

A Review of Oral/Pharyngeal Cancer
and
A Review of 328 Cases of Lingual Squamous Cell Carcinoma

By

Carol Oakley

D.D.S., University of Alberta, 1978
Dip. Periodontics, University of British Columbia, 1990
Ph.D., University of British Columbia, 1995

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF DENTAL SCIENCE

in
THE FACULTY OF GRADUATE STUDIES
THE FACULTY OF DENTISTRY

(Department of Oral Biological and Medical Sciences)

We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

July 1997

© Carol Oakley, 1997

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Faculty of Dentistry
The University of British Columbia
Vancouver, Canada

Date Nov 6/87

Abstract

Between 1979 and 1994, 328 cases of squamous cell carcinoma (SCC) of the tongue were admitted to the BCCA, Vancouver Branch. These cases were reviewed by Drs. Hay, Epstein and van der Meij who also established the data fields that were used in statistical analyses performed by Dr. Le. The analyzed data set comprises Chapter 3, Results, and the data set is critiqued in Chapter 4, Discussion.

The major component of the thesis is Chapter 1, Introduction which offers a broad overview of oral/pharyngeal cancer with respect to carcinogenesis, premalignancy, and treatment. In addition, Chapter 1 includes a brief review of epidemiological concepts and provides a perspective for the balance of the thesis in terms of understanding the strengths and shortcomings of previous investigations into the diagnosis, epidemiology, treatments and outcomes of oral/pharyngeal SCC. Conclusions across studies over time for trend data of oral/pharyngeal cancer cannot easily be drawn because of inconsistencies in criteria defining cancer cases and risk factors, and in data analyses and reporting. Nevertheless, analyses of the 328 lingual SCCs revealed that this case series was consistent with other reports of lingual SCC with respect to patient demographics, risk factors, tumour characteristics and survival. Among 328 patients, the mean age was 61 years with a male:female ratio of 1.5:1.0. The majority of patients had a history of alcohol and tobacco use, and the most common symptom was a sore tongue. Most cancers were early stage, well-differentiated keratinizing tumours of the oral tongue. Treatment was primarily radiation alone, followed by a combination of surgery and radiation; complications included necrosis of the soft tissues (10%) and mandible (6%). Patient follow-up ranged from 0 to 154 months. The overall all-cause survival was 41% and the stage of disease was significantly ($p < 0.001$) related to survival. SCCs of the tongue have a good prognosis if they are detected early; consequently, screening and case finding strategies are essential and should concentrate efforts on individuals at high risk due to alcohol and tobacco use.

TABLE OF CONTENTS

Abstract	ii
Table of Contents	iii
List of Figures	ix
List of Tables	x
Acknowledgements	xii

CHAPTER 1 - INTRODUCTION

I. Overview	1
II. Principles of Epidemiology	4
A. Definitions and Taxonomy	4
B. Descriptive Studies	7
1. Correlational Studies	8
2. Case Reports and Series	9
3. Cross-Sectional Surveys	10
C. Analytic Observational Studies	10
1. Case Control Studies	10
2. Cohort Studies	11
D. Measures of Disease Frequency	11
1. Ratios, Proportions and Rates	14
2. Prevalence and Incidence	15
a. Prevalence	15
b. Incidence	15
3. Crude, Category-Specific and Adjusted Rates	16
a. Crude Rates	17
b. Category-Specific Rates	17
c. Adjusted Standardized Rates	18
E. Measures of Association	19
1. Relative Risk	21
2. Odds Ratios	24
3. Attributable Risk	25
F. Chance, Confounding and Bias	26
1. Chance	26
a. p values	27
b. Confidence Interval Estimates	28
2. Confounding	29
3. Bias	30
a. Selection Bias	31
b. Observation Bias	34

