderate sized tumours those at risk of early metastatic spread.

The extent of metastasis within the cervical lymphatic system, indicated by the number and anatomical level of the involved nodes, and the presence and degree of extracapsular spread, have an important influence on prognosis (Carter *et al.*, 1987). Patients with extensive lymphatic spread are at risk of both regional recurrent disease and blood-borne distant metastases, although the relative prognostic importance of the different pathological findings is still uncertain (Stell *et al.*, 1983; Vikram *et al.*, 1984a; Grandi *et al.*, 1985; Carter *et al.*, 1985 and 1987; Richard *et al.*, 1987). The importance of a detailed histological examination of neck dissection specimens in patients with oral cancer has recently been acknowledged by the American Joint Committee on Cancer, and an assessment of both further treatment needs and prognosis based on the pathological, rather than the clinical, stage is now recommended (American Joint Committee on Cancer, 1988a).

The aim of the present study is to characterize the different patterns of lymphatic spread seen in oral cancer and to determine the extent to which selected clinical, histological, and morphometric features of the primary tumour correlate with the pattern and extent

of metastatic spread throughout the neck.

In order to answer these questions, three separate but related investigations have been carried out:

(i) detailed observations were made on the pattern of lymphatic spread in the surgical neck dissection specimen, in a series of 60 patients undergoing unilateral or bilateral neck dissection simultaneous with resection of an intra-oral primary squamous cell carcinoma (Chapter 4). In addition, the accuracy of the clinical assessment of the metastatic status and the efficacy of the surgical procedure in the treatment of actual or potential metastatic spread were appraised (Chapter 5).

(ii) greatest surface-dimension and thickness measurements, histological malignancy grading scores, and assessment of perineural and vascular invasion were made of a series of 45 primary tumours of the tongue or floor of mouth. Clinical and histological features of the primary tumour were evaluated against the actual metastatic status of the cervical lymph nodes and logistic regression analysis was used to develop a simple prognostic method for predicting cervical lymph node metastasis in an individual patient (Chapter 6).

(iii) morphometric analysis of the nucleus (stereological estimation of the volume-weighted mean nuclear volume) was made of the same 45 primary tumours to establish whether an objective assessment of nuclear size correlated with the actual metastatic status of the cervical nodes (Chapter 7).

Before describing the methodology and reporting the results of these investigations, the incidence and aetiology of oral cancer and factors influencing its prognosis will be reviewed (Chapter 2). In

Chapter 3, the anatomy of the cervical lymphatic system is described; the mode, distribution and the clinical diagnosis of metastases are discussed, and the different surgical procedures designed to deal with actual or potential lymphatic spread are outlined.

Chapter 2.

ORAL CANCER - INCIDENCE, AETIOLOGY AND PROGNOSIS.

1. Introduction.
2. Incidence of Oral Cancer.
3. Aetiology of Oral Cancer.
4. Prognosis of Oral Cancer.

INTRODUCTION

In order to understand the significance of my investigations, it is important to review the extent to which oral cancer is present in the community, and to understand the factors influencing its occurrence and its outcome.

INCIDENCE OF ORAL CANCER

The incidence of oral cancer varies greatly in different regions of the world. In the areas of highest incidence - India and other parts of Southern and South-Eastern Asia - oral cancer accounts for over 40% of all cancers and is the first or second most common site for malignancy (Daftary *et al.*, 1991). In Northern Europe, 5,700 new cases were reported in 1980, representing 2% of all cancer registrations (Parkin *et al.*, 1988). Although oral squamous cell carcinoma accounts for less than 2% of all cancer registrations in the United Kingdom (1,900 new cases in 1984), the high mortality rate (960 deaths each year) means it is still a significant problem (Cancer Research Campaign Atlas of Cancer Incidence in England and Wales, 1991). Based on these figures, the death/registration (D/R ratio) is 0.50 - significantly worse than the D/R ratio for malignant melanoma (0.35) - which is traditionally regarded as a highly lethal neoplasm (American Cancer Society, 1983).

The prevalence of oral cancer increases with increasing age. In the Western World, 98% of cases are over 40 years of age and 85% over 50 years. The incidence rises from an average at all ages of 3-4 per 100,000 per annum to 100 cases per 100,000 per annum in those aged

over 75 years (Langdon *et al.*, 1977; Johnson, 1990). In high prevalence areas of the world, however, it is relatively common in younger age groups. Recently, an increasing incidence in the third and fourth decades, especially in males, has been reported in many Western countries (MacFarlane *et al.*, 1987; Moller, 1989).

The sex distribution also varies in different areas of the world. In industrialised countries, males are affected more frequently than females, the sex difference being especially marked for some sites. In recent years, however, the sex ratio has decreased as male rates have fallen proportionately more than female rates and, in the United Kingdom, the male:female sex ratio has reduced from 2.0:1 in 1962-1967 to 1.7:1 in 1984 (Hindle and Nally, 1991). In high prevalence areas such as India, the incidence for females is equal to, or greater than, that for males (Parkin *et al.*, 1988).

In all Western Countries, intra-oral cancer most commonly affects the lateral border of the tongue and the floor of mouth, followed in order of frequency, by the buccal mucosa, lower alveolar ridge, retromolar trigone, and the soft palate (Johnson, 1990). In high incidence areas of South-Eastern Asia, the buccal, retromolar, and commissural mucosae are the most cancer-prone sites. In Europe and North America, cancers of the hard palate (Pindborg, 1980), and dorsum of the tongue (Delemarre and van der Waal, 1973) are rare.

AETIOLOGY OF ORAL CANCER

Like cancer at most other sites, the global difference in incidence rates of oral cancer, and how these rates continue to change

with time, reflect different patterns of exposure to environmental risk factors. The aetiology and pathogenesis of cancer is, however, multifactorial, and it is probable that both environmental and host factors are involved.

There is no doubt that tobacco is the most important of all known intra-oral carcinogens, and its method of use accounts for both the different incidence rates worldwide and the different sex and site distribution patterns (International Agency for Research on Cancer, 1985; Daftary *et al.*, 1991). Evidence suggests there is a dose/time relationship between cigarette smoking and intra-oral cancer (Wynder *et al.*, 1977; Mashberg *et al.*, 1981). In parts of Asia, the use of bidis is associated with a high incidence of carcinoma of the labial commissure, (Pindborg *et al.*, 1967), while, in other areas, the habit of reverse smoking results in a greatly increased incidence of palatal precancer and cancer (Reddy *et al.*, 1975). Pipe smoking is linked to cancer of the lip (Levin *et al.*, 1950; Wynder *et al.*, 1977). Smokeless tobacco - either in the form of chewing tobacco, snuff, or as part of the betel quid - contains known carcinogens and has been shown to increase the risk of oral cancer (International Agency for Research on Cancer, 1985). Recently, the increasing use of smokeless tobacco, particularly in young age groups in the United States of America and Europe, is a matter of growing concern (Mattson and Winn, 1989).

Alcohol is also an important risk factor since it potentiates the effect of other topical carcinogens, particularly those from tobacco (Blot *et al.*, 1988). Heavy drinkers experience a two to six times higher risk than non-drinkers, depending on the amount of tobacco smoked. When compared to non-drinking/non-smoking controls,

the risk for heavy alcohol and tobacco users is fifteen times higher (Rothman, 1975). The role of alcohol in non-smokers is less clear, but in some Western countries, the recent rise in oral, pharyngeal, and oesophageal cancers correlates with an increased alcohol consumption (Moller, 1989).

Other aspects of the diet also influence the incidence of oral cancer (Marshall *et al.*, 1992). Nutritional deficiency resulting in epithelial atrophy may render the oral mucosa more susceptible to local carcinogens. The most important association is that of iron deficiency and cancer of the upper digestive tract (Wynder *et al.*, 1957).

The role of micro-organisms in oral carcinogenesis is uncertain at present. It is not known if the Candidal organisms frequently seen within the epithelium in oral cancer and precancer are aetiologically important or just secondary invaders (Arendorf *et al.*, 1983), although enzymes produced by *Candida albicans* are capable of producing carcinogens by nitrosination (Krogh *et al.*, 1987). Similarly, an aetiological role for Human Papilloma and Herpes simplex viruses is unproven (Syrjanen *et al.*, 1988; Maitland *et al.*, 1989; Scully, 1992).

Although environmental factors appear to be of major importance in oral cancer, genetic factors, particularly those which influence the immune response, are probably also involved (de Vries, 1990). Immunosuppression, either drug induced or in Human Immunodeficiency Virus infection, is associated with an increased incidence of malignancy and this may be associated with infection by oncogenic viruses (Silverman *et al.*, 1986; Greenspan *et al.*, 1990).

PROGNOSIS OF ORAL CANCER

The extent of the disease at presentation is the major factor influencing the prognosis. The TNM Classification (American Joint Committee on Cancer, 1988a and 1988b) of the clinical extent or stage of the disease is based on the three significant events in the life history of a cancer - growth of the primary tumour (T), spread to the regional lymph nodes (N), and distant blood borne metastases (M). This concept provides a convenient framework for the present discussion. The influence of more general factors, such as age and sex, will also be considered. Finally, second primary tumours and their influence on prognosis will be discussed.

(1) Prognostic Factors related to the Primary Tumour (T)

(a) Tumour Surface Size

Clinical experience has shown that the prognosis of oral cancer worsens as the size of the tumour at presentation increases. Maddox (1984) reported that for carcinomas of the anterior two-thirds of the tongue less than 1.0cm. in greatest surface dimension, the ten-year survival rate was 100%, while for carcinomas 3.0-4.0cm. in size, the survival rate was 46%. Several independent reports of studies using multivariate analysis (regression method of Cox, 1972) confirm that large tumour size at presentation is predictive of poor survival (Platz *et al.*, 1983; Maddox, 1984; Crissman *et al.*, 1984).

The size (T stage) of the primary tumour affects both the choice and outcome of treatment. The size of the primary tumour is an important factor in determining the surgeon's ability to obtain tumour-free margins (Scholl *et al.*, 1986). In addition, several

authorities (Ildstad *et al.*, 1983; Shaha *et al.*, 1984; Scholl *et al.*, 1986) have reported a higher rate of local recurrence with increasing T stage. In patients treated by radiotherapy, tumour size is an important determinant of the dose necessary to effect a cure (Fletcher, 1973).

Tumour size is an important predictor of cervical metastasis, and this is a major factor in the correlation between tumour size and prognosis (Lund *et al.*, 1975a and 1975b; Maddox, 1984). Hibbert *et al.* (1983) attempted to study the effect of tumour size alone on prognosis, and their results showed that in patients without cervical metastasis, tumour size was not significantly related to five-year survival.

(b) Tumour Depth of Infiltration

Clinical assessment of the depth of infiltration into muscle, bone and adjacent structures has some prognostic value, with increased infiltration indicating a worse prognosis (Krause *et al.*, 1973; Platz *et al.*, 1983). However, the actual depth of infiltration or stage of invasion is more accurately assessed histologically, and Moore *et al.* (1986a and 1986b) reported a good correlation between the histological stage of invasion and survival. The stage of invasion can also be expressed by a direct micrometer measurement of tumour thickness, which also correlates with survival (Urist *et al.*, 1987). The histological stage of invasion and tumour thickness will be appraised more critically in Chapter 6.

(c) Histological Grade of the Primary Tumour

Several authorities (Arthur and Farr, 1972; Shear *et al.*, 1976; Langdon *et al.*, 1977) have reported a correlation between the degree

of differentiation (Broders, 1920) and survival. More recent reports (Anneroth and Hansen, 1984; Anneroth *et al.*, 1987; Bryne *et al.*, 1989) suggest a multifactorial histological malignancy grading system is a more accurate predictor of prognosis. Details of the multifactorial grading system and the prognostic significance of other histological features will be discussed in Chapter 6.

(d) Tumour Site

In carcinoma of the oral cavity and pharynx, the prognosis depends on the site of the primary tumour. The D/R ratio of 0.15 for cancer of the vermillion border of the lip is significantly different from the D/R ratio of 0.62 for intra-oral cancers (Hindle and Nally, 1991). The specific site within the oral cavity has a smaller, but significant, influence on prognosis, with a gradual decrease in the five-year cure rate for more posteriorly located tumours (Arthur and Farr, 1972; Farr *et al.*, 1980). However, the relationship between site and prognosis is complex, since other important factors, such as stage at presentation (Spiro and Strong, 1974; Ildstad *et al.*, 1983); risk of nodal metastasis (Shear *et al.*, 1976; Farr *et al.*, 1980); and histological differentiation (Arthur and Fenner, 1966; Arthur and Farr, 1972; Shear *et al.*, 1976; Langdon *et al.*, 1977) also vary significantly at different intra-oral sites.

Because of variations in local anatomy, the significance of size and the degree of local spread to adjacent structures varies according to the site. For example, carcinomas of the alveolar ridge may involve bone at an early stage (McGregor and MacDonald, 1988). Also, the site of the tumour may influence prognosis indirectly through its influence on the choice of treatment, since it is known that the rate of local

recurrence following surgery varies at different intra-oral sites (Langdon *et al.*, 1977; Henk and Langdon, 1985a).

(e) Velocity of Tumour Growth

Evans *et al.* (1982) reported that an assessment of the velocity of growth of the primary tumour had significant prognostic value, with slow growing tumours having a better prognosis. Velocity of growth was estimated by dividing the surface area of the tumour at presentation by the time elapsed since symptoms were first noted. However, assessment of the delay between the onset of symptoms and definitive treatment relies on the memory of the patient and is, therefore, highly subjective, and the prognostic significance of velocity of growth derived in this way has not been confirmed by other authorities.

(2) Prognostic Factors related to Cervical Lymph Nodes (N)

Several authorities (Farr and Arthur, 1972; Krause *et al.*, 1973; Langdon *et al.*, 1977; Hibbert *et al.*, 1983) have reported that patients with clinically positive nodes at presentation have a significantly worse prognosis, in terms of survival, than patients with clinically negative nodes. However, the clinical diagnosis of cervical metastasis is frequently inaccurate (Ali *et al.*, 1985) and this problem will be discussed in detail in Chapter 3. Hence, for the rest of the present discussion, only histologically diagnosed metastatic disease will be considered.

(a) Presence of Nodal Metastasis

The single most important factor indicating a poor prognosis is the presence of histologically confirmed nodal metastasis. For

example, carcinoma of the anterior two-thirds of the tongue without evidence of lymph node metastasis has a five-year survival rate of between 50% and 65%, while the rate falls to between 18% and 34% when metastasis is present (Ildstad *et al.*, 1983; Maddox, 1984; Callery *et al.*, 1984).

(b) Number of Metastatic Nodes

The number of nodes involved by tumour is of prognostic value. In the study reported by Kalnins *et al.* (1977), the five-year survival rate was 75% when lymph nodes were histologically negative, and fell to 49%, 30% and 13% in the groups with one, two and three or more positive nodes, respectively. There was a direct correlation between the number of involved nodes and the incidence of both recurrence in the operated neck and distant metastasis. These findings have since been confirmed by several independent authorities (Snow *et al.*, 1982; Grandi *et al.*, 1985; Richard *et al.*, 1987; Carter *et al.*, 1987).

(c) Anatomical Position of the Metastatic Node(s)

Although patients with positive nodes low in the neck tend to have multiple positive nodes at several anatomical levels, an assessment of the numerically highest level of involvement is of some additional prognostic value (Stell *et al.*, 1983; Grandi *et al.*, 1985; Carter *et al.*, 1987). Spiro *et al.* (1974) reported that the five-year survival rate decreased from 45% for patients with involvement of the submental/submandibular nodes (level I) to 18% for patients with metastatic nodes at the inferior cervical level (level IV), while no patient with involvement of the posterior triangle (level V) survived five years. Schuller *et al.* (1980) confirmed that metastasis to a posterior triangle node, with or without involvement of other nodes,

is indicative of a very poor prognosis. Involvement of non-contiguous nodal sites, and bilateral or contralateral metastasis, also indicate a poor prognosis (Spiro *et al.*, 1974; Schuller *et al.*, 1980; Grandi *et al.*, 1985).

(d) Size of the Metastatic Node

The correlation between lymph node size and the presence of histologically confirmed metastasis is well recognised (Spiro *et al.*, 1974; Cachin *et al.*, 1979; Snow *et al.*, 1982; Richard *et al.*, 1987). It has been suggested that the size of the largest metastatic node has some prognostic value (Spiro *et al.*, 1974; Cachin *et al.*, 1979). However, these studies refer to the size of the node as estimated on clinical examination. When lymph nodes are measured following dissection of the surgical specimen, the size of the largest positive node appears to have no independent prognostic value (Sessions, 1976; Schuller *et al.*, 1980).

(e) Extracapsular Spread of Metastatic Carcinoma

The tendency for metastatic squamous cell carcinoma of the head and neck to breach the capsule of the involved node and infiltrate surrounding tissues was described by Willis in 1930. The clinical implications were only recognised more recently and several authorities (Snow *et al.*, 1982; Carter *et al.*, 1985 and 1987; Richard *et al.*, 1987) now regard extracapsular spread as the second most important prognostic factor after the presence of histologically confirmed nodal metastasis.

Extracapsular spread is reported to be present in between 23% and 86% of all patients with histological evidence of nodal metastasis (Kalnins *et al.*, 1977; Carter *et al.*, 1985). It is more commonly

associated with large metastatic nodes, being present in 75% of nodes greater than 3.0 cm. (Johnson *et al.*, 1981; Snow *et al.*, 1982). The spread may be microscopic or macroscopic in extent (Carter *et al.*, 1985). In the study reported by Carter *et al.* (1987), there was a ten-fold difference in the rate of regional recurrence in patients with macroscopic extracapsular spread compared to those with disease confined to the node or only microscopic extracapsular spread.

(f) Tumour Emboli within Cervical Lymphatic Vessels

The presence of emboli within the perinodal lymphatic vessels provides additional prognostic information, since their presence indicates an increased risk of further nodal involvement and/or regional recurrent disease (Sancho *et al.*, 1977; Cachin *et al.*, 1979; Richard *et al.*, 1987).

(3) Distant Metastasis (M) and Prognosis

Distant blood-borne metastasis is usually a late event in oral cancer. Extensive locoregional disease, at presentation or following treatment failure, is an important risk factor (Merino *et al.*, 1977; Vikram *et al.*, 1984b; Byers, 1985; Kotwall *et al.*, 1987). The lungs are the most common site of the metastatic spread, with bone, liver, and mediastinal nodes also frequently involved (Vikram *et al.*, 1984b; Kotwall *et al.*, 1987). In the study reported by Merino *et al.* (1977), of the patients developing clinical evidence of distant disease, the metastases became apparent within nine months of initial treatment in 48% of cases, and within two years in 80%. Langdon *et al.* (1977) reported that 1.5% of patients had clinical evidence of distant disease at presentation. The prognosis of patients with distant

metastasis is extremely poor. Ninety per cent died within two years of diagnosis in the study reported by Probert *et al.* (1974), resulting in a five year actuarial survival of only 8%. In the study reported by Snow *et al.* (1982), all patients with distant disease died within five years. The use of chemotherapy has not yet been shown to significantly improve survival (Wright *et al.*, 1988).

(4) TNM Stage and Prognosis

Details of the American Joint Committee on Cancer (1988b) TNM classification for staging oral cancer are given in Appendix 1. Stage I and Stage II patients, with localised disease, have the best prognosis. Stage III patients, with extensive local and/or the presence of regional disease, have a poorer prognosis. Patients with extensive locoregional disease and/or distant metastasis have stage IV disease and the worst prognosis.

(5) Recurrence of Locoregional Disease and Prognosis

Failure of locoregional control is the primary cause of death in 70% of patients dying of oral cancer (Farr *et al.*, 1980). Residual disease at the primary site is indicative of a particularly poor prognosis (Futrell *et al.*, 1971). Local intra-oral recurrence is reported in between 28% and 73% of patients (Farr and Arthur, 1972; Elbrond *et al.*, 1973). Failure in the neck is more important (Henk and Langdon, 1985b) and may result from growth of occult metastases when initial treatment has been confined to the primary site, or it may reflect metastasis to nodes outside the original field of treatment, or recurrence in the operated or irradiated field. In the study

reported by Cunningham *et al.* (1986), 42% of patients with T1/T2 tumours of the oral tongue/floor of mouth treated by local therapy alone later developed clinically overt nodal disease. The reported frequency of recurrent disease in the operated neck ranges from 15-50% of cases (Byers *et al.*, 1988; Farr *et al.*, 1980). Factors influencing the rate of recurrence include the pathological extent of the metastatic disease at the time of initial surgery; the type of surgical neck dissection procedure; and the use of adjuvant therapy, such as post-operative radiotherapy (Vikram *et al.*, 1988a; Byers *et al.*, 1988; Spiro *et al.*, 1988). Ninety per cent of recurrences present within 18 months. The cure rates for salvage procedures in patients with recurrent regional disease are poor (Ildstad *et al.*, 1983; Henk and Langdon, 1985b; Wright *et al.*, 1988).

(6) Age and Prognosis

Any correlation between the age of the patient at presentation of the carcinoma and prognosis is difficult to assess. Virchow (1863) suggested malignancies behave more aggressively in young patients and this has been confirmed by later authorities (Venables and Craft, 1967; Byers, 1975; Son and Kapp, 1985). Other authorities (Martin *et al.*, 1940; Spiro and Strong, 1971; Lindquist, 1979) have suggested older patients are less able to tolerate major surgery and radiotherapy, and, hence, have a worse prognosis. Platz *et al.* (1983), using calculated survival curves and multivariate analysis, showed patients aged over 50 years had a worse prognosis than younger patients, with those aged 70 or more having a particularly poor

prognosis. However, this was not confirmed by Hibbert *et al.* (1983) or Callery *et al.* (1984).

(7) Sex and Prognosis

There are numerous reports such as those by Arthur and Fenner (1966), Easson and Russell (1968), Waterhouse, (1974), and Langdon *et al.* (1977) suggesting female patients have a better prognosis. Langdon and Rapidis (1979) showed the improved survival was not related to age, site, stage or degree of differentiation of the tumour, or to a difference in tobacco or alcohol consumption. However, most of the more recent studies (Evans, 1978; Hibbert *et al.*, 1983; Platz *et al.*, 1983; Callery *et al.*, 1984), using calculated survival curves and multivariate analysis have failed to show a significant correlation between sex and prognosis, suggesting the apparent improved survival of females is explained by the high mortality due to diseases other than cancer in elderly males.

(8) Immunological Reactivity in Non-Metastatic Nodes and Prognosis

Numerous authorities have attempted to analyse and quantify the reactive changes occuring in non-metastatic cervical lymph nodes, and assess the prognostic significance of the different morphological patterns. Such studies have resulted in conflicting opinions: firstly, as to the value of such an assessment (Bennett *et al.*, 1971; Noone *et al.*, 1974; Gilmore *et al.*, 1978; Carter *et al.*, 1987), and secondly, as to the type of morphological pattern associated with a good prognosis (Malicka, 1971; Berlinger *et al.*, 1976; Zoller *et al.*, 1978; Ring *et al.*, 1985). The largest study was that reported by Carter *et*

al. (1987) in which 7,000 non-metastatic nodes were assessed. Although there were differences in the morphological patterns, both in patients with and without metastatic disease, and after radiotherapy, assessment of these was of no prognostic value.

(9) Multiple Primary Tumours

Despite advances in head and neck oncology leading to better control of local and regional disease, long term survival of oral cancer patients has not increased significantly during the past two decades (Goepfert, 1984; Stell and McCormick, 1985; Hindle and Nally, 1991). The occurence of further primary tumours has been increasingly responsible for this lack of progress (Carr and Langdon, 1989; Cooper *et al.*, 1989; Kotwall *et al.*, 1989). The occurrence of multiple intra-oral cancers was first described in detail by Slaughter *et al.* (1953). They used the term 'field cancerisation' to describe an area of altered epithelium at risk of developing multifocal carcinoma. The likely mechanism was further explained by Willis (1964) who suggested that following exposure to a carcinogen, neoplasia develops initially at the site of maximum stimulus, and may later become manifest in neighbouring sites subjected to a lower dose of the same original carcinogen.

The reported frequency of second primary tumours in patients with oral cancer ranges from 7% (Berg *et al.*, 1970) to 33% (Shaha *et al.*, 1984). In most recent reports (Hordijk and de Jong, 1983; Black *et al.*, 1983; de Vries *et al.*, 1986), the frequency is greater than 17%. Between 4.4% and 17% of patients have simultaneous or synchronous cancers (Hsairi *et al.*, 1989; McGuirt *et al.*, 1982). These are sited

in the oral cavity or pharynx in approximately 50% of patients (Shaha *et al.*, 1988). Prospective studies show a further 6.5-14% of patients develop metachronous tumours (Shaha *et al.*, 1988; Vikram *et al.*, 1984c). Most of these are sited in the lungs and the oesophagus and are diagnosed within three years of the index tumour diagnosis (Cahan *et al.*, 1976; Shaha *et al.*, 1988).

The prognosis of patients with multiple primary cancers is extremely poor (Gluckman and Crissman, 1983). The average survival after diagnosis of the second tumour was only 7.2 months in the study reported by Carr and Langdon (1989). Even in studies concerning only patients treated for cure of the second cancer, McCullough *et al.* (1988) reported an absolute disease-free five-year survival rate of only 14%. The site of the second cancer has an important influence on survival, since even index tumours of the pharynx, lungs, and oesophagus have a very poor prognosis (Skinner, 1983).

It is likely that multiple tumours within the oral cavity and upper aerodigestive tract are aetiologically related. Of the known or suspected external carcinogens, continued use of tobacco and alcohol appears to be associated with greatest risk, but opinion varies about which is the more influential (Silverman and Griffith, 1972; Schottenfeld *et al.*, 1974; Hsairi *et al.*, 1989). The aetiological role of factors related to the treatment of the index tumour, particularly radiotherapy, is uncertain (Shons and McQuarrie, 1985; Parker and Enstrom, 1988; Cooper *et al.*, 1989; Ogden, 1991). It is possible that the immune response to environmental carcinogens is abnormal in patients with multiple tumours (de Vries *et al.*, 1987a and 1987b).

Chapter 3.

METASTATIC SPREAD IN ORAL CANCER.

1. Introduction.
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INTRODUCTION

For the diagnosis and treatment of metastatic disease in oral cancer, a detailed knowledge of the anatomy and physiology of the cervical lymphatic system is essential. Clinical experience over many years has shown most cancers at specific intra-oral sites exhibit similar patterns of biological behaviour. The distribution of lymphatic metastases has proved consistent enough to allow the design of standard surgical procedures to deal with the primary tumour and actual or potential lymphatic spread. The use of such procedures has been one of the most important advances in the management of oral cancer (Conley, 1967a). More recently, several authorities have reported the results of histological studies of neck dissection specimens, allowing a more accurate visualisation of the actual extent and patterns of metastatic spread.

THE MECHANISM OF LYMPHATIC SPREAD

While a number of morphological and behavioural characteristics are used to differentiate between benign and malignant neoplasms, the only absolute criterion is the ability of a malignant neoplasm to invade surrounding tissue and to colonise distant sites (Woolf, 1986). Invasion of small lymphatic vessels during the early stages of local spread is characteristic of carcinomas. Once the malignant cells have gained access to the lymphatic vessel, they can grow along the lumen as a continuous cord, and this permeation may extend to the regional lymph node. In addition, following invasion of the lymphatic vessels, tumour emboli are formed and carried by the stream of lymph to the

regional node. The embolic cells may be destroyed, remain dormant, or establish a growing focus with gradual replacement of the node. The new deposit has no continuity with the primary lesion and is termed a metastasis (Woolf, 1986). In addition to the local spread of the metastatic deposit, further dissemination by permeation or embolisation may occur within the lymphatic system. Also, tumour cells within lymph nodes may gain access to the bloodstream by invasion of small intra-nodal or extra-nodal blood vessels, opening up of small lymphatico-venous communications, or directly via the thoracic duct, hence increasing the likelihood of distant blood-borne metastastic spread.

In carcinoma of the oral mucosa, most lymphatic metastases arise by embolic spread and lymphatic permeation is usually confined to the immediate vicinity of the primary tumour (Willis, 1973b). Initially, tumour emboli settle in a single, or two or more adjacent nodes, in the first group or level of nodes draining the primary site. Once the first group of nodes is completely replaced by tumour, emboli may then pass to the next group lower in the neck, where the process is repeated. The presence of only microscopic foci in the first and subsequent groups of nodes is unusual in oral cancer (McKelvie, 1976).

ANATOMY OF THE CERVICAL LYMPHATIC SYSTEM

The oral mucosa is drained by a network of small lymphatic capillaries, which, in turn, drain into collecting vessels, which have numerous valves, elastic fibres and smooth muscle within their walls (Feind, 1972). Larger lymphatics follow the course of veins and drain

into lymph nodes grouped along their course. The structure of lymph nodes allows them to function as efficient filters, with lymph entering the peripheral sinuses via the afferent lymphatics and then draining, via the medullary sinuses, into the efferent lymphatic which leaves the node at the hilum.

It has been estimated that 300 of the 800 lymph nodes in the body are located within the head and neck (Feind, 1972; Haagensen, 1972; McKelvie, 1976). The anatomical groups of nodes having a particular relevance in intra-oral cancer are:

(i) The Submental and Submandibular Nodes

The submental nodes - usually three or four - lie within the adipose tissue of the submental triangle located between the anterior bellies of the digastric muscles and the hyoid bone. They receive lymph from the lower lip, anterior lower alveolar ridge, anterior floor of mouth and tip of tongue. Efferent trunks drain to the submandibular nodes or directly to the deep cervical chain.

The submandibular nodes - usually three to seven - lie along the lower border of the mandible close to the submandibular salivary gland and facial artery. Afferent vessels arise in the submental and facial nodes, labial and buccal mucosae, palate, anterior two-thirds of tongue, and floor of mouth. Efferents drain to the deep cervical chain.

(ii) The Deep Cervical Chain (or Jugular Chain) of Nodes

These nodes, usually 20 to 30, are situated along the internal jugular vein and all lymphatic channels arising in the oral mucosa drain, directly or indirectly, into them (DiTroia, 1972). The nodes are uniform in their distribution along the vein (Fisch and Sigel,

1964) but for convenience can be divided into three groups: the superior, middle, and inferior cervical groups (DiTroia, 1972).

The superior cervical (jugulodigastric) nodes are located parallel to the vein just below the posterior belly of the digastric muscle. Afferents arise in the submental, submandibular, occipital, posterior auricular, parotid, and retropharyngeal nodes, and directly in most sites in the oral cavity and pharynx.

The mid cervical (jugulocarotid) nodes are located at the level of the bifurcation of the common carotid artery. In addition to draining the superior cervical nodes, some afferents drain directly from the mid portion of the tongue.

The inferior cervical (jugulo-omohyoid) nodes are located where the anterior belly of omohyoid crosses the internal jugular vein. This group drains the more superior deep cervical nodes, and nodes of the posterior triangle. Some afferents arise directly in the tip of the tongue, and the anterior floor of mouth.

(iii) The Posterior Triangle Nodes

Anatomically grouped as the transverse cervical and spinal accessory chains, approximately 30 small nodes are found within the fat of the posterior triangle. They receive afferents from the occipital and posterior auricular nodes, and from the scalp and skin of the neck. They drain into the deep cervical chain.

At the lower end of the deep cervical chain, the vessels join to form one major trunk, which, on the right, empties into the subclavian vein, and, on the left, empties into the thoracic duct directly.

The lymphatic drainage of the tongue is complex and merits detailed consideration (Rouviere, 1938; Feind, 1972). The superficial

mucosal and deep muscular networks join to form four collecting trunks. The apical trunks, from the tip of the tongue, drain into the jugulo-omohyoid nodes, with an inconstant route draining bilaterally to the submental nodes. The marginal trunks, from the lateral one-third of the tongue, drain into the submandibular, jugulodigastric and jugulocarotid nodes. The central trunks, from the medial part of the tongue, drain bilaterally into the submandibular nodes and nodes at all three levels of the jugular chain. Finally, the basal trunks, from the posterior one-third of the tongue, drain bilaterally to the jugulodigastric and jugulocarotid nodes.

TOPOGRAPHICAL DISTRIBUTION OF CERVICAL METASTASES

In carcinoma of the oral cavity and oropharynx, metastatic spread is usually confined to one side of the neck at initial presentation (Lindberg, 1972). Histological examination of neck dissection specimens shows that nodes of the jugulodigastric (superior cervical) group are most frequently involved (Cachin *et al.*, 1967; Carter *et al.*, 1987; Byers *et al.*, 1988). Although many patients in the series reported by Carter *et al.* (1987) had advanced primary tumours, 53% of patients had metastases confined to a single nodal group, and the median number of positive nodes for the whole series was two. Nevertheless, 10% of their patients showed widespread disease involving four or more different anatomical groups of nodes. Also, it should be noted that 66% of the patients in their series had received pre-operative radiotherapy to the neck, and this is known to influence the pathological findings (Tanner *et al.*, 1980; Carter and Clifford,

1985).

In carcinoma of the tongue, any nodal group, at either side of the neck, may be the initial site of a metastatic deposit (Lyall and Shetlin, 1952; Droulias and Whitehurst, 1976). This is readily explained by a detailed knowledge of the anatomy of the tongue's lymphatic drainage system (Rouviere, 1938; Feind, 1972). The incidence of bilateral/contralateral spread ranges from 4% for tumours at the tip of the tongue to 25% for lesions at the base (DiTroia, 1972).

Metastatic spread from carcinomas of the anterior floor of mouth most frequently involve ipsilateral submandibular nodes (Shaha *et al.*, 1984). However, 25% of tumours that cross the midline ultimately develop bilateral/contralateral metastases, usually involving the contralateral jugulodigastric group (Keim and Lowenberg, 1970; DiTroia, 1972). Carcinomas of the posterior floor of mouth usually metastasise directly to the jugulodigastric and jugulocarotid nodes (DiTroia, 1972).

Involvement of the submental nodes, even in carcinoma of the anterior floor of mouth, tip of tongue, and lower lip, is rare (Sharpe, 1981; Shingaki *et al.*, 1985). Involvement of posterior triangle nodes in intra-oral cancer is also rare, and only occurs in the presence of metastasis in the jugular chain (Sharpe, 1981; Shaha *et al.*, 1984; Carter *et al.*, 1987). Metastasis to the posterior triangle, however, is more likely in carcinoma of the oropharynx and pharyngolarynx (Bataini *et al.*, 1985; Byers *et al.*, 1988). In addition, carcinoma of the soft palate, retromolar trigone, base of tongue, and pharynx may spread directly to the retropharyngeal nodes (Ballantyne, 1964).

In the presence of nodal metastasis, collateral or retrograde lymphatic flow may result in bizarre patterns of metastatic spread (Willis, 1973a). In addition, the distribution of metastases may be modified by previous radiotherapy or surgery (Sharpe, 1981; Carter and Clifford, 1985).

CLINICAL DIAGNOSIS OF CERVICAL METASTASIS

The clinical diagnosis of cervical metastasis is notoriously inaccurate. In 1906, Crile reported that 'palpable glands may be inflammatory and unpalpable glands may be carcinomatous'. The limit of palpability depends on both the location and consistency of the node. In addition to size, the shape, consistency, mobility/fixation, and the assymetrical or unilateral position of the enlargement are important clinical signs (Henk and Langdon, 1985b).

The accuracy of the pre-operative clinical assessment can be judged by a consideration of the incidence of false-positive and false-negative evaluations. The reported incidence of false-positive (clinically positive but histologically negative) assessments ranges from 8% (Beahrs and Barber, 1962) to 56% (Crissman *et al.*, 1980). Similarly, the reported false-negative rates (clinically negative, but histologically positive) range from 4% (Beahrs and Barber, 1962) to 60% (Lyall and Schetlin, 1952). In most studies, there is agreement between the clinical assessment of the patient, in terms of presence or absence of metastasis, and the histological reality in 70-76% of cases (Spiro *et al.*, 1974; Cachin *et al.*, 1979; Grandi *et al.*, 1985). This is similar to the accuracy of clinical axillary node assessment

in patients with breast cancer (Fisher *et al.*, 1975). In oral cancer, false-negative assessments account for the majority of the discordancies (Vandenbrouck *et al.*, 1980; Ali *et al.*, 1985). For example, in the study reported by Grandi *et al.* (1985), the incidence of false-positive clinical assessments was 22% and the incidence of false-negative assessments was 28%. Significantly, 12% of clinically negative necks showed extracapsular spread of metastatic tumour. Cachin *et al.* (1979), Byers *et al.* (1988), and Spiro *et al.* (1988) also commented on extracapsular spread of tumour from clinically negative nodes. The accuracy of the clinical assessment of the neck is greatest in patients with large, fixed nodes (Richard *et al.*, 1987). When the term 'fixed' is reserved for nodes that are immobile, extracapsular spread of tumour is always confirmed histologically (Spiro *et al.*, 1974).

Cady and Catlin (1969) showed the accuracy and sensitivity of clinical examination was related to the anatomical level of the nodes. While 70% of pathologically involved nodes at the inferior cervical level were detected clinically, the equivalent figure for submental and submandibular nodes was only 44%. Ali *et al.* (1985) found the incidence of false-positive nodes decreased progressively with an increase in the histological grade of the primary tumour. The number of false-positive and false-negative assessments, however, showed no relationship to the tumour stage, and even small primary tumours were found to have a high incidence of clinically occult metastases.

Enlargement of cervical nodes due to reactive hyperplasia accounts for the majority of the false-positive assessments. Grandi *et al.* (1985) showed that 17.2% of nodes clinically larger than 3.0cm.

were histologically free of metastatic disease. In addition, several anatomical structures may be mistaken for enlarged nodes. These include the greater cornu of the hyoid, the transverse process of the sixth cervical vertebra, a prominent carotid bulb, and a tortuous carotid artery (Martin *et al.*, 1951; Henk and Langdon, 1985b). An enlarged salivary gland may also be a source of confusion (Crissman *et al.*, 1980; Byers, 1985; Grandi *et al.*, 1985). In addition, the direct extension of a large primary tumour into the neck may be mistaken for metastatic disease (Byers, 1985). Following radiotherapy, fibrosis (Carter and Clifford, 1985) and keratin granulomas within cervical nodes or perinodal tissues (Tanner *et al.*, 1980) are frequently misdiagnosed as residual or recurrent metastatic disease.

Fine needle aspiration of cervical nodes is a rapid, safe and cost-effective method of confirming the presence of metastatic disease. Accuracy levels greater than 90% have been reported (Eneroth, 1973; Frable and Frable, 1979; Weymuller *et al.*, 1983). Such a high degree of accuracy, however, is only achieved when the technique is applied to accessible, easily palpable and clinically suspicious nodes. In these circumstances, false-positive results are virtually never obtained, but a small number of false-negative results is inevitable since the needle tip may fail to locate the actual tumour cells within the node (Henk and Langdon, 1985b). Also, scarring from previous therapy may pose technical difficulties (Frable and Frable, 1979), and, on occasions, the sample may be inadequate due to widespread necrosis or cystic degeneration (Conley, 1967b). Hence, as with all minimal sampling techniques, only a positive result is contributory. Implantation of cancer cells within the needle tract

does not seem to occur (Conley, 1967b; Frable and Frable, 1979).

In the past, various imaging techniques such as lymphography (Fisch and Sigel, 1964) and scintigraphy (Schwab, 1967) have been used in an attempt to detect early nodal metastases or to confirm the metastatic nature of a palpable mass. None of these techniques proved reliable (Henk and Langdon, 1985b). However, three of the newer imaging techniques currently in use merit brief comment. These are ultrasound, computed tomography, and nuclear magnetic resonance.

Small parts ultrasound has been used to detect and delineate enlarged cervical nodes (Hajek *et al.*, 1986). However, is is not always possible to distinguish reactive from metastatic nodes and accurate definition of the extent of invasion of perinodal structures is the most useful aspect.

In recent years, computed tomography (CT) has been used to determine the size and gross morphology of lymph nodes in the neck and diagnostic criteria for both metastasis and extranodal extension are now established (Mancuso *et al.*, 1983; Friedman *et al.*, 1984; Close *et al.*, 1989; Stern *et al.*, 1990; Hillsamer *et al.*, 1990). Some authorities claim that CT assessment of the clinically negative neck is highly sensitive. For example, sensitivity rates of 84% and 90%, respectively, have been reported by Hillsamer *et al.* (1990) and Friedman *et al.* (1984). However, in the study reported by Feinmesser *et al.* (1987), the sensitivity of the CT assessment was no greater than that of physical examination alone (62% and 60%, respectively).

Reports by Hillsamer *et al.* (1990) and Friedman *et al.* (1990) suggest the accuracy of the pre-operative assessment can be improved still further by the use of magnetic resonance imaging (MRI), which

has better resolution properties than CT. In the study reported by Hillsamer *et al.* (1990), MRI had an overall efficiency predictive value of 90%, compared with 81% for CT, and 78% for physical examination. By using both CT and MRI, the incidence rate of pre-operative occult metastases was reduced to 7%. Although these initial results are promising, it will be some time before the equipment and expertise become widely available for routine use.

NECK DISSECTION - TYPES OF PROCEDURE

Surgical control of neck metastases dates back to 1906 when Crile described the first anatomically based neck dissection. Since then, there have been many modifications of this procedure. Neck dissections as currently performed may be classified according to:

(i) the extent of the procedure (radical/functional/partial; unilateral/bilateral).

(ii) the timing of the procedure in relation to the treatment of the primary tumour (simulataneous/delayed). Simultaneous dissections may be further classified as 'in continuity' or 'in discontinuity'.

(iii) the indications for the procedure (elective/therapeutic/salvage).

(1) The Extent of the Neck Dissection

In the standard radical neck dissection (Crile, 1906), the submandibular nodes, the jugular chain of nodes and the posterior triangle nodes are removed, *en bloc*, with sacrifice of the sternomastoid muscle, the internal jugular vein, the spinal accessory

nerve, the submandibular salivary gland and the tail of the parotid gland. Following this procedure, patients suffer from several temporary or permanent disabilities (Barnes and Johnson, 1986b). Transection of the spinal accessory nerve results in loss of motor function to the trapezius muscle. Removal of the sternomastoid muscle produces a cosmetic deformity and decreased ability to move the neck. Sacrifice of the internal jugular vein results in temporary congestion of the face.

The standard radical neck dissection does not, by definition, include removal of the retropharyngeal, paratracheal, parotid, suboccipital, and/or upper mediastinal nodes. When one or more of these groups are likely to be involved by tumour, the procedure can be extended to include them. This then constitutes the extended radical neck dissection (Goepfert and Jesse, 1982). In the case of extensive metastatic disease in the jugular chain, the standard radical neck dissection can be extended to include platysma and/or the stylohyoid and digastric muscles (Feind, 1972; Lore, 1973).

In an attempt to reduce the post-operative morbidity of the standard radical neck dissection, Bocca and Pignataro (1967) described the modified radical neck dissection (the functional neck dissection). In this procedure, the sternomastoid muscle, spinal accessory nerve and the internal jugular vein are spared. Most authorities believe that when the disease in the neck is either occult or confined to mobile lymph nodes, a functional neck dissection is oncologically safe and results in cure rates similar to the standard radical neck dissection (Bocca, 1975; Jesse *et al.*, 1978; Carenfelt and Eliasson, 1980; Bocca *et al.*, 1984). A functional neck dissection is contra-

indicated in the presence of fixed nodes or recurrent disease in the neck following surgery or radiotherapy (Bocca *et al.*, 1984).

The partial (regional or selective) neck dissection is tailored to remove those nodes at greatest risk in a particular patient. Byers (1985) classified these partial procedures as suprahyoid, supra-omohyoid, anterior modified, posterior, and lower neck dissections. Partial procedures are based upon the premise that cervical metastases from each primary site are predictable in their distribution and orderly in their progression through the lymphatic system. However, the complexity of the intra-oral and cervical lymphatic drainage systems means that partial procedures such as the suprahyoid neck dissection are of limited value, even as staging procedures (Donegan *et al.*, 1982; Barnes and Johnson, 1986b).

Neck dissection is usually a unilateral procedure. If bilateral standard radical neck dissections are indicated, one procedure must be delayed to avoid venous engorgement of the head (Martin *et al.*, 1951). Alternatively, a functional neck dissection may be performed on the less-affected side of the neck (Henk and Langdon, 1985b). Bilateral functional neck dissections can be effected as a simultaneous procedure with no untoward consequences (Bocca *et al.*, 1984).

(2) Timing of the Neck Dissection

Treatment of the neck as a simultaneous procedure during resection of the primary tumour has several surgical and oncological advantages over a delayed (staged) procedure (Lyall and Shetlin, 1952; Henk and Langdon, 1985b). When possible, the intra-oral resection should be in continuity with the neck dissection.

(3) Indications for Neck Dissection

Therapeutic neck dissection is indicated for clinically positive, resectable lymph nodes (Henk and Langdon, 1985b).

The decision whether the patient with a clinically negative neck should be treated by elective (prophylactic) neck dissection, or merely followed up and surgery performed only if overt metastases develop, is one of the most controversial issues in head and neck oncology (Maddox, 1984). The decision whether to treat the clinically negative neck or not is frequently based on the statistical risk of the presence of occult cervical metastases (Farr *et al.*, 1980; Henk and Langdon, 1985b). However, the evidence that patients benefit from elective neck dissection is inconclusive. Spiro and Strong (1973), Lee and Krause (1975), Shingaki *et al.* (1985), and Cunningham *et al.* (1986) reported that the rate of recurrence in the operated neck was lower following elective neck dissection than following therapeutic and salvage procedures. However, Stell and Green (1976) found no significant difference in the survival rates of matched pairs of patients following either elective neck dissections or a 'wait and see' policy. Similarly, Vandenbrouck *et al.* (1980) reported no difference in survival following a random clinical trial of elective versus therapeutic neck dissection. Mendelson *et al.* (1976) found that the high cure rate in their elective procedure group was due entirely to the subgroup of patients with histologically negative necks. Jesse *et al.* (1970) reported that only 2-5% of patients actually benefit from a prophylactic neck dissection when such factors as uncontrolled local disease, distant metastases, second primary tumours, and contralateral neck disease are considered.

However, proponents of elective neck dissection argue that functional and partial (selective) procedures are followed by little, if any, morbidity, and avoid the risk of a radical operation at a later date (Farr *et al.*, 1980; Byers *et al.*, 1988). In addition, accurate pathological staging of the neck dissection specimen identifies patients at risk of cervical recurrence who may benefit from post-operative radiotherapy (Henk and Langdon, 1985b; Byers *et al.*, 1988).

Technical indications for elective neck dissection are less controversial. Most surgeons recommend a functional or partial dissection when the neck is entered to facilitate access for the removal of the intra-oral tumour, microvascular anastomosis, or elevation of skin flaps (Nahun *et al.*, 1977; Farr *et al.*, 1980; Callery *et al.*, 1984; Byers *et al.*, 1988). Elective procedures are also indicated if clinical assessment of the neck is difficult (Farr *et al.*, 1980; Callery *et al.*, 1984), or if follow-up of the patient is likely to be unsatisfactory (Nahun *et al.*, 1977; Farr *et al.*, 1980; Byers *et al.*, 1988).

A salvage procedure is indicated if neck disease persists or recurs after radiotherapy or surgery (Henk and Langdon, 1985b). The management of overt metastases in the ipsilateral previously untreated neck, or contralateral neck, with or without recurrence of intra-oral disease, can also be classified as a salvage procedure. In such circumstances, standard or extended radical neck dissections are usually necessary, and cure rates are low (Henk and Langdon, 1985b).

SUMMARY

In this and the preceding chapter, the role of the cervical lymphatic system in the natural history of oral cancer, and the significance of metastatic spread in determining the outcome of this important disease have been discussed. From this extensive review, it is apparent that uncertainties still exist in relation to the histological incidence and extent of metastatic spread in oral cancer. Of particular concern is the incidence of clinically occult metastases and extracapsular spread of tumour from small nodes. The relative importance of the different pathological features of metastatic nodes in predicting regional recurrence and blood-borne metastases is still contentious. In addition, the precise clinical and histological features of the primary tumour that correlate with the presence of metastatic disease are uncertain. In particular, the relative importance of tumour thickness and tumour surface area is unresolved and the value of a multifactorial histological malignancy grading system in predicting metastasis is uncertain.

My proposed investigations, therefore, are relevant to current limitations in the state of knowledge. As outlined in the Introduction to this thesis, three separate but related investigations have been carried out.

Firstly, the patterns of metastatic spread in a series of neck dissection specimens from patients with oral squamous cell carcinoma, have been observed, and these observations will be detailed in the next chapter (Chapter 4). The related questions of the accuracy of the clinical assessment of the metastatic status and the efficacy of the surgical neck dissection procedure in the management of actual or

potential lymphatic spread will be addressed in Chapter 5.

The second investigation, to assess the extent to which selected clinical and histological features of the primary intra-oral tumour correlate with the presence of cervical metastasis will be described in Chapter 6.

Finally, in Chapter 7, the third investigation, to assess the value of nuclear morphometry in predicting the nodal metastatic status, will be reported.

Chapter 4.

THE INCIDENCE AND PATTERN OF NODAL METASTASIS IN NECK DISSECTIONS PERFORMED SIMULTANEOUSLY WITH SURGICAL RESECTION OF THE PRIMARY ORAL SQUAMOUS CELL CARCINOMA (SERIES I).

1. Introduction.
2. Surgical Cases.
3. Methods.
4. Results.
5. Discussion.
6. Summary.

INTRODUCTION

The aim of the present study is to investigate the histological incidence and pattern of cervical lymph node metastasis in a series of neck dissections, performed simultaneously with resection of the primary intra-oral/oropharyngeal squamous cell carcinoma. The pattern of metastasis will be detailed by investigating the anatomical level of metastatic involvement; the number, size and extent of replacement of positive lymph nodes; and the incidence and extent of extracapsular spread of metastatic carcinoma. The related questions of the accuracy of the clinical assessment of the metastatic status of the cervical nodes and the efficacy of the surgical neck dissection procedure in dealing with actual or potential metastatic spread will be addressed in the next chapter (Chapter 5).

SURGICAL CASES

Between November, 1989, and November, 1992, 175 sides of neck dissection were submitted to the Oral Pathology Diagnostic Histology Service at the University of Liverpool School of Dentistry. All the surgical procedures had been carried out or closely supervised by one of two Consultants (Mr E. D. Vaughan and Mr J. S. Brown), at the Mersey Region Maxillofacial Unit at Walton Hospital, Liverpool. On reception into the Oral Pathology Laboratory, a standard protocol was followed, and all gross dissections and histological examinations were made and reported by the author. These neck dissections provided the material for the present study and for the studies reported in Chapters 5, 6 and 7. A brief resume of the clinical details, the surgical procedure and the histological findings in each of the 175 sides of neck dissection (in chronological order of their submission) is presented in Appendix 2.

Only neck dissections which were performed simultaneously with surgical resection of the primary carcinoma were considered for entry into the present study (Series I). In addition, four other criteria had to be met:

(i) The primary carcinoma should have arisen from the mucosa lining the oral cavity or oropharynx, excluding the lips.

(ii) The clinical history should show no previous chemotherapy, radiotherapy or surgery to the head and neck, other than routine dento-alveolar procedures and/or a recent diagnostic biopsy procedure.

(iii) The clinical history should show no evidence of

immunodeficiency, including drug-induced immunosuppression.

(iv) The surgical neck dissection procedure should have been either a standard or extended radical neck dissection, a functional neck dissection or a supra-omohyoid neck dissection. Both unilateral and bilateral procedures were acceptable.

The radical neck dissection (Crile, 1906; Martin *et al.*, 1951) and the functional neck dissection (Bocca and Pignataro, 1967) are described in Chapter 3 (Neck Dissection - Types of Procedures). The supra-omohyoid neck dissection is a partial (selective) neck dissection that removes, *en bloc*, the contents of the submental and submandibular triangles, the lymph nodes of the deep cervical chain from the jugulodigastric to the jugulo-omohyoid groups, inclusive, and the lymph node-bearing tissues located anterior to the cutaneous branches of the cervical plexus (Medina and Byers, 1989).

The first 40 unilateral neck dissections and the first 20 bilateral (40 sides) neck dissections which met all the necessary criteria were selected from the neck dissections listed in Appendix 2 and entered into the present study which thus comprised 80 sides of neck dissection (Series I).

The following clinical information was available for each of the neck dissections accepted into the study:

(i) The sex of the patient.

(ii) The age of the patient at the time of the neck dissection.

(iii) The specific site of the primary tumour.

The oral cavity was divided into the following seven

subsites (American Joint Committee on Cancer, 1988b): buccal mucosa, lower alveolar ridge, upper alveolar ridge, retromolar trigone, hard palate, floor of mouth, and oral tongue (anterior two-thirds of the tongue). The oropharynx was considered as a single site, with the base (posterior one-third) of tongue forming its anterior wall and the inferior surface of the soft palate and uvula forming its superior wall. Detailed definitions of these eight sites are presented in Appendix 3. Oral tongue, floor of mouth, and oropharynx were designated as sites at high risk of metastasis. The other five sites were designated as 'low-risk' sites.

(iv) The stage of the primary tumour at initial presentation as recorded in the case notes. Definitions of the four T categories (American Joint Committee on Cancer, 1988b) are presented in Appendix 1.

(v) The pre-operative clinical metastatic status of the cervical lymph nodes, as determined by the results of two investigations: the Consultant Surgeon's examination of the neck under general anaesthesia during the initial clinical staging assessment and imaging of the neck by Computed Tomography (CT). The neck was recorded as clinically N positive if either or both investigations suggested that metastatic disease was present. All other necks were recorded as clinically N negative.

METHODS

(1) Operating Theatre Procedures and Fixation

The following protocol was agreed between the author and the members of the surgical team to ensure that specimens reached the Oral Pathology Laboratory in a well-preserved state, with the minimum of tissue distortion.

Immediately following surgery, the resected specimen was pinned or sutured to a block of polystyrene. In functional and supra-omohyoid dissections, marker sutures were placed to aid orientation and to denote the position of the jugulodigastric nodes. Other anatomical structures were labelled if their origin and/or orientation were not obvious. In addition, the Surgeon provided a labelled sketch of the resected specimen on the accompanying Pathology Request Form. Sheets of gauze were placed between the specimen and the polystyrene to aid absorption of the fixative, and the polystyrene block was then inverted into a large container of fixative, taking care that the specimen was completely immersed. In all cases, the fixative was 10% buffered formalin.

(2) Gross Dissection and Sampling

The resected specimens were examined in the Laboratory after approximately 24-48 hours of fixation (Figures 4.1a and 4.1b). If the neck dissection was in continuity with the resection of the intra-oral primary tumour, pathological description and trimming of the intra-oral part was carried out immediately prior to examination and trimming of the neck dissection. In bilateral dissections, each side of the neck was described and dissected separately.

The neck dissection was orientated to its *in vivo* position. Major components were described and measured, firstly, from the superficial aspect, and then from the deep aspect. The sequence of the dissection depended on the type of surgical procedure. In radical dissections, the contents of the posterior triangle were dissected before removal of the sternomastoid muscle and dissection of the jugular chain of nodes. In functional and supra-omohyoid dissections, the bed of the internal jugular vein and the sternomastoid muscle, if discernible, were used as aids in the identification of the different anatomical groups of nodes. Lymph nodes greater than 0.3cm. in maximum dimension were identified by palpation and visual inspection. Clearing techniques were not used.

In the case of nodes with obvious metastatic involvement, the following protocol was used to describe the presence and extent of macroscopic extracapsular spread. Firstly, any fixation to perinodal fat, muscles and vessels and the need for sharp, as opposed to blunt, dissection in order to expose and remove the metastatic node(s) was recorded. Secondly, if extracapsular spread had resulted in fusion (matting) of adjacent nodes, these were dissected out and recorded as a single metastatic mass. Thirdly, the extent of any macroscopic extracapsular spread (from either metastatic masses or discrete positive nodes) was recorded by reference to the perinodal tissues and/or anatomical structures involved.

Metastatic masses and discrete positive nodes were removed from the gross specimen, measured and divided into slices, each approximately 0.4cm. in thickness. The presence and extent of any cystic change was noted. Those slices which appeared to show the

maximum extent of extracapsular spread were selected for processing.

Lymph nodes not obviously involved by tumour were also removed from the gross specimen, together with a small amount of perinodal fibro-adipose tissue. The maximum dimension of the node was recorded. Nodes larger than 0.5cm. were bisected in their long axis, through the hilum, if this could be identified. Depending on the shape of the node, further longitudinal cuts were sometimes made to ensure tissue blocks were sufficiently thin (0.4-0.5cm.) to allow optimal tissue processing. Smaller lymph nodes were not bisected prior to processing.

During the macroscopic examination and dissection of the specimen, lymph nodes were assigned to one of the five major anatomical groups, described in Chapter 3 (Anatomy of the Cervical Lymphatic System). The submandibular/submental group was designated as 'level I', the jugulodigastric (superior cervical) group as 'level II', the jugulocarotid (mid cervical) group as 'level III', the jugulo-omohyoid (inferior cervical) group as 'level IV', and the posterior triangle group as 'level V'.

Lymph nodes which had been bisected or sliced were placed in cassettes, and labelled and charted on a diagram, in such a way that their anatomical position within the nodal group was recorded as accurately as possible. Smaller nodes from the same anatomical group were processed together in one or more cassettes.

Any areas of the sublingual, submandibular and parotid salivary glands that appeared macroscopically to be involved by tumour were selected for processing. In the absence of obvious involvement by tumour, representative blocks of the salivary glands were processed.

(3) Histological Methods

The tissue blocks were routinely processed for embedding in paraffin wax. From each block, one complete section was cut at five microns thickness, stained with haematoxylin and eosin, and histologically examined.

Step-serial sections (100 microns apart) were prepared of those lymph nodes greater than 2.4cm. in maximum dimension that appeared free of tumour on histological assessment of the initial section. In addition, in some nodes with obvious metastatic involvement, further sectioning was necessary in order to clarify the extent of the extracapsular spread.

Lymph nodes were recorded as negative or positive for metastatic carcinoma. In the case of positive nodes, the extent to which the normal nodal architecture had been replaced by the metastatic deposit was subjectively graded and assigned to one of three categories:

(i) Minimal: up to 5% replacement (Figure 4.2a).

(ii) Partial: between 5% and 80% replacement (Figure 4.2b).

(iii) Total: more than 80% replacement (Figure 4.2c).

Step-serial sections (100 microns apart) were prepared of those nodes showing minimal replacement on initial assessment, and, if necessary, the extent-of-replacement grade was adjusted.

In the case of metastatic masses and discrete nodes showing macroscopic extracapsular spread, histological assessment was carried out in order to confirm the extent of spread. In the case of other positive nodes, the presence and extent of microscopic extracapsular spread was assessed and each positive lymph node was assigned to one of three categories:

(i) No extracapsular spread: metastatic deposit confined by the node capsule (Figure 4.3a).

(ii) Microscopic embolisation and/or permeation of perinodal lymphatics (Figure 4.3b).

(iii) Microscopic extracapsular spread to perinodal tissues and/or anatomical structures (Figure 4.3c).

The anatomical position of the lymph nodes, and their approximate size, were recorded on a topographical diagram. Negative lymph nodes were shown in blue ink, while positive nodes were marked in red ink. Extracapsular spread was indicated by arrows radiating from the involved node. Examples of these diagrams are given in Figure 4.4a, which shows a unilateral dissection, and Figure 4.4b, which relates to a bilateral dissection.

(4) Statistical Methods

The results of the study were analysed using the SPSS-X statistical package on the University of Liverpool IBM computer. The following tests were used.

(a) The Two Sample t Test

This test is for the comparison of two independent groups of data. The test is appropriate when the data is both continuous and approximately Normal in its distribution, and when the two groups have similar variances (Altman, 1991a).

The test statistic, t, is calculated from the formula

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{s^2(1/n_1 + 1/n_2)}}$$

where n_1 and n_2 are the number of observations in the two groups, $\bar{x}_1$

and $\bar{x}_2$ are the means of the observations in the two groups and s^2 is the common variance.

The result of the test is presented as the t value, the degrees of freedom (d.f.), and P, the associated probability (two-tailed) of obtaining such a t value by chance under the null hypothesis.

(b) The Chi Squared Test

This test analyses categorical data that can be presented as a frequency or contingency table. The test is based on observed row and column totals and compares the observed frequencies with the expected frequencies assuming, as the null hypothesis, that the categorical variables denoting the rows and columns are independent (Altman, 1991b).

The test statistic, X^2, is obtained from the observed and expected frequencies, O and E respectively, by calculating the sum of the quantities

$$\frac{(O - E)^2}{E}$$

for all the cells in the table.

The expected frequency in each cell is the product of the relevant row and column totals divided by the total sample size.

When the categorical variables are independent, the test statistic has a Chi squared distribution. Hence, the null hypothesis can be evaluated from the test statistic by reference to standard tables. The result of the test is presented as the test statistic, X^2, the degrees of freedom (d.f.), and the associated probability, P, of obtaining such a X^2 value by chance under the null hypothesis.

(c) Yates'-Corrected Chi Squared Test

When the sample size is small, the use of the continuous Chi squared distribution to approximate frequencies introduces some bias into the Chi squared calculation, resulting in a Chi squared test statistic, X^2, that is too large (Altman, 1991b). The Yates'-Corrected Chi Squared Test uses a continuity correction to remove the bias:

each (O-E) is moved nearer to zero by ½.

Hence, (O-E) in the equation given for the standard Chi squared test statistic becomes (O-E)-½ in the Yates'-Corrected Chi Squared Test.

The Yates' Corrected Chi Squared Test is used when the expected cell frequency is less than five, and the result is presented as the Yates'-Corrected Test Statistic, X^2_{YATES}, the degrees of freedom (d.f.), and the associated probability, P, of obtaining such a value of X^2_{YATES} by chance under the null hypothesis.

(d) Chi Squared Test For Trend

This test, a modification of the standard Chi squared test, is used to compare frequencies or proportions among groups that have an ordering. The Chi Squared Test For Trend uses the ordering to increase the power of the statistical analysis (Altman, 1991b).

Variation among groups can be subdivided into that due to a trend in the proportions across the groups and the remainder (Altman, 1991b). In the Chi Squared Test For Trend, regression analysis is used to evaluate a trend, and the result is tested against the null hypothesis that there is no trend across the groups.

The result of the test is presented as the test statistic, X^2_{TREND}, the degrees of freedom (d.f.), and the associated probability, P, of obtaining such a value of X^2_{TREND} by chance under

the null hypothesis.

(e) Fisher's Exact Test

This test analyses categorical data that can be presented as a frequency or contingency table. The test is appropriate when the sample size and, hence, the expected number of observations falling in each category or cell, is small (Altman, 1991b).

The test is based on the observed row and column totals and evaluates the probability associated with all possible 2 2 tables which have the same row and column totals as the observed data, assuming the null hypothesis is correct.

The probability of obtaining the cell frequencies a, b, c and d is given by:

$$\frac{(a+b)!\ (a+c)!\ (b+d)!\ (c+d)!}{N!a!b!c!d!}$$

where x! denotes 'x factorial', and N is the total number of observations.

The result of the test, P, is the overall probability of obtaining a difference between the groups as large as the observed difference under the null hypothesis. The P value (for one tail) can be doubled to give a two-tailed test result (Altman, 1991b) and this latter value is given in each case.

RESULTS

I. CLINICAL DETAILS OF THE SURGICAL CASES

(1) The Simultaneous Unilateral Procedure Patients

Clinical details of the 40 patients undergoing unilateral neck dissection are presented in Table 4.1. Here, the cases have been grouped according to the specific site of the primary tumour. Also shown in Table 4.1 is the type of neck dissection (radical/functional/supra-omohyoid) and the histological metastatic status of the cervical nodes (negative/positive).

Twenty-three (58%) of the 40 patients were male, with a mean age of 60 years (SD 12.3, range 37-81). The mean age of the 17 female patients was 64 years (SD 11.8, range 33-81). A summary of the details of the site and stage of the primary tumour in the 40 unilateral procedure patients is presented in Table 4.2.

(2) The Simultaneous Bilateral Procedure Patients

Clinical details of the 20 patients undergoing bilateral neck dissection are presented in Table 4.3. For each patient, the clinical and histological status of the left and right neck is shown. Details concerning the type of surgical procedure are also presented separately for the left and right sides of the neck.

Fourteen (70%) of the 20 patients were male, with a mean age of 58 years (SD 9.8, range 43-73). The mean age of the six female patients was 64 years (SD 8.0, range 57-79). A summary of the details of the site and size of the primary tumour in the 20 bilateral procedure patients is presented in Table 4.4.

(3) The Simultaneous Procedure Patients (Series I)

A summary of the details of the site and stage of the primary

tumour in all 60 neck dissection patients (40 unilateral procedure patients, 20 bilateral procedure patients) is presented in Table 4.5, which thus summarises the data of Tables 4.2 and 4.4. Thirty-seven (62%) of the 60 patients were male, with a mean age of 59 years (SD 11.4, range 37-81). The mean age of the 23 female patients was 64 years (SD 10.7, range 33-81). As shown in Table 4.5, the site of the primary tumour was oral tongue or floor of mouth in 39 (65%) of the 60 patients: oral tongue in 21 (35%) and floor of mouth in 18 (30%). In the other 21 patients, the primary tumour was sited on the buccal mucosa (six cases, 10%); lower alveolar ridge (seven cases, 12%); retromolar trigone (two cases, 3%); and oropharynx (six cases, 10%). In 16 (27%) of the 60 patients, the primary tumour was staged T1; in 21 patients (35%), T2; in seven patients (12%), T3; and in the remaining 16 patients (27%), T4.

II. PATHOLOGICAL ASSESSMENT OF THE NECK DISSECTION SPECIMENS

(1) Pathological Findings in the Unilateral Neck Dissection Specimens

(a) Frequency of Metastasis

Lymph node metastasis was evident on histological assessment in 18 (45%) of the 40 patients undergoing unilateral neck dissection.

(b) Frequency of Metastasis in relation to Clinical Features

There was no significant difference in the frequency of metastasis in relation to sex. Metastasis was present in 12 (52%) of the 23 males and in six (35%) of the 17 females (Fisher's Exact Test, $P = 0.46$). The mean age of the 18 patients with metastasis was 65 years (SD 9.1, range 43-81). This was not significantly different from the mean age, 59 years (SD 13.9, range 33-81), of patients without

metastasis (Two Sample t Test, t = 1.52, 17 d.f., P = 0.14).

The frequency of histological metastasis in relation to the site and stage of the primary tumour is shown in Table 4.2. Metastasis was present in three (21%) of the 14 tumours arising at low-risk sites and in 15 (58%) of the 26 tumours at high-risk sites, and the difference in frequency of metastasis in relation to site was statistically significant (Fisher's Exact Test, P = 0.05). However, there was no significant difference in the frequency of metastasis in relation to the T stage of the primary tumour (Chi-squared, X^2 = 3.81, 3 d.f., P = 0.28; Chi-squared for Trend, X^2_{TREND} = 1.48, 1 d.f., P = 0.22). Also, there was no significant difference in the frequency of metastasis in tumours staged T1 and tumours staged T2/T3/T4 (Fisher's Exact Test, P = 0.14), nor in tumours staged T1/T2 and tumours staged T3/T4 (Fisher's Exact Test, P = 0.84).

(c) Frequency of Extracapsular Spread of Metastatic Carcinoma

In 14 of the 18 positive dissections, the metastatic carcinoma was associated with discrete lymph nodes. Macroscopic extracapsular spread was present in two of the 14 dissections and microscopic extracapsular spread was diagnosed in another five. In the remaining seven dissections, metastatic carcinoma was confined by the capsule of the involved node(s). The other four unilateral procedure patients with metastasis had more advanced disease, with at least one mass of fused nodes (and, therefore, macroscopic extracapsular spread) evident on examination of the gross specimen. Hence, in total, eleven (61%) of the 18 unilateral procedure patients with metastasis showed extracapsular spread. In six patients (33%), this was macroscopic in extent, and in five patients (28%), microscopic in extent (Table 4.6).

(d) Number and Distribution of Metastatic Deposits

The number and anatomical position of positive lymph nodes/ metastatic masses in each of the 18 positive dissections is shown in Figure 4.5.

The metastatic deposit(s) involved a single anatomical level in eight (44%) of the 18 positive dissections (level I in four cases, and level II in four cases). Two levels were involved in four dissections (22%); three levels in three dissections (17%); four levels in two dissections (11%); and all five anatomical levels were involved in one dissection (6%).

Anatomical level II was the most frequent site of metastasis. It was involved in 13 (72%) of the 18 positive dissections. Level I was involved in eight dissections (44%), as was level III. Level IV was involved in six dissections (33%), and level V was involved in three dissections (17%). In the three patients with a primary carcinoma of the buccal mucosa, the positive lymph nodes were all located at level I. In the other 15 patients (in whom the primary carcinoma was sited on the oral tongue, floor of mouth or oropharynx), metastasis involved node(s) of the deep cervical chain (levels II-IV). In five of these patients, node(s) at level I were involved also (Figure 4.5).

Five of the 14 patients with metastatic disease associated with discrete lymph nodes had a single positive node. Six patients had two positive nodes, two patients had three positive nodes, and in the remaining patient, five positive nodes were evident on histological assessment. Hence, the average number of positive nodes in the 14 dissections with disease associated with discrete nodes was 2.0 (SD 1.1, median 2). Two of the four patients with advanced metastatic

disease had one fused nodal mass. The other two patients each had four discrete masses of fused nodes. In addition, all four patients had at least one positive discrete node (Figure 4.5). In total, the 18 positive unilateral neck dissections yielded ten metastatic masses and 44 discrete positive nodes for detailed histological assessment (Table 4.7).

Also recovered from the 40 unilateral neck dissections were 1,288 discrete lymph nodes showing no evidence of metastasis on histological assessment. Details of their anatomical distribution, size, and yield in relation to the type of surgical procedure, will be presented in Chapter 5.

(2) Pathological Findings in the Bilateral Neck Dissection Specimens

(a) Frequency of Metastasis

Lymph node metastasis was evident on pathological assessment in 13 (65%) of the 20 patients undergoing bilateral neck dissection. Three of the 13 patients with metastasis had histological evidence of disease in both sides of the neck. Hence, in total, 16 (40%) of the 40 bilateral procedure sides of neck dissection were positive for metastatic disease.

(b) Frequency of Metastasis in relation to Clinical Features

There was no significant difference in the frequency of metastasis in relation to sex. Metastasis was present in ten (71%) of the 14 males and in three (50%) of the six females undergoing bilateral neck dissection (Fisher's Exact Test, P = 0.67). The mean age of the 13 patients with metastasis was 56 years (SD 8.6, range 43-68). This was significantly lower than the mean age of 66 years (SD

8.1, range 57-79) in the seven patients without metastasis (Two Sample t Test, t = 2.39, 18 d.f., P = 0.03). The frequency of histological metastasis in relation to the site and stage of the primary tumour is shown in Table 4.4. The single patient with a tumour arising at a low-risk site had no evidence of metastasis. In contrast, metastasis was present in 13 (68%) of the 19 patients with tumours at high-risk sites. However, the difference in frequency of metastasis in relation to site did not achieve statistical significance (Fisher's Exact Test, P = 0.76). Also, there was no significant difference in the frequency of metastasis in relation to the T stage of the primary tumour (Chi-squared, X^2 = 0.38, 3 d.f., P = 0.94; Chi-squared for Trend, X^2_{TREND} = 0.14, 1 d.f., P = 0.71; T1 versus T2/T3/T4, Fisher's Exact Test, P = 1.0; T1/T2 versus T3/T4, Fisher's Exact Test, P = 0.95).

Two of the three patients with bilateral metastases (ND106/107 and ND114/115) had primary tumours sited on the floor of mouth (staged T1 and T2, respectively). The third patient with bilateral spread (ND141/142) had two synchronous T1 tumours of the oropharynx.