G. Measurement Reliability	36
1. Pearson Correlation Coefficients	37
2. Kappa	38
H. Measurement Validity	40
1. Sensitivity and Specificity of Measurement Techniques	41
2. Diagnostic Tests	43
J. Early Diagnosis and Survival of Disease	49
K. Summary	53
III. Development and Anatomy of the Tongue	59
A. Embryological Development	59
B. Clinical Anatomy of the Tongue	60
C. Structural Anatomy of the Tongue	62
1. Mucosa	62
a. Lining Mucosa	62
b. Gustatory Mucosa	63
2. Musculature of the Tongue	63
a. Extrinsic Muscles	64
i. Genioglossus	64
ii. Styloglossus	64
iii. Hyoglossus	65
iv. Palatoglossus	65
b. Intrinsic Muscles	65
i. Vertical	66
ii. Superior Longitudinal	66
iii. Inferior Longitudinal	66
iv. Transverse	66
3. Innervation of the Tongue	66
a. Sensory Innervation	66
i. Tactile	66
ii. Gustatory	67
b. Motor Innervation	67
c. Autonomic Innervation	67
4. Vasculature of the Tongue	67
a. Arterial	67
b. Venous	68
5. Lymphatic Tissues	68
6. Lymphatic Vessels and Drainage	69
7. Papillae and Taste Buds	70
a. Papillae	70
i. Filiform	70
ii. Fungiform	70
iii. Foliate	71
iv. Circumvallate	71
b. Taste Buds	71
8. Lingual Salivary Glands	72

D. Functions of the Tongue	72
1. Speech	72
2. Mastication	73
3. Taste	73
4. Swallowing	74
5. Protective Reflexes	74
IV. Epithelium	77
A. General Characteristics	77
B. Protective Role of Oral Epithelium	78
1. Desquamation	80
2. Permeability Barriers	81
3. Cellular Defense Mechanisms	84
C. Differentiation of Oral Epithelium	85
1. Cornified Masticatory Epithelium	86
a. Stratum Basale	87
b. Stratum Spinosum	87
c. Stratum Granulosum	88
d. Stratum Corneum	88
i. Orthokeratin	88
ii. Parakeratin	88
2. Noncornified Lining Epithelium	89
3. Keratins as Markers of Differentiation	89
4. Influence of Retinoids on Epithelial Differentiation	91
D. Epithelial Proliferation	95
1. Cell Cycle	96
2. Control of the Cell Cycle	97
a. Intrinsic Mechanisms	97
i. Restriction Point	97
ii. p53 Gene and Protein	98
iii. myc Genes	98
iv. Apoptosis	99
v. Telomeres	99
b. Extrinsic Mechanisms	100
i. Regulation by Cell-Matrix Interactions	100
ii. Regulation by Cell-Cell Interactions	101
iii. Regulation by Growth Factors	102
iv. Regulation by Subepithelial Connective Tissue	104
V. Carcinogenesis	111
A. Models of Transformation	112
1. Monoclonal Transformation	112
2. Polyclonal Transformation and Field Cancerization	112
3. Contiguous Transformation	113
B. Tumour Initiation, Promotion and Progression	113
C. Molecular Basis and Biomarkers for Squamous Cell Carcinoma of the Head and Neck	116
1. Tumour Suppressor Gene p53 and Protein p53	119

a. p53 Protein	119
b. p53 Gene	120
2. c-erb B Gene, Epidermal Growth Factor Receptor and its Growth Factors	122
3. ras Oncogene Family	123
4. myc Oncogene Family	124
5. Bcl-1 and Bcl-2 Oncogenes	124
6. TGF β Growth Factor and Attachment Receptors	125
7. Retinoic Acid Receptors and Keratin Markers	125
8. DNA Content	127
D. Tumour Kinetics	128
1. Implications for Radio and Chemo Therapy	130
E. Invasion and Metastasis	133
F. Risk Factors Associated with Oral/Pharyngeal Squamous Cell Carcinoma	136
1. Tobacco	137
a. Smoking Tobacco	138
b. Smokeless Tobacco	141
2. Alcohol	142
a. Alcoholic Beverages	142
b. Alcohol-Containing Mouthwashes	144
3. Tobacco Smoking and Alcohol	144
4. Diet, Oral Health, Dental Restorations and Socioeconomic Class	147
5. Candida	149
6. Viruses	150
a. Epstein-Barr Virus	150
b. Herpes Simplex Virus	151
c. Human Papillomavirus	151
7. Genetic Factors	154
VI. Oral Premalignancy	165
A. Histopathology	165
B. Clinical Features	170
1. Premalignant Lesions	170
a. Leukoplakia	171
i. Homogeneous Leukoplakias	172
ii. Nonhomogeneous Leukoplakias	172
b. Erythroplakia	173
c. Epidemiology of Premalignant Oral Lesions	173
d. Management of Premalignant Oral Lesions	177
2. Premalignant Conditions	180
a. Lichen Planus	180
b. Submucous Fibrosis	184
c. Tertiary Syphilis	186
d. Plummer-Vinson Syndrome	187
e. Dyskeratosis Congenita	187
VII. Oral Squamous Cell Carcinoma	190
A. Histopathology	191