(c) Frequency of Extracapsular Spread of Metastatic Carcinoma

In nine of the 13 bilateral procedure patients with metastasis, the metastatic carcinoma was associated with discrete nodes. In three of the nine patients, macroscopic extracapsular spread was present; in another three patients, microscopic extracapsular spread was diagnosed; and in another single case, permeation and embolisation of the perinodal lymphatics was present. Tumour was confined by the capsule of the node(s) in the remaining two patients. Four bilateral procedure patients had more advanced metastatic disease, with at least one metastatic mass of fused nodes. Hence, in total, eleven (85%) of

the 13 bilateral procedure patients with metastasis showed extracapsular spread. In seven patients (54%), this was macroscopic in extent, and in four patients (31%), microscopic in extent (Table 4.6).

One of the three patients with bilateral metastasis (ND141/142) had fused nodal masses on both sides of the neck. The second patient (ND106/107) had bilateral microscopic spread from discrete positive nodes. The third patient (ND114/115) had a fused nodal mass on one side but no evidence of extracapsular spread on the other side.

(d) Number and Distribution of Metastatic Deposits

The number and anatomical position of the positive lymph nodes/ metastatic masses in each of the 13 bilateral procedure patients with metastasis (16 positive sides of neck dissection) is shown in Figure 4.6.

In eight of the ten patients with metastasis confined to one side of the neck, the metastatic deposits involved a single anatomical level. In five patients, this was level I and in three patients, level II. In the other two patients with unilateral metastatic spread, two anatomical levels were involved (levels I and II in one case, and levels II and IV in the other case).

One of the three patients with bilateral metastatic spread had bilateral involvement of level I; one had bilateral involvement of level II; and the third had involvement of level I on one side and involvement of levels I-III on the other side.

In bilateral procedure patients, anatomical level I was the most frequent site of metastasis. It was involved in ten (63%) of the 16 positive sides of neck dissection. Level II was involved in eight dissections. Level III was involved in one dissection, as was level

IV. Level V was not involved in any of the bilateral procedure dissections. In six of the ten patients with a primary carcinoma of the oral tongue or floor of mouth, metastasis was confined to level I. The other four patients with tongue or floor-of-mouth tumours showed involvement of nodes of the deep cervical chain (levels II-IV) with or without involvement of level I. In the three patients with a primary carcinoma of the oropharynx, the positive nodes were located at level II (Figure 4.6).

In eleven of the 16 positive sides of neck dissection, metastasis was associated with discrete lymph nodes. In six of these dissections, there was a single positive node; in four, two positive nodes; and in one dissection, three positive nodes were present (Figure 4.6). Hence, the average number of positive nodes in dissections with disease associated with discrete nodes was 1.5 (SD 0.7, median 1). Four of the five sides of neck dissection showing advanced metastatic disease contained one metastatic mass of fused nodes. The other dissection contained two metastatic masses. One dissection contained, in addition, three positive discrete nodes. Hence, in total, the 16 positive sides of neck dissection in the 13 bilateral procedure patients with metastasis yielded six metastatic masses and 20 discrete positive nodes for detailed histological assessment (Table 4.7).

Also recovered from the 40 bilateral procedure sides of neck dissection were 906 discrete lymph nodes showing no evidence of metastasis on histological assessment. Details of these will be presented in Chapter 5.

(3) Pathological Findings in Simultaneous Neck Dissection Specimens (Series I)

Since only three of the 20 bilateral procedure patients had histological evidence of bilateral metastastic spread, the unilateral and bilateral neck dissections will be grouped together for detailed analysis of the results of my investigations.

(a) Frequency of Metastasis

A summary of the histological metastatic status in all 60 patients in Series I is presented in Table 4.6, and a summary of the pathological findings in the 80 sides of neck dissection is presented in Table 4.7. Thiry-one (52%) of the 60 patients and 34 (43%) of the 80 sides of neck dissection had histologically confirmed lymph node metastasis.

(b) Frequency of Metastasis in relation to Clinical Features

Histologically diagnosed lymph node metastasis was present in 22 (59%) of the 37 males and in nine (39%) of the 23 females. The small difference in frequency of metastasis in relation to sex was not significant (Fisher's Exact Test, $P = 0.21$).

The mean age of the patients with metastasis was 61 years (SD 9.8, range 43-81). This was similar to the mean age, 60 years (SD 12.9, range 33-81), of patients without metastasis (Two Sample t Test, $t = 0.14$, 58 d.f., $P = 0.89$).

As shown in Table 4.5, none of the seven patients with tumours sited on the lower alveolar ridge, nor either of the two patients with tumours of the retromolar trigone had histological evidence of metastasis. However, metastasis was present in three (50%) of the six patients with tumours of the buccal mucosa; in twelve (57%) of the 21

patients with tumours of the oral tongue; in twelve (67%) of the 18 patients with tumours of the floor of mouth; and in four (67%) of the six patients with tumours of the oropharynx. There was a significant difference in the frequency of metastasis in relation to the metastatic risk of the primary site. Metastasis was present 28 (62%) of the 45 tumours at high-risk sites and in only three (20%) of the 15 tumours at low-risk sites (Fisher's Exact Test, P = 0.01).

Metastasis was present in six (38%) of the 16 patients with T1 tumours; in 13 (62%) of the 21 patients with T2 tumours; in three (43%) of the seven patients with T3 tumours; and in nine (56%) of the 16 patients with T4 tumours (Table 4.5). However, the difference in frequency of metastasis in relation to tumour stage did not achieve significance (Chi-squared, X^2 = 1.59, 3 d.f., P = 0.47; Chi-squared for Trend, X^2_{TREND} = 0.50, 1 d.f., P = 0.48). Also, there was no significant difference in the frequency of metastasis in tumours staged T1 and tumours staged T2/T3/T4 (Fisher's Exact Test, P = 0.30), nor in tumours staged T1/T2 and tumours staged T3/T4 (Fisher's Exact Test, P = 1.0).

(c) Frequency of Extracapsular Spread of Metastastic Carcinoma

Metastatic carcinoma was associated with discrete lymph nodes in 25 (74%) of the 34 positive dissections (Table 4.7). Extracapsular spread was present in 15 of the 25 dissections. Macroscopic spread was present in five dissections, microscopic spread in nine, and permeation and embolisation of perinodal lymphatics in one. The other nine positive dissections contained at least one metastatic mass of fused nodes. Hence, in total, 24 (71%) of the 34 positive dissections showed extracapsular spread: 14 dissections (41%) showed macroscopic

spread, nine dissections (26%) showed microscopic spread, and one dissection (3%) showed microscopic involvement of the perinodal lymphatics (Table 4.7).

(d) Number and Distribution of Metastatic Deposits

The 34 positive sides of neck dissection yielded a total of 80 metastatic deposits: 64 positive discrete nodes and 16 metastatic masses of fused nodes (Table 4.7). Their anatomical distribution and size is shown in Table 4.8, and a summary of their anatomical distribution is presented in Table 4.9.

In 21 (62%) of the 34 positive dissections, the metastatic deposit(s) involved a single anatomical level. Two anatomical levels were involved in six dissections (18%); three levels in four dissections (12%); four levels in two dissections (6%); and all five anatomical levels were involved by the metastatic carcinoma in one dissection (3%).

Anatomical level II was the most frequent site of metastasis. It was involved in 21 (62%) of all positive dissections and in twelve (92%) of the 13 necks showing involvement of multiple anatomical levels. Level I was involved in 18 dissections (53%), level III in nine dissections (26%), and level IV in seven dissections (21%). Level V was involved in only three dissections (9%), and in all three cases, nodes at all levels of the jugular chain were also involved.

The results of my observations in the 34 positive sides of neck dissection show that, in nearly all patients, anatomical level I or level II is the site of the initial metastatic deposit(s). Sequential involvement of lymph nodes of ascending numerical levels may follow. As shown in Figure 4.7, level I or level II was involved in all 21

dissections with metastasis confined to a single anatomical level (level I in twelve cases, level II in nine cases).

Anatomical level I was involved in six of the 13 dissections with positive nodes at multiple anatomical levels: in two cases, node(s) at levels I and II were involved; in three cases, node(s) at levels I, II and III were involved; and in one case, the metastatic deposits involved all five anatomical levels (Figure 4.7).

Anatomical level II was involved in twelve of the 13 dissections showing involvement of multiple anatomical levels. Excluding the six cases outlined above, in which both level I and level II were involved, the metastatic pattern in the other six dissections was as follows: involvement of levels II and III in one case; involvement of levels II, III and IV in one case; involvement of levels II, III, IV and V in two cases; and involvement of level II and level IV, with 'skipping' of level III in two cases. In the remaining dissection with positive nodes at multiple anatomical levels, involvement of levels III and IV, without involvement of either level I or level II, was observed (Figure 4.7).

The number of metastatic deposits (discrete positive nodes and/or fused nodal masses) per dissection ranged from one to ten (mean 2.4, SD 1.9, median 2). Fourteen (41%) of the 34 positive dissections contained a single metastatic deposit (Figure 4.8).

In the 25 dissections containing only discrete nodes, the number of positive nodes ranged from one to five (mean 1.8, SD 0.9, median 2). Twenty-one (84%) of the 25 dissections contained only one or two positive nodes. One positive node was present in eleven dissections; two in ten dissections; three in three dissections; and five in one

dissection.

In the nine dissections containing fused nodes, the number of metastatic deposits ranged from one to ten (mean 3.4, SD 3.1, median 4). Three dissections contained a single metastatic deposit; two dissections contained five; and the other four dissections contained either two, four, six or ten deposits.

Discrete positive nodes were recovered most frequently from anatomical level I, from where 24 (38%) of the 64 positive discrete nodes were recovered (Table 4.9). Eighteen positive nodes (28%) were recovered from level II; eight (13%) from level III; and seven (11%) from each of the two remaining levels (level IV and level V).

Metastatic masses of fused nodes were recovered most frequently from anatomical level II, from where seven (44%) of the 16 metastatic masses were recovered. A single mass was recovered from both level I and level III, and two masses were recovered from level IV. The other five nodal masses each involved several anatomical levels. However, for simplicity, in Tables 4.8 and 4.9, they have been assigned to the first (lowest numerical) level of involvement. One mass extended from level I to level IV; one from level II to level V; two from level II to level IV; and one from level IV to level V. Hence, in total, level II was involved by eleven (69%) of the 16 metastatic masses.

(e) Lymph node Size and Metastasis

In total, 2,258 discrete lymph nodes (64 of which were involved by metastatic carcinoma) and 16 metastatic masses of fused nodes were recovered from the 80 sides of neck dissection. The anatomical distribution of all 2,258 discrete nodes, and their size, is shown in Table 4.10.

The size of discrete lymph nodes ranged from 0.3-4.5cm. The largest nodes were located at anatomical level II, where 9% of nodes measured 2.0cm. or more. In contrast, only 4% of nodes at levels I and III, and 3% of nodes at level IV, measured 2.0cm. or more. All 553 nodes located at level V measured less than 2.0cm. Nevertheless, the median nodal size (between 0.5-0.9cm.) was similar at levels I-IV, inclusive. At level V, the median nodal size was less than 0.5cm.

The relationship between the size of the node and the frequency of metastasis is shown in Table 4.10, and summarised in Table 4.11. The percentage of positive nodes rose with increasing nodal size. Forty-six (2.1%) of the 2,162 nodes less than 2.0cm. in size, and 18 (18.8%) of the 96 nodes 2.0cm. or more in size, were positive. The difference in frequency of metastasis in relation to nodal size was highly significant (Yates' Corrected Chi-squared Test, $X^2 = 86.28$, 1 d.f., $P = <0.001$). Nevertheless, only three (25%) of the twelve discrete nodes measuring 3.0cm. or more were positive for metastatic disease. Hence, nine nodes, 3.0cm. or more in maximum dimension, showed no evidence of carcinoma on histological examination of step-serial sections. The largest negative node (in case ND55) was the site of an inactive tuberculous infection. The other eight negative nodes measuring 3.0cm. or more showed only non-specific reactive hyperplasia (with follicular hyperplasia as the dominant histological pattern).

The size of the 64 positive discrete nodes ranged from 0.3-3.0cm., with a mean of 1.4cm. (SD 0.7, median 1.3). Twenty (31%) measured less than 1.0cm., and 46 (72%) measured less than 2.0cm. The metastatic masses of fused nodes were significantly larger (Two Sample t Test, $t = 3.97$, 15 d.f., $P = 0.001$). Their size ranged from 2.0-

12.0cm., with a mean 3.8 cm. (SD 2.4, median 3.0).

On macroscopic assessment, most of the metastatic masses appeared to have been formed by fusion of four or five nodes, and, on histological assessment, all showed total replacement of the nodal architecture by the metastatic carcinoma. Macroscopic cystic change was seen in six (38%) of the 16 metastatic masses. The mean size of the cystic masses was 4.8cm. (SD 3.8, range 2.0-12.0, median 3.7). In three, the centre of the cyst cavity contained clear or blood-stained gelatinous material with cheesy, cream material towards the periphery (Figure 4.9a). Histologically, cystic change had occurred within the centre of large masses of keratin, and/or within areas of necrotic tumour. In all cases, viable carcinoma was seen lining the cyst, although this was often reduced to a thin, and, at times, incomplete layer (Figure 4.9b). The other three cystic metastatic masses were recovered from a single dissection (ND108), and on cutting, multiple papillary processes were seen projecting into the cyst lumen. Histological examination showed the cyst lumen contained necrotic debris. The papillary processes were formed by neoplastic epithelium supported by a lymphoid stroma. The epithelium was non-keratinising and resembled transitional epithelium (Figure 4.10).

The relationship between the size of the discrete positive lymph nodes and the extent of their replacement by metastatic carcinoma is shown in Table 4.12. Thirty-five (55%) of the 64 nodes were partially replaced by the metastatic deposit. Nineteen nodes (30%) were totally replaced. In the remaining ten positive nodes (16%), tumour was present as emboli in the peripheral (subcapsular) sinuses with minimal replacement of the nodal architecture (Figure 4.2a). All three degrees

of replacement were seen in both small and large nodes, and there was no discernible relationship between nodal size and extent of replacement (Chi-squared, X^2 = 4.62, 2 d.f., P = 0.09; Chi-squared Test for Trend, X^2_{TREND} = 0.38, 1 d.f., P = 0.54). Macroscopic cystic change was present in nine (14%) of the 64 positive discrete nodes. The mean size of the cystic nodes was 2.4cm. (SD 0.6, range 1.2-3.0cm., median 2.5). Seven (78%) of the nine cystic nodes measured 2.0cm or more. On histological assessment, all were totally replaced by the metastatic carcinoma, with necrotic debris and keratin partially filling the cyst lumen and viable carcinoma lining the cyst wall (Figure 4.11).

(f) Extracapsular Spread: Histological Features

(i) Extracapsular Spread From Discrete Lymph Nodes

Thirty-two (50%) of the 64 positive discrete nodes showed extracapsular spread of metastatic carcinoma (Table 4.7).

In twelve positive nodes (19%), the extracapsular spread was macroscopic in extent. Sharp dissection was needed to expose and remove the nodes from the gross specimen. Tumour was seen spreading into perinodal fibrofatty tissue from five of them and into the fascia surrounding the posterior belly of the digastric muscle from one of them. In 17 positive nodes (27%), extracapsular spread was only evident on microscopic assessment and it was confined to the immediate perinodal fibrofatty tissue (Figure 4.3c). In three positive nodes (5%), the extent of the extracapsular spread was limited to microscopic embolisation and permeation of the perinodal lymphatics (Figure 4.3b). Permeation of perinodal lymphatics was seen also in four of the positive nodes showing more extensive extracapsular

spread.

The relationship between the anatomical level of the positive node and the incidence and extent of extracapsular spread is shown in Table 4.13. Extracapsular spread was seen in association with nodes located at all anatomical levels. Its incidence ranged from 33% of positive nodes at level II to 58% of positive nodes at level I. However, the small variation in incidence at the different anatomical levels was not statistically significant (Chi-squared, $X^2 = 2.95$, 4 d.f., $P = 0.57$; Chi-squared Test for Trend, $X^2_{TREND} = 0.0085$, 1 d.f., $P = 0.93$).

The relationship between the size of the node and the incidence and extent of extracapsular spread is shown in Table 4.14. Extracapsular spread was seen in 23 (50%) of the 46 positive nodes measuring less than 2.0cm., and in nine (50%) of the 18 nodes measuring 2.0cm. or more. Hence, there was no discernible relationship between lymph node size and the incidence of extracapsular spread. Macroscopic extracapsular spread was present in seven (15%) of the 46 nodes measuring less than 2.0cm., and in five (27%) of the 18 nodes measuring 2.0cm. or more (Fisher's Exact Test, $P = 0.42$).

The relationship between the extent of lymph node replacement by the metastatic deposit and the incidence and extent of extracapsular spread is shown in Table 4.15. Macroscopic extracapsular spread was usually seen in nodes totally replaced by the metastatic deposit: eleven (92%) of the twelve discrete nodes with macroscopic spread were totally replaced by tumour. Microscopic spread was frequently seen in nodes partially replaced by tumour: 14 (82%) of the 17 nodes showing microscopic spread were only partially replaced by the metastatic

deposit. Nine (28%) of the 32 positive nodes without extracapsular spread showed only minimal replacement of the nodal architecture.

Extracapsular spread from nodes minimally replaced by tumour was limited to embolisation and permeation of the perinodal lymphatics, which was seen in one of the ten minimally-replaced nodes. Seventeen (49%) of the 35 partially-replaced nodes showed extracapsular spread, but in all except one, this was only microscopic in extent. Fourteen (74%) of the 19 totally-replaced nodes showed extracapsular spread, and in eleven (58%), this was macroscopic in extent. Three of the five totally-replaced nodes without extracapsular spread showed extensive cystic change. The other two totally-replaced/no extracapsular spread nodes were both small (measuring 1.2cm.).

(ii) Metastatic Masses of Fused Nodes

Two of the eight patients with advanced cervical metastatic disease (cases ND108 and ND110) had presented clinically with sinuses on the skin of the upper neck, behind and below the angle of the jaw. On investigation, both were found to have intra-oral/oropharyngeal carcinomas with cervical node metastasis and they proceeded to surgery. On pathological assessment of the surgical resection specimens, in each case, carcinoma was seen spreading from metastatic masses involving nodes of the deep cervical chain, into the sternomastoid muscle and subcutaneous adipose tissue anterior to the muscle, through the dermis, with ulceration of the epidermis (Figure 4.12). Metastatic carcinoma was involving, also, major vessels and nerves.

In another of the eight patients with advanced regional disease (ND84), carcinoma spreading from a metastatic mass at anatomical level

I was resorbing the outer cortex of the mandibular body.

In the remaining five patients, including the patient with advanced bilateral disease, (ND39, ND72, ND114, ND135, ND141/142), the metastatic masses were fixed to major vessels and muscle (deep surface of sternomastoid and/or posterior belly of digastric). Histological assessment showed tumour invading the fascia around the muscles, but invasion of the muscle fibre bundles was not demonstrated. Involvement of the fibrous sheath around the major blood vessels was seen in all five cases and spread into the lumen (Figures 4.13a and 4.13b) was demonstrated in three cases (ND39, ND114, ND135).

DISCUSSION

In the initial part of my study, I have investigated the patterns of metastatic spread in surgical neck dissection specimens in a series of patients undergoing neck dissection simultaneous with resection of a primary squamous cell carcinoma of the intraoral/oropharyngeal mucosa. Although there are numerous reports in the literature on different aspects of the metastatic spread of oral cancer, there are few reports of histological investigations into the precise pattern and extent of nodal involvement. The present study differs in several significant aspects from, and includes three main advantages over, most previous investigations.

Firstly, in all cases, the primary tumour was a histologically diagnosed squamous cell carcinoma arising at a known site within the oral cavity or oropharynx. Many of the previous investigations have not shown such a restriction in the site of the primary tumour, and have included, for example, cutaneous carcinomas (Snow *et al.*, 1982) and carcinomas of the larynx, cervical oesophagus, nasopharynx and paranasal sinuses (Cachin *et al.*, 1967; Carter *et al.*, 1985 and 1987; Richard *et al.*, 1987). The inclusion of carcinomas arising at different sites within the head and neck increases the range of variables, and, therefore, makes conclusions more difficult to achieve. In particular, it is highly likely that the site of origin of the primary tumour will determine different patterns of metastatic spread, because of the involvement of different anatomical lymphatic drainage routes.

Secondly, all the patients in the present study underwent neck dissection simultaneous with initial treatment of the primary tumour.

None had undergone previous head or neck surgery, other than a diagnostic biopsy, nor received pre-operative radiotherapy or chemotherapy. In addition, any patient with a history of immunosuppression was excluded. Hence, the effect of previous therapy on the observed pattern of metastatic spread could be eliminated in all patients. In contrast, most of the patients in the series reported by Carter *et al.* (1985 and 1987) were secondary referrals undergoing salvage surgery. Although the latter authors discuss how pre-operative radiotherapy influenced the pathological findings, no consideration was given to the possible effects of previous surgery.

The third advantage of the present study is that it was a prospective study, restricted to material from a single surgical unit, with all aspects of the pathological assessment (excluding purely technical work) performed by the author. Hence, although clinical material is inherently variable, there was consistency in the manner in which this material was handled, both clinically/surgically and in the Pathology Laboratory. Most of the previous investigations, including those reported recently by Byers *et al.* (1988), Spiro *et al.* (1988) and Shah *et al.* (1990), have been retrospective studies based on information recovered from patients' medical records. Only major pathological findings have been reported, usually in relation to prognosis, and the authors have given no details of the number of surgeons and pathologists involved. The investigations by one group of workers (Tanner *et al.*, 1980; Carter *et al.*, 1985 and 1987) are exceptional in stating that one author performed or supervised all the pathological assessments.

On the other hand, in common with previous reports, the present

study has limitations. For example, my decision to include supra-omohyoid neck dissections could be criticised on the grounds that level V nodes are not available for pathological assessment in this type of surgical procedure. Several independent authorities (Sharpe, 1976; Skolnik *et al.*, 1976; Shaha *et al.*, 1984; Byers *et al.*, 1988; Spiro *et al.*, 1988) have reported that metastasis to nodes in the posterior triangle is rare in oral cancer, and only occurs in conjunction with metastatic disease at all levels of the deep cervical chain. In view of these reports, the surgeons at the Walton Unit support the trend towards selective (partial) neck dissection, as an elective procedure, and, on occasions, for treatment of clinically-limited neck disease. Thus, it was decided that supra-omohyoid dissections could be included in the present study without influencing the validity of my findings. More limited procedures, such as the suprahyoid and the extended suprahyoid dissections, were excluded.

Another criticism of the present study is the relatively small size of the series. Since histological evidence of metastasis was present in less than 50% of the 80 sides of neck dissection, the actual number of metastatic deposits available for detailed histological assessment was limited. Also, some intra-oral sites were represented by only a few cases. On the other hand, patients with disease of all T stages were represented, in contrast to the series reported by Carter *et al.* (1985 and 1987) which included 'a large proportion' of patients with Stage III and Stage IV disease.

A further criticism of the present study may be that the site and stage of the primary tumour had been recorded by the surgical team, rather than the author. The site of origin may be difficult to

determine in voluminous cancers invading multiple anatomical regions. Nevertheless, the study had to assume that the site specified in the patient's case notes was correct. The pre-operative staging assessment was carried out under general anaesthesia by one of the two Consultants, who are both experienced Head and Neck Oncologists.

The remainder of the discussion will be arranged in two sections, the first concerned with the accuracy of the pathological assessment and the second dealing with the incidence and patterns of metastatic spread.

(1) The Accuracy of the Pathological Assessment

(a) Orientation and Fixation of the Gross Specimen

Adequate fixation of the surgical specimen is a pre-requisite for an accurate histological diagnosis of the extent of metastatic disease (Barnes and Johnson, 1986b; Rhys Evans *et al.*, 1987). In addition, correct orientation of the specimen is necessary for a precise clinicopathological correlation, and this has both therapeutic and prognostic significance. By following the protocol agreed with the surgical team, no problems were encountered with shrinkage and distortion. Pinning or suturing onto polystyrene blocks was a simple and efficient way of orientating the specimens. The provision of a sketch by the surgical team was also found to be helpful in orientation and identification of the nodal groups, particularly in functional and supra-omohyoid dissections. Penetration of the fixative solution was sufficient to allow adequate fixation of large metastatic deposits, even in bulky radical dissections. As the study progressed, personal experience showed that by prolonging the fixation period up to 48 hours, small nodes could be palpated more readily and removed

with less risk of crushing.

(b) Nodal Yield

It is possible that more nodes could have been recovered from the gross specimens by using a clearing technique, such as that described by De La Pava and Pickren (1967). Average yields of 50 and 68 nodes, respectively, have been reported in cleared radical neck dissections by De La Pava and Pickren (1967) and Feind (1972). Although the yield achieved without clearing is smaller (an average yield of 44 nodes was reported by Byers, 1985), the difference was not considered to be sufficiently large to justify the use of the more time consuming and expensive technique of clearing.

In the present study, two categories of metastatic deposits - discrete positive nodes and fused nodal masses - were described in an attempt to provide a realistic account of the extent of metastatic involvement. However, each fused mass was counted as a single deposit. It might have been more meaningful to adopt an arbitrary number, based on the average observation (say, four), and to have used this figure, rather than one, when describing the number of metastatic deposits. Another practical problem in relation to some fused nodal masses was the difficulty in assigning the mass to a specific anatomical level. In these cases, the mass was assigned to the first (lowest numerical) group of nodes which it involved, followed by a description of its extent.

(c) Histological Sampling of Lymph Nodes

If a meticulous search of the gross specimen is made and all the identified nodes are dissected out, failure to diagnose metastatic tumour due to a gross sampling error is unlikely. However, much more

likely is the possibility of a false-negative histological assessment due to inadequate sampling of individual nodes. The accuracy of the histological diagnosis of metastatic disease depends on the size of the lymph node, the size of the metastatic deposit, the location of the deposit within the node, and the number of histological sections examined (Wilkinson and Hause, 1974). Squamous cell carcinoma generally metastasises to the periphery of nodes and this reduces the theoretical probability of identifying small deposits in a single histological section (Wilkinson and Hause, 1974). In the present study, the protocol for histological sampling was a compromise between the routine diagnostic assessment of a single hilar (equatorial) section and systematic serial sectioning. It has been reported that serial sectioning and immunohistochemistry increase the detection rate of micrometastases in breast cancer (International (Ludwig) Breast Cancer Study Group, 1990; Springall *et al.*, 1990) and, more recently, in head and neck cancer (McKenna *et al.*, 1991). The presence of micrometastases results in a reduction in the disease-free and overall survival rates in breast cancer (International (Ludwig) Breast Cancer Study Group, 1990). Their significance in head and neck cancer is uncertain.

In the present study, the degree of nodal replacement was assessed in an attempt to provide detailed information on the patterns and mechanisms of extracapsular spread. The accuracy of the assessment was limited. For example, the percentage of the nodal architecture that had been replaced by metastatic carcinoma was assessed subjectively. In most nodes, the assessment was made by examination of a single histological section. Hence, it may not represent accurately

the volume of nodal replacement. Nevertheless, nodes showing only minimal replacement on initial assessment were sampled more thoroughly before the definitive degree-of-replacement grade was assigned. The boundaries of the three designated categories of nodal replacement were chosen arbitrarily.

The diagnosis of permeation/embolisation of the perinodal lymphatics (Figure 4.3b) was facilitated, in the present study, by including some of the investing fibro-adipose tissue with each node as it was removed from the gross specimen. Accurate diagnosis of involvement of the perinodal lymphatics is desirable since its presence is associated with a reduced prognosis (Sancho *et al.*, 1977; Cachin *et al.*, 1979; Richard *et al.*, 1987).

(2) The Incidence and Patterns of Metastatic Spread

The incidence and extent of metastatic spread observed in the present study is broadly in agreement with previous reports (Snow *et al.*, 1982; Shingaki *et al.*, 1985; Carter *et al.*, 1985 and 1987; Spiro *et al.*, 1988; Shah *et al.*, 1990). The patterns that were observed are characteristic of the pathophysiology of squamous cell carcinoma involving the cervical lymphatic system (Cachin *et al.*, 1967; McKelvie, 1976; Batsakis, 1979). My observations confirm the generally held view that tumour emboli usually settle in the first lymph node they drain into, and, as the disease advances, progressively more nodes and more anatomical levels of nodes become involved.

(a) Frequency of Metastasis in relation to Clinical Features

In the present study, there was no significant difference in the frequency of metastasis in relation to the T stage of the primary

tumour (Table 4.5), and this is in agreement with several other reports (Moore *et al.*, 1986a and 1986b; Mohit-Tabatabai *et al.*, 1986; Spiro *et al.*, 1986). However, my results confirm the well-established concept of 'high-risk' and 'low-risk' sites.

(b) Topographical Distribution of Cervical Metastases

My findings (Figures 4.5 and 4.6) confirm previous reports (Byers *et al.*, 1988; Spiro *et al.*, 1988) that cervical metastases from each primary site are predictable in their distribution. My results show that submental/submandibular nodes (level I) are the initial site of metastasis in tumours of the buccal mucosa (Figure 4.5). Initial involvement of level I nodes is also seen in some tumours of the floor of mouth and oral tongue. In the present study, patients with tumours of the anterior/midline floor of mouth underwent bilateral neck dissection and involvement of nodes at level I was usual (Figure 4.6). Floor-of-mouth tumours in unilateral procedure patients tended to be more posterior in location, and, consequently, level I nodes were involved less frequently (Figure 4.5). My results show, also, that tumours of the oropharynx, posterior floor of mouth, and, in some cases, the tongue, metastasised, initially, to the superior cervical nodes (Figures 4.5 and 4.6). It is well known that lymphatics from some intra-oral sites and the oropharynx drain directly to the jugulo-carotid and jugulo-omohyoid groups (Feind, 1972; DiTroia, 1972), and that metastasis may occur via these 'long-range, fast' pathways (McKelvie, 1976). Nevertheless, involvement of nodes low in the jugular chain, without involvement of nodes at level II, was seen in only a single case in the present study (Case ND9, primary tumour of tongue). However, another four patients, also with tumours of the

tongue (ND52, ND120, ND122, ND144), showed only minimal involvement of nodes at multiple anatomical levels, suggesting tumour emboli had reached nodes low in the jugular chain simultaneously (via different lymphatic pathways), rather than progressively. Another possible explanation is that the pattern of metastatic involvement observed in the latter patients reflects a specific property of either the cancer cells and/or the host's immune response.

Positive nodes were found in the posterior triangle in only three dissections in the present study. In all cases, the neck was clinically positive, and histological examination showed metastatic disease throughout the jugular chain, thus confirming the findings reported by Byers *et al.* (1988), Spiro *et al.* (1988), and Shah *et al.* (1990).

Only six (24%) of the 25 supra-omohyoid neck dissections in the present study showed histological evidence of metastasis. In four cases, this was limited to node(s) at level I. One case had a single positive node at level II, and the remaining case had a positive node at three different levels (levels I, II and III). The limited extent of metastastic disease observed in the supra-omohyoid dissections justifies my decision to include them in the present study, since it is reasonable to assume that none of the patients had involvement of level V nodes.

Five per cent of head and neck cancer patients are reported to have bilateral metastatic spread at presentation (Snow *et al.*, 1982). A further 5-10% develop bilateral spread during the course of their disease (McQuarrie *et al.*, 1977). In the present study, simultaneous bilateral metastatic spread was rare: it was seen in only three

patients. In contrast, Byers *et al.* (1988) found bilateral metastases in 27% of simultaneous bilateral procedure patients. The low incidence in the present series may be explained by the criteria for entry into the study. At the Walton Unit, many patients with clinical evidence of bilateral spread receive pre-operative chemotherapy, and, hence, were excluded from the present study.

(c) Lymph Node Size and the Frequency and Extent of Metastatic Involvement

My results show that the size of negative lymph nodes is related to their anatomical position, with only small nodes being located in the posterior triangle (Table 4.10). At other levels, the size of negative nodes was more variable. Some of the difference in size is explained by the difference in the shape of nodes at different anatomical levels (Feind, 1972). Since the measurement given is the maximum dimension, it may not reflect accurately the volume or actual size of bean-shaped and multilobular nodes. Variability in the size of nodes located at the same anatomical site may be a reflection of a different functional status, with only some of the nodes at a specific anatomical level draining the oral cavity. For example, in my study, 20% of the nodes harvested from level II measured less than 0.5cm. Some of these nodes were located within the tail of the parotid or within fat under the superior attachment of sternomastoid, rather than within the fascial sheath which encloses and compartmentalises the deep cervical nodes (Bocca *et al.*, 1984). Hence, it is possible that some of the small nodes harvested at level II, in the present study, were not functionally a part of the intra-oral mucosal lymphatic drainage system.

The association between the clinical size of a lymph node and the histological presence of metastatic disease is well known (Cachin *et al.*, 1979; Snow *et al.*, 1982; Grandi *et al.*, 1985; Richard *et al.*, 1987). In the present study, all lymph nodes were measured following their dissection from the gross specimen, and the frequency of metastasis rose with increasing lymph node size (Tables 4.10 and 4.11). However, nine nodes measuring 3.0cm. or more did not contain tumour. All of these nodes were located at level II and were bean-shaped or multilobular. Hence, the measurement given cannot be assumed to indicate closely the nodal volume. Nevertheless, my results show that negative nodes may be large, and, hence, readily palpable, and this is discussed in Chapter 5.

My observations on the relationship between the size of a positive node and the extent of its replacement (Table 4.12) suggest that tumour usually replaces the normal architecture of the node before forming an expanding mass. The presence of minimal deposits in nodes showing reactive hyperplasia suggests either the nodal enlargement is a reaction to the tumour deposit, or recent metastasis to an already hyperplastic node.

Gross cystic change was seen in 38% of the metastatic masses and in 14% of positive discrete nodes in the present study. Some of the nodes showing cystic change were only partially replaced by tumour. It is possible that the size of the metastatic node increases more rapidly once cystic change has occurred, with clinically overt disease as the likely outcome. Cystic degeneration within metastatic cervical nodes has been reported previously (Ackerman, 1968; Shear and Ichilcik, 1973). Necrosis due to an inadequate blood supply is the

most likely predisposing factor (Ackerman, 1968).

(d) Extracapsular Spread of Nodal Metastastic Carcinoma

In the present study, extracapsular spread of metastatic carcinoma was a frequent finding, and was present in 24 (71%) of the 34 positive neck dissections. The reported incidence is wide, ranging from 23-86% of positive dissections (Kalnins *et al.*, 1977; Carter *et al.*, 1985). The wide range may be explained, in part, by the lack of uniformity in defining its morphological extent. In the present study, as in the studies reported by Carter *et al.* (1985 and 1987), extracapsular spread was described as macroscopic or microscopic. Macroscopic spread was diagnosed more frequently than microscopic spread (Table 4.7), and the percentage of positive dissections showing macroscopic spread was similar to that reported by Carter *et al.* (1987).

Nine (26%) of the 34 positive dissections in the present study contained fused nodal masses, consistent with long-standing metastatic disease. Shah *et al.* (1990) reported that 10% of simultaneous procedure patients had matted nodes. At the Walton Unit, many patients presenting with gross neck disease receive pre-operative chemotherapy. Hence, my data underestimates the incidence of late-presenting (stage IV) disease. Late presentation of head and neck cancer in Merseyside has been commented on previously by Stell and McCormick (1985).

In some dissections in the present study, macroscopic extracapsular spread was diagnosed on the basis of difficulty in dissecting the node from its surrounding fibro-adipose tissue, even though the extent of tumour was still well defined and not macroscopically involving adjacent anatomical structures. In some

cases, the difficulty in removing the node may have been due, at least in part, to reactive fibrosis around the capsule. Nevertheless, extracapsular spread was subsequently confirmed histologically in these cases.

In the present study, macroscopic extracapsular spread in dissections with metastasis associated with discrete lymph nodes only was confined to the perinodal fibro-adipose tissue in all except one case. However, contiguous structures were involved by the metastatic carcinoma, to some extent, in all nine dissections with fused nodes. The internal jugular vein and the sternomastoid muscle were the structures involved most frequently, and this is in agreement with previous reports (Tanner *et al.*, 1980; Carter *et al.*, 1985). The presence of sinuses on the skin of the neck at initial presentation, seen in two cases (ND108 and ND110) in the present study, appears to be rare. Carter *et al.* (1985) reported involvement of the skin in four out of 59 patients with gross spread of extracapsular carcinoma. However, most of the patients in their study were salvage cases following failed radiotherapy and/or surgery. The histological appearance of the malignant epithelium lining the sinus tracts, and the appearances of the metastatic nodes, in Case ND108 in the present study (Figure 4.9), are characteristic of a variant of oropharyngeal carcinoma known as the cystically-metastasising tonsillar carcinoma (Micheau *et al.*, 1974; Marlowe *et al.*, 1984; Foss *et al.*, 1991).

Carter *et al.* (1985) described direct spread of metastatic carcinoma into salivary gland in three cases. In the present study, the submandibular gland was frequently found to be resistant to invasion so that spread was confined to the gland capsule, and

invasion of glandular parenchyma by metastatic carcinoma was not demonstrated. Muscular arteries were also resistant to invasion and not infrequently were seen as the only recognisable structure in a 'sea' of tumour. Involvement of the mandible by metastatic carcinoma spreading from a submandibular node, seen in one case in the present study, has been reported previously (Carter *et al.*, 1985).

Microscopic extracapsular spread was sometimes difficult to diagnose with certainty, especially in those carcinomas with abundant fibrous stroma, which frequently merged with the node capsule. Serial sections and histochemical stains for reticulin and collagen fibres did not always resolve this problem. It is uncertain whether the diagnosis of extracapsular spread in these marginal cases is important or not. Carter *et al.* (1987) reported a strong association between the presence of macroscopic extracapsular spread and subsequent regional recurrent disease. The presence of microscopic extracapsular spread did not apparently affect the chances of recurrence compared to patients with no extracapsular spread. This contrasts with other investigations (Noone *et al.*, 1974; Kalnins *et al.*, 1977; Shingaki *et al.*, 1985; Richard *et al.*, 1987) which have shown the prognosis is reduced in patients with capsular involvement or microscopic spread when compared to those with metastasis confined to the node.

My results (Table 4.15) show that extracapsular spread was observed in one node showing minimal and 17 nodes showing partial replacement by tumour. Carter *et al.* (1985 and 1987) also observed that extracapsular spread may be associated with nodes only partially replaced by tumour, but gave no further details. The process whereby carcinoma breaks through the node capsule, and why this is

characteristic of squamous cell carcinoma metastatic to cervical nodes as opposed to other anatomical sites (Willis, 1930), is not yet fully elucidated. The most obvious suggestion is mechanical disruption by the expanding tumour mass, possibly aided by secretion of collagenase by the tumour cells and their stroma (Carter *et al.*, 1985; Burman and Carter, 1985). Such a mechanism fails to explain extracapsular spread from small nodes only minimally or partially replaced by tumour. However, my observations provide some support for the mechanism proposed by Toker (1963) that, on occasions, a tumour embolus in the afferent lymphatics may lodge initially within the capsular sinuses. Its subsequent expansion at that site would lead to extracapsular spread at a stage when the rest of the node was only minimally or partially replaced. In the present study, extracapsular spread from minimally-replaced nodes was limited to microscopic embolisation and permeation of perinodal lymphatics, but the histological appearances of some of the partially-replaced nodes with microscopic extracapsular spread suggested this mechanism had operated.

SUMMARY

The histological incidence and extent of lymph node metastasis has been investigated in a series of 80 sides of neck dissection, from 60 patients with primary intra-oral/oropharyngeal squamous cell carcinoma.

Metastasis was present in 34 (43%) of the 80 sides of neck dissection. There were no significant differences in the incidence of metastasis in relation to the sex and age of the patient, nor in relation to the T stage of the primary tumour. However, metastasis was more frequent in tumours sited on the oral tongue, floor of mouth and oropharynx.

Eighty metastatic deposits (64 discrete positive nodes, 16 masses of fused nodes) were recovered from the 34 positive dissections. In 21 (62%) of positive dissections, the metastatic deposit(s) involved a single anatomical level and involvement of all five anatomical levels was demonstrated in only one dissection. Level II (superior cervical) was the most frequent site of metastasis: it was involved in 62% of all positive dissections and in 92% of dissections showing involvement of multiple anatomical levels.

Twenty (31%) of the 64 discrete positive nodes measured less than 1.0cm., and 46 (72%) measured less than 2.0cm. In ten nodes, tumour was present as emboli in the peripheral sinuses with minimal replacement of the nodal architecture.

Extracapsular spread of metastatic carcinoma was demonstrated in 24 (71%) of the 34 positive dissections, and, in nine dissections, metastatic masses of fused nodes were present. Extracapsular spread was demonstrated in 34 (50%) of the 64 positive discrete nodes. There

was no discernible relationship between the incidence of extracapsular spread and the anatomical level or size of the node, and microscopic extracapsular spread was seen frequently in nodes only partially replaced by tumour. Extracapsular spread from discrete positive nodes was limited to the pericapsular fibro-adipose tissue in all except one case, but extracapsular spread was more extensive in dissections with matted nodes and frequently involved major blood vessels and the sternomastoid and/or digastric muscles.

Histological examination of all the lymph nodes removed during a neck dissection procedure, together with an examination of the perinodal fibro-adipose tissue, is essential for an accurate assessment of the actual metastatic status.

Chapter 5.

CLINICAL AND SURGICAL IMPLICATIONS OF THE PATHOLOGICAL FINDINGS IN SERIES I NECK DISSECTIONS.

1. Introduction.
2. Results.
3. Discussion.
4. Summary.

INTRODUCTION

In the present chapter, the two questions raised in the previous study which described the histological incidence and pattern of cervical lymph node metastasis in simultaneous neck dissections (Chapter 4) will be addressed.

The first question concerns the accuracy of the clinical assessment of the metastatic status. This will be investigated by comparing the pre-operative clinical status (N positive/N negative) with the histological metastatic status (positive/negative) for each of the 80 sides of neck dissection in Series I. In cases where there is a discrepancy between the clinical and histological diagnoses, any pathological findings in the surgical specimen that may account for the inaccurate clinical assessment will be described.

The second question concerns the efficacy of the surgical neck dissection procedure in dealing with actual or potential metastatic spread, and will be addressed in two ways. Firstly, the yield and anatomical distribution of discrete lymph nodes (excluding nodes harvested from within the parotid gland) will be reported in relation to the type of surgical procedure. Neck dissections containing metastatic masses of fused nodes will be excluded. Hence, only 71 of the 80 sides of neck dissection in Series I will be available for this study. Secondly, the relevant clinical and surgical implications of the results presented in Chapter 4 will be discussed.

Details of the Surgical Cases and the Methods, presented in Chapter 4, are applicable to the present investigations.

RESULTS

I. ACCURACY OF THE CLINICAL ASSESSMENT OF THE METASTATIC STATUS

(1) The Simultaneous Unilateral Procedure Patients

The pre-operative clinical metastatic status and the histological metastatic status in each of the 40 unilateral procedure patients in Series I is shown in Table 4.1. The clinical assessment was in agreement with the histological assessment in 22 (55%) of the 40 patients. As shown in Figure 5.1, metastasis was diagnosed histologically in nine of the 22 patients with clinically negative necks (false-negative incidence rate, 41%), and there was no histological evidence of metastasis in nine of the 18 patients with clinically positive necks (false-positive incidence rate, 50%).

(2) The Simultaneous Bilateral Procedure Patients

The clinical and histological metastatic status in each of the 40 sides of neck in the 20 bilateral procedure patients is shown in Table 4.3. The accuracy of the clinical assessment was significantly higher in the bilateral procedure group than in the unilateral procedure group (Chi Squared Test, $X^2 = 4.53$, 1 d.f., $P = 0.03$). There was agreement between the clinical assessment and the histological assessment in 31 (78%) of the 40 bilateral procedure necks. As shown in Figure 5.2, metastasis was diagnosed histologically in five of the 25 clinically negative necks (false-negative incidence rate, 20%), and there was no histological evidence of metastasis in four of the 15 clinically positive necks (false-positive incidence rate, 27%).

(3) The Simultaneous Procedure Patients (Series I)

The clinical assessment of the metastastic status was in agreement with the histological assessment in 53 (66%) of the 80 sides

of neck (Figure 5.3). Metastasis was evident on histological assessment in 14 of the 47 clinically negative necks. Hence, the accuracy of a clinically negative assessment was 70%, with a false-negative incidence rate of 30%. Thirteen of the 33 clinically positive necks showed no histological evidence of nodal metastasis. Hence, the accuracy of a clinically positive assessment was 61%, with a false-positive incidence rate of 39%.

The accuracy of the clinical assessment was appraised in relation to the site and T stage of the primary tumour, and the results are presented in Table 5.1. The clinical assessment was correct in 11 (69%) of the 16 necks associated with tumours at low-risk sites and in 42 (66%) the 64 necks associated with tumours at high-risk sites. Hence, there was no significant difference in the accuracy in relation to the metastatic risk of the site of the primary tumour (Chi Squared Test, $X^2 = 0.056$, 1 d.f., $P = 0.81$). The clinical assessment was correct in 18 (82%) of the 22 neck dissections associated with T1 tumours. Two of the four clinical misdiagnoses were false-negative assessments. The clinical assessment was correct in only 15 (54%) of the 28 necks associated with T2 tumours, and nine of the 13 misdiagnoses were false-negative assessments. In necks associated with both T3 and T4 tumours, the clinical assessment was correct in 67% of cases and false-positive assessments accounted for most of the misdiagnoses (Table 5.2). The small differences in accuracy, and in the frequency of false-positive and false-negative assessments, in relation to the T stage of the primary tumour did not achieve statistical significance (for accuracy: Total Chi Squared, $X^2 = 4.40$, 3 d.f., $P = 0.22$, Chi Squared Test for Trend, $X^2_{TREND} = 0.44$,

1 d.f., P = 0.51; for frequency of false-positive assessments, Yates'-Corrected Chi Squared Test, X^2_{YATES} = 0, 1 d.f., P = 1.0; for frequency of false-negative assessments, X^2_{YATES} = 0.049, 1 d.f., P = 0.83).

(4) Pathological Findings in the False-Positive Neck Dissections

A summary of the pathological findings in the 13 false-positive neck dissections is presented in Table 5.2. As shown here, metastasis was diagnosed clinically at anatomical level I in nine cases, and at anatomical level II in the other four cases. Table 5.2 also indicates additional pathological findings revealed at laboratory dissection, but not evident clinically, which might have contributed to the clinical impression of nodal metastasis. In particular, reactive hyperplasia was confirmed histologically in six (46%) of the 13 false-positive neck dissections. Other cases showed direct extension of the primary tumour into the submandibular triangle (two cases, 15%), or salivary gland pathology (five cases, 38%).

(5) Pathological Findings in the False-Negative Neck Dissections

A summary of the pathological findings in the 14 false-negative neck dissections is presented in Table 5.3. A total of 25 metastatic nodes were recovered from the 14 dissections. The number of positive nodes per dissection ranged from 1-5 (mean 1.8, SD 1.1, median 1.5), but seven necks contained only a single positive node. This was located at anatomical level I in three cases, and at level II in four cases. Multiple anatomical levels were involved in three of the seven necks with multiple positive nodes. In two cases, the positive nodes were located at three different anatomical levels (levels I, II and III). None of the false-negative dissections showed involvement of

level V, and level IV was involved in only one case. The size of the 25 positive nodes ranged from 0.3-2.5cm., with a mean size of 1.4cm. (SD 0.6, median 1.4). Eight (32%) of the 25 nodes measured 1.0cm. or less. Histological examination showed that five (20%) of the 25 nodes were totally replaced by the metastatic deposit. Thirteen nodes (52%) were partially replaced, and in the other seven nodes (28%), metastatic tumour was seen as emboli in the peripheral sinuses with minimal replacement of normal nodal architecture.

Extracapsular spread of metastatic carcinoma was present in eight (57%) of the 14 false-negative neck dissections - 17% of the 47 clinically negative necks in Series I. In all cases, the extracapsular spread was only microscopic in extent: in seven of the eight cases, invasion of the perinodal fibro-adipose tissue was seen, while in the eighth case, extracapsular spread was limited to embolisation and permeation of the perinodal lymphatics. In two of the eight dissections, positive nodes at two different anatomical levels showed microscopic extracapsular spread, and, in total, extracapsular spread was diagnosed in twelve (48%) of the 25 positive nodes (microscopic, ten nodes; involvement of the perinodal lymphatics, two nodes).

II. YIELD AND DISTRIBUTION OF LYMPH NODES IN SURGICAL NECK DISSECTION SPECIMENS

The yield and anatomical distribution of lymph nodes in the 71 Series I sides of neck dissection containing only discrete nodes are shown in Tables 5.4-5.8. Eleven (15%) of the 71 dissections were radical procedures. The other 60 (85%) were modified procedures: 35 (49%) functional dissections and 25 (35%) supra-omohyoid dissections.

The sex ratio of the patients undergoing radical and modified procedures was similar. Seven (64%) of the eleven radical procedure patients and 39 (65%) of the 60 modified procedure patients were male. The age of patients undergoing a radical neck dissection ranged from 43-79 years, with a mean age of 60 years (SD 11.1). This was similar to the mean age, 61 years (SD 11.2, range 33-81), of patients undergoing a modified procedure (Two Sample t Test, t = 0.261, 69 d.f., P = 0.79). Also, there was no significant difference in the age of patients undergoing functional (mean 60 years, SD 11.6, range 33-77) and supra-omohyoid (mean 63 years, SD 10.5, range 43-81) dissections (Two Sample t Test, t = 0.992, 58 d.f., P = 0.67).

In the eleven radical neck dissections, the yield of nodes ranged from 31-68, with a mean of 45.0 (SD 11.1, median 41). Fewer nodes were recovered from the 35 functional dissections (range 18-54, mean 30.3, SD 9.2, median 28), and from the 25 supra-omohyoid dissections (range 7-38, mean 16.4, SD 6.9, median 14). As shown in Table 5.5, the differences in nodal yield in the three types of neck dissection achieved significance when tested by the Two Sample t Test (P = <0.0001).

The mean number of nodes recovered at level I was 4.5, and there was no significant difference in the yield achieved at level I in the three types of neck dissection procedure (Table 5.4).

The mean number of nodes recovered at level II in the radical dissections was 11.1 (SD 4.3, range 4-17, median 11). This was significantly higher than the yield achieved in functional (P = 0.01) and supra-omohyoid dissections (P = 0.0006), where the mean was 6.9 and 5.8 nodes, respectively (Table 5.3). The small difference in nodal

yield in functional and supra-omohyoid dissections did not achieve significance (Table 5.5).

At level III, there were significant differences in the nodal yield in the three types of neck dissection (Table 5.5). The mean number of nodes recovered from the radical dissections was 8.7 (SD 3.7, range 3-15, median 10). In contrast (Tables 5.4 and 5.5), in the functional dissections, the mean yield at level III was 4.6 nodes (SD 2.5, range 1-12, median 4), and in the supra-omohyoid dissections, the mean yield was only 3.1 nodes (SD 1.9, range 1-7, median 2).

At level IV, there was no significant difference in the nodal yield achieved in radical (range 1-14, mean 6.4, SD 3.4, median 6), and functional (range 1-13, mean 5.3, SD 2.8, median 5) dissections. However, significantly fewer nodes were recovered at level IV in the supra-omohyoid dissections (range 1-10, mean 3.4, SD 2.7, median 2).

At level V, the mean yield in the radical dissections was 13.8 nodes (SD 5.5, range 7-23, median 12). In the functional dissections, the mean yield was 9.0 nodes (SD 5.5, range 3-32, median 9), and the difference was statistically significant (Table 5.5). Lymph nodes at level V (posterior triangle) are not included in the supra-omohyoid neck dissection.

When levels II-IV are considered as a single anatomical site (the 'deep cervical chain'), there were significant differences in the yield of nodes in the three types of surgical neck dissection (Table 5.5). As shown in Table 5.4, the mean yield at levels II-IV in radical dissections was 26.2 nodes (SD 9.4, range 13-43, median 26). In contrast, the mean yield in functional dissections was 16.7 nodes (SD 5.8, range 7-31, median 16) and, in supra-omohyoid dissections, 12.4

nodes (SD 6.8, range 5-35, median 14).

The yield of nodes in each type of neck dissection was higher when the dissection was carried out as a unilateral surgical procedure than when it was carried out as part of a bilateral procedure (Tables 5.6-5.8). The difference in nodal yield in relation to whether the surgical procedure was unilateral or bilateral achieved significance ($P = 0.02$) in the functional neck dissections (Table 5.7).

DISCUSSION

The results reported in the present chapter relate to, and are complementary to, the study reported in Chapter 4. The present discussion will be arranged in two sections, the first concerned with the accuracy of the clinical assessment of the metastatic status, and the second dealing with the efficacy of the surgical neck dissection procedure in the management of oral cancer.

(1) Accuracy of the Clinical Assessment of the Metastatic Status

The present study sought to determine with a high level of accuracy the validity of the clinical assessment of positive or negative cervical nodes in patients presenting with oral cancer. The neck was recorded as clinically N positive if examination under general anaesthesia and/or CT imaging suggested metastatic disease was present. Series I included a large number of cases (47, that is 59% of the 80 sides of neck dissection), clinically assessed as N negative in which elective neck dissections were performed to allow pathological staging of the metastatic status and for access to the neck for microvascular anastomosis.

In the present study, the accuracy rate of the clinical assessment was 66%. The accuracy of the clinically positive assessment was slightly lower than the accuracy of the clinically negative assessment (61% and 70%, respectively). In addition, the clinical assessment was less accurate in unilateral procedure patients than in bilateral procedure patients (55% and 78%, respectively). The most likely explanation is that this is a spurious result due to the relatively small series size.

In the present study, there was no statistical relationship

between the metastatic risk of the site of the primary tumour and the accuracy of the clinical assessment. This is in agreement with a previous report (Ali *et al.*, 1985).

Moore *et al.* (1986a) suggested that false-positive assessments are more likely in patients with large tumours, because of the examiner's expectation of metastatic nodes, as well as the increased likelihood of reactive hyperplasia due to local factors. This is borne out to a certain extent by my findings. As shown in Table 5.1, the clinical assessment was correct in 82% of necks associated with T1 tumours, compared to 54-67% of necks associated with larger tumours, and a higher percentage of false-positive assessments were made in T3/T4 cases than in T1 cases. However, in the present study, the high incidence of clinically occult metastases in T2 tumours had an equally important influence in the relationship between accuracy of the clinical assessment and tumour stage.

In the present study, none of the patients had received previous therapy, either at the site of the primary tumour or in the neck, hence, scarring and fibrosis can be eliminated as potential causes of an inaccurate clinical assessment. As shown in Figure 5.3, false-positive assessments were made in 13 (39%) of the 33 clinically positive necks in the present study. In most reports of studies confined to previously untreated patients, the incidence of false-positive assessments is between 19.5% and 39% (Ali *et al.*, 1985; Medina and Byers, 1989; Shah *et al.*, 1990). However, Crissman *et al.* (1980) reported a very high incidence of false-positive assessments (56%) in their series of patients with carcinoma of the floor of mouth, and it was suggested that sialadenitis of the submandibular

gland was the usual cause of the discrepancy in their patients. In the present study, false-positive assessments were made more often in relation to submandibular nodes (nine cases), than in relation to nodes of the deep cervical chain (four cases). However, as shown in Table 5.2, histological evidence of sialadentitis was present in only four of the nine necks with false-positive assessments at level I. In these four cases, the submandibular gland was noted to be firm and shrunken during macroscopic assessment of the gross specimen. Hence, it is likely that a firm structure had been palpable on clinical examination, giving rise to the pre-operative diagnosis of nodal metastasis. Histologically, acinar atrophy and fibrosis of the gland was seen, and, this was complicated by pus and mucus plugs in one case, and by multiple calculi in interlobular ducts in a further case. In one patient, a large mucous extravasation cyst, located deep to the sublingual gland, was evident on pathological assessment. In a further two patients, direct spread of the primary tumour into the submandibular salivary gland was demonstrated pathologically, and, in one case, this was complicated by a large abscess which had formed within necrotic/cystic areas of the tumour. It is likely that in all these seven cases, the pathological changes in anatomical structures in the submandibular triangle led the clinician to an erroneous conclusion that carcinoma was present in nodes at this site.

In the remaining cases in which metastasis was erroneously diagnosed clinically at level I (two cases), and at level II (four cases), pathological assessment of the gross specimen revealed multiple, enlarged lymph nodes. In one patient, one node was densely calcified, and a histological diagnosis of inactive tuberculosis was

made. In the other cases, the histological diagnosis was reactive hyperplasia of the lymph node. Follicular hyperplasia was the dominant histological pattern in most nodes. However, in one patient, the paracortex of some nodes contained discrete, non-caseating epithelioid cell granulomata, similar to the sarcoid type of granulomatous reaction reported as an occasional finding in head and neck cancer by Lennert (1967) and by Noone *et al.* (1974). The patient with granulomatous changes in the present study had no evidence of systemic sarcoidosis.

The number and the size range of the lymph nodes in the six dissections in which non-metastatic nodal enlargement was considered to be the cause of the clinical misdiagnosis is shown in Table 5.2. In each case in the present series, the largest node exceeded the normal parameters of 0.7cm. and 2.0cm. cited as the upper limit for the size of normal submandibular and superior cervical nodes, respectively (Feind, 1972). Even though the diagnosis of metastasis by clinical palpation and CT imaging does not depend solely on the size of the node (Henk and Langdon, 1985b; Som, 1992), the extent of the nodal enlargement and/or the number and grouping of nodes in these patients were suggestive of metastatic disease. Patients were not routinely treated with antibiotics before the clinical assessment was made, and ulceration and sepsis of the primary tumour, or sepsis elsewhere in the mouth or pharynx, are probably responsible for the reactive hyperplasia of the nodes that so frequently occurs in these patients. Passage of tumour antigens, or even the presence of occult micrometastases, are other possible explanations for the reactive changes.

False-negative clinical assessments were made in 14 (30%) of the 47 clinically negative necks in the present study. The reported incidence of false-negative clinical assessments for similar series of previously untreated patients is wide and ranges from 15-49% (Spiro *et al.*, 1974; Vandenbrouck *et al.*, 1980). However, three recent studies, each based on a large series of patients, have reported a false-negative incidence rate of between 25-34% (Byers *et al.*, 1988; Spiro *et al.*, 1988; Shah *et al.*, 1990).

In the present study, metastasis to nodes in the posterior triangle (level V) was not identified in any of the 47 patients with clinically negative necks, but a node at level IV was positive in one case (2% of clinically negative necks), and this is similar to the incidence rate reported by Shah *et al.* (1990).

Extracapsular spread of metastatic carcinoma was seen in eight (17%) of the 47 clinically negative necks in the present study. In most previous reports, the incidence of extracapsular spread in clinically negative necks has ranged from 4-6% (Cachin *et al.*, 1979; Byers *et al.*, 1988; Spiro *et al.*, 1988). However, Grandi *et al.* (1985) reported a higher incidence (12%), more in keeping with my findings. In the previous reports, the extent of the extracapsular spread was not specified. In the present study, tumour was confined to the immediate pericapsular fibro-adipose tissue, and spread into adjacent structures, such as major veins and muscles was not demonstrated in any patient with a clinically negative neck, despite meticulous assessment at the histological level.

The histopathological assessment provided possible explanations for the inaccurate clinical assessment in 10 (71%) of the 14 false-

negative neck dissections in the present study. In one patient (ND2), the positive node occupied a depression within the lingual aspect of the mandible and it is likely that the node was not detected due to this unusual anatomical arrangement. In three cases (ND5, ND52, ND115), the positive node(s) contained only micrometastases: tumour was seen as emboli within the peripheral sinuses with minimal replacement of normal nodal architecture (Figure 4.2a). Due to the small size of the deposit, it is most unlikely that these positive nodes could have been detected pre-operatively. In another six cases (ND106, ND107, ND109, ND129, ND144, ND152), the positive node(s) were partially or totally replaced by metastatic carcinoma. However, the nodes were not enlarged (all measured 1.7cm. or less in maximum dimension) and, therefore, it is likely that they escaped detection due to their small size. In the remaining four false-negative neck dissections (ND51, ND111, ND120, ND136), the largest positive node measured 2.0cm. or more, and no features were identified during the pathological assessment to account for the clinical misdiagnosis.

All the patients in the present study underwent CT imaging of the neck as part of the pre-operative assessment. Recently, several authorities (Close *et al.*, 1989; Friedman *et al.*, 1990; Hillsamer *et al.*, 1990) have reported a low incidence of false-negative assessments (between 12-16%) following CT imaging. It is possible that the CT scans in the present study were less accurate than the scans in the studies reported by Close *et al.* (1988), Friedman *et al.* (1990) and Hillsamer *et al.* (1990) due to technical differences, such as the thickness of the image sections. However, another possible explanation for the low incidence of false-negative assessments reported by some

authors (Close *et al.*, 1988; Friedman *et al.*, 1990; Hillsamer *et al.*, 1990) is that, in their studies, the pathological assessment was less thorough than in the present study, and, hence, some microscopic metastatic deposits may have missed detection in their patients. It is generally accepted that detection by CT imaging is impossible when the tumour deposits within a node are only microscopic in extent (Feinmesser *et al.*, 1987; Stern *et al.*, 1990; Som, 1992). It has been estimated that more than one million malignant cells are needed to create a mass of one cubic millimetre (Tannock, 1988). Although a deposit of this size is easily visible using the light microscope, it is unlikely to be detectable on gross examination, and, in practical terms, impossible to detect by sectional imaging of lymph nodes in the clinical setting. Therefore, CT imaging is only useful in identifying gross (that is, macroscopic) metastatic disease and for detecting and delineating extracapsular spread (Mancuso *et al.*, 1983; Feinmesser *et al.*, 1987; Stern *et al.*, 1990; Som, 1992). Hence, a detailed histological assessment of all the lymph nodes removed during a neck dissection procedure is the most reliable, currently available method of assessing the actual metastatic status of the cervical nodes.

(2) <u>The Efficacy of the Surgical Neck Dissection Procedure in the Management of Oral Cancer</u>

(a) <u>Nodal Yield</u>

To date, only a small number of authorities have reported data on the number of nodes recovered from different types of surgical neck dissection specimens. My average yield of 45 nodes in radical dissections is similar to the average yields (39 and 44 nodes,

respectively) reported by Shah *et al.* (1990) and Byers (1985). The latter author reported an average yield of 31 nodes for functional dissections, which is almost identical to the yield achieved in the present study (30 nodes).

As discussed in Chapter 3, the radical neck dissection (Crile, 1906) was designed to remove, *en bloc*, all the cervical lymph channels and nodes draining the oral cavity and oropharynx, and its introduction is still recognised as a major advance in the treatment of head and neck cancer (Conley, 1967a). The functional neck dissection (Bocca and Pignataro, 1967) was based on an increased understanding of the anatomy of the cervical lymphatic system, and its principal use is as an elective procedure, or in patients clinically assessed to have a single, mobile positive node (Byers, 1985). It is the only modification of the standard radical neck dissection designed to remove all the principal nodal groups (Byers, 1985). Selective (partial or regional) neck dissections, such as the supra-omohyoid, are designed to remove only those nodes at highest risk of metastasis (Byers, 1985). Their principal uses are as an elective or staging procedure in patients with clinically negative necks (Spiro *et al.*, 1988), or as a therapeutic procedure in the treatment of clinically-limited neck disease (Byers *et al.*, 1988).

A recent review article (DeSanto and Bearhs, 1988) compares radical and functional dissections, in terms of their effectiveness in controlling cervical metastatic disease and the post-operative functional and aesthetic results. However, no mention is made of the number of lymph nodes recovered from the gross specimens in the two types of procedures. Although a comparison of the number of nodes

recovered from the gross specimens is only a crude indication of whether or not the functional dissection achieves its aim of eradicating the principal nodal groups, my results merit further consideration. Also, the yield of nodes in the supra-omohyoid dissections will be critically appraised in an attempt to assess its effectiveness as an elective/staging/limited therapeutic procedure.

Three precautions were taken in the present study in an attempt to improve the validity of the comparison in nodal yield in the three types of surgical neck dissection. Firstly, dissections containing matted lymph nodes were excluded from the study. The disadvantage of this precaution was that only eleven of the 17 radical dissections in Series I were available for the present investigation. Secondly, lymph nodes harvested from within the parotid gland were not included in the nodal yield. There were two reasons for this decision. Firstly, the parotid gland is not included routinely in any of the three types of neck dissection practiced by the surgeons at the Walton Unit. My observations, made on the gross dissection specimens, suggested that anatomical/pathological factors, such as the size of the parotid gland and the site of the primary carcinoma, influenced whether or not parotid tissue was included in the neck dissection. The second reason for excluding parotid nodes was based on the anatomy of the lymphatic system. Mucosal lymphatics do not normally drain directly to intra-glandular parotid nodes (Feind, 1972), and, hence, they are not relevant to the present investigation. The third precaution taken in the present study was that anatomical levels II, III and IV were considered as individual sites and as one combined site - the 'deep cervical chain'. This precaution was taken because the distinction

between levels II and III, and levels III and IV, is somewhat arbitrary (Fisch and Sigal, 1964; DiTroia, 1972), particularly in functional and supra-omohyoid dissections.

My results (Table 5.4) show a wide range in the nodal yield in all three types of surgical neck dissection. This can be explained by individual variation in the anatomy of the cervical lymphatic system, and other factors, such as the age of the patient. Nevertheless, there were significant differences in the nodal yield at anatomical levels II-IV in the three types of surgical procedure (Table 5.5). The mean yield achieved in functional and supra-omohyoid dissections was only 65% and 46%, respectively, that achieved in radical dissections. Hence, my findings suggest strongly that modified dissections are less efficient at removing the principal groups of cervical nodes draining the intra-oral and oropharyngeal mucosa.

The clinical relevance of my findings will be considered in detail. The obvious concern is that when a modified dissection is used, lymph nodes outside the field of dissection may contain occult metastases. If so, the likely outcome is that the patient will present with late regional disease, and, consequently, have a less favourable prognosis. However, the presence of occult metastases in nodes outside the original field of dissection is less important if the original dissection specimen also contained one or more metastastic nodes. In such cases, the treatment regime followed at most centres would include post-operative radiotherapy which is likely to be effective in killing any small metastatic deposits in nodes outside the field of dissection (Fletcher, 1972 and 1973; Jesse and Lindberg, 1975; Byers, 1985; Byers *et al.*, 1988).

If a modified neck dissection is used as a staging (rather than an elective or therapeutic) procedure, it must sample adequately all nodal drainage areas at risk of metastatic spread. What constitutes an 'adequate sample' is uncertain. My results (Table 5.4) suggest that approximately one-half of nodes at anatomical levels II-IV are sampled in the supra-omohyoid procedure. Hence, the value of using a supra-omohyoid dissection as a staging procedure may be limited. Nevertheless, several authorities (Morgan *et al.*, 1983; Byers *et al.*, 1988; Spiro *et al.*, 1988) have reported that the failure rate following supra-omohyoid neck dissection is low (in the region of 10-15%).

It is possible that modified dissections remove all lymph nodes located along the routes draining the lymphatics of the oral cavity, and that the additional nodes removed in a radical dissection are not part of the intra-oral drainage system. This is one possible explanation for the reported clinical success of modified dissections. Another possible explanation of my findings is that the modified neck dissections carried out by the Surgeons at the Walton Unit are less extensive procedures than those practiced by other authorities, and my results give conflicting evidence in relation to this possibility. On the one hand, the yield of nodes in functional dissections in my study was almost identical to the yield achieved at the M.D.Anderson Hospital (Byers, 1985), suggesting a similar technique is practiced at both units. To date, no data is available on nodal yield in supra-omohyoid dissections at the M.D.Anderson Unit. On the other hand, the fact that I recovered more nodes from each type of surgical neck dissection specimen when the operation was carried out as a unilateral

procedure, than when it was part of a bilateral procedure (Tables 5.6-5.8) provides some evidence that the nodal yield in modified dissections is influenced by technical factors. For example, the desire to reduce to a minimum the duration of the surgical operation may result in a less-extensive dissection of the second neck in a bilateral procedure. The exact clinical relevance and surgical implications of my findings cannot be assessed fully until long-term follow up data on all Series I patients is available. Nevertheless, it is evident that further studies are required to assess the efficiency of modified neck dissections in relation to sampling of nodes in staging procedures and eradication of nodal groups in therapeutic procedures.

(b) Clinical and Surgical Implications of the Histopathological Patterns of Metastatic Spread in the Cervical Lymph Nodes

As reported in Chapter 4, my investigations into the patterns of metastatic spread confirm previous reports that metastasis to nodes in the posterior triangle is rare in oral cancer. In my study, positive nodes were found in the posterior triangle in only three dissections. In all cases, the neck was clinically positive, and histological examination showed metastatic disease throughout the deep cervical chain. Hence, my findings support the use of surgical procedures which preserve the posterior triangle in patients with no or only limited neck disease on pre-operative assessment. Nevertheless, it emerges strongly from my findings that anatomical levels I-IV must be included in all neck dissections when the oral tongue, floor of mouth or oropharynx is the site of, or involved by, the primary tumour. This is in agreement with the findings of Chu and Strawitz (1978), Donegan *et*

al. (1982) and Byers *et al.* (1988). The true metastatic status is unknown if limited modified dissections, such as the supra-hyoid or the extended supra-hyoid procedures, are used. A delay in the removal of the complete deep cervical chain of nodes may necessitate a more extensive salvage procedure, with a reduced chance of cure. In addition, it could be surmised that when the lymph nodes removed in a limited dissection are histologically negative, the surgical team might be lulled into a false sense of security, and consequently adopt a less intensive programme of follow-up examinations.

Other findings in the Series I neck dissections, in particular, that most false-negative clinical assessments can be explained by the presence of micrometastases or metastasis to small nodes, and that microscopic extracapsular spread is frequently seen in small nodes and nodes only partially replaced by tumour, have several important clinical implications, in relation to:

(i) The staging of nodal disease.

(ii) The selection of patients for elective neck dissection.

(iii) The use of standard radical or modified neck dissection procedures.

(iv) The indications for post-operative radiotherapy.

My results suggest there is no threshold size for metastatic involvement of a node or extracapsular spread. Even small nodes can be involved by tumour (Table 4.11), and, once involved, are equally likely to show extracapsular spread (Table 4.14). Extracapsular spread was frequently observed in nodes partially replaced by tumour (Table 4.15), and in nodes at all anatomical levels (Table 4.13). An important finding is that microscopic extracapsular spread was

diagnosed in eight (17%) of the 47 clinically negative necks (Table 5.3). In all cases, tumour was confined to the pericapsular fibro-adipose tissue (up to 0.3cm. distant from the involved nodes), and spread into adjacent structures, such as major veins and muscles, was not demonstrated in any patient with a clinically negative neck in the present study. My observations on the extent and direction of spread of extracapsular tumour in all 34 positive neck dissections in Series I suggest that a modified dissection is a safe procedure in patients with clinically negative necks. However, because of the apparent ease with which tumour can spread into the adventitia of the internal jugular vein, a radical neck dissection is likely to be a safer procedure if there is clinical evidence of metastasis in a deep cervical node. An alternative approach is to perform modified dissections supplemented by post-operative radiotherapy (Bocca *et al.*, 1983), and, currently, this is the approach favoured by the Surgeons at the Walton Unit. In patients with clinical evidence of extracapsular spread, a standard radical procedure is usually necessary for technical reasons in addition to therapeutic reasons.

SUMMARY

The accuracy of the clinical assessment of the metastatic status of the cervical lymph nodes was assessed in a series of 80 sides of neck in 60 patients undergoing simultaneous neck dissection. The clinical assessment was in agreement with the histological assessment in 53 (66%) of the 80 necks. Metastasis was evident on histological assessment in 14 (30%) of the 47 clinically negative necks. Pathological examination showed a possible explanation for the clinical misdiagnosis in ten necks (micrometastases only: three cases; largest positive node measuring 1.7cm. or less: six cases; positive node within bony depression: one case). Thirteen (39%) of the 33 clinically positive necks showed no histological evidence of nodal metastasis. Pathological assessment revealed reactive nodal hyperplasia (five cases), calcification of node (one case), direct spread of primary tumour (two cases), chronic sialadenitis of submandibular salivary gland (four cases), and mucous extravasation cyst (one case) as possible causes of the clinical misdiagnosis. Thus, the detailed pathological findings in both the false-negative and the false-positive neck dissections showed that the accuracy of pre-operative staging, even when supplemented by imaging techniques, is limited.