B. Clinical Features	195
1. Adjuvative Diagnostic Investigations	200
C. Classification and Staging of Oral/Pharyngeal Cancers	202
D. Epidemiology of Oral/Pharyngeal Squamous Cell Carcinoma	204
1. Overview	204
2. Cancer Statistics for Canada	210
3. Cancer Statistics for British Columbia	212
E. Treatment Modalities for Oral/Pharyngeal Cancer	213
1. Surgical Resection	214
2. Radiotherapy	217
a. Mechanisms of Action	217
i. Radiation Physics	217
ii. Radiation Biology	220
b. Clinical Considerations	226
c. Complications of Radiation Therapy	227
i. Acute Complications	227
ii. Chronic Complications	228
d. Clinical Applications of Radiotherapy for Oral Cancer	230
e. Radiotherapy for Lingual Cancer	232
i. Oral Tongue	232
ii. Base of Tongue	232
3. Chemotherapy	233
a. Mechanisms of Action	233
b. Clinical Applications	235
4. Treatment of Tongue Cancer at the Memorial Sloan-Kettering Cancer Center	236
F. Follow-up and Prevention	240
G. Summary	245
CHAPTER 2 - METHODS	249
CHAPTER 3 - RESULTS	252
I. Descriptive/Association Statistics	252
A. Cases by Year and Sample Demographics	252
1. Cases by Year	252
2. Gender, Age and Stage of Disease	252
B. Risk Factors	253
1. Alcohol	253
2. Tobacco	254
C. Symptoms	254
D. Tumour Characteristics	255
1. Tumour Location	255
2. Cell Type	256

3. Cell Differentiation	257
4. Tumour Status, Node Status and Stage of Disease	257
E. Treatment	258
1. Treatment Modalities	258
2. Complications of Treatment	259
II. Survival Analyses	277
A. Overall All-Cause Survival	277
B. Disease-Specific Survival	278
C. Local Recurrence	278
D. Regional Recurrence	278
E. Distant Metastasis	279
III. Summary of Results	279
 CHAPTER 4 - DISCUSSION	 286
I. Descriptive Associations	286
A. Demographics, Stages of Disease, Symptoms and Risk Factors	286
B. Tumour Characteristics	287
C. Treatment	288
1. Treatment Modalities	288
2. Complications of Treatment	289
II. Survival	292
 CHAPTER 5 - FUTURE DIRECTIONS	 301
 NOMENCLATURE	 307
 BIBLIOGRAPHY	 310

List of Figures

Figure Number	Title	Page
 <u>CHAPTER 1</u>		
Section II		
Figure 1.1	Taxonomy of Epidemiologic Studies	54
Figure 1.2	Designs of Case-Control Study and Cohort Study	55
Figure 1.3	The Natural History of a Disease	58
 Section III		
Figure 1.4	The Dorsal Surface of the Tongue	76
 Section IV		
Figure 1.5	The Cell Cycle	107
Figure 1.6	Control of the Cell Cycle	108
 <u>CHAPTER 3</u>		
Section I		
Figure 3.1	Number of Cases Admitted to Vancouver Branch, BCCA, by Year, 1979 - 1994	262
Figure 3.2	Gender and Age Distribution of Patients	263
Figure 3.3	Distribution of Stage of Disease by Age Groups	264
Figure 3.4	Distribution of Stage of Disease by Gender	265
Figure 3.5	Patient Symptoms and Duration of Symptoms	270
Figure 3.6	Distribution of Patient Symptoms by Tumour Location	271
Figure 3.7	Distribution of Patients by T Status and Clinically Positive Neck Nodes	274