The yield and anatomical distribution of lymph nodes in relation to the type of surgical procedure were studied in the 71 Series I sides of neck dissection containing discrete lymph nodes only. There were significant differences in the nodal yield in the three types of neck dissection (radical: mean 45.0 nodes; functional: mean 30.3 nodes; supra-omohyoid: mean 16.4 nodes). The mean yield at anatomical

levels II-IV in functional and supra-omohyoid dissections was only 65% and 46%, respectively, that achieved in radical dissections. Hence, modified dissections may not achieve their aims in either eradicating or sampling adequately the principal groups of cervical nodes.

The important clinical implications of my investigations into the histological patterns of spread within the cervical lymph nodes are:

(i) For tumours of the tongue, floor of mouth and oropharynx, the surgical neck dissection procedure should include anatomical levels I-IV, even when the neck is negative on pre-operative assessment.

(ii) The posterior triangle should be preserved in elective neck dissections.

(iii) A standard radical dissection should be performed when extracapsular spread is evident on pre-operative assessment.

(iv) Modified dissections should only be performed when there is provision for post-operative radiotherapy.

Chapter 6.

THE RELATIONSHIP OF SELECTED CLINICAL AND HISTOLOGICAL FEATURES OF THE PRIMARY TUMOUR TO THE INCIDENCE OF CERVICAL METASTASIS.

1. Introduction.
2. Surgical Cases.
3. Methods.
4. Results.
5. Discussion.
6. Summary.

INTRODUCTION

From my earlier review of the literature, it is evident that the incidence of cervical lymph node metastasis is related to the site and size of the primary tumour and to its histological degree of differentiation (Arthur and Fenner, 1966; Arthur and Farr, 1972; Shear *et al.*, 1976; Yamamoto *et al.*, 1984; Spiro *et al.*, 1986; Moore *et al.*, 1986a and 1986b). Although statistically significant differences in each of these variables are found between patients with and without metastasis when large numbers of cases are studied, as yet individual cases cannot be confidently assigned to the metastatic or non-metastatic groups solely on the basis of these described characteristics of the primary tumour. My aim, in the present study, is to improve the prognostic efficiency of the histological assessment of the primary tumour in predicting cervical lymph node metastasis in an individual patient. Before describing my investigations, some of the histological malignancy grading systems that have been proposed previously will be reviewed. Also, the prognostic value of vascular and perineural invasion will be appraised, and, finally, recent reports on the prognostic value of the depth of invasion and tumour thickness will be considered.

(1) Malignancy Grading in Oral and Oropharyngeal Squamous Cell Carcinoma

Numerous authorities over many years have attempted to grade neoplastic lesions in the hope that a combined assessment of the clinical stage and the histological features would give a more precise prediction of the likely outcome and so determine the type and extent of treatment necessary to effect a cure.

Hansemann published the pioneer work on the relationship between the histological features of cancer and clinical malignancy (Hansemann, 1890, 1891, 1892, 1893 and 1902). He postulated that epithelial cells changed into cancer cells by the process of 'anaplasia' - literally, 'backward to form' - and he observed that cancers showing the most anaplasia exhibited the greatest tendency to metastasise. Broders (1920) initiated quantitative histological grading of cancer, based on Hansemann's principle of cell differentiation. Reporting on his studies of squamous cell carcinoma of the lip, Broders proposed four grades of carcinoma based on the proportion of highly differentiated, keratinising cells present within the entire tumour. Broders' grading system became an important factor in treatment planning (Broders, 1926 and 1941), and, in a modified form, it is still widely used today. Most pathologists now apply the terms 'well-differentiated', 'moderately-differentiated' and 'poorly-differentiated', rather than a specific numerical grade, with keratin production, nuclear and cellular pleomorphism, and the mitotic count being assessed subjectively to arrive at the final grade of differentiation (Walter and Israel, 1979).

There have been conflicting reports on the usefulness of Broders' grading system. Arthur and Fenner (1966) reported a correlation between tumour grade and radiocurability and survival in cancer of the tongue. Arthur and Farr (1972), reporting on a series of patients with cancer of the mouth and pharynx, established a clear relationship between the tumour grade and the site of the primary tumour, the clinical stage of disease, cervical node metastasis, and survival. Shear *et al.* (1976) and Langdon *et al.* (1977) also reported

that the histological grade was related to survival. However, Stoddart (1966), Jakobbson *et al.* (1973), Shah *et al.* (1976), Anneroth and Hansen (1984), Anneroth *et al.* (1987), and Bryne *et al.* (1989 and 1992) reported that Broders' system was of little value, and even proponents of the system recognised that the correlation between histological grade and outcome was not absolute (Arthur and Farr, 1972; Shear *et al.*, 1976).

In their study of laryngeal cancers, McGavran *et al.* (1961) reported a significant correlation between the type of growth pattern at the periphery of the tumour and the frequency of metastasis. They proposed that assessment of the pattern of invasion, in addition to Broders' degree of differentiation, would improve the accuracy of histological grading. The importance of this proposal was endorsed by Eneroth *et al.* (1972). The latter authors reported a higher incidence of nodal metastasis and poor survival in patients with squamous carcinoma of the palatal mucosa exhibiting a diffuse growth pattern and no keratinisation, than in patients with tumours showing invasion by well defined cords of neoplastic cells and abundant keratinisation. Eneroth *et al.* (1972) made two other important observations: firstly, that some carcinomas were highly differentiated and keratinising at the surface, but poorly differentiated at the invasive front, and secondly, that discrepancies between the growth pattern of the tumour and the degree of cellular differentiation may occur. For example, invasion by single or small groups of cells was sometimes observed in cytologically well-differentiated tumours. These observations, and similar observations made in laryngeal cancer, formed the basis of the multifactorial grading system which they proposed in 1973 (Jakobbson

et al., 1973). This grading system considered both the tumour cell population and its relationship to the adjacent tissues. Four morphological characteristics were evaluated in assessment of the tumour cell population: structure (a reflection of the cohesion of the tumour cells); differentiation (measured by the amount of keratinisation); nuclear polymorphism; and number of mitoses. Four morphological characteristics were also evaluated in assessment of the tumour-host relationship: pattern of invasion; stage (depth) of invasion; presence of vascular invasion; and lymphoplasmacytic response. Because this system incorporated strict point scoring, it was felt to be more objective than any previous attempts at grading. However, it could be argued that this 'improved objectivity' is, in fact, spurious, since points are still allocated on a subjective basis.

Jakobbson and co-authorities also applied the multifactorial grading system to carcinomas of the palate (Eneroth and Moberger, 1973); the lip (Lund *et al.*, 1975a) and the tongue (Lund *et al.*, 1975b). They reported a statistically significant correlation between the microscopic score and the following independent variables: T stage (Lund *et al.*, 1975a); frequency of local recurrence (Lund *et al.*, 1975a); nodal metastasis (Lund *et al.*, 1975a and 1975b); and death rate (Eneroth and Moberger, 1973; Lund *et al.*, 1975a).

Modifications of the multifactorial grading system were proposed by Willen *et al.* (1975), Crissman *et al.* (1980 and 1984), Anneroth and Hansen (1984) and Anneroth *et al.* (1987). In the system proposed most recently (Anneroth *et al.*, 1987), the histological features 'structure' and 'vascular invasion' have been deleted, hence a total

of six morphological characteristics are assessed.

Most human neoplasms consist of heterogeneous cell populations with different biological characteristics (Hepner, 1984; Nicolson, 1987), and it is likely that only a small population of tumour cells has the ability to metastasise (Nicolson, 1987). Hence, the success of any grading system depends on whether the area selected for grading is representative of the total tumour cell population and whether the most aggressive cells, with the capacity to metastasise, can be detected. Nicolson (1987) reported that the phenotype of cells from the deeply invasive parts of a tumour more closely resemble the metastatic phenotype, than do cells from the less invasive areas. Hence, it is possible that the morphological characteristics relating to the tumour cell population are better assessed at the invasive front. Evidence in favour of this suggestion has been provided by Bryne *et al.* (1989 and 1992), who reported that the validity of the multifactorial grading system, in predicting survival, is improved if only the most anaplastic areas at the invasive margin are selected for grading ('invasive cell grading').

Reproducibility of the malignancy score - both between pathologists and by the same pathologist at different times - is another factor important in determining the success of any grading system. Helweg-Larsen *et al.* (1978) reported that the reproducibility of the Jakobbson system was too unreliable for individual prognostic purposes. The inter-examiner agreement for the features 'mitoses' and 'vascular invasion' was reported to be particularly poor (Graeme *et al.*, 1980). However, more recent reports (Bryne *et al.*, 1989 and 1991a) suggest the reproducibility is improved if the modified system

proposed by Anneroth *et al.* (1987), with its more strictly defined grading criteria, is employed. The reproducibility and accuracy may be improved further with increased familiarity with the grading criteria and calibration of pathologists (Bryne *et al.*, 1991a).

The multifactorial grading system proposed by Jakobbson *et al.* (1973) and the modified systems (Willen *et al.*, 1975; Crissman *et al.*, 1980 and 1984; Anneroth and Hansen, 1984; Anneroth *et al.*, 1987) assume all the morphological characteristics are of equal importance in arriving at the overall evaluation of the degree of malignancy. However, several independent authorities (Crissman *et al.*, 1984; Frierson and Cooper, 1986; Anneroth *et al.*, 1987; Okamoto *et al.*, 1988) have reported that some histological features are better prognostic indicators than others. Currently, however, there is no agreement over which features are the more important and no satisfactory weighted scoring system has been proposed.

It has been recognised for many years (Ackerman, 1948) that carcinomas with well defined, pushing borders, such as verrucous carcinomas, seldom metastasise, and, hence, are associated with a good prognosis. In contrast, carcinomas that invade in a non-cohesive pattern of single or small aggregates of cells have a higher frequency of regional lymph node and distant metastases (McGavran *et al.*, 1961; Crissman *et al.*, 1984; Yamamoto *et al.*, 1984; Frierson and Cooper, 1986; Okamoto *et al.*, 1988). Using multivariate analysis to assess the independent prognostic value of each of the morphological characteristics assessed in multifactorial grading systems, both Johnson (1976 and 1977) and Crissman *et al.* (1984) reported that the pattern of invasion was the single most important characteristic in

predicting survival. This confirmed the findings of earlier authorities (Jakobbson, 1973; Willen *et al.*, 1975), who also reported that an assessment of the pattern of invasion was a good predictor of clinical outcome. An increased frequency of mitoses also showed a good correlation with poor survival in the studies reported by Crissman *et al.* (1980 and 1984). However, other authorities (Jakobbson, 1973; Willen *et al.*, 1975; Johnson, 1977; Frierson and Cooper, 1986) reported that an assessment of the mitotic rate had no significant independent prognostic value. Also, the characteristic 'nuclear polymorphism' showed a good correlation with survival, in the studies reported by Jakobbson (1973) and Willen *et al.* (1975), but not in the studies reported by Johnson (1977) and Crissman *et al.*, (1984).

The Multifactorial Grading System as a Predictor of Nodal Metastasis

The value of the multifactorial grading system in predicting nodal metastasis, rather than survival, has been assessed by several authorities. Willen *et al.* (1975), Lund *et al.* (1975a and 1975b), Holm *et al.* (1982) and Tytor *et al.* (1987) reported a significant correlation between the total malignancy score and the presence of nodal metastasis, but this was not confirmed by Helweg-Larsen *et al.* (1978) and Crissman *et al.* (1980 and 1984). However, in the study of floor-of-mouth tumours reported by Crissman *et al.* (1980), the stage (depth) of invasion was of value in predicting metastasis. Also Yamamoto *et al.* (1984); Frierson and Cooper (1986); Okamoto *et al.* (1988); and Shingaki *et al.* (1988) have reported that the pattern or mode of invasion has significant independent prognostic value. Hence, the importance of the total malignancy score and the scores of the individual morphological characteristics in predicting cervical

metastasis are still uncertain.

(2) Prognostic value of Vascular Invasion

An assessment of the presence and extent of vascular invasion was one of the original morphological characteristics assessed in the multifactorial grading system proposed by Jakobbson *et al.* (1973). However, Willen *et al.* (1975), Anneroth and Hansen (1984), and Anneroth *et al.* (1987) omitted this characteristic in their modified grading systems, since they considered it was difficult to define and recognise with certainty. In particular, retraction artefact around groups of tumour cells often mimics vascular invasion (Underwood, 1992). Nevertheless, the relationship between a neoplasm and the vascular system is routinely assessed in the diagnosis and prognosis of carcinomas of the thyroid (Graham, 1924) and kidney (Mostofi, 1967). Also, the association between the presence of tumour emboli in endothelial-lined channels and increased regional and distant metastases and decreased survival, is well documented. For example, it has been demonstrated in carcinomas of the breast (Baak *et al.*, 1982); stomach (Kodama *et al.*, 1983); uterine cervix (Friedell *et al.*, 1967); and in oral and pharyngeal tumours by Poleksic and Kalwaic (1978), Crissman *et al.* (1984), and Shingaki *et al.* (1988). However, McGavran *et al.* (1961) failed to show such an association in laryngeal carcinomas.

Reports of animal experiments (Butler and Gullino, 1975; Weiss *et al.*, 1982) and clinical studies (Sugarbaker, 1979) suggest that the majority of malignant cells within vascular spaces and the peripheral circulation are destroyed, with only a small proportion successfully establishing a metastatic deposit. However, the presence of vascular

invasion in random tissue sections statistically implies that a considerable number of cells are entering the vascular compartment, thus increasing the likelihood of successful metastatic growth. Hence it is desirable to include such an assessment when grading oral carcinomas, even though this is time-consuming (Fisher, 1975).

(3) Prognostic Value of Perineural Invasion

Perineural invasion of cancer cells is a well-recognised route of spread (Ernst, 1905; Ballantyne *et al.*, 1963; Larson *et al.*, 1966). Within the head and neck, perineural spread is particularly associated with adenoid cystic carcinoma of the major and minor salivary glands, but it is also a common feature of squamous cell carcinoma of the skin and mucosae (Dodd *et al.*, 1970; Mendenhall *et al.*, 1989). For example, histological evidence of perineural spread in the vicinity of the primary tumour has been reported in 50% of cancers arising in the oral cavity (Carter *et al.*, 1982).

Skin cancers with perineural invasion are more likely to recur locally and to metastasise to the regional lymph nodes and distant sites (Goepfert *et al.*, 1984). A significant association between perineural invasion and nodal metastasis has also been reported for cancers of the larynx (McGavran *et al.*, 1961); lip (Byers *et al.*, 1978; Frierson and Cooper, 1986); and oral tongue (Maddox, 1984). Maddox (1984) reported that perineural invasion was particularly useful in predicting the presence of nodal metastasis in tumours between 1.0cm. and 3.0cm. in size. However, Carter *et al.* (1982) failed to demonstrate an association between perineural spread and nodal metastasis in patients with advanced cancers of the upper aero-digestive tract.

Although an assessment of perineural invasion is recommended in grading cancers of the skin (Lever and Schaumburg-Lever, 1990a) and lip (Frierson and Cooper, 1986), such an assessment does not form part of the established multifactorial grading systems for intra-oral cancer (Jakobbson *et al.*, 1973; Anneroth *et al.*, 1987).

(4) Prognostic Value of Histological Depth of Invasion and Tumour Thickness Measurements

An assessment of the depth of invasion is part of the multifactorial grading system proposed by Jakobbson *et al.* (1973) and the modified schemes proposed by Willen *et al.* (1975); Crissman *et al.* (1980 and 1984); Anneroth and Hansen (1984); and Anneroth *et al.* (1987). In these grading systems, histological depth is expressed by reference to the anatomical deep structures reached by the advancing edge of the tumour.

Histological depth of invasion showed a good correlation with survival in the study of carcinomas of the upper aerodigestive tract reported by Moore *et al.* (1986b). In addition, tumour depth has been reported to be a better predictor of nodal metastasis than tumour greatest surface-dimension in floor-of-mouth cancers (Crissman *et al.*, 1980), and in cancers of the oral cavity (Moore *et al.*, 1986a) and upper aerodigestive tract (Moore *et al.*, 1986b). Greater depth of invasion also increases the risk of local recurrence (Willen *et al.*, 1975; Crissman *et al.*, 1980; Moore *et al.*, 1986a and 1986b; Urist *et al.*, 1987).

However, since there are differences in the thickness of the lamina propria and submucosa, and in the depth of muscles, salivary glands, and bone at different anatomical sites within the oral cavity,

measurement of the vertical growth of the tumour by reference to the anatomical structures reached by the advancing edge is open to criticism. For example, in the lower lip, the orbicularis oris muscle may be located immediately beneath the labial mucosa and, hence, may be infiltrated by very early carcinomas with low metastatic potential (Frierson and Cooper, 1986). A similar problem was encountered when staging cutaneous malignant melanomas by the Clark level of invasion system (Clark *et al.*, 1969), and led to the introduction of a tumour thickness measurement by Breslow (1970). This direct micrometer measurement proved to be more objective and reproducible than an assessment of the Clark level of invasion (Breslow, 1975), and it is now routinely used in treatment planning and as a major prognostic indicator in cutaneous melanomas (Lever and Schaumburg-Lever, 1990b).

The success of the Breslow grading system prompted pathologists to look more critically at the thickness of squamous cell carcinomas of the skin in relation to lymph node metastasis and prognosis (Friedman *et al.*, 1985). The technique was soon applied to lip and intra-oral squamous cell carcinomas (Frierson and Cooper, 1986; Spiro *et al.*, 1986; Mohit-Tabatabai *et al.*, 1986; Moore *et al.*, 1986a and 1986b; Urist *et al.*, 1987; Shingaki *et al.*, 1988; Nathanson and Agren, 1989). The results of these studies show that prognosis - in terms of local recurrence, nodal metastasis, and survival - is related to tumour thickness, but the critical thickness differs widely in the different reports. For example, in their study of carcinomas of the lower lip, Frierson and Cooper (1986) proposed the critical thickness for predicting metastatic potential was 6mm., with only 4% of carcinomas less than, but 74% greater than 6mm. metastasising. Spiro

et al. (1986) studied a series of patients with carcinoma of the tongue and floor of mouth, and reported a critical thickness of 2mm., with nearly 40% of thicker tumours metastasising. A similar critical thickness (1.5mm.) was reported for T1-T2 tumours of the floor of mouth by Mohit-Tabatabai *et al.* (1986). For carcinoma of the buccal mucosa, Urist *et al.* (1987) reported a critical thickness of 6mm. The latter series included some verrucous carcinomas, and it is possible that inclusion of these low grade malignancies resulted in an exaggeration of the critical thickness measurement. A difference in the technique used to obtain the thickness measurement, or a true difference in the critical thickness at different intra-oral sites, are alternative explanations for the range of values that have been reported. Hence, although further studies are needed, reports in the literature to date suggest that a measurement of the tumour thickness is an important indicator of prognosis, and the American Joint Committee on Cancer (1988a) have recently recommended that a thickness measurement is included in the pathological staging of resection specimens.

Summary

In summary, the present situation is that several pathological grading systems are in current use, and although these are useful in predicting patterns of outcome in large numbers of patients with oral cancer, their prognostic value for an individual patient is very limited. Also, it is uncertain whether or not the prognostic value is improved by using a combination of different grading systems. In view of these uncertainties, the aim of the present study, to improve the

prognostic efficiency of predicting lymph node metastasis in an individual patient, is relevant. My investigations will identify those clinical and histological features in the primary tumour most frequently associated with cervical metastasis and these will be incorporated into a simple prognostic method to be recommended for future use in the initial assessment of oral carcinoma.

SURGICAL CASES

Material selected from the Series I neck dissections detailed in Chapter 4 formed the basis of the present study. It may be recalled that only neck dissections performed simultaneously with resection of the primary intra-oral carcinoma and meeting four other criteria were entered into Series I. In the present study, in order to reduce the number of variables with a possible influence on the rate of metastasis, cases were restricted by the site of the primary tumour. Only primary tumours arising on the oral tongue and the floor of mouth, sites already established as carrying a high risk of metastasis (Spiro *et al.*, 1974; Farr *et al.*, 1980), were included. In addition, a further criterion, namely, that the surgical resection should include the entire primary tumour, had to be met.

Thirty-six cases in Series I met the additional criteria and were accepted into the present study. A further five of the cases listed in Appendix 2, which were not included in Series I, met the criteria for entry into the present study. In addition, four cases of tongue/floor-of-mouth carcinoma in which the neck dissection was delayed and performed some time after surgical resection of the primary tumour (Cases ND40, ND48, ND74/89, ND145), met all other criteria for entry into the present study. In the latter four cases, there was no evidence of intra-oral recurrent or additional malignant disease in the period between resection of the primary tumour and the neck dissection. These cases were, therefore, accepted into the study. Hence, in total, 45 cases of carcinoma of the oral tongue/floor-of-mouth were entered into the present study, and these 45 cases will be referred to as Series II.

The following clinical information was available for each patient in Series II:

(i) The sex of the patient.

(ii) The age of the patient at the time of resection of the primary tumour.

(iii) The specific site of the primary tumour, that is, either oral tongue or floor of mouth, as defined by the American Joint Committee on Cancer (1988b). Detailed definitions of the two sites are presented in Appendix 3.

(iv) The clinical stage of the primary tumour at initial presentation. Definitions of the four T categories (American Joint Committee on Cancer, 1988b) are presented in Appendix 1.

(v) The tobacco usage and alcohol consumption habits of the patient. This information was elicited by one member of the surgical team using a protocol designed to overcome the known tendency to understate tobacco and alcohol usage (Pernanen, 1974). Current usage was recorded as the number of cigarettes smoked and the number of units of alcohol consumed per day. Heavy usage was defined as more than 24 cigarettes and more than six units of alcohol per day.

METHODS

(1) Gross and Histological Sampling Techniques and Laboratory Methods

The protocol detailed in Chapter 4 (Operating Theatre Procedures and Fixation), which was designed to ensure that the specimens reached the Oral Pathology Laboratory in a well preserved state with the minimum of tissue distortion, was used, also, in the present study.

The resected specimens were examined after approximately 24-48 hours of fixation in 10% buffered formalin. The macroscopic appearance of the primary tumour, its site and size were recorded. The tumour was cut into 3-4mm. thick slices. The cuts were made perpendicular to the surface, in either a coronal or a sagittal plane, hence producing vertical sections (Baddeley et al, 1986; Gundersen et al, 1988a). The cuts were positioned so that an accurate measurement of the macroscopic greatest surface-dimension (D) could be made, either directly or indirectly by combining the measurement of adjacent blocks of tissue. No compensation was made for shrinkage during fixation of the tissues, as, for comparative purposes, this was assumed to apply equally throughout the series. The block(s) of tissue showing the maximum cross-sectional area and depth of tumour were selected for processing, together with tissue blocks showing the surgical margins and the relationship of the tumour to these. In most cases, the number of tissue blocks processed was representative of the size of the tumour. The tissue blocks were routinely processed for embedding in paraffin wax. From each block, one complete section was cut at five microns thickness, stained with haematoxylin and eosin, and histologically assessed and reported as part of the routine diagnostic service.

For the purposes of the present study, from each block containing tumour tissue, five further sections, approximately 300 microns apart, were cut and stained with Harris's haematoxylin and 1% eosin. The exact distance between the sections was chosen to ensure even sampling throughout the block. The following precautions were taken in order to reduce the risk of technical factors influencing the results of the histological assessment:

(i) The same microtome was used for cutting all the sections.

(ii) Care was taken to avoid stretching the paraffin-wax sections when floating-out and mounting.

(iii) All the staining was carried out in consecutive sessions using the same solutions for an identical time period.

Following staining, the sections were coded by laboratory technical staff to ensure that the case number and the metastatic status were not known to the author during the histological assessment.

(2) Histological Assessment

(a) Measurement of Tumour Thickness and Greatest Surface-Dimension

The histological thickness of each tumour was determined by measuring vertically from the surface of the tumour to the point of maximum invasion, using an optical micrometer. The measurement was recorded to the nearest millimetre. Surface keratin, parakeratin and inflammatory exudate were excluded from the measurement, but superficial layers of viable cells with open nuclei were included.

In ulcerated tumours, measurement was made from the floor of the ulcer to the deepest extent of growth (T_A). In addition, a second

measurement from a theoretically reconstructed normal mucosal line (T_R) was recorded (Figure 6.1a). T_A and T_R measurements were recorded, also, for tumours with an exophytic pattern of growth (Figure 6.1b). The tumour thickness was measured in each histological section. When histological assessment of all the sections was complete, the sections were re-grouped into surgical cases and the greatest thickness measurement was recorded as the definitive tumour thickness of that case. In addition, if there was doubt concerning the accuracy of the macroscopic assessment of the tumour's greatest surface-dimension (D), this was re-assessed histologically at this time. Particular attention was paid to the periphery of small and/or thin tumours, and only invasive carcinoma was included in the D measurement.

(b) Assessment of Epithelial Dysplasia adjacent to the Primary Tumour

The undersurface-of-tongue and floor-of-mouth mucosae included in the surgical resection, but not directly involved by the primary tumour, were examined histologically for evidence of epithelial dysplasia. The diagnostic criteria proposed by the World Health Organisation Collaborating Centre for Oral Precancerous Lesions (World Health Organisation, 1978) were used and the results were recorded as 'associated dysplasia identified' or 'associated dysplasia not identified'.

(c) Assessment of the Histological Malignancy Grade

The histological malignancy grade was determined by a modification of the multifactorial grading system proposed by Anneroth *et al.* (1987). In the present study, six histological features were assessed (Table 6.1). Three features represented the tumour cell population: degree of keratinisation, amount of nuclear polymorphism,

and frequency of mitotic figures. The other three features represented the tumour-host relationship: pattern of invasion, stage (depth) of invasion, and intensity of the lymphoplasmacytic infiltrate. For each of the six features, a grade or score of between 1 and 4 points was assigned, resulting in a possible maximum score of 24 points. In an assessment where the grade was equivocal or borderline, the lower point score was recorded.

The criteria for assessment of the six histological features were as follows:

(i) Degree of Keratinisation

The degree of keratinisation of tumour cells within the islands or tongues at the advancing front was assessed by evaluating the presence and number of keratin pearls and the amount of keratinisation of individual tumour cells. Four grades were designated according to the following criteria:

Grade 1: A high degree of keratinisation, as evident by well-formed keratin pearls (Figure 6.2a).

Grade 2: A moderate degree of keratinisation, as evident by attempts at pearl formation (Figure 6.2b).

Grade 3: A poorly keratinising tumour, with no attempted pearl formation, but with evidence of individual cell keratinisation (Figure 6.2c).

Grade 4: A tumour with no evidence of keratinisation (Figure 6.2d).

(ii) Nuclear Polymorphism

The most dysplastic areas at the advancing front of the tumour were selected for assessment of nuclear polymorphism. Nuclear

polymorphism was measured by a subjective and combined assessment of the variation in size and shape of tumour cell nuclei; the nuclear/cytoplasmic ratio; the presence of hyperchromatic and multiple nuclei; the number, size and shape of nucleoli; and the presence of atypical mitoses. Four grades of nuclear polymorphism were designated according to the following criteria:

Grade 1: A relatively homogeneous tumour cell population with small nuclei, almost uniform in size; and only a few nuclear aberrations (Figure 6.3a).

Grade 2: A tumour cell population with large nuclei; a high nuclear/cytoplasmic ratio; distinct, and often multiple, nucleoli; and moderately abundant nuclear aberrations (Figure 6.3b).

Grade 3: A tumour cell population with large nuclei; distinct nucleoli; and abundant nuclear aberrations, including some cells with multiple or large anaplastic nuclei and/or atypical mitoses (Figure 6.3c).

Grade 4: A tumour cell population with extreme nuclear aberrations, including numerous cells with multiple or large anaplastic nuclei and/or atypical mitoses (Figure 6.3d).

(iii) Frequency of Mitosis

The number of mitotic figures was counted in one field, using 400x magnification, in the most dysplastic area at the invasive front of the tumour. The field was selected to include a high ratio of neoplastic epithelium to stroma (subjectively estimated as more than 80%). Mitotic counts were then made in nine additional contiguous

tumour-containing fields. If the microscopic field was subjectively assessed to contain only 50% of neoplastic epithelium or less, the actual mitotic count for that field was doubled. For each histological section, four sets of ten high power fields (400x magnification) were counted and the maximum number in any one set was recorded. Point scores were then designated:

Grade 1: 0-15 mitoses per set of 10 high power fields.

Grade 2: 16-35 mitoses per set of 10 high power fields.

Grade 3: 36-55 mitoses per set of 10 high power fields.

Grade 4: More than 55 mitoses per set of 10 high power fields.

The presence of distinguishable chromosomes, or hairy nuclear projections in the absence of a nuclear membrane, were used as the criteria for diagnosis of mitosis (Baak and Oort, 1983a). Cells with triangular or spiky nuclei and cytoplasmic eosinophilia were designated as pyknotic cells and excluded from the mitotic count. Any equivocal structures were also excluded. No attempt was made to categorise the phase of mitosis.

(iv) Pattern of Invasion

The pattern of invasion at the advancing front of the tumour was assessed and graded according to the following criteria:

Grade 1: A well delineated, pushing border (Figure 6.4a).

Grade 2: Invasion by broad strands and solid cords of tumour cells (Figure 6.4b).

Grade 3: Invasion by thin cords or small islands of tumour cells, each containing more than 15 cells (Figure 6.4c).

Grade 4: Invasion by single tumour cells or small islands, each

containing less than 15 cells, resulting in poor delineation of the advancing front (Figure 6.4d).

(v) Stage of Invasion

The stage or maximum depth of invasion was recorded by reference to the anatomical structures reached by the advancing front of the tumour, and graded according to the following criteria:

Grade 1: Carcinoma-in-situ or borderline invasion.

Grade 2: Distinct invasion, involving the lamina propria only.

Grade 3: Invasion below the lamina propria, into the submucosa.

Grade 4: Invasion below the submucosa, into muscle and/or adjacent structures, including bone.

(vi) Lymphoplasmacytic Infiltrate

The intensity of the lymphoplasmacytic infiltrate was assessed in the stroma in close relationship to the tumour cells at the advancing front, and graded according to the following criteria:

Grade 1: A dense infiltrate of lymphocytes and plasma cells, producing a dense continuous band surrounding the tumour (Figure 6.5a).

Grade 2: A moderate infiltrate of lymphocytes and plasma cells, producing numerous large patches (Figure 6.5b).

Grade 3: A mild infiltrate of lymphocytes and plasma cells, producing either small, sparse patches or a sparse but diffuse infiltrate (Figure 6.5c).

Grade 4: No lymphocytes and plasma cells detected (Figure 6.5d).

Whenever possible, the assessment was not made in stroma close to areas of ulceration. Other inflammatory cells, such as eosinophils and foreign-body giant cells, were disregarded when making the

assessment.

Derivation of the Total Histological Malignancy Grade

A grade or point score was assigned for each of the six described histological features. Each histological section was initially graded independently, in a random sequence. The results were then grouped into surgical cases. For each surgical case, the highest grade for each feature was selected. Following confirmation, by further histological assessment, that this grade was an accurate record of the histological appearances, it was recorded as the definitive grade of that feature for that surgical case. The total histological malignancy grade for each case was derived by summation of the six definitive grades. In an attempt to prevent inaccuracies arising from inconsistent point scoring, all the histological assessments were carried out within a single three-month period.

(d) Assessment of Perineural and Vascular Invasion

While assessing the histological malignancy grade, each histological section was examined, also, for evidence of perineural and vascular invasion, both within the tumour and in the host stroma at the advancing front. Contiguous fields of the tumour and the tumour-host interface were examined at 200x magnification and perineural and vascular invasion were each recorded as 'identified' or 'not identified'.

Perineural invasion was defined as infiltration of the perineural space by tumour cells (Figure 6.6). Islands of carcinoma present in the stroma adjacent to nerves but lacking unequivocal perineural infiltration were disregarded.

Vascular invasion was defined as the presence of aggregates of

tumour cells within endothelial-lined channels (Figure 6.7a) or invasion of the media of a vessel, with ulceration of the overlying intima (Figure 6.7b). Equivocal examples of vascular invasion were disregarded.

When the histological sections were grouped into surgical cases on completion of the malignancy grading procedure, both perineural and vascular invasion were recorded as 'Identified' or 'Not Identified' for each surgical case, with no attempt to record the number or size of the nerves and vessels involved.

(e) Pilot Study

Before the commencement of the principal study, a pilot study was carried out to assess the consistency of both the histological malignancy grading system and the assessment of perineural and vascular invasion.

In the Pilot Study, 30 histological sections were selected at random from the sections prepared in readiness for the principal study. Each section was graded using the criteria detailed above. The point score for each of the six features assessed in the grading procedure, and the presence or absence of perineural and vascular invasion, were recorded for each section. Histological assessment of the same 30 sections was repeated after a one-month interval, and the two sets of results were compared.

The results of the Pilot Study are shown in Tables 6.2 and 6.3. The consistency of point scoring for the assessment of the six features in the multifactoral grading system ranged from 87% for 'Lymphoplasmacytic Infiltrate' and 'Pattern of Invasion' to 100% for 'Stage of Invasion'. The identification of both perineural and

vascular invasion was consistent in 90% of the sections examined.

On the basis of these results, it was decided that there was sufficient intra-observer consistency to proceed with the principal study.

(3) Statistical Methods

The results of the study were analysed using the SPSS-X and SAS statistical packages on the University of Liverpool IBM computer. The following tests were used:

(a) The Two Sample t Test

(b) The Chi Squared Test

(c) Yates'-Corrected Chi Squared Test

(d) Chi Squared Test For Trend

(e) Fisher's Exact Test

Details of Statistical Tests (a) to (e), inclusive, are presented in Chapter 4 (Statistical Methods).

(f) The Mann-Whitney U Test

This test is a non-parametric alternative to the Two Sample t Test for comparing data from two independent groups. It is appropriate when the two groups of observations are of unequal variance or for comparing data that are scores rather than measurements (Altman, 1991b).

The Mann-Whitney U test is based on the rank order of observations and the U statistic is derived from the formula:

$$U = n_1 n_2 + \tfrac{1}{2} n_1 (n_1 + 1) - T$$

where n_1 and n_2 are the numbers of observations in each group, and T is the sum of the ranks in the smaller group.

The result of the test is presented as the U statistic and P, the associated probability (corrected for tied ranks) of obtaining such a value by chance under the null hypothesis.

(g) Pearson's Correlation Test

This test assesses the relationship between two variables. The Pearson correlation coefficent, r, measures the degree of 'straight line' association between the values of two variables plotted as a scatter diagram. The correlation coefficient has a value of +1 or -1 if all the points lie on a perfect straight line. The coefficient is positive (between 0 and +1) if higher values of one variable are associated with higher values of the other, and negative (between 0 and -1) if one variable tends to be lower as the other becomes higher. A coefficient of zero indicates the points are widely scattered with no linear relationship between the two variables. Pearsons's correlation test is appropriate if both variables are continuous and have a Normal distribution (Altman, 1991c).

$$r = \frac{(x_i-\bar{x})\ (y_i-\bar{y})}{\sqrt{[\Sigma(x_i-\bar{x})^2]\ [\Sigma(y_i-\bar{y})^2]}}$$

where x_i and y_i and $\bar{x}$ and $\bar{y}$ are the pairs and the means of the observations, respectively, and Σ denotes 'the sum of'.

The null hypothesis test can be applied to r, and a probability value, P, obtained by reference to standard tables.

The result of the test is given as the Pearson coefficent, r, and its associated probability (two-tailed), P.

(h) Spearman's Rank Correlation Test

This test assesses the relationship between two continuous or ordered categorical variables. This is a non-parametric method which

gives similar information to Pearson's Correlation Test. In addition to its wider application, it also has the advantage of assessing any, not just a linear, association between the variables (Altman, 1991c).

Spearman's rank correlation coefficient, r_s, is obtained by ranking in order the values of each of the two variables, and then calculating the product moment (Pearson's) correlation of the ranks. Hence,

$$r_s = \frac{\text{sum of products about mean of ranks}}{\text{sum of squares of ranks for first variable} \times \text{sum of squares of ranks for the second variable.}}$$

The r_s statistic can be used to test the null hypothesis that the variables are independent and a probability value, P, is obtained by reference to standard tables.

The result of the test is given as the Spearman Coefficient, r_s, and its associated probability (two-tailed), P.

(i) Multiple Linear Logistic Regression Analysis

This is a variant of multiple linear regression, and in order to explain the indications for and the technique of a logistic regression, an initial brief explanation of ordinary least squares linear regression is necessary.

The technique of ordinary least squares linear regression is used to estimate the numerical relationship between two continuous variables. The technique can be used to predict the value of one variable (the outcome or dependent variable) when the value of the second (predictor or independent) variable is known (Altman, 1991c). Data on the two variables is presented as a scatter diagram (with the outcome variable on the vertical Y axis and the predictor variable on the horizontal X axis) so that a straight line which best fits the

data can be constructed. From the regression line, the value of Y can be estimated from the regression equation:

$$Y = a + bX$$

where a is the intercept (the value of Y when X = 0), and b is the gradient (the regression coefficient of Y on X).

The accuracy of the prediction depends on how well the regression line fits the data. The goodness-of-fit is shown by the residual, which is the difference between an observed value, Y_o, and the fitted value, Y_{fit}. The residual standard deviation can be used to calculate the standard error of the slope, and this value is used to indicate the accuracy of the regression.

Multiple linear (or ordinary least squares multivariate) regression analysis produces a regression model in which the value of the outcome (dependent) variable can be predicted from a combination of the values of a group of explanatory (independent) variables (Altman, 1991d).

Logistic regression is a modification of multiple linear regression which is used when the outcome variable is dichotomous in nature, such as the absence or presence of a characteristic (for example, metastatic spread in the present instance), rather than a continuous variable (Walsh, 1990; Altman, 1991d). Ordinary multiple linear regression analysis cannot be used with non-continuous data since it may result in predictions outside the range of possibility, that is, less than zero and more than one, and, hence, it is necessary to perform a logistic regression.

In the present study, the logistic regression analysis was performed using the SAS statistical package. The logistic regression

model predicts a transformation of the proportion of subjects with the dependent characteristic (or, equivalently, a transformation of the probability of an individual having the dependent characteristic) for any combination of explanatory variables in the regression model.

In the regression analysis, if p is the proportion of individuals with the dependent characteristic, then (1-p) is the proportion without. The ratio p/(1-p) is called the odds, and the logit transformation is the log odds.

Thus,

$$\text{logit}(p) = \log_e \frac{p}{1-p}$$

The numerical value of logit(p) is derived from the regression coefficients of the explanatory variables in the logistic regression model.

The value of p can be derived from logit(p) and it always lies within the range of 0 to 1.

$$p = \frac{e^{(\text{logitp})}}{1 + e^{(\text{logitp})}}$$

Hence, the estimated probability or risk (p) of an individual having the dependent characteristic can be calculated for any combination of explanatory variables in the logistic regression model.

The statistical significance of each explanatory variable in the regression model is shown by the standard error of the regression coefficent, the model Chi Squared Test statistic and its associated probability value. In the SAS logistic regression model, a significant Chi Squared value indicates that the explanatory variable has significant predictive value (Walsh, 1990). The 'best' model uses the combination of explanatory variables that most accurately predicts the

outcome variable, subject to the number of variables not amounting to 'overfitting'. At present, there is no accepted way of determining the maximum number of variables in any given model but two methods have been suggested (Altman, 1991d): firstly, that the number of variables should not exceed the square root of the sample size, and secondly, that the number of variables should not exceed n/10, where n is the sample size.

The accuracy of the regression model is assessed by the percentage of correct classifications which also indicates the sensitivity and specificity of the model.

In the present study, for each of the explanatory variables in the regression model, the results of the regression are presented as the regression coefficient (Beta), the standard error of Beta, the Chi Squared test statistic (X^2) and its associated probability, P. Also, for each regression model, the classification table and associated sensitivity and specificity values, are presented. In addition, for the chosen 'best' model, the estimated probability or risk of each individual in Series II having the dependent characteristic is given.

RESULTS

I. CLINICAL DETAILS OF THE SURGICAL CASES

Clinical details of 45 Series II patients are presented in Table 6.4. The patients were divided into two groups on the basis of the metastatic status of the cervical lymph nodes, as revealed by histological examination of nodes harvested from the neck dissection specimen. Cervical lymph node metastasis was diagnosed in 27 (60%) of the 45 patients, and Table 6.5 shows the number and location of the positive nodes.

(1) Sex of the Patient

Thirty (67%) of the 45 patients were male. There was no significant difference in the frequency of metastasis in relation to sex. Metastasis was present in 19 (63%) of the 30 males, and in eight (53%) of the 15 females (Yates'-Corrected Chi Squared Test, X^2_{YATES} = 0.10, 1 d.f., P = 0.75).

(2) Age of the Patient

The age of the 45 patients ranged from 37-81 years, with a mean of 60 years (SD 10.5). The mean age of 62 years (SD 9.6, range 42-81) in patients with metastasis was not significantly different from the mean age of 59 years (SD 11.8, range 37-79) in patients without metastasis (Two Sample t Test, t = 0.86, 31 d.f., P = 0.39).

(3) Site of the Primary Tumour

Oral tongue was the site of the primary tumour in 22 (49%) of the 45 cases, with the other 23 cases (51%) sited on the floor of mouth. There was no discernible association between sex and the site of the primary tumour. Fourteen (64%) of the 22 patients with tongue tumours and 16 (70%) of the 23 patients with floor-of-mouth tumours

were male (Yates' Corrected Chi Squared Test, $X^2_{YATES} = 0.11$, 1 d.f., $P = 0.92$).

The frequency of metastasis was similar at the two sites. Metastasis was present in 13 (59%) of the 22 patients with tongue tumours and in 14 (60%) of the 23 patients with floor-of-mouth tumours (Yates'-Corrected Chi Squared Test, $X^2_{YATES} = 0.00$, 1 d.f., $P = 1.00$).

(4) Clinical T Stage of the Primary Tumour

Sixteen (36%) of the primary tumours were staged T1 on clinical assessment. Nineteen tumours (42%) were staged T2, four tumours (9%) were staged T3, and the remaining six (13%) were staged T4. Tumours of clinical stages T1, T2, and T3/T4 were represented at both of the selected intra-oral sites.

Metastasis was present in seven (44%) of the 16 T1 tumours, in 13 (68%) of the 19 T2 tumours, in two (50%) of the four T3 tumours, and in five (83%) of the six T4 tumours. However, the difference in frequency of metastasis in relation to tumour stage did not achieve statistical significance (Chi Squared Test, $X^2 = 3.85$, 3 d.f., $P = 0.28$; Chi Squared Test for Trend, $X^2_{TREND} = 2.31$, 1 d.f., $P = 0.26$). Also, there was no significant difference in the frequency of metastasis in T1/T2 and T3/T4 tumours (Fisher's Exact test, $P = 0.73$).

(5) Tobacco and Alcohol Usage

The number of cigarettes smoked and the amount of alcohol consumed per day is shown in Table 6.6. Thirty-two (71%) of the 45 patients were regular smokers and drinkers, six patients (13%) were regular smokers but non-drinkers, and another two patients (4%) were regular drinkers but non-smokers. The remaining five patients (11%) were non-smokers and non-drinkers. Twelve patients (27%) were both

heavy smokers and heavy drinkers, seven patients (16%) were heavy smokers and moderate or non-drinkers, and four patients (9%) were heavy drinkers but only moderate smokers.

There was no significant difference in the site of the primary tumour in relation to either tobacco usage (Mann-Whitney U Test, U = 218.5, P = 0.43) or alcohol consumption (Mann-Whitney U Test, U = 212.0, P = 0.35).

There was no significant difference in the frequency of metastasis in heavy smokers and moderate/non-smokers (Fisher's exact test, P = 0.14). Also, there was no significant difference in the frequency of metastasis in heavy drinkers and moderate/non-drinkers (Fisher's Exact Test, P = 0.18).

II. PATHOLOGICAL FEATURES

The average number of histological sections assessed in each of the 45 Series II cases was 15 (range 5-35).

(1) Tumour Greatest Surface-Dimension

As shown in Table 6.7, the tumour greatest surface-dimension ranged from 4-65mm. (mean 24.3, SD 14.5).

There was some discrepancy between the clinical T stage and the laboratory-measured greatest surface-dimension, with tumours assigned clinically to both higher and lower T categories. Two of the T1 tumours measured more than 2.0cm. In one of the T2 tumours, the dimension of invasive carcinoma was only 8mm., and four T2 tumours measured 2.0cm. The dimension of the six T4 tumours ranged from 15-65mm., and all were staged T4 because of suspected bone involvement.

Bone involvement was confirmed histologically in five of the six cases.

There was a significant difference in the tumour greatest surface-dimension (D) in patients with and without metastasis (Two Sample t test, t = 2.13, 38 d.f., P = 0.04). In patients with metastasis, D ranged from 5-65mm., with a mean of 27.9mm. (SD 14.3). In patients without metastasis, D ranged from 4-44mm., with a mean of 18.9mm. (SD 13.3).

The mean D for tumours of the tongue, 28.7mm. (SD 13.0, range 7-60) was significantly greater than the mean D, 20.0mm. (SD 14.8, range 4-65), for tumours of the floor of mouth (Two Sample t Test, t = 2.09, 43 d.f., P = 0.04).

At each of the two selected sites, D was greater in patients with metastasis (Figure 6.8).

For tongue tumours, the mean D was 31.9mm. (SD 12.6, range 9-60) in patients with metastasis, and 24.1mm. (SD 12.8, range 7-44) in patients without metastasis (Two Sample t Test, t = 1.42, 17 d.f., P = 0.17). Metastasis occured in eleven (79%) of the 14 tongue tumours with a D of 22mm. or more. In contrast, only two (25%) of the eight tongue tumours with a D of less than 22mm. metastasised.

For tumours of the floor of mouth, the mean D was 24.1mm. (SD 15.2, range 5-65) in patients with metastasis, and 13.8mm. (SD 12.4, range 4-43) in patients without metastasis (Two Sample t Test, t = 1.77, 20 d.f., P = 0.09). Metastasis occured in ten (83%) of the twelve floor-of-mouth tumours with a D of 16mm or more, In contrast, only four (36%) of the eleven floor-of-mouth tumours with a D of less than 16mm. metastasised.

(2) Tumour Thickness

As shown in Table 6.7, the actual tumour thickness (T_A) ranged from 2-25mm. (mean 9.4, SD 6.0).

There was a significant difference in T_A in patients with and without metastasis (Two Sample t Test, t = 2.65, 42 d.f., P = 0.008). In patients with metastasis, T_A ranged from 2-25mm., with a mean of 11.2mm. (SD 6.1). In patients without metastasis T_A ranged from 2-18mm, with a mean of 6.7mm. (SD 4.8).

As shown in Table 6.7, the reconstructed tumour thickness (T_R) ranged from 1-25mm. (mean 9.8, SD 6.4). T_R differed from T_A in 21 patients. In 13 patients, T_R was greater than T_A indicating an ulcerated tumour. The estimated depth of the ulcer ranged from 1-6mm. (mean 2.8, SD 1.5). In eight patients, T_R was less than T_A indicating the tumour had an exophytic component. The estimated value of the exophytic component ranged from 1-4mm. (mean 1.9, SD 0.9).

There was a significant difference in T_R in patients with and without metastasis (Two Sample t Test, t = 2.88, 39 d.f., P = 0.005). In patients with metastasis, T_R ranged from 2-25mm., with a mean of 11.9mm. (SD 6.2). In patients without metastasis, T_R ranged from 1-18mm., with a mean of 6.7mm. (SD 5.5). As shown in Table 6.8, when the Two Sample t test is used to compare each of the three measurements relating to tumour size in the groups of patients with and without metastasis, the associated probability, P, is lowest for the T_R measurement.

Tumours sited on the tongue were thicker than tumours sited on the floor of mouth. For tongue tumours, the mean T_A was 12.8mm. (SD 5.8, range 2-25), and the mean T_R was 13.5mm. (SD 6.3, range 1-25).

The equivalent measurements for floor-of-mouth tumours were 6.1mm. (SD 4.1, range 2-17) and 6.3mm. (SD 4.2, range 1-15), respectively. The difference in tumour thickness in relation to site was highly significant (Two Sample t Test: for T_A, t = 4.45, 43 d.f., P = 0.0001; for T_R, t = 4.53, 21 d.f., P = 0.0002).

The T_A and T_R measurements were greater in patients with metastasis at each of the two selected sites, and the T_R in relation to site and metastatic status is shown in Figure 6.9.

For tongue tumours, the mean T_A was 15.1mm. (SD 5.3, range 4-25) in patients with metastasis, and 9.6mm. (SD 5.3, range 2-18) in patients without metastasis (Two Sample t Test, t = 2.40, 17 d.f., P = 0.03). The mean T_R measurements were 15.8mm. (SD 5.6, range 3-25) and 10.2 (SD 5.9, range 1-18), respectively (Two sample t Test, t = 2.24, 17 d.f., P = 0.04). Metastasis occured in ten (83%) of the twelve tongue tumours with a T_R of 15mm. or more. In contrast, only three (30%) of the ten tongue tumours with a T_R of less than 15mm. metastasised.

For floor-of-mouth tumours, the mean T_A was 7.6mm. (SD 4.6, range 2-17) in patients with metastasis, and 3.8mm. (SD 1.4, range 2-6) in patients without metastasis (Two Sample t Test, t = 2.94, 16 d.f., P = 0.009). The mean T_R measurements were 8.3mm. (SD 4.2, range 2-15) and 3.2mm. (SD 1.7, range 1-6), respectively (Two Sample t Test, t = 4.04, 19 d.f., P = 0.001). All nine floor-of-mouth tumours with a T_R measurement greater than 6mm. metastasised. In contrast, metastasis occured in only five (36%) of the 14 floor-of-mouth tumours with a T_R measurement equal to or less than 6mm.

There was a significant positive association between tumour

thickness (represented by T_R) and tumour dimension, D, in the 45 cases in Series II (Pearson's Correlation Test, r = 0.64, P = <0.001). Further analysis of the data (presented in Figure 6.10) showed a significant association between T_R and D in patients with metastasis (r = 0.75, P = <0.001) but not in patients without metastasis (r = 0.30, P = 0.22). As shown in Figure 6.10, three of the eight tumours with a D of 40mm. or more did not metastasise: one was sited on the floor of mouth and had a T_R of only 2mm; two were sited on the tongue, and had T_R measurements of 7mm., and 14mm.

(3) Epithelial Dysplasia adjacent to the Primary Tumour

Epithelial dysplasia adjacent to the primary tumour was identified in 24 (53%) of the 45 patients (Table 6.6).

There was no significant difference in the frequency of dysplasia in relation to metastasis (Yates'-Corrected Chi Squared Test, X^2_{YATES} = 0.30, 1 d.f., P = 0.58). Dysplasia was identified in 13 (48%) of the 27 patients with metastasis and in eleven (61%) of the 18 patients without metastasis.

There was no significant difference in the frequency of dysplasia at the two intra-oral sites. Dysplasia was present in association with ten (44%) of the 22 tongue tumours and with 14 (61%) of the 23 floor-of-mouth tumours (Fisher's Exact Test, P = 0.46).

The relationship between epithelial dysplasia and the size of the primary tumour was also investigated. In tumours of the tongue, there was no significant difference in tumour D in patients with and without dysplasia (mean 30.5mm., SD 8.3, range 17-42 and mean 27.3mm., SD 16.1, range 7-60, respectively; Two Sample t Test, t = 0.60, 9

d.f., P = 0.56). Also, there was no significant difference in tumour thickness (represented by T_R) in patients with and without dysplasia (mean 13.2mm., SD 5.0, range 1-17, and mean 13.0, SD 7.6, range 2-25, respectively; Two Sample t Test, t = 0.07, 21 d.f., P = 0.94). However, for tumours of the floor-of-mouth, both the tumour D and tumour T_R measurements were significantly lower in patients with dysplasia compared to patients without dysplasia. The mean D was 15.1mm. (SD 11.3, range 4-43) in patients with dysplasia, and 27.7mm. (SD 17.0, range 8-65) in patients without dysplasia (Two Sample t Test, t = 2.13, 21 d.f., P= 0.04). The mean T_R was 4.8mm. (SD 3.5, range 1-12) in patients with dysplasia, and 9.3mm. (SD 4.1, range 3-15) in patients without dysplasia (Two Sample t Test, t = 2.64, 20 d.f., P = 0.02).

My results show a significant association between heavy smoking combined with heavy drinking and the presence of epithelial dysplasia (Table 6.9). Eleven of the 24 patients with dysplasia were both heavy smokers and heavy drinkers and another five were either heavy smokers or heavy drinkers. Only one of the twelve patients who were both heavy smokers and heavy drinkers did not have dysplasia (Total Chi Squared Test, X^2 = 10.31, 2 d.f., P = 0.005; Chi Squared Test For Trend, X^2_{TREND} = 9.78, 1 d.f., P = 0.002).

(4) Histological Malignancy Grade

(a) Total Histological Malignancy Score

The total malignancy score of the 45 tumours ranged from 10-23 points. The score was similar at the two selected sites. For tongue tumours, it ranged from 12-22 points and for floor-of-mouth tumours,

it ranged from 10-23 points (Mann-Whitney U Test, U = 242.5, P = 0.81).

In the 27 patients with metastasis, the total malignancy score ranged from 13-23 points (Table 6.10, Figure 6.11). In the 18 patients without metastasis, the score was lower and ranged from 10-19 points (Table 6.11, Figure 6.11). The difference in the malignancy score in the two groups of patients was highly significant (Mann-Whitney U Test, U = 42.0, P = <0.0001). Twenty-four (89%) of the 27 tumours scoring 16 or more points metastasised, compared with only three (17%) of the 18 tumours scoring 15 points or less. At both of the selected sites (Figure 6.12), the malignancy score was significantly higher in patients with metastasis (for tongue tumours: Mann-Whitney U Test, U = 11.0, P = 0.001; for floor-of-mouth tumours, Mann-Whitney U Test, U = 12.0, P = 0.001).

The relationship between the histological malignancy grade and the clinical T stage of the tumour is shown in Figure 6.13. Although there was no significant difference in the malignancy grade in relation to T stage, within each T category, the score was greater in patients with metastasis.

The relationship between the histological malignancy grade and tumour dimension is shown in Figure 6.14. The Spearman's Rank Correlation Test showed a significant positive association between the malignancy grade and tumour D (r_s = 0.37, P = 0.01). The relationship between the malignancy grade and tumour thickness (represented by T_R), is shown in Figure 6.15. The Spearman's Rank Correlation Test showed a significant positive association between the grade and tumour T_R (r_s = 0.47, P = 0.001).

(b) Assessment of the Prognostic Value of the Individual Component Features of the Histological Malignancy Grade

A comparison by the Chi Squared Test of the scores for each of the six histological features in patients with and without metastasis is shown in Table 6.12.

(i) Degree of Keratinisation

The frequency of the four point scores for the degree of keratinisation is shown in Table 6.13. Higher point scores were more frequent in patients with metastasis and the difference in frequency of the four point scores was highly significant (Chi Squared Test, X^2 = 14.17, 3 d.f., P = 0.003). Also, there was a significant difference in the frequency of point scores 1+2 and 3+4 (Fisher's Exact Test, P = 0.0005).

(ii) Nuclear Polymorphism

The frequency of the four point scores for assessment of nuclear polymorphism is shown in Table 6.14. There was no significant difference in the scores between the two groups of patients (Chi Squared Test, X^2 = 3.07, 3 d.f., P = 0.38; Fisher's Exact Test, point scores 1+2 x 3+4, P = 0.86).

(iii) Frequency of Mitosis

The frequency of the four point scores for assessment of the number of mitotic figures is shown in Table 6.15. Higher point scores were more frequent in patients with metastasis and the difference was significant (Chi Squared Test, X^2 = 11.20, 3 d.f., P = 0.01). Also, there was a significant difference in the frequency of point scores 1+2 and 3+4 (Fisher's Exact Test, P = 0.005).

(iv) Pattern of Invasion

The frequency of the four point scores for assessment of the pattern of invasion is shown in Table 6.16. Higher point scores were more frequent in patients with metastasis and the difference was highly significant (Chi Squared Test, X^2 = 25.00. 3 d.f., P= 0.00002). Also, the difference in the frequency of point scores 1+2 and 3+4 was highly significant (Fisher's Exact test, P = 0.00003).

(v) Stage of Invasion

All tumours scored three or four points on assessment of the stage of invasion (Table 6.17). The higher point score was more frequent in patients with metastasis (Chi Squared Test, X^2 = 8.35, 1 d.f., P = 0.004).

(vi) Lymphoplasmacytic Infiltrate

The frequency of the four point scores for assessment of the lymphoplasmacytic infiltrate is shown in Table 6.18. Although higher point scores were more frequent in patients with metastasis, the difference did not achieve statistical significance (Chi Squared Test, X^2 = 7.06, 3 d.f, P = 0.07; Fisher's Exact test, point scores 1+2 x 3+4, P = 0.09).

(c) Correlation between the Component Features of the Histological Malignancy Grade

Correlation between the scores of the six components of the histological malignancy grading system was tested by Spearman's Rank Correlation Test and the results are presented in Table 6.19. All six components showed a statistically significant positive correlation with the total malignancy score. The correlation between nuclear polymorphism and the total score was weaker (P = 0.02) than the

correlation between each of the other five features and the total score (P = or <0.002). Also, nuclear polymorphism showed no significant correlation with any other component feature. In contrast, four of the other five features (degree of keratinisation, frequency of mitosis, pattern of invasion, and intensity of the lymphoplasmacytic infiltrate) were significantly correlated with each other. The stage of invasion showed a significant correlation with the pattern of invasion and the intensity of the lymphoplasmacytic infiltrate.

(5) Perineural Invasion

Perineural invasion was identified in 24 (53%) of the 45 primary tumours in Series II. Perineural invasion was identified in 15 (68%) of the 22 tumours sited on the tongue, and in nine (39%) of the 23 tumours sited on the floor of mouth (Yates' Corrected Chi Squared Test, $X^2_{YATES} = 2.74$, 1 d.f., P = 0.09).

The relationship between perineural invasion and the size of the tumour was also investigated. Spearman's Rank Correlation Test showed a significant positive association between the presence of perineural invasion and both tumour dimension, D, ($r_s = 0.40$, P = 0.007), and tumour thickness, (for T_R, $r_s = 0.61$, P = <0.001).

Tumours of the tongue with perineural invasion were larger than tumours of the tongue without perineural invasion. The mean D was 32.1mm. (SD 11.9, range 13-60) in the 15 tongue tumours with perineural invasion, and 21.6mm. (SD 13.1, range 7-44) in the seven tongue tumours without perineural invasion (Two Sample t Test, t = 1.87, 20 d.f., P = 0.07). The mean T_R measurements were 15.9mm. (SD

5.0, range 5-25) and 7.1mm. (SD 5.5, range 1-16), repectively, in tumours with and without perineural invasion (Two Sample t Test, t = 3.71, 20 d.f., P = 0.001).

In the case of floor-of-mouth tumours, an association between size and perineural invasion could be discerned, but the differences did not achieve statistical significance. The mean tumour D was 25.2mm. (SD 18.8, range 4-65) in the nine tumours with perineural invasion, and 16.7mm. (SD 11.1, range 5-43) in the 14 tumours without perineural invasion (Two Sample t Test, t = 1.22, 8 d.f., P = 0.25). The mean T_R measurements were 8.2mm. (SD 4.1, range 2-15), and 5.1mm. (SD 3.9, range 1-12), respectively, in tumours with and without perineural invasion (Two Sample t Test, t = 1.84, 21 d.f., P = 0.07).

As shown in Table 6.20, perineural invasion was identified in 19 (70%) of the 27 patients with metastasis and in five (28%) of the 18 patients without metastasis. The difference in frequency of perineural invasion in relation to metastasis was statistically significant (Yates' Corrected Chi Squared Test, X^2_{YATES} = 6.30, 1 d.f., P = 0.01). Perineural invasion was identified in twelve (92%) of the 13 tongue tumours with metastasis, and in only three (33%) of the nine tongue tumours without metastasis (Fisher's Exact Test, P = 0.013). In floor-of-mouth tumours, the association between perineural invasion and metastasis was less evident. Perineural invasion was identified in seven (50%) of the 14 floor-of-mouth tumours with metastasis, and in 2 (22%) of the nine floor-of-mouth tumours without metastasis (Fisher's Exact Test, P = 0.37).

(6) Vascular Invasion

Vascular invasion was identified in 23 (51%) of the 45 primary tumours in Series II. Vascular invasion was identified more frequently in tumours sited on the tongue (14 or 64% of the 22 cases) than in tumours sited on the floor-of-mouth (nine or 39% of the 23 cases). However, the difference in frequency of vascular invasion in relation to site did not achieve statistical significance (Yates' Corrected Chi Squared Test, $X^2_{YATES} = 1.81$, 1 d.f., $P = 0.18$).

The relationship between vascular invasion and the size of the tumour was also investigated. Spearman's Rank Correlation Test showed a highly significant positive association between vascular invasion and both tumour D ($r_s = 0.57$, $P = <0.001$), and tumour thickness (for T_R, $r_s = 0.67$, $P = <0.001$).

At both of the selected sites, there was a significant difference in the size of tumours with and without vascular invasion.

For tumours of the tongue, the mean D was 32.9mm. (SD 11.8, range 13-60) in the 14 tongue tumours with vascular invasion, and 21.4mm. (SD 12.1, range 7-44) in the eight tongue tumours without vascular invasion (Two Sample t Test, $t = 2.19$, 20 d.f., $P = 0.03$). The mean T_R measurements were 16.8mm. (SD 4.0, range 9-25), and 7.9mm. (SD 5.5, range 1-16), respectively, in tumours with and without vascular invasion (Two Sample t Test, $t = 4.39$, 20 d.f., $P = 0.0003$).

For floor-of-mouth tumours, the mean D was 29.9mm. (SD 15.1, range 16-65) in the nine floor-of-mouth tumours with vascular invasion, and 13.7mm. (SD 10.9, range 4-43) in the 14 floor-of-mouth tumours without vascular invasion (Two Sample t Test, $t = 2.98$, 21 d.f., $P = 0.007$). The mean T_R measurements were 9.6mm. (SD 3.8, range

2-15), and 4.1mm. (SD 2.9, range 1-10), respectively, in tumours with and without vascular invasion (Two Sample t Test, t = 3.96, 21 d.f., P = 0.0008).

Correlation between vascular and perineural invasion on the one hand, and the histological malignancy grade and its six component features on the other hand, was tested by Spearman's Rank Correlation Test and the results are presented in Table 6.21. Vascular invasion showed a highly significant positive correlation (P = or <0.001) with perineural invasion, the total malignancy grade, and four of its component features (degree of keratinisation, frequency of mitoses, pattern of invasion, and stage of invasion). Correlation between vascular invasion and intensity of the lymphoplasmacytic infiltrate was significant (P = 0.01), but there was no significant correlation between vascular invasion and nuclear polymorphism (P = 0.65). Perineural invasion showed a highly significant positive correlation with pattern of invasion (P = 0.007) and a significant correlation with stage of invasion and the total malignancy grade (P = 0.02).

As shown in Table 6.20, vascular invasion was identified in 21 (78%) of the 27 patients with metastasis but in only two (11%) of the 18 patients without metastasis. The difference in the frequency of vascular invasion in relation to metastasis was statistically highly significant (Yates Corrected Chi Squared Test, X^2_{YATES} = 16.63, 1 d.f., P = 0.00005). Vascular invasion was identified in twelve (92%) of the 13 tongue tumours with metastasis, and in two (22%) of the nine tongue tumours without metastasis (Fisher's Exact Test, P = 0.006). Nine (64%) of the 14 floor-of-mouth tumours with metastasis, and none

of the nine floor-of-mouth tumours without metastasis, showed vascular invasion (Fisher's Exact Test, P = 0.004).

(7) Logistic Regression Analysis

Logistic regression analysis was used to assess which clinical and histological features were predictive of lymph node metastasis. Hence, presence of lymph node metastasis was the dependent variable, and there were 19 potential predictor or explanatory variables: site, and stage, of the primary tumour; sex, and age, of the patient; number of cigarettes smoked; amount of alcohol consumed; tumour greatest surface dimension, D; actual tumour thickness, T_A; reconstructed tumour thickness, T_R; presence of epithelial dysplasia adjacent to the primary tumour; the histological malignancy grade, and each of its six components (degree of keratinisation, amount of nuclear polymorphism, frequency of mitotic figures, pattern of invasion, stage of invasion, and intensity of the lymphoplasmacytic infiltrate); presence of perineural invasion; and, finally, presence of vascular invasion.

During the study, the relationship of each of these variables to the dependent variable was analysed in turn and the results are presented in the previous sections of this chapter. A summary of my findings is presented in Table 6.22. As shown here, there were significant differences at the 5% level between patients with and without metastasis in ten of the 19 variables: tumour D, tumour T_A, tumour T_R, histological malignancy grade, degree of keratinisation, frequency of mitosis, pattern of invasion, stage of invasion, presence of perineural invasion, and presence of vascular invasion. In addition, for a further variable, intensity of the lymphoplasmacytic

infiltrate, the difference was almost significant (P = 0.07).

These eleven histological features were entered into the initial logistic regresion model. To facilitate the regression, it was necessary to transform the raw data into binary data for the following variables: degree of keratinisation, frequency of mitosis, pattern of invasion, stage of invasion, and intensity of the lymphoplasmacytic infiltrate (Table 6.23). Tumour D, tumour T_A and tumour T_R, and the histological malignancy grade were entered into the regression model as continuous data. The data on perineural and vascular invasion was already in binary form (0 = not identified, 1 = identifed).

The results of the initial logistic regression model are shown in Table 6.24, and its associated classification table is presented in Table 6.25. Because of the relatively small number of cases in the present study, a model with eleven independent variables grossly 'overfits' the data. In addition, it is possible that interactions between variables which are highly correlated may result in a regression model with inflated standard errors (Walsh, 1990; Altman, 1991d). Hence, the number of variables was reduced in a stepwise manner.

Firstly, the number of variables relating to tumour size was reduced. T_R was selected to represent tumour size, since it had the lowest associated probability value when the three measurements relating to tumour size were analysed individually (Table 6.22), and tumour D and tumour T_A were removed from the logistic regression at this stage. The result - a model with nine explanatory variables - is shown in Table 6.26. Its associated classification table was identical to that presented in Table 6.25.

Next, the five variables representing the individual components of the histological malignancy grade were removed, and the regression model containing the four remaining variables is shown in Table 6.27. Its associated classification table is shown in Table 6.28.

In the final steps of the regression, the variable contributing least to the regression model (that is, the variable with the highest probability value) was removed at each step. The model containing three variables is shown in Table 6.29, and the final model containing just two variables, histological malignancy grade and vascular invasion, is shown in Table 6.30. The percentage of correct classifications was identical in the models containing four, three, and two variables (Table 6.28).

An alternative two-variable model, with pattern of invasion replacing histological malignancy grade is shown in Table 6.31. This alternative model resulted in a lower percentage of correct classifications (Table 6.32), and the two-variable model containing histological malignancy grade and vascular invasion (Table 6.30) was chosen as the 'best' model.

Transformation of the Beta coefficient of the two variables in the final model shows the predictive value of the logistic regression. For example, if the histological malignancy grade is 20 points and vascular invasion is present, then

$$p = \frac{e^{(\text{logitp})}}{1 + e^{(\text{logitp})}}$$

where logitp = (0.639 x 20 + 1.947 - 10.729) as shown in Table 6.30. Hence, the estimated probability or risk of cervical lymph node metatasis, p = 0.98.

If vascular invasion is not identified, and the histological

malignancy grade is 15 points, then

$$p = \frac{e^{(\text{logitp})}}{1 + e^{(\text{logitp})}}$$

where logitp = (0.639 x 15 - 10.729) as shown in Table 6.30.

Hence, the estimated probability or risk of metastasis, p = 0.24.

Table 6.33 shows the estimated probability or risk of metastasis for each of the 45 cases in the present study.

DISCUSSION

The aim of the present study was to improve the prognostic efficiency of the histological assessment of the primary tumour in predicting lymph node metastasis, by identifying those features most frequently associated with metastasis, and incorporating these into a simple prognostic method which could be recommended for future use in the initial clinical assessment and surgical planning of oral cancer. Although there are numerous reports in the literature on the histological grading of squamous carcinoma of the oral mucosa and its value in predicting survival, only a small number of these previous studies have assessed which, if any, histological features accurately predict lymph node metastasis.

The present study has several advantages over some of the previous investigations. Firstly, the metastatic status of the cervical lymph nodes was determined by pathological assessment, and, in all cases, the gross examination of the neck dissection specimen and the histological examination of the lymph nodes was carried out by the author. In some previous investigations, for example those reported by Arthur and Fenner (1966), Arthur and Farr (1972), and Langdon *et al.* (1977), the metastatic status was determined by clinical examination. It is known that clinical examination results in a high incidence of false-positive and false-negative assessments (Ali *et al.*, 1985). Indeed, the level of this source of inaccuracy was amply demonstrated by my investigations and reported in Chapter 5. Therefore, the accuracy of reports based on the clinical status of cervical nodes is questionable, since the true metastatic status remains unknown in patients treated by radiotherapy. The other

advantages of the present study concern several precautions taken to reduce the number of variables that may influence the development of metastasis, namely, the site of the primary tumour and the effect of previous therapy.

When evaluating the prognostic importance of histological features it is important, for two main reasons, to assess groups of tumours from well-defined sites. Firstly, restricting the site reduces the risk of anatomical differences in the local lymphatic drainage influencing the rate of metastasis. Secondly, some histological features, such as the degree of keratinisation, are characteristic of the site of the tumour (Anneroth and Hansen, 1984). Hence, when assessing the predictive value of malignancy grading, it is desirable to compare only tumours from a similar site. In the present study, all the tumours were sited on the tongue or floor of mouth. It is reasonable to consider these anatomically closely-related areas as a single site when assessing the predictive value of histological features. The risk of metastasis is similar at both sites (Spiro *et al.*, 1974; Farr *et al.*, 1980). Furthermore, large tumours often involve both sites, making the exact site of origin uncertain. Both the tongue and the floor of mouth are drained by a rich plexus of lymphatic capillaries (Feind, 1972), and the mucosa covering the ventral/lateral tongue is similar in structure to that covering the floor of mouth (Sicher, 1962).

None of the patients in the present study had received radiotherapy, chemotherapy, or surgery (other than a diagnostic biopsy) prior to the resection of the primary tumour. This is important because treatment can alter the cytological features of the

malignant cells and the pattern of spread of the tumour. For example, radiotherapy and some chemotherapeutic agents may increase the degree of keratinisation (Safaii and Azar, 1966; Wright *et al.*, 1988) and block the mitotic cycle resulting in an exaggerated mitotic count (Underwood, 1992). Patients were also excluded from the present study if their medical and/or drug histories indicated that their immune system was compromised, since this may influence the development of metastasis (Scully, 1982), and the assessment of the local immune response. In addition, cases in which the surgical resection specimen did not include the entire primary tumour were excluded from the study, since pathological assessment of the entire tumour is necessary for an accurate assessment of tumour size, and accurate 'invasive front' grading of histological features.

However, there is a disadvantage in adopting strict entry criteria for any study, as it inevitably results in a reduced sample size. This was the case in the present investigation. Nevertheless, the results of the present study do allow firm conclusions to be drawn about the relative value and importance of several patient/tumour variables in prognosis. For example, my results clearly show that histological features are more important than clinical features, such as the age and sex of the patient, or the clinical stage of the tumour, in predicting the presence of lymph node metastasis. My results also show that perineural invasion and vascular invasion, features not included in most previous multifactorial systems for grading squamous carcinoma of the oral cavity (Anneroth *et al.*, 1987; Bryne *et al.*, 1989) are important predictors of metastasis. On the other hand, the amount of nuclear polymorphism, which is traditionally

regarded as one indicator of the degree of differentiation (Broders, 1926; Walter and Israel, 1979) has no predictive value in the present study.