List of Tables

Table Number	Title	Page
 <u>CHAPTER 1</u>		
Section II		
Table 1.1	Contingency Table to Aid in the Calculation of Measures of Association	56
Table 1.2	Comparison of a New Diagnostic Test to the Gold Standard	57
 Section IV		
Table 1.3	Distribution and Classes of Keratin Filaments in Oral Mucosa	105
Table 1.4	Summary of Some Effects of Retinoic Acid on Oral Epithelium	106
Table 1.5	Some Factors that Enhance Proliferation of Epithelial Cells	109
Table 1.6	Some Factors that Suppress Proliferation of Epithelial Cells	110
 Section V		
Table 1.7	Chromosomal Alterations in Squamous Cell Carcinoma of the Head and Neck	156
Table 1.8	Biomarkers and Possible Roles in Carcinogenesis	157
Table 1.9A	Keratin Markers in Oral Epithelial Dysplasia	159
Table 1.9B	Keratin Markers in Oral Squamous Cell Carcinoma	160
Table 1.10	Risk Factors, Odds Ratios and Relative Risks Associated with Oral and Pharyngeal Cancer	161
 Section VI		
Table 1.11	Examples of Transformation Rates of Oral Leukoplakia to Malignancy	189
 Section VII		
Table 1.12	TNM Classification of the Lips, Oral Cavity and Pharynx	246
Table 1.13	Summary of Cancer Statistics for Canada	247
Table 1.14	Summary of Cancer Statistics for British Columbia	248

CHAPTER 2

Table 2.1	Criteria for Clinical Status at Last Follow-up used in Survival Analyses	251
-----------	--	-----

CHAPTER 3**Section I**

Table 3.1	Cases of Lingual SCC by Year and Patient Demographics	260
Table 3.2	Risk Factors Associated with Patients	266
Table 3.3	Alcohol and Tobacco Consumption and Percentage Distribution by Age, Stage of Disease and Tumour Location	267
Table 3.4	History of Patient Symptoms	268
Table 3.5	Tumour Characteristics	272
Table 3.6	Summary of Treatments for SCC of the Tongue by Stage of Disease	275
Table 3.7	Surgical Treatment, Radiation Therapy, Surgical Pathology and Complications	276

Section II

Table 3.8	Prognostic Factors for Survival and p Values	280
Table 3.9	Prognostic Factors and Survival Proportions at 5 years	281
Table 3.10	Summary of Patient and Tumour Characteristics, Treatment and Survival of Patients with SCC of the Tongue Admitted to Vancouver Branch, BCCA Between 1979-1994	283

CHAPTER 4

Table 4.1	Survival of SCC of the Oral Tongue and BOT by Study, Site, Stage of Disease and Treatment Focus	296
Table 4.2	Complications of Radiotherapy by Study, Site and Treatment Focus	299

Acknowledgements

I would like to acknowledge the Master of Dental Science Thesis Committee, Dr. N. Le, Dr. J. Epstein, and Dr. B. Blasberg as well as Dr. L. Zhang who withdrew from the Committee but kindly agreed to act as Faculty Examiner of the thesis.

In particular, I am indebted to Dr. Nhu Le for his analysis not only once, but twice of the data set that comprised the Epstein BCCA case series! I would like to thank Dr. Joel Epstein for his review of the thesis and for his helpful comments. I would also like to thank and acknowledge the contributions of Dr. J. Epstein and Dr. E. van der Meij for generating the data set that was provided to Dr. Le for analysis, and subsequently to myself for review and critique.