The present discussion will be arranged in four sections: the first concerned with the prognostic value of tumour size; the second dealing with epithelial dysplasia adjacent to the primary tumour; the third concerned with the histological malignancy grading system, and perineural and vascular invasion; and the fourth dealing with the logistic regression model. Before taking up these detailed considerations, it is worth reiterating that all my studies are directed at finding means to improve the prognostic efficiency of predicting metastasis, and that this is different from predicting survival, although the two are ultimately related.

(1) The Prognostic Value of Tumour Size

In the present study, the clinical assessment of tumour size (T Stage) was not useful in predicting the presence of lymph node metastasis. However, all three of the laboratory measurements representing tumour size showed significant differences in patients with and without metastasis. The difference was greatest for the reconstructed tumour thickness measurement, and this finding is in agreement with several recent reports which state that tumour thickness is more important than surface-dimension in predicting nodal metastasis and survival (Moore *et al.*, 1986b; Spiro *et al.*, 1986; Mohit-Tabatabai *et al.*, 1986; Nathanson and Agren, 1989). An explanation for this has been given by DiTroia (1972) who points out that since the superficial lymphatics are small in calibre, it is

difficult for tumour emboli to form within them. However, as the tumour invades deeper, it has access to the lymphatic collecting trunks, which have a much larger calibre, and this favours embolisation. Hence, nodal metastasis is more frequent in association with more deeply invasive tumours.

Tumour surface-dimension has the advantage that it can be assessed clinically, and, therefore, it is more readily available as a potential early predictor of metastasis than a tumour thickness measurement, with the result that the clinical staging procedure is based on an assessment of the surface-dimension (American Joint Committee on Cancer, 1988b). For this reason, I have investigated the relationship between tumour surface-dimension and tumour thickness, and my results show that a significant positive association was present only in patients with metastasis. In patients without metastasis, there was a poor correlation, with some extensive tumours having small thickness measurements. Hence, my results show that tumour surface-dimension is an unreliable indicator of both tumour thickness and nodal metastasis.

In addition to the intrinsic limitations of using tumour surface-dimension as a prognostic indicator, clinical assessment of the surface-dimension during the pre-operative staging procedure may be inaccurate due to difficult access, difficulty in measuring a curved surface, and difficulty in assessing induration due to abnormal muscle and/or tissue tone. In the present study, shrinkage of the fixed specimen accounts for the discrepancy between clinical stage and the laboratory-measured surface-dimension in four tumours (Cases ND13, ND18, ND31 and ND135). However, two tumours staged T1 (Cases ND9 and

ND22) had laboratory measurements of more than 2.0cm., and this is probably due to an inaccurate clinical assessment. A sudden increase in tumour size immediately prior to surgery is an alternative explanation. Clinical staging is also inaccurate when areas of leukoplakia or erythroplakia are mistaken for carcinoma. This occured in one patient staged T2 in the present study (Case ND42/43). In this case, on histological assessment, the surface-dimension of the carcinoma was only 8mm. In addition, clinically suspected bone involvement, which is not confirmed histologically, may result in some tumours being misclassified as T4 tumours. This was the case in one patient (Case ND51) in the present study.

A tumour thickness measurement conveys more information than an assessment of the histological stage of invasion, where the depth of invasion is compared to the anatomical structures reached by the invasive front. A thin tumour arising on the tongue is likely to involve the lingual intrinsic muscle, whereas tumours of similar or greater thickness arising on the floor of mouth may only involve the submucosal glands. For example, in the present study, one tumour sited on the floor of mouth (Case ND51) had a thickness measurement of 10mm., but, on histological assessment of the stage of invasion, was confined to the submucosa (scoring 3 points in the multifactorial grading system). In contrast, a tumour measuring 2mm. in thickness sited on the tongue (Case ND47) was already invading muscle (scoring 4 points in the multifactorial system). These results, therefore, reinforce the suggestion that a tumour thickness measurement, rather than an assessment of the stage of invasion, should be incorporated into histological malignancy grading systems. In addition, my results

support the suggestion by Moore *et al.* (1986b) that tumour thickness measured from a theoretically reconstructed mucosal line is a more accurate prognostic indicator than an actual tumour thickness measurement. Hence, it is recommended that a reconstructed tumour thickness measurement is used in the pathological staging of oral cancer.

My results show that the 'critical' thickness in relation to metastasis is significantly different at the two selected intra-oral sites, even though the sites are anatomically closely-related. All nine floor-of-mouth tumours with a reconstructed thickness measurement greater than 6mm. metastasised. In contrast, the mean reconstructed thickness measurement of the nine non-metastasising tumours sited on the tongue was 10.2mm., and in six of the nine tumours, the reconstructed thickness measurement was greater than 10.0mm. However, a much larger sample size is necessary in order to define accurately the 'critical' thickness for each site, and until such information is available, the value of using a tumour thickness measurement to predict metastasis in an individual patient is limited.

(2) Epithelial Dysplasia adjacent to the Primary Tumour

The occurence of multiple primary tumours and/or areas of dysplasia in patients with oral cancer has been recognised for many years (Slaughter *et al.*, 1953; Wright and Shear, 1985). Epithelial dysplasia was identified in the oral mucosa adjacent to the primary tumour in 24 (53%) of the 45 patients in the present study. Although the number of cases was small, there was a significant association between the presence of epithelial dysplasia and heavy smoking

combined with heavy drinking. Heavy drinking was defined as more than six units of alcohol per day, which is equivalent to more than twice the recommended safe weekly limit for men of 21 units (Health Education Authority, 1989). Nine of the 16 heavy drinkers in the present study had a macrocytic anaemia and developed the alcohol withdrawal syndrome in the post-operative period.

Tobacco and alcohol usage are known risk factors for the development of multiple oral and oropharyngeal tumours but the relative importance of each is uncertain (Ogden, 1991). Heavy alcohol consumption was the most important risk factor in a logistic regression model reported by Hsairi *et al.* (1989). Previous work in Liverpool (Valentine *et al.*, 1985) has shown that both heavy drinking and heavy smoking are associated with epithelial atrophy in clinically normal oral mucosa. It is reasonable to assume that the structural changes are accompanied by functional changes and an increased vulnerability to carcinogens is the likely outcome.

In the present study, two patients with dysplasia (Cases ND106/107 and ND176) were lifetime non-smokers/non-drinkers, and a third patient (Case ND22) was a lifetime non-smoker but consumed a small amount of alcohol (one unit per day). All three patients were female and the presence of atrophic changes affecting large areas of the oral mucosa was noted during the clinical examination. One patient (Case ND106/107) had a five-year history of Primary Sjogren's Syndrome, and the floor-of-mouth carcinoma developed in an area of persistent soreness. In the other two patients, the aetiology of the epithelial atrophy was unknown.

It has been reported (Bouquot *et al.*, 1988) that carcinomas

associated with leukoplakias are more likely to be superficially invasive with fewer metastases and a better prognosis than similar carcinomas not associated with leukoplakia. In the present study, floor-of-mouth tumours with an associated dysplasia were significantly thinner with a smaller surface-dimension than floor-of-mouth tumours without an associated dysplasia. Also, dysplasia was identified more frequently in patients without metastasis, although the rate was not significantly different from that in patients with metastasis. Hence, my results provide some evidence in support of the findings of Bouquot *et al.* (1988). Whether carcinomas with an associated dysplasia are inherently less aggressive is uncertain. It may be that dysplasia is not seen around some tumours simply because of their rapid growth and larger size.

(3) The Histological Malignancy Grading System and Perineural and Vascular Invasion

(a) The Multifactorial Histological Malignancy Grading System

The histological malignancy grading system used in the present study was a modification of the multifactorial system proposed by Anneroth *et al.* (1987). There were two main modifications in the present study. Firstly, the point scoring criteria for some of the features were re-defined to improve the consistency and reproducibility of the system. The criteria used in the present study evolved after a period of evaluating the scoring systems recommended by other authorities. The results of the Pilot Study (Table 6.2) show that a satisfactory level of consistency was achieved using the more strictly defined and/or simplifed criteria recommended in Table 6.1.

Secondly, the histological assessment was made in the most dysplastic areas at the invasive front of the tumour ('invasive cell grading'), a modification first recommended by Bryne *et al.* (1989).

The six histological features assessed in the multifactorial grading system will be discussed in turn. In each case, the criteria used in point scoring and general observations noted during the assessment will be discussed first, followed by a discussion on the independent prognostic importance of the feature and its contribution to the total malignancy score.

(i) Degree of Keratinisation

During the period of evaluation of recommended grading schemes, I found that the consistency of scoring the degree of keratinisation was poor when the criteria recommended by Anneroth *et al.* (1987) were used. However, the consistency was improved when the grading criteria defined in Table 6.1 were applied. Thus, in the present study, highly keratinising tumours were graded according to the number and appearance of the keratin pearls forming at the invasive front, and poorly keratinising tumours were graded according to the presence or absence of individual cell keratinisation. Crissman *et al.* (1984) recommended a similar modification. Even with the modified criteria, I found that the assessment of some poorly keratininsing tumours was difficult. For example, it was sometimes difficult to detect individual cell keratinisation with certainty. However, the protocol dictated that the lower point score was assigned in these circumstances, and consistency of scoring was achieved in 90% of sections in the Pilot Study (Tables 6.2 and 6.3).

My results show that tumours with metastasis were less well

keratinised than tumours without metastasis, and the difference in the frequency of the different point scores was highly significant. In contrast, several other authorities (Johnson, 1977; Crissman *et al.*, 1980 and 1984; Okamoto *et al.*, 1988) have reported that an assessment of the degree of keratinisation has no significant independent prognostic value.

The degree of keratinisation is undoubtedly an important indicator of cellular differentiation and the original system for grading squamous cell carcinomas (Broders, 1920 and 1926) was based solely on an assessment of this feature. Although most squamous cell carcinomas of the oral mucosa are well differentiated tumours (Maddox, 1984), my results show an assessment of the amount of keratin formed by cells at the advancing front of the tumour has significant independent value in predicting metastasis, and such an assessment should be included in multifactorial histological malignancy grading schemes for oral cancer.

(ii) Nuclear Polymorphism

In order to achieve consistency, it was necessary to re-define and simplify the scoring criteria recommended by Anneroth *et al.* (1987). In the present study (Table 6.1), tumours scoring one and two points were distinguished by the size of the nuclei and the number and appearance of the nucleoli: Grade 1 nuclei were small and uniform with no distinct nucleoli and Grade 2 nuclei were larger, on subjective assessment, with distinct nucleoli. Within the Grade 2 category, the nuclei showed a variable degree of polymorphism and hyperchromatism, ranging from mild to severe. The assignment of three and four points depended on the presence and number of multinucleated cells or cells

with bizarre anaplastic nuclei.

My results show there was no significant difference in the amount of nuclear polymorphism in patients with and without metastasis. Most tumours were cytologically well-differentiated and only four tumours (Cases ND51, ND118, ND120 and ND170) showed extreme nuclear aberrations with numerous anaplastic and giant cell forms (point score 4). Nevertheless, metastasis was present in all four of these cases.

An assessment of nuclear polymorphism was included in the original multifactorial grading sysytem (Jakobbson *et al.*, 1973) as an indicator of cellular differentiation, and an assessment of this feature is recommended in all the modified schemes reviewed by Anneroth *et al.* (1987). Jakobbson *et al.* (1973) and Willen *et al.* (1975) reported that nuclear polymorphism was a good predictor of metastasis and survival. However, my finding, that this feature has no significant independent prognostic value, is in agreement with several other authorities (Johnson, 1977; Crissman *et al.*, 1980 and 1984; Frierson and Cooper, 1986).

In the present study, simple criteria were used to define the point score categories for assessment of nuclear polymorphism. This was necessary in order to achieve a satisfactory level of consistency. Subjective assessment of nuclear polymorphism is crude (Baak and Oort, 1983b). It is possible that there are differences in the nuclear size and shape, and in the amount of polymorphism, in tumours which metastasise, but that these differences are too subtle to be detected by routine histological assessment. Such differences, if they exist, may be detected by morphometry, and this possibility will be

investigated in Chapter 7.

(iii) Frequency of Mitoses

Anneroth *et al.* (1987) recommended counting mitoses above the basal cell layers in as many fields as possible. In the present study, new criteria were defined in an attempt to compensate for the variation in the proportion of the microscopic field occupied by malignant epithelial cells and to standardise the number of microscopic fields examined. In addition, all epithelial cell mitoses were counted since different cell layers cannot always be identified in small islands of tumour. In the Anneroth system, point scores were awarded on the average number of mitoses per high power field. In the present study, the boundaries of the four point categories were so placed that each category contained approximately ten times the number of mitoses allowed in the equivalent category in the Anneroth system (Table 6.1).

My results show the frequency of mitotic figures was significantly greater in patients with metastasis and an assessment of this feature had independent prognostic value in predicting lymph node metastasis. Crissman *et al.* (1980 and 1984) reported that the frequency of mitoses was a good predictor of survival, but this feature has not emerged as an independent prognostic indicator in other studies (Willen *et al.*, 1975; Johnson, 1977; Frierson and Cooper, 1986).

Excessive proliferation is one of the most important features characterising the malignant phenotype. Tumours with high proliferation rates have a worse prognosis, and poorer response to radiotherapy and chemotherapy, than neoplasms with low rates of

proliferation (Tannock, 1987). Rapidly dividing tumours have an increased capacity for tumour cells to detach from the main body of the neoplasm, and this facilitates metastasis (Weiss, 1964; Franks, 1973).

An assessment of the mitotic rate is made in several established malignancy grading systems (Baak and Oort, 1983a) and the mitotic index (the proportion of cells in mitosis) is the single best predictor of survival in breast cancer (Baak *et al.*, 1985). Nevertheless, assessment of the proliferation rate of a tumour from the number of mitoses counted in histological sections (the mitotic count) is open to criticism. Firstly, no consideration is given to cell size, tumour/stroma ratio, or tumour heterogeneity. Hence, the technique may be inherently inaccurate (Quinn and Wright, 1990). Secondly, the number of cells in mitosis at any given time is a function of the rate of entry of cells into mitosis and the duration of the mitotic cycle (Ojo and MacDonald, 1988). Hence, a high mitotic count may refect an increase in the duration of mitosis, rather than increased cell proliferation. In malignant tumours, abnormalities in the mitotic cycle may result in a block in cell division, and produce a discrepancy between the number of visible mitotic figures and real tumour growth (Ojo and MacDonald, 1988). Thirdly, factors such as temperature, inadequate tissue oxygenation, hormones, and circadian biorhythms influence the number of mitoses (Dallenbach and Komitowski, 1982; Donhuijsen *et al.*, 1990). In the absence of oxygen, cells may complete their mitotic cycle and no more cells enter mitosis. Thus, a delay in fixation can reduce the mitotic count (Bullough, 1950). Finally, the reproducibility of mitotic counts is reported to be poor

since mitoses may be confused with pyknotic nuclei (Norris, 1976).

In the present study, the criteria used for the identification of mitotic figures were those recommended by Baak and Oort (1983a). The method of compensating for a low epithelium/stroma ratio was simple, but crude, since it depended on a subjective assessment of the proportion of the microscopic field that was occupied by malignant epithelium. Although other techniques, such as immunostaining for the proliferation antigens Ki-67 and Proliferating Cell Nuclear Antigen (Cyclin), may result in a more accurate assessment of cell proliferation (Underwood, 1992), my results suggest simple mitotic counts are satisfactory for use in a multifactorial grading system.

(iv) Pattern of Invasion

In the present study, the criteria recommended by Anneroth *et al.* (1987) were used for assessment of the pattern of invasion. Islands of tumour cells were seen infiltrating between muscle fibres, splitting fibre bundles, in both pattern 3 and pattern 4, and the criterion used to distinguish between these two grades was the number of cells in the infiltrating islands. In the assessment of some extensive tumours, it was noted that the pattern of invasion tended to score higher in sections where the tumour was invading lingual muscle, than in sections where the tumour was invading the floor-of-mouth submucosa or sublingual glands. In the latter areas, the periphery of the tumour tended to be better delineated and 'pushing' rather than infiltrative.

In the present study, assessment of the pattern of invasion had significant independent value in predicting lymph node metastasis and this finding is in agreement with other recent reports (Yamamoto *et*

al., 1984; Okamoto *et al.*, 1988; Shingaki *et al.*, 1988).

A logical explanation can be offered to account for the relationship between the pattern of invasion and nodal metastasis. Large cohesive sheets of tumour cells (pattern of invasion grade 1 and grade 2) push or expand into the adjacent connective tissues of the host and are unlikely to gain access to the host's lymphatic vessels. In contrast, non-cohesive tumour cells have the capacity to invade the host stroma as single cells or small aggregates (pattern of invasion grade 3 and grade 4), at least as may be discerned from histological examination. Therefore, they have direct access to the host's lymphatic vessels, and, in comparison with cohesive tumour cells, they are more capable of penetrating these. This explanation is supported by experimental evidence, since the cohesiveness of neoplastic cells cultured in vitro is inversely related to the ability to invade and metastasise (Crissman, 1986). Other experiments show the pattern of invasion correlates with a loss of contact inhibition, tumour cell motility, and secretion of proteolytic enzymes such as collagenases and plasminogen activator (Crissman, 1986). In the present study, I have shown a highly significant positive correlation between the pattern of invasion and the presence of both vascular and perineural invasion.

Hence, although the biological behaviour, and, in particular, the invasive nature, of neoplasms is complex and not yet fully elucidated, it is reflected by the histological pattern of invasion. Thus, a simple assessment of this feature is of considerable prognostic importance in predicting metastasis and forms an important part of a multifactorial grading system.

(v) Stage of Invasion

The point scoring criteria recommended by Anneroth *et al.* (1987) were used for assessment of this feature.

My results show a significant difference in the frequency of point scores in patients with and without metastasis. However, as discussed earlier in this chapter, the depth of invasion is more precisely defined by a tumour thickness measurement. Hence, replacing the stage of invasion assessment by a reconstructed tumour thickness measurement is likely to improve the prognostic efficiency of histological malignancy grading.

(vi) Lymphoplasmacytic Infiltrate

The grading criteria used in the present study were a modification of those recommended by Crissman *et al.* (1984). The Pilot Study showed the consistency of point scoring for this feature was lower than that achieved for most other features. In some histological sections, it was difficult to distinguish between an immune response and a mixed inflammatory response to ulceration or the biopsy procedure. In some cases, foreign-body giant cells were present, often in large numbers, around keratin pearls at the advancing front of the tumour, and this also contributed to the difficulty in making an accurate assessment.

Oral carcinomas show a wide variation in the composition and density of the inflammatory cells in the stroma at the invasive front of the tumour, and in the proximity of this to the malignant cells. A positive association between the intensity of the lymphoplasmacytic infiltrate at the tumour-host interface, indicative of an immunological reaction, and survival, has been reported by several

authorities including Jones and Coyle (1969), Enneroth and Moberger (1973), Noone *et al.* (1974), and Johnson (1977). Assessment of the lymphoplasmacytic infiltrate was recommended in the multifactorial grading system proposed by Jakobbson *et al.* (1973), and this feature has been included, also, in the more recent modified grading systems (Anneroth *et al.*, 1987).

My results show that the lymphoplasmacytic infiltrate was less intense in patients with metastasis, although the difference in the frequency of the different point scores did not achieve significance at the 5% level. Hence, in the present study, the grade assigned for the intensity of the lymphoplasmacytic infiltrate contributed to the total malignancy score but it did not have significant independent value in predicting metastasis, and this finding is in agreement with other authorities (Crissman *et al.*, 1980; Frierson and Cooper, 1986; Shingaki *et al.*, 1988).

Monoclonal antibodies can be used to identify the types of cells and lymphocyte subsets within the tumour-associated inflammatory cell infiltrate (Hiratsuka *et al.*, 1984; Rabin *et al.*, 1984), and it is possible that, in the future, precise characterisation of the local immune response to tumour antigens will provide more valuable prognostic information than a routine assessment of the intensity of the lymphoplasmacytic infiltrate.

(vii) Total Multifactorial Histological Malignancy Grading Score

In summary, the total malignancy score achieved on multifactorial grading was useful in predicting lymph node metastasis (Figure 6.11). Setting the critical score at 16 points would have resulted in six misclassifications in the present series: three false-

positives (Cases ND16, ND41 and ND42/43), and three false-negatives (Cases ND31, ND94 and ND145). Furthermore, my results suggest that replacement of the stage of invasion grade with a more precise tumour thickness measurement and removal of the feature 'Nuclear Polymorphism' would further enhance the predictive value of the multifactorial grading system.

(b) Perineural Invasion

Perineural invasion was assessed in the present study because of its reported prognostic value in predicting nodal metastasis in cancers of the skin (Lever and Schaumburg-Lever, 1990a); lower lip (Frierson and Cooper, 1986); and oral tongue (Maddox, 1984). In the present study, no attempt was made to describe the size and number of nerves invaded by the tumour. However, in cases where perineural invasion was identified, a pattern of spread around multiple small nerves was usually seen. In some cases, deposits were present around nerves at some distance from the invasive front of the tumour. Foci of lymphocytes or foreign-body giant cells were frequently seen adjacent to the involved nerve (Figure 6.6).

Perineural invasion was identified in 24 (53%) of the 45 tumours in the present study, and this is in agreement with the findings of Carter *et al.* (1982). In the present study, there was a significant difference in the frequency of perineural invasion in patients with and without metastasis. In contrast, Carter *et al.* (1982) failed to find an association between perineural invasion and lymph node metastasis. My results show that perineural invasion was identified more often in highly invasive tumours with individual cells or small islands at the invasive front (pattern of invasion grade 3 and grade

4), although it was also seen in two tumours with a more favourable pattern of invasion (grade 2). It is of interest to note, however, that neither of these tumours metastasised.

The frequency of perineural invasion in squamous cell carcinomas at other sites is much lower. For example, the frequency is reported to be 2-11% in cancers of the lower lip (Byers *et al.*, 1978; Frierson and Cooper, 1986) and less than 5% in skin cancers (Lever and Schaumburg-Lever, 1990a). My results show that perineural invasion is frequently seen in carcinomas of the oral mucosa, including some which subsequently were shown not to have metastasised. Hence, its value in predicting metastasis is less than that reported at extra-oral sites. Nevertheless, my results show that an assessment of perineural invasion has significant independent prognostic value in predicting nodal metastasis.

(c) Vascular Invasion

In the present study, an assessment of vascular invasion was made but no attempt was made to distinguish between lymphatics and blood vessels. In those cases where vascular invasion was identified, it was seen in approximately half of the total number of histological sections examined. Either emboli of tumour cells were seen in small, often superficial, thin-walled vessels, or invasion of the media of a larger vessel was noted. Invasion of larger vessels was easier to detect than embolisation, because the fibrin in the thrombus associated with the intimal ulceration was usually strongly eosinophilic (Figure 6.7b). The presence and amount of retraction artefact was variable, and, in all cases, an equivocal diagnosis of vascular invasion was disregarded.

Although an assessment of vascular invasion was included in the multifactorial grading system proposed by Jakobbson *et al.* (1973), it was not included in later modifications (Anneroth *et al.*, 1987) because of the difficulty in recognising it with certainty in routinely-stained sections (Willen *et al.*, 1975; Anneroth *et al.*, 1987), and the amount of time involved in making the assessment (Fisher, 1975). However, in the present study, vascular invasion was one of the two most important features in predicting the presence of lymph node metastasis. Therefore, it is important that this feature is assessed in histological malignancy grading. Since the results of the assessment cannot be presented as multicategorical data comparable to the other features assessed in histological grading, it is difficult to assign a specific numerical score for this feature. However, a statement concerning the identification of vascular, and, also, perineural, invasion would increase the prognostic efficiency of the multifactorial grading system.

Shingaki *et al.* (1988) also reported that vascular invasion was an important predictor of metastasis in oral and oropharyngeal carcinomas. Conversely, other authorities have found no significant association between the identification of vascular invasion and nodal metastasis (Crissman *et al.*, 1980; Frierson and Cooper, 1986). Failure to identify vascular invasion, rather than its true absence, may account for the uncertainty about its importance as a prognostic indicator. Detection of vascular invasion may be improved by examination of multiple histological sections, the use of special fixatives, or special staining techniques.

Poleksic and Kalwaic (1978) recommend examination of at least

five histological sections from each specimen. Whether this is an adequate number is uncertain. It is possible that the detection rate increases as more sections are examined.

Willen *et al.* (1983) reported that vascular invasion could be detected more readily in biopsies from the uterine cervix when phosphate-buffered formalin (10% formalin and 0.17ml. phosphate buffer at pH 7) was used as the fixative. The osmolarity of this solution resulted in minimal tissue shrinkage and optimal 'opening' of vessels. There are no reports in the literature, to date, of a similar study on material from the oral cavity.

Recent reports show staining for vascular markers such as *Ulex europeaus* lectin and Factor VIII-related antigen can improve the detection rate of vascular invasion in several tumours including breast carcinoma (Lee *et al.*, 1986) and thyroid carcinoma (Stephenson *et al.*, 1986) by facilitating the recognition of intra-tumoral vessels. It is likely that such techniques would also aid the detection of vascular invasion in cancers of the oral mucosa. In addition, staining for vascular markers prevents retraction artefact being mistaken for vascular invasion, hence reducing the risk of false-positive assessments (Larsen *et al.*, 1990).

(4) Logistic Regression Analysis

Logistic regression is a technique which analyses the effects of a set of explanatory or independent variables on a dichotomous dependent variable with minimal statistical bias and loss of information (Walsh, 1990). The development of a prognostic index for prediction of the dependent variable from a set or group of

explanatory variables is one of the specific uses of logistic regression analysis (Altman, 1991d), Hence, it was considered to be the ideal technique for analysing the results of the present study.

Logistic regression analysis has several advantages over other types of multiple regression analysis (Walsh, 1990; Altman, 1991d). It can be used with a smaller sample size than discriminant analysis, which is the older method of using several variables to classify individuals into the correct group. In addition, the results of logistic regression are presented in a simple way, as the estimated probability or risk of an individual having the dependent (outcome) characterisitic. However, the most important advantage of logistic regression analysis is its ability to cope with categorical or dichotomous data (Altman, 1991d).

Ordinary linear (or least squares regression) is inappropriate for dichotomous dependent variables since it can result in predictions outside the range of possibility. The true representation of the effect of an independent explanatory variable on a dichotomous dependent variable is a sigmoid curve. This arises because small changes in the explanatory variable at the extremes of the range make no difference in the probability of the outcome variable, but a small change in the middle of the range radically affects the outcome.

Because the aim of the regression in the present study was to identify the important predictor variables, it was appropriate to include in the initial model only those variables which were significant, or nearly significant, when their simple relationship to the outcome variable was tested. It could be argued that the initial selection should have been more lax. For example, Altman (1991d)

suggests that all variables with associated probability values of less than 0.2 should be included, on the grounds that some variables may contribute to the regression in unforeseen ways due to complex inter-relationships amongst the independent variables. In the present study, the only variables with probability values of less than 0.2 which were not included in the initial regression analysis were tobacco usage and alcohol consumption (Table 6.22). The accuracy of the information relating to these habits is dependent on how truthfully the patient responded to the questions asked by the member of the surgical team responsible for eliciting their clinical and social histories. Hence, the information must be regarded as approximate. Therefore, it is unlikely that either variable could make a useful contribution to the regression analysis. In the present study, the major constraint was the sample size and the need to avoid 'overfitting'. As argued previously, in this method, a more cautious approach demands the use of fewer, rather than more, variables, when the sample size is relatively small. In practice, I found that the regression models containing four, three, and two variables all resulted in the same number of correct classifications. Hence, the model containing two variables was chosen as the 'best' model for a sample of this size.

Since the 'critical thickness' in relation to metastasis was different at the two selected intra-oral sites (Figure 6.9), another logistic model was tried, allowing for interaction between site and the reconstructed thickness (that is, allowing the thickness effect to be different at the two sites). However, neither the site effect nor its interaction with thickness were significant. Thus, there is no strong evidence that the two-variable model already described as the

'best' model is invalid.

In the present study, six patients were misclassified in the 'best' regression model. Three patients without evidence of lymph node metastasis (Cases ND41, ND42/43, ND134) were misclassified as node positive, and three patients with metastasis (Cases ND31, ND51 and ND145) were misclassified as node negative. Four of the six patients (Cases ND41, ND51, ND134 and ND145) were misclassified in all five regression models.

One of the three false-positive predictions (Case ND41) died of local disease, with radiographic evidence of pulmonary metastases, three months after surgery. Hence, the high score achieved on histological grading of the primary tumour (19 out of a possible 24 points, plus perineural and vascular invasion) was accurate in predicting aggressive behaviour. However, the development of distant metastases in the absence of lymph node metastasis is unusual (Vikram *et al.*, 1984b; Kotwall *et al.*, 1987). Hence, it is possible that a nodal metastasis was missed due to a sampling error, or even at surgery, since the neck dissection was a functional procedure. The implications of restricted surgical procedures have been dealt with in full in Chapters 4 and 5.

The second false-positive prediction (Case ND42/43) also developed a local recurrence. In this patient, the primary tumour was small (the greatest surface-dimension was only 8mm.). Nevertheless, a local recurrence with extensive involvement of the anterior mandible was diagnosed one year post-operatively. Hence, also in this patient, histological assessment of the primary tumour was correct in predicting aggressive behaviour and a poor prognosis. The third

patient with a false-positive prediction (Case ND134) is alive with no evidence of recurrent disease nor metastasis. However, the post-operative period is short (six months to date).

As shown in Table 6.10, in the three false-negative predictions (Cases ND31, ND51 and ND145), the histolgical pattern of invasion was unfavourable (scoring three points), and in one case (ND31), perineural and vascular invasion were identified. However, assessment of other histological features, for example, the degree of keratinisation and the intensity of the lymphoplasmacytic infiltrate, resulted in low scores. Hence, the final histological malignancy grade was low. This illustrates one of the disadvantages of using a multifactorial grading system where equal weight is given to each component, since my results (Table 6.12) suggest that not all the components are equally good at predicting nodal metastasis. A modified histological malignancy grade, for example, excluding nuclear polymorphism, may improve further the predictive value of the logistic regression model.

Two of the three patients with false-negative predictions (Cases ND31 and ND51) are alive and well, more than two years after surgery. The third patient (Case ND145) presented with cervical metastasis 28 months after resection of a T1 tumour of the floor of mouth with an elective suprahyoid neck dissection (Case ND24). On presenting with overt neck disease (ND145), there was no evidence of intra-oral carcinoma - either a recurrence or a second primary tumour. However, extensive dysplasia was seen around the original carcinoma, and the patient is a heavy smoker and a heavy drinker. Hence, it is possible that a covert (second) primary tumour, rather than the floor-of-mouth

tumour, gave rise to the nodal metastasis. On the other hand, the metastastic mass consisted of several fused nodes with extensive cystic change, which is suggestive of long-standing metastatic disease (Shear and Ichilick, 1973).

A logistic regression model is likely to be over-optimistic with respect to the importance of each variable and the goodness-of-fit (Altman, 1991d). Although it is desirable to test the model on a new independent set of data, this ideal is difficult to achieve in research based on clinical material. Also, it is important to remember that, theoretically, the correct prediction of a dichotomous dependent variable could be achieved in 50% of cases by simple random guesswork. Nevertheless, my results suggest that the histological malignancy grade and vascular invasion are important predictors of nodal metastasis (Table 6.33). Although the present study was based on resection specimens, it is likely that the model is equally applicable to biopsy material which includes the invasive front of the tumour (Bryne, 1991; Bryne *et al.*, 1992). Hence, the important conclusion to be drawn from this study is that when the histological malignancy grade is high (for example, 18 points or more) and vascular invasion is identified in the biopsy specimen, cervical metastasis is almost certainly present, and, therefore, neck dissection or radiotherapy should be included in the definitive treatment plan.

SUMMARY

The relationship between selected clinical and histological features of primary squamous cell carcinoma of the tongue/floor of mouth and the histological metastatic status of the cervical lymph nodes has been investigated in a series of 45 patients.

There were no significant differences in clinical features, such as sex and age, and clinical T stage of the tumour, in patients with and without metastasis. However, there were significant differences in several histological features.

Firstly, all three laboratory-made measurements of tumour size (greatest surface-dimension, actual tumour thickness and reconstructed tumour thickness) were greater in patients with metastasis.

Secondly, the histological malignancy grading score (an assessment of six features at the advancing tumour front) was greater in patients with metastasis. Four features (degree of keratinisation, frequency of mitosis, pattern of invasion, and stage of invasion) had significant independent prognostic value.

Thirdly, perineural and vascular invasion were identified more frequently in patients with metastasis.

Logistic regression analysis was used to develop a prognostic index for the prediction of lymph node metastasis. During the regression analysis, the 'Total Histological Malignancy Grade' and 'Vascular Invasion' emerged as the two most important predictor variables. The 'best' logistic regression model correctly classified 39 (87%) of the 45 cases.

In a multifactorial malignancy grading system, strict criteria for grading all the component histological features are essential in

order to achieve consistent and reproducible results. Some features such as nuclear polymorphism and the lymphoplasmacytic infiltrate, may be better assessed by special techniques, such as morphometry or immunohistochemistry, which may identify differences too subtle to be detectable on routine histological assessment.

In conclusion, the results of the present study show that a detailed histological assessment of the primary tumour is useful in predicting lymph node metastasis in an individual patient. A similar assessment of the biopsy specimen would provide important information on the likely metastatic status of the cervical nodes and, therefore, would be of considerable help to the surgeon when formulating the definitive treatment plan for any particular patient.

Chapter 7.

THE RELATIONSHIP OF NUCLEAR VOLUME IN THE PRIMARY TUMOUR TO THE INCIDENCE OF CERVICAL METASTASIS.

1. Introduction.
2. Methods.
3. Results.
4. Discussion.
5. Summary.

INTRODUCTION

In the previous chapter, my results showed that detailed histological assessment of the primary tumour was useful in predicting lymph node metastasis in an individual patient. However, the degree of nuclear polymorphism, which was one of the features assessed in the multifactorial grading system, showed no correlation with the presence of metastasis, and a routine assessment of this feature made no useful contribution to the total malignancy score. Nevertheless, the histological appearance of the nucleus reflects cellular behaviour (Collan *et al.*, 1987), and it is possible that differences exist in the nuclei of tumours which metastasise, but that these differences are too subtle to be detected by subjective assessment in routine histological examination. In view of these uncertainties, the nuclear grade in the 45 tumours studied in Chapter 6 (Series II) was re-assessed using a more precise morphometric technique, namely, stereological estimation of the mean nuclear volume, based on a sample of nuclei from the volume-weighted distribution (the volume-weighted mean nuclear volume).

Before describing these investigations, the histological structure of the nucleus and the changes that occur in neoplasia will be outlined. In addition, some of the other morphometric techniques which are widely used in diagnostic histopathology, and, in particular, in malignancy grading, will be reviewed.

(1)) Structure of the Nucleus

Most of the deoxyribonucleic acid (DNA) in eukaryotic cells is located in the nucleus. Together with binding proteins such as histones, the DNA is organised into chromosomes which constitute the

chromatin of interphase nuclei. This can be seen as haematoxyphilic, granular material on histological examination of routinely-prepared tissue sections (Underwood, 1990).

Each nucleus contains at least one subsidiary structure detectable on routine histology: the nucleolus. The nucleolus is formed around nucleolar organising regions (NORs) which are loops of DNA located on the acrocentric chromosomes (Underwood, 1990). The NORs possess the ribosomal ribonucleic acid (rRNA) genes which are important in protein synthesis. The nucleolus is not a constant structure (Underwood and Giri, 1988), and the size and number of nucleoli and NORs reflect cellular and nuclear activity (Ploton *et al.*, 1986).

The shape of the nucleus is determined by characterisitics of the nuclear envelope, specifically, the fibrous lamina of the inner nuclear membrane (Underwood, 1990). The physical nuclear volume reflects both the DNA content and a range of nuclear biochemical events, such as the amount of inactive DNA (heterochromatin) and active DNA (euchromatin) (Nielsen *et al.*, 1989). In addition, nuclear volume may reflect the local metabolic state of the tissue (Mattfeldt *et al.*, 1988).

(2) Nuclear Abnormalities in Malignant Cells

Enlargement of the nucleus and variation in nuclear size (anisonucleosis), abnormal and variable nuclear shape (nuclear polymorphism/pleomorphism), and multiple nuclei are characteristics of malignant cells and such aberrations reflect disturbances in nuclear ploidy and DNA synthesis (Underwood, 1990). In addition, malignant nuclei frequently display hyperchromatic, coarse chromatin and

abnormally large or irregular or abundant nucleoli.