I would like to thank and acknowledge Mr. Jack Zolty who very kindly contributed his computer expertise to create the seven graphs in Chapter 3. I would also like to acknowledge the helpful librarians at the BCCA who provided numerous journal articles for review in the thesis. I am grateful to Dr. Don Brunette, Associate Dean, Faculty of Dentistry, for providing generous access to a Macintosh computer with Word Perfect Software and a laser printer, as well as for providing the photocopied versions of this thesis for the use of the Committee members.

I am indebted to Dr. Michelle Williams, Dr. Helen Scott and Ms. Bonnie Craig for their friendship and moral support. Finally, I would like to express my gratitude to my family and especially to my husband, John. There have been too many sacrifices made and priorities misplaced; it has been a long, hard lesson but it was well-learned.

CHAPTER 1

INTRODUCTION

I. Overview

In the United States, oral/pharyngeal cancers represent 3% (Wingo et al., 1995) to 4% (Boring et al., 1991) of all body cancers in males and 2% of all body cancers in females (Boring et al., 1991; Wingo et al., 1995). For all cancer-associated deaths in the United States, oral/pharyngeal cancer is attributable for 2% of deaths in males and 1% of deaths in females (Boring et al., 1991; Wingo et al., 1995). In Canada, cancer statistics demonstrate similar trends: in 1991, there were a total of 3017 new cases of oral/pharyngeal cancers, representing 2.8% of all body cancers, and the number of Canadian deaths attributable to oral/pharyngeal cancer in 1993 was 995 cases which represented 1.8% of all deaths attributable to cancer (National Cancer Institute of Canada 1996). For 1996, the estimated age-standardized incidence rate for oral/pharyngeal cancer was 15 per 10⁵ Canadian male population and 5 per 10⁵ Canadian female population (National Cancer Institute of Canada 1996). In general, incidence rates of oral/pharyngeal cancer are 2.5 times as high in males as in females and tumours typically occur in the fifth and sixth decades of life (Shaha and Strong, 1995). The 5-year survival rates for oral/pharyngeal cancer “average 52%” and “rates have shown little significant change over the last 15 years” (Garfinkel 1995a). However, survival rates vary considerably for different sites with lips having the most favourable survival rate at 90%, the mouth 53%, the tongue 42%, and the pharynx 32% (Garfinkel 1995a).

Squamous cell carcinoma (SSC) is a malignant neoplasm of the stratified squamous epithelium that lines the oral cavity and pharynx. SCC accounts for 80% to 90% of all oral/pharyngeal malignancies (Krolls and Hoffman, 1976; van der Waal and Pindborg, 1986), and nearly half of all SCCs of the upper aerodigestive tract occur in the oral cavity (Shaha and Strong, 1995). Excluding lip lesions, between 25% and 40% of all oral SCCs occur on the tongue (Regezi and Scuibba, 1989; Spitz 1994). In the United States, 6400 new cases of tongue cancer and 1820 deaths from tongue cancer were estimated for 1997 (Parker et al., 1997). In British Columbia,

Canada, 81 new cases of tongue cancer and 32 deaths from tongue cancer were estimated for 1996 (BC Cancer Agency et al., 1995-1996 Annual Report).

In the 16-year period between 1979 and 1994, 328 patients with SCC of the tongue were admitted to the Vancouver branch of the British Columbia Cancer Agency (BCCA). The purpose of this thesis is to provide a retrospective, descriptive analysis of that case series (Chapters 2, 3, 4). In addition, Chapter 1 of the thesis provides a general overview of oral/pharyngeal cancer and tongue cancer in particular. Chapter 1 was written with the intent of compiling an instructional aid for graduate students or dental residents who have an interest in oral oncology from either a clinical- or laboratory-based research focus. In addition to providing a broad understanding of topics such as cell biology, carcinogenesis, precancer, and cancer treatment, an introduction to Epidemiology was included to provide a perspective for the balance of the thesis in terms of understanding the strengths and shortcomings of previous investigations into oral/pharyngeal cancer, and for appreciating the potential problems encountered in integrating laboratory-based research with the clinical setting.

Chapter 1 begins with an introduction to fundamental epidemiological concepts (Section II) that should provide a perspective for subsequent sections which discuss the diagnosis, epidemiology, treatments and outcomes of oral SCC. A critique of the oral/pharyngeal cancer literature is included in Section II to illustrate certain epidemiological concepts as well as the strengths and shortcomings of previous investigations. As epidemiology is the study of disease in a population, its distribution and determinants, it is also helpful to understand the biology underlying the disease being studied. Cancer is basically a disease of cells characterized by the loss of control mechanisms that govern cell proliferation and differentiation; but in order to appreciate these aberrant processes, the normal functions and structure of the affected tissues must first be understood. Hence, the development, anatomy and function of the tongue are reviewed in Section III; the structure and function of oral epithelium and its normal proliferation and differentiation are

reviewed in Section IV. Carcinogenesis, biomarkers of malignancy and risk factors for oral/pharyngeal cancer are reviewed in Section V; oral premalignancy is discussed in Section VI, and oral/pharyngeal SCC and lingual SCC in particular are reviewed in Section VII.

In Section II, the reader will become aware of different terminologies and different methodologies that have been employed by the numerous investigators of head and neck cancers. For example, the terms “oral”, “pharyngeal” or “oropharyngeal” cancer often appeared in the literature without further clarification or specification of which anatomic sites were included by the investigators. The descriptors “oral cancer” and “pharyngeal cancer” can designate a variety of different anatomic sites which may or may not include the lips, nasopharynx, oropharynx or hypopharynx, and may or may not include tumours of the salivary glands. As well, the descriptor “tongue cancer” may or may not include both the anterior two-thirds and posterior third of the tongue, and again, details were not consistently provided by the investigators (Section II. D). Throughout this thesis, the term “oral/pharyngeal” was used as a general term encompassing the upper aerodigestive tract, but details about the specific sites included in an investigation were included if they were described by the authors, and if that information was relevant to the discussion. In similar manner, the authors’ phrases/descriptors to report results and conclusions were used; consequently, if some statements appear ambiguous or lack detail, it is because additional information to enable better understanding of the data was not provided by the authors of the cited study. In addition, if results were stated to be statistically significant, p values or confidence intervals were included if they were available in the cited study.

Throughout this thesis, figures and tables are numbered consecutively in order of appearance in the text within each chapter, hence figure or table 1.1, 1.2, etc. for Chapter 1; 3.1, 3.2 for Chapter 3, etc. Within each chapter, the figures and tables are included, in order of appearance, at the end of the section in which they are cited.

II. Principles of Epidemiology

This Section will provide definitions and descriptions of the epidemiological strategies that are encountered in the subsequent sections of Chapter 1. The intent of this section is not to provide a comprehensive review of epidemiology but, rather to provide a perspective or point of reference for interpreting the literature on oral/pharyngeal cancer.

A. Definitions and Taxonomy

Epidemiology is predicated on two assumptions; first, that human health and disease are not randomly distributed and secondly, that human disease has causal and preventive factors that can be identified (Hennekens and Buring, 1987). Implicit in these assumptions is the premise that the diseased state can be clearly distinguished from the healthy state so that the treatment that will provide the best prognosis can be selected; consequently, the classification of disease is also a predictive process and it is related to different concepts of disease (Wulff, 1976; Hennekens and Buring, 1987). Wulff (1976) distinguished two major concepts of disease, the nominalistic or patient-oriented concept, and the essentialistic concept which emphasizes disease as an independent entity.

The nominalistic concept (Wulff 1976) is based on the view that "disease" does not exist as an independent entity and that disease classification is really a classification of sick people or patients. Thus, a particular "disease" is defined by the group of characteristics which occur more often in the patients of concern than in other people; patients will have a pattern of symptoms that resemble each other, and their prognosis and treatment have some common features. The nominalistic approach does not require a definition of "normality" and recognizes that definitions of disease may vary between different societies (Wulff 1976).

The essentialistic view (Wulff 1976) is closely related to a modern concept of disease termed biochemical fundamentalism (reviewed by Dabelsteen and Mackenzie, 1987) which is based upon

the idea that disease can be described in terms of biochemistry and molecular biology. Diseases are assumed to follow regular patterns and once the underlying biochemical events are understood, the course of the disease can theoretically be predicted. Hence, the classification of disease becomes a matter of biotechnology yet defining the “normal” state is avoided by relying upon statistical terms to define disease. That is, disease is defined by the distribution of certain features in a particular population of people, and the extent to which that distribution differs from a similar assessment of a group whom the investigators consider “healthy” or not diseased (Wulff 1976; Dabelsteen and Mackenzie, 1987). This statistical approach forms the basis for utilizing biomarkers as diagnostic or screening tests, and as prognostic indicators (Sections II.H and II.J).

Patients, clinicians and researchers generally agree that disease infers a derangement in anatomy, biochemistry, physiology or psychology, yet they rarely agree on the exact criteria defining the disease or disorder that is the target of the diagnostic process. Patients, as a result of having the target disorder, exhibit symptoms which are manifestations of the disorder that they themselves perceive, and signs which are manifestations perceived by the clinician (Sackett et al., 1991). Sackett et al. (1991) suggest that the cluster of symptoms and signs comprise the “illness”, but the illness is also associated with social, psychological and economic factors of the patient’s environment and they are collectively known as the “predicament”. Thus, the act of clinical diagnosis focuses on the illness in order to identify the target disorder or disease, but it must do so in the context of the predicament (Sackett et al., 1991).

The need to correctly assess the disease or target disorder appears self-evident, yet it remains a problem in many epidemiological studies including those investigating oral/pharyngeal cancer (eg. see Section II.D). Significantly, the failure to achieve clear definitions and accurate diagnoses of the target disease will cause systematic errors in the classification of the disease which in turn, distorts the natural history of the disease, its etiology (determinants), outcomes and treatments of the disease. As well, the suspected determinants or exposures of interest must be clearly defined

and assessed. Risk markers are determinants that cannot be modified and they are intrinsic characteristics of an individual such as age, gender, genetics, race, etc. Risk factors are determinants that can be modified such as lifestyle behaviours of smoking, diet, exercise, etc.

Epidemiology is the study of the distribution, frequency and determinants of a disease in human populations. Distribution addresses the “who, when and where” aspects of the disease, and frequency involves quantifying the existence or occurrence of the disease (Hennekens and Buring, 1987). The distribution and frequency of disease are essential to formulating and testing hypotheses about the determinants or the causal/preventive factors of the disease to which individuals in a population are exposed. An epidemiologic hypothesis begins with a suspicion concerning the possible influence of a particular factor on the occurrence of a particular disease (Hennekens and Buring, 1987). This suspicion may arise from disease patterns in a population, from clinical practice, from laboratory research or even from theoretical speculation, but in each instance, the hypothesis must make biological and scientific sense. While basic research provides biological understanding of why an exposure causes or prevents disease, only epidemiology can quantify the magnitude of the exposure-disease relationship (risk) in humans and subsequently offer the possibility of altering risk through intervention (Hennekens and Buring, 1987).

The taxonomy of epidemiological studies differs in specifics but generally separates studies according to whether they focus on describing the distribution of the disease or elucidating determinants of the disease by testing specific hypotheses (Figure 1.1). Descriptive epidemiology is a series of observations about the distribution (who, where, when) of the disease and how the frequency of disease varies over time. The observer may have a suspicion about the relationship between variables (i.e. the exposure to causative agents and the disease distribution) but the observer is passive and does not interfere or manipulate the variables. The data obtained from descriptive/observational studies are used to formulate hypotheses (*a posteriori* hypotheses). Analytic epidemiology focuses on determinants of disease by testing hypotheses (*a priori*

hypotheses) that were generated by descriptive/observational studies. In analytic studies there is an explicit comparison of exposure and outcome status and the use of an appropriate comparison group permits investigation into whether exposure to particular agent(s) causes or prevents the outcome. Depending upon the degree of intervention and control of the variables by the investigator, analytic studies can be observational or experimental (Figure 1.1), (Hennekens and Buring, 1987; Sheps 1995; Brunette 1996). The degree of exposure to a particular causative/preventive agent may change over time in a cohort study (Section