It is now more than 100 years since Hansemann (1890) first postulated that abnormalities in nuclear morphology in tumour cells might be correlated with their biological properties and clinical behaviour, and histological assessment of the nucleus is now used routinely in the diagnosis and grading of several types of neoplasms. Some grading schemes, such as that proposed by Mittal *et al.* (1988) for grading endometrial adenocarcinomas, utilise nuclear features exclusively. However, most grading schemes, such as those recommended for breast adenocarcinomas (Bloom and Richardson, 1957); pancreatic adenocarcinomas (Kloppel *et al.*, 1985); laryngeal squamous cell carcinomas (Jakobbson *et al.*, 1973); and oral squamous cell carcinomas (Anneroth *et al.*, 1987) incorporate an assessment of nuclear grade, together with an assessment of cellular differentiation and tumour architecture. In general, malignancy grading schemes correlate successfully the nuclear grade with prognosis (Underwood, 1990). However, most schemes are prone to a significant degree of observer error because the appraisal of nuclear features, such as size and shape, by routine histological examination is highly subjective.

(3) Morphometry and its Applications in Diagnostic Pathology

Morphometry was defined originally by Weibel (1969) as 'the quantitative description of a structure', and, more recently, as 'the measurement of structures by any method, including stereology' (Weibel, 1979a). While the structures may be macroscopic or microscopic in size, generally, the term is used in a restricted sense to denote the quantitative description of microscopic images and features (Baak and Oort, 1983b).

Several morphometric techniques and methods are available to provide quantitative data (Baak and Oort, 1983a). For example, the following methods are frequently used: the counting of elements such as mitotic figures or nucleoli; planimetry, the assessment of quantitative features of structures in a two-dimensional plane, although these structures themselves may not be two-dimensional (Baak and Oort, 1983a); and stereology, a body of mathematical methods relating three-dimensional parameters defining the structure to two-dimensional measurements obtainable on sections of the structure (Weibel, 1979b). Other morphometric techniques include scanning densitometry, digital image processing, flow cytometry, and static cytofluorometry. However, these latter techniques are less widely applied in routine diagnostic pathology.

Morphometry has three main advantages over routine histological assessment, namely, objectivity, reproducibility, and detection possibility (Baak and Oort, 1983b). For example, accurate objective measurements of cellular and nuclear size and shape eliminate observer error due to random or systematic shifts in the subconscious diagnostic thought-making process (Langley, 1978). Although the eye easily detects all-or-none phenomena, it is poor at assessing continuous variables such as size and density (Stephenson, 1990). Hence, morphometry allows the detection of differences which escape subjective judgement. A further advantage of morphometry is that the numerical data produced are readily communicated, and are suitable for stastistical analysis (Stephenson, 1990).

Planimetric measurements of nuclear profiles, as seen in routinely-prepared histological sections, are easily obtained using

equipment which is available in most diagnostic laboratories (Stephenson, 1990). Measurements of the profile area, perimeter, and greatest diameter are usually used to represent nuclear size, and the standard deviation of these measurements is used to represent the variation in nuclear size. In addition, nuclear shape and the variation in shape can be described by the 'Nuclear Contour Index', 'Form PE', and the standard deviation of 'Form PE' (Schrek, 1972; Stephenson, 1990).

Routinely-prepared histological sections are thin (4-5 microns), and, therefore, they can be regarded as two-dimensional preparations of three-dimensional tissue structures (Baak and Oort, 1983a). This dimension reduction results in important technical complications in morphometry of nuclear profiles. For example, nuclei in tissue sections are frequently sectioned by the microtome resulting in slices of nuclei in different equatorial planes (Fu and Hall, 1985). In planimetric quantitation of nuclear size, the profiles of these incomplete nuclei will be included in the analysis. Although correction factors to compensate for inclusion of incomplete structures are available for regular shapes such as spheres (DeHoff, 1962; Saltykov, 1967), there is no satisfactory method to compensate for irregular structures, such as nuclei in neoplasia (Gundersen *et al.*, 1988a), and the relationship of the two-dimensional profile to the three-dimensional volume remains unknown. Hence, the validity of planimetric measurements of nuclear profiles is uncertain (Gundersen *et al.*, 1988a and 1988b; Stephenson, 1990; Sorensen, 1991).

Stereology is a set of simple and efficient methods for the quantitation of three-dimensional microscopic structures which is

specifically tuned to provide reliable data from histological sections (Gundersen *et al.*, 1988a). In 1983, Gundersen and Jensen developed a simple, 'design-based' stereological method, namely, point-sampling of linear intercepts, for estimating the volume-weighted mean nuclear volume of arbitrary particles in three-dimensional space. Further developments (Sterio, 1984; Gundersen and Jensen, 1985; Baddeley *et al.*, 1986) allow the method to be applied simply and efficently to a single, routinely-prepared histological section, provided this is an isotropic, uniform random plane, or the tissue itself is statistically isotropic. Vertical sections of tissues with a natural plane, such as the skin and oral mucosa, fulfill this requirement (Gundersen *et al.*, !988a). Using point-sampled intercepts, a realistic, unbiased estimate of the mean nuclear volume is achieved without any assumptions of shape, making it well-suited for the quantitative description of nuclear size.

(4) Nuclear Morphometry and Malignancy Grading

During the past two decades, numerous authorities have shown that selective morphometry, in which the pathologist uses his full diagnostic knowledge to select areas, cells or nuclei of interest (Baak and Oort, 1983c), is useful in the diagnosis and grading of neoplasms. For example, the combination of morphometry and conventional prognosticators (tumour size and axillary lymph node status) gives a significant improvement in the prognosis prediction in breast cancer (Stenkvist *et al.*, 1981; Baak *et al.*, 1985). Both groups of authors used planimetry, rather than stereology, and, of the morphometric features studied, the mitotic activity index and the mean and standard deviation of the nuclear profile area were the most

powerful predictors of recurrence and survival. Other neoplasms for which grading by nuclear planimetry has been successful include chondrosarcoma (Kreicbergs *et al.*, 1981); papillary carcinoma of the thyroid (Ambros *et al.*, 1989); and carcinoma of the prostate (Diamond *et al.*, 1982a and 1982b).

Recent reports indicate that stereological estimates of nuclear volume have significant prognostic value in a range of neoplasms. For example, mean nuclear volume estimates predicted successfully the clinical course in retrospective studies of carcinomas and non-invasive tumours of the urinary bladder (Nielsen *et al.*, 1986 and 1989); and uterine cervix (Sorensen *et al.*, 1991a). In addition, nuclear volume estimates have been recommended for predicting survival and disease-free period in malignant melanomas (Sorensen, 1989; Sorensen *et al.*, 1991b).

However, the value of nuclear morphometry in grading carcinomas arising from the mucosa of the upper aerodigestive tract is less well documented (Bryne, 1991). A recent report by Reifen *et al.* (1992) suggests that nuclear planimetry and stereology may have prognostic value in predicting lymph node metastasis in nasopharyngeal carcinomas, and a preliminary report by Sorensen *et al.* (1989) suggests that mean nuclear volume estimates may be of value in predicting prognosis, in terms of survival, in supraglottic laryngeal carcinoma. Attempts to grade lesions of the oral mucosa using planimetry and stereology have met with a variable degree of success. For example, planimetry has been used successfully to differentiate between various white lesions and to predict the behaviour of leukoplakias (Keszler and Cabrini, 1983; Shabana *et al.*, 1987). On the

other hand, in a study reported recently (Briggs *et al.*, 1992), planimetric assessment of nuclear shape in primary carcinomas sited on the floor of mouth could not distinguish between patients with and without cervical node metastasis. In addition, Bryne *et al.* (1991b) have reported that a stereological assessment of mean nuclear volume is less useful than a subjective grading of nuclear polymorphism in predicting survival in oral cancer.

In view of the uncertainties regarding the prognostic value of nuclear features and the value of nuclear morphometry in grading squamous cell carcinoma of the oral mucosa, my aim, in the present study, is to assess the efficiency of stereological estimation of the volume-weighted mean nuclear volume in predicting lymph node metastasis. This will be done by comparing the mean nuclear volume of the primary tumour in groups of patients with and without metastasis in order to identify any differences which could be used in the initial assessment of the primary tumour.

METHODS

(1) Surgical cases

The 45 primary tumours of oral tongue/floor of mouth forming Series II, which were described in detail in Chapter 6 and summarised in Table 6.4, provided the material for the present study.

(2) Histological Sampling and Stereological Technique

(a) Histological Sampling

For each of the 45 surgical cases, one histological section was selected from the multiple sections already prepared for, and used during, the study described in Chapter 6. As stated in Chapter 6 (Gross and Histological Sampling Techniques and Laboratory Methods), these histological sections fulfilled the requirements for vertical sections, as detailed by Baddeley *et al.* (1986). The criterion used to select the section for use in the present study was the 'Nuclear Polymorphism' point score detailed in Chapter 6. For the present study, for each surgical case, one histological section was chosen at random from the sections which were available and representative of the point score already assigned to the case.

For the purposes of the present study, the advancing front of the tumour was re-examined histologically, and the area showing the greatest degree of nuclear polymorphism on subjective assessment was selected and identified using a black ink marker. Also, the more superficial two-thirds of the tumour were masked by black ink, leaving the deepest one-third clear for further microscopic assessment. The 45 sections were then re-coded by an independent technician and the stereological estimations were made without knowledge of the

metastatic status of the patient.

(b) Estimation of Mean Nuclear Volume by Point-Sampling of Linear Intercepts

In order to explain the practical methods used in the present study, it is necessary to consider theoretical aspects of the technique in more detail.

(i) Theoretical Aspects

Four basic stereological probes are available for estimating the different geometric characteristics of three-dimensional objects (Gundersen *et al.*, 1988b). The probe for estimating volume is the point. When the probe - a series of points - is thrown onto a histological tissue section, the probe hit particles within the tissue with a probabilility which is directly proportional to the volume of the particles. Hence, if only particles hit by the probe are sampled, a volume-weighted sample of particles (or a sample of particles from the volume-weighted distribution) is studied (Gundersen and Jensen, 1983 and 1985). The points of the probe are random inside the particles they have fallen on, and the volume-weighted mean particle volume can be estimated from the linear intercepts drawn through the points.

The technique uses a sampling probe consisting of a test grid of systematic points on lines in any fixed direction. When a point falls within a particle profile, the linear intercept (l_o) is measured in a three-dimensionally isotropic direction, that is, in a way that all orientations and all positions in space are equally likely (Weibel, 1979a). According to Gundersen and Jensen (1983), the mean of the third power of the observed point-sampled intercept lengths multiplied

by $\pi/3$ is an unbiased estimator of the volume-weighted mean particle volume, V_v. That is,

$$V_v = \frac{\pi}{3} \cdot l_o^3$$

where V_v = volume-weighted mean particle volume

and l_o = observed point-sampled intercept length.

In this technique, a particle which is hit by two or more points must be sampled two or more times. The only requirement for the estimation is that an individual particle (in the present study, nuclei of malignant keratinocytes) can be unambiguously identified by its profile on isotropic uniform random (IUR) or vertical sections for the measurement of intercepts (Baddeley *et al.*, 1986; Sorensen, 1991).

As stated above, the linear intercepts through the sampled particles must be measured in random directions. These random directions are generated with a non-uniform distribution of orientation in the section plane, with a probability proportional to the sine of the angles between the test lines and the vertical axis of the test system (Sorensen, 1991). In practical terms, a sine-weighted 'orientation frame' or 'direction finder' can be used to generate the three-dimensional isotropic test-line directions on vertical sections, and the intercept lines can be measured using a l_o^3 or cubic ruler (Gundersen *et al.*, 1988a and 1988b; Moss *et al.*, 1989; Sorensen, 1991). Alternatively, as in the present study, test-line directions can be generated and measured using an interactive image analysis system with a computer software package designed for this purpose (Moss *et al.*, 1989).

(ii) Practical Aspects

In the present study, measurement of point-sampled linear

intercepts and estimation of the volume-weighted mean nuclear volume were carried out using an interactive image analysis system. A schematic diagram showing the component parts of the system is presented in Figure 7.1. The computer software package is available commercially ('Fenestra', Confocal Technology Limited, Liverpool, U.K.).

In addition to the tasks outlined below, the software performed two other important functions. Firstly, it acquired a digitized image of the histological section. Secondly, once calibrated, it converted the intercept length to physical dimensions, with automatic compensation for any geometric distortion of the digitized image (Moss *et al.*, 1989).

Measurement of the point-sampled intercepts and estimation of mean nuclear volume for each of the 45 cases in Series II proceeded as follows. The black ink mark identifying the area of greatest nuclear polymorphism was used to determine the first microscopic field. An oil immersion objective lens (x100) was used to facilitate the optimum definition of the ditized image of the nuclear profiles displayed on the monitor screen. A test grid which sampled approximately 50% of nuclear profiles in each microscopic field was selected and overlaid on the digitized image. The linear intercepts of all the keratinocyte nuclear profiles sampled by the test grid were measured. The direction of the test line in each of the sampled nuclei was generated automatically. Once the cursors were correctly positioned at the intercepts of the test line and the boundary of the nuclear profile, the observed intercept length, l_o, (Figure 7.2) was measured and recorded. The next field was selected by moving the microscope stage

slightly in one direction with the image out of focus. The image on the screen was then re-focussed and the observed linear intercepts of all the point-sampled nuclear profiles displayed on the monitor screen were measured, as before. This procedure was repeated until a minimum of 100 intercepts (in approximately eight to twelve microscopic fields) had been measured. The microscope stage was moved in the same lateral direction throughout. Fields containing areas of necrosis and heavy inflammation were avoided.

For each case in the study, the results are presented as the mean nuclear volume (based on a minimum of 100 point-sampled intercepts), and the associated co-efficient of error.

(3) Pilot Study

Before the commencement of the principal study, a Pilot Study was carried out to assess, firstly, the appropriate number of observed point-sampled linear intercepts, and, secondly, the consistency of the stereological estimation of the mean nuclear volume.

For use in the Pilot Study, five histological sections were selected at random from the 45 sections prepared in readiness for the principal study.

The appropriate number of observed intercept lengths was determined by measuring 140 point-sampled intercepts while observing the graphical display of the co-efficient of error on the computer monitor screen. In each of the five cases, after 80 intercepts had been measured, the co-efficient of error was consistently less than 0.2. Hence, 100 intercepts was chosen as the appropriate number for the principal study.

The consistency of the stereological estimation of the mean nuclear volume was assessed by estimating the mean nuclear volume, based on 100 point-sampled intercepts, according to the protocol detailed above, in each of the Pilot Study cases. The procedure was repeated after two weeks and the two sets of estimates were compared.

The results of the Pilot Study are shown in Table 7.1. In all cases, the difference between the two sets of estimates was less than 10%. On the basis of these results, it was decided that there was sufficient consistency to proceed with the principal study.

(4) Statistical Methods

The results of the present study were analysed using the SPSS-X statistical package on the University of Liverpool IBM computer.

The Two sample t Test (Chapter 4, Statistical Methods) was used to evaluate the difference between the distributions of the two groups: tumours in patients with metastasis and tumours in patients without metastasis.

RESULTS

In the 45 tumours in Series II, the volume-weighted mean nuclear volume ranged from 224.8μm^3 to 1340.0μm^3, with a mean of 698.4μm^3 (SD 294.7). In all cases, the co-efficient of error was less than 0.2.

Figure 7.3 shows the mean nuclear volume of each of the 45 tumours grouped according to the 'Nuclear Polymorphism' point score assigned on histological multifactorial malignancy grading during the study reported in Chapter 6. The mean nuclear volume was lower in the six tumours scoring one point (mean 430.0μm^3, SD 131.1, range 224.8-566.8), than in the 39 tumours scoring two or more points (mean 739.7μm^3, SD 291.9, range 239.5-1340.0), and the difference in mean nuclear volume in relation to the 'Nuclear Polymorphism' point score was significant when tested by the Two Sample t Test ($t = 4.36$, 5 d.f., $P = 0.008$).

In the 27 patients with metastasis (Table 7.2, Figure 7.4), the mean nuclear volume ranged from 224.8μm^3 to 1305.0μm^3, with a mean of 716.0μm^3 (SD 292.0). In the 18 patients without metastasis (Table 7.3, Figure 7.4), the mean nuclear volume ranged from 239.5μm^3 to 1340.0μm^3 with a mean of 672.1μm^3 (SD 305.2). Hence, there was no significant difference in the mean nuclear volume in relation to metastasis (Two Sample t Test, $t = 0.49$, 43 d.f., $P = 0.64$).

DISCUSSION

The aim of the present study was to evaluate the efficiency of a stereological estimation of the volume-weighted mean nuclear volume in predicting lymph node metastasis in squamous cell carcinoma of the oral mucosa. Changes in the size, shape, and staining patterns of the nucleus characterise malignant cells and reflect changes in cellular behaviour (Underwood, 1990). For many years, a subjective assessment of the nucleus has been used in the diagnosis and grading of neoplasms (Hansemann, 1890). More recently, new 'design-based' stereological techniques have facilitated precise objective quantitation of nuclear features (Gundersen, 1986; Gundersen *et al.*, 1988a and 1988b). Several authorities (Nielsen *et al.*, 1986 and 1989; Sorensen, 1989; Sorensen *et al.*, 1991a and 1991b) now recommend that these techniques are used to predict prognosis in a range of neoplasms. However, no studies, to date, have shown that stereological estimation of nuclear features has predictive value in assessing the behaviour of squamous cell carcinoma of the oral mucosa.

The present discussion will be arranged in two sections, the first concerned with the stereological technique used, and the second concerned with my findings.

(1) Estimation of the Mean Nuclear Volume: Theoretical and Practical Aspects

The volume-weighted mean nuclear volume based on point-sampling of linear intercepts is an efficient, unbiased estimator of the three-dimensional nuclear volume (Sorensen, 1991). An important advantage, in respect of malignancy grading and prognosis prediction, is that the measurement procedure is simple to perform and suitable for routinely-

prepared vertical or isotropic uniform random histological sections (Sorensen, 1991). However, the major advantage of the technique is the shape-independent sampling probe: points which sample particles proportional to their three-dimensional volume. Hence, the assumption, that all particles have the same simple geometric shape, which is necessary with both planimetric and 'model-based' stereological techniques, is avoided. Thus, the point-sampling technique is well suited to estimating the volume of nuclei, which are known to vary in shape and frequently show anisotropism, that is, they exhibit different properties in different directions of space (Sorensen, 1991). For example, the nuclei in various simple epithelia are elongated, often with a clearly polar orientation (Rigaut *et al.*, 1985). In addition, nuclear shape becomes considerably more variable following malignant transformation (Sorensen, 1991). Hence, in maligancy grading, the use of a shape-independent sampling probe is even more desirable.

In addition to the benefits proffered by the shape-independent probe, estimation of mean nuclear volume by point-sampling of linear intercepts offers several other advantages. For example, the older 'model-based' stereological methods are less efficient, requiring a minimum of several hundred physical measurements to give a stable estimate of particle size (Moss *et al.*, 1989). Also, the point-sampling technique combines information on size and the relative variability of size without the need for multiple histological sections (Gundersen *et al.*, 1988b). The volume-weighted sample, produced by point-sampling, contains a larger fraction of big nuclei than a uniform sample produced by the optical disector (Sterio, 1984)

might, making the point-sampling technique more sensitive to subtle changes in nuclear size (Gundersen, 1986). Hence, it is clear that the special qualities of the point-sampling technique, and its advantages compared to planimetric and other stereological methods, combine to make the volume-weighted mean nuclear volume an attractive description of nuclear size in malignant tissues.

In the present study, an interactive image analysis system was used to estimate the mean nuclear volume. Such a system allows both the operator and the computer to perform the tasks to which each is best suited. For example, the human visual field can segment a low contrast image very rapidly, even when there is missing information such as incomplete boundaries, while the computer can control sampling protocols and data handling in a rapid and efficient manner (Browne and Gaydecki, 1987; Moss *et al.*, 1989). The generation of three-dimensional isotropic test-lines by the computer was an important advantage of the present study, since the reliability of the estimation of mean nuclear volume by point-sampling of linear intercepts is totally dependent on a correctly-designed and executed sampling regime (Gundersen *et al.*, 1988b; Sorensen 1991). In addition, with the software package used in the present study, the direction of the test-line was changed for each intercept, rather than for each field as is usual with manual methods (Gundersen *et al.*, 1988b), thus, improving the efficiency of the 'computer-aided' estimator.

The validity of any quantitative study is dependent on correct specimen sampling. Weibel (1969) described the representative sample as the value which faithfully reflects the composition of the material. An excessive sample size is inefficient and, therefore,

undesirable. On the other hand, an inadequate sample size may lead to results which are not representative of the population under study. In the present study, stereological analysis was confined to nuclei in the deepest third of the tumour, with the area showing the greatest polymorphism on routine assessment determining the first microscopic field. This sampling procedure was chosen because of the reported improvement in the prognostic accuracy of histological malignancy grading schemes when selective invasive cell grading is used (Bryne *et al.*, 1989). However, it could be argued that a more systematic sampling scheme, where consideration is given to regional variability of nuclear volume within one tumour, is preferable. Nevertheless, the results of my Pilot Study show that the protocol used resulted in reproducible estimates of the mean nuclear volume, since, in all five Pilot Test cases, the original and repeat nuclear volume estimates were within the limit of 10% considered acceptable in stereological studies (Schroeder and Munzel-Pedrazzoli, 1970; Moss *et al.*, 1989).

In the present study, the number of observed intercept lengths on which to base the estimation of the mean nuclear volume was determined by the co-efficient of error. The volume of a sphere is an identity because the radius is a constant, whereas the volume-weighted mean nuclear volume is an estimator with a certain co-efficient of error (Gundersen *et al.*, 1988b). However, if the observed intercept length, l_o, is measured in a sufficient number of isotropic directions, the mean value of the estimator comes arbitrarily close to the true volume and the co-efficient of error goes to zero. Gundersen *et al.* (1988b) reported that the co-efficient of error depends on the tumour type and the malignancy grade. In the present study, in all

cases, the co-efficient of error was less than 0.2, and this is within the range (0.1-0.3) considered satisfactory for routine use in individual patients and for scientific purposes (Gundersen *et al.*, 1988b; Sorensen, 1991).

In the present study, the point density of the test grid was selected so that approximately 50% of the keratinocyte nuclear profiles in each microscopic field were sampled, as recommended by Gundersen and Jensen, (1985). The actual number of profiles sampled in each microscopic field, and, hence, the number of fields necessary to provide a minimum of 100 intercept lengths, was different in each case. It depended on the tumour/stroma ratio, the histological pattern of invasion, and whether areas of inflammation and/or necrosis were present or not. Fields were selected for analysis with the image slightly out of focus in order to reduce any observer bias. However, while moving the microscope stage, the blurred image was observed on the monitor screen in order to prevent any overlapping of consecutive fields.

(2) The Results of the Stereological Estimation and Their Prognostic Value

My results, in the present study, show that an estimation of the volume-weighted mean nuclear volume by point-sampling of linear intercepts had no prognostic value in predicting lymph node metastasis in carcinoma of the tongue and floor of mouth. The range of estimated nuclear volumes was large in the two groups of patients, and the mean value of the two groups differed by less than 10% (Figure 7.4).

Data on the volume-weighted mean nuclear volume in oral cancer

has been reported recently by Bryne *et al.* (1991b). They also reported that the range of estimated nuclear volume was very wide. Their mean value ($608\mu m^3$) differed from the mean value for the 45 cases in the present study ($698\mu m^3$) by approximately 15%. However, Bryne *et al.* (1991b) used a manual, rather than a computer-aided technique, and the tumours in their study were sited on the buccal mucosa. When these and other differences in methodology are considered, the similarity of their results and my results in the present study, supports the view of Gundersen *et al.* (1988b) and Sorensen (1991) that estimates of the mean nuclear volume by the point-sampling technique are robust.

My results show an association between the estimated volume-weighted mean nuclear volume and the 'Nuclear Polymorphism' point score assigned during histological malignancy grading. The mean value of the estimated mean nuclear volume for the group of tumours scoring one point was significantly lower than the mean value for the group of tumours scoring two or more points. This result is expected since one of the criteria for point scoring (detailed in Chapter 6, Gross and Histological Sampling Techniques and Laboratory Methods) was size of the nucleus as perceived on subjective histological assessment of the tissue section. Sorensen *et al.* (1989) failed to demonstrate a relationship between the estimated volume-weighted mean nuclear volume and the morphologically assessed degree of nuclear polymorphism in supraglottic laryngeal squamous cell carcinoma. However, they scored 'Nuclear Polymorphism' according to the criteria used by Lund *et al.* (1977), which are less strictly defined than the criteria I used.

Bryne *et al.* (1991b) found no correlation between the estimated volume-weighted mean nuclear volume and prognosis, in terms of

survival, in buccal carcinoma. Nevertheless, they found that patients scoring one point on subjective grading of nuclear polymorphism had significantly better survival than patients scoring two or more points (as defined by Anneroth *et al.*, 1987). Hence, Bryne *et al.* (1991b) concluded that a subjective assessment of nuclear grade was more useful in predicting prognosis than nuclear volume estimates. The reason for this, they surmise, is that nuclear pleomorphism, and variation in chromatin content and pattern, are considered, in addition to nuclear size, in subjective grading. As reported in Chapter 6, I found that a subjective assessment of 'Nuclear Polymorphism' has no prognostic value in predicting cervical node metastasis. In addition, in the present study, I have shown that a stereological estimate of the volume-weighted mean nuclear volume has no prognostic value. Hence, my results suggest that neither the nuclear characteristics which are assessed by a subjective examination of routine histological sections, nor those characteristics which are estimated by simple stereological techniques, are useful in predicting cervical node metastasis in oral cancer.

SUMMARY

The relationship between the volume-weighted mean nuclear volume (estimated by point-sampling of linear intercepts) in primary squamous cell carcinoma of the tongue/floor of mouth and the actual metastatic status of the cervical lymph nodes (as revealed by histological examination of nodes yielded from surgical neck dissection specimens) has been investigated in a series of 45 patients.

Estimates of the volume-weighted mean nuclear volume by the point-sampling technique provide an attractive method of describing the three-dimensional nuclear volume in malignant tissues. The 'design-based' technique is an unbiased, objective estimator of the mean nuclear volume, which is independent of nuclear shape. The method is simple and efficient, and can be applied to a single routinely-prepared histological section, making it suitable for malignancy grading of small biopsy specimens.

In the present study, an interactive image analysis system was used to generate the three-dimensional isotropic test-lines for measurement of the point-sampled linear intercepts, and to estimate the mean nuclear volume of neoplastic cells at the advancing front of the tumour.

My results show that the range of estimated mean nuclear volume was wide in groups of patients with and without metastasis, and the mean value for the two groups differed by less then 10%. Hence, my findings show that estimation of the volume-weighted mean nuclear volume has no prognostic value in predicting the metastatic status of the cervical nodes in carcinoma of the tongue and floor of mouth.

Chapter 8.

CONCLUSIONS AND SUGGESTIONS FOR FURTHER STUDIES.

Oral cancer is a serious and important disease. Although it is a relatively rare disease in the United Kingdom, the incidence is increasing in females and younger age groups. The disease and its treatment may have a profound effect on both the aesthetics and the functions of the face and upper aerodigestive tract. Despite the advances in surgery and radiotherapy in recent years, the prognosis remains poor. Even if the disease is eradicated, both locally in the mouth and regionally in the neck, survivors face the prospect of developing further primary tumours and/or distant metastases. Exact details of the natural history and progression of the disease are uncertain. For example, large tumours may have a short history, and *vice versa*. Some tumours are locally extensive, but show no regional or systemic spread. Conversely, the presence of regional metastasis in association with a small primary tumour is not an infrequent finding.

My study has given me the opportunity to observe, both macroscopically and microscopically, a large number of surgical resection specimens. The aims of my study were broad - to investigate the incidence and extent of cervical node metastasis in patients presenting with oral cancer, and to determine the extent to which selected clinical, histological, and morphometric features of the primary tumour influence the development of lymphatic spread. Some of my findings have direct and immediate clinical relevance, and others are potentially important, once long-term follow-up data is available. In this chapter, I will reiterate some of the more important aspects of my investigations, and discuss, briefly, possible future studies.

Accurate pathological assessment of surgical neck dissection specimens demands that the specimen is correctly pinned out prior to

fixation. This prevents distortion due to contraction of the sternomastoid muscle and aids in the identification of the different anatomical levels of nodes within the deep cervical chain. For logistic reasons, the surgical team had to pin out and fix the specimens. However, I took responsibility for this aspect of their training.

Personal experience, gained during the study, has led to minor modifications in my protocol for gross dissection and sampling. For example, I have found that small nodes can be identified more easily, and removed with less risk of crushing, if fixation is prolonged to 48 hours. The bed of the sternomastoid muscle can be identified in most functional dissections and this is helpful in defining the limits of the posterior triangle. Also, I have found that the bed of the internal jugular vein can be discerned in most modified dissections, by careful visual inspection and palpation. One of the most important aspects of my technique was the inclusion of some fibro-adipose tissue with each node as it was dissected from the gross specimen. Emboli and larger cords of tumour cells were identified within perinodal lymphatic channels in several dissections (Figure 4.3b), and, in some cases, these emboli were the only evidence that tumour had spread beyond the metastatic node. Similarly, inclusion of some perinodal tissue is essential for determination of the extent of microscopic extracapsular spread. Hence, my observations suggest that an accurate histological assessment may not be possible if nodes are removed from the specimen by blunt dissection at the plane of the node capsule, as is usually recommended (Barnes and Johnson, 1986b; Rhys Evans *et al.*, 1987; Rosai, 1989).

My results show that histological examination of all the lymph nodes which were harvested from the gross specimen is essential for an accurate pathological assessment of the metastatic status. Metastatic deposits may be present in small nodes, while larger nodes, within the same anatomical group, are histologically free of tumour.

An accurate pathological assessment allows clinicopathological correlation of the extent of metastatic spread, and this has immediate therapeutic significance, in relation to post-operative radiotherapy. The prognostic importance of my findings will be assessed once follow-up data is available. The relative importance of the number and level of positive nodes, the presence and extent of extracapsular spread, and the presence of 'micrometastases' - nodes showing only minimal replacement - will be studied in relation to recurrent disease in the neck and systemic metastasis.

My findings in relation to the number of nodes harvested from the gross surgical specimens raise questions concerning the efficacy of modified dissections. To date, few authorities have published data on nodal yield, and although the clinical relevance of my results is uncertain until follow-up data is available, it is apparent that this aspect of my study merits further investigation.

My results show that clinical assessment of the neck is frequently inaccurate, even when examination under general anaesthesia is supplemented by CT scanning. However, and more importantly, I have shown that, on most occasions, the inaccurate clinical diagnosis can be explained by pathological findings in the surgical neck dissection specimen. The most important example is my observation that metastatic carcinoma may be present only as micrometastases, and, hence,

undetectable on pre-operative assessment given the limitations of current scanning equipment. Hence, this source of inaccuracy in the clinical assessment is unavoidable.

My results show that histological assessment of the primary tumour, using a multifactorial grading system, is useful in predicting the presence of lymph node metastasis in an individual patient. However, it must be remembered that the grading was performed on resection, rather than biopsy, specimens. Also, interexaminer variability, an important source of inaccuracy in any histological grading system, was not considered in my study. The logistic regression model is inherently over-optimistic and its true potential can only be judged on a new set of cases. In addition, it should be remembered that the correct metastatic status could be predicted in 50% of cases by simple random guesswork.

Nevertheless, my results show that several components of the multifactorial grading system have significant independent value in predicting nodal metastasis. In addition, perineural and vascular invasion, two features not assessed in the Anneroth system (Anneroth *et al.*, 1987) were useful predictors of metastasis in my study, and my results suggest that the multifactorial grading system would be enhanced by their inclusion.

Vascular invasion was one of the two most important predictor variables in the final regression model (Table 6.30). It is possible that vascular invasion could be detected more readily if a phosphate buffer is added to the fixative solution, as is the case in the cervix (Willen *et al.*, 1983). This may be particularly helpful in the assessment of biopsy specimens, and a study to investigate this is

planned.

Recent reports (Nicolson, 1987; Fidler and Balck, 1987; Fidler, 1989 and 1991; Aznavoorian *et al.*, 1993) suggest that angiogenesis plays an important role in the pathogenesis of metastasis. Invasion of the microvasculature (both lymph vessels and blood vessels), in or near the primary tumour, is recognised as a critical step. Endothelial cells can be visualised in paraffin-embedded tissue sections by immunohistochemical staining directed against Factor VIII or platelet/endothelial cell adhesion molecule (CD31). In addition, the microvasculature can be assessed morphometrically. A positive correlation between the surface area of vessels at the base of the primary tumour and the presence of metastasis has been reported in cutaneous melanoma (Srivastava *et al.*, 1988), and the morphology of the vasculature is an important prognostic factor for patient survival following enucleation in uveal melanoma (Folberg *et al.*, 1992). Other reports suggest that assessment of the vasculature may have prognostic value in breast (Weidner *et al.*, 1991; Horak *et al.*, 1992) and lung (Macchiarini *et al.*, 1992) cancers. This aspect of metastasis development appears of interest, and a morphometric assessment of the microvasculature in oral cancer and its relationship to lymph node metastasis and survival is at the planning stage.

In my study, tumour size was represented by three laboratory-made measurements. My results show that tumour surface-dimension is an unreliable indicator of both tumour thickness and nodal metastasis. The results show, also, that the 'critical thickness' in relation to metastasis is different in tumours of the tongue and tumours of the floor of mouth, even though the two sites are anatomically closely

related. Hence, further studies are needed to determine the critical thickness at each intra-oral site.

Of the six histological features assessed in the multifactorial malignancy grading system, only two, 'Nuclear Polymorphism' and 'Lymphoplasmacytic Infiltrate' had no independent prognostic value in predicting metastasis (Table 6.22). In addition, 'Nuclear Polymorphism' showed no correlation to other histological features. The morphometric study of nuclear volume was undertaken to assess the predictive value of an objective assessment of nuclear features. However, my results show that a simple stereological estimation of the volume-weighted mean nuclear volume showed no correlation with the metastatic status. When follow-up data on Series II patients is available, the relationship between the estimated nuclear volume and survival will be investigated. However, it seems likely that nuclear features are less important in predicting the behaviour in squamous cell carcinomas than in other types of tumours, such as cutaneous melanomas (Sorensen, 1989; Sorensen *et al.*, 1991b). One possible explanation is that the tumour architecture (invasion by islands and tongues of tumour cells) and cytoplasmic differentiation (keratin production), characteristics of squamous cell carcinomas, prevent the accurate identification of, and assessment of nuclear features in, tumour cells which have maintained the capacity to divide and metastasise. The technique of estimation of the volume-weighted mean nuclear volume by point-sampling of linear intercepts demands that all the sampled cells are measured. Thus, it is likely that cells no longer capable of division (and metastasis) are included in the sample, and so effectively mask any differences in the nuclear volume

of cells capable of entering the mitotic cycle and/or metastasising.

In conclusion, the first part of my study enables me to state that there are at least two patterns of spread of metastatic carcinoma within the cervical lymphatic system. In the majority of cases, metastasis involves, initially, one or a small number of nodes, at the submandibular or superior cervical levels. Extracapsular spread of metastatic carcinoma is common, and often occurs before the metastatic deposit has completely replaced and/or expanded the node. Further spread of the metastatic carcinoma may result in fusion of adjacent nodes, to form a metastatic mass, and/or progressive involvement of other nodes, lower in the cervical chain. In a minority of cases, however, a different pattern of metastatic spread is seen. In these cases, micrometastases are present in nodes at several or all levels of the deep cervical chain, in the absence of a macroscopic metastatic focus. The factors determining the different patterns of nodal involvement are uncertain. The second part of my study shows that histological assessment of the primary tumour is useful in predicting the presence of lymph node metastasis in an individual patient. However, the accuracy is less than 100%. Further studies should be directed at tumour thickness, the pattern of invasion (including perineural invasion), and the microvasculature within and around the tumour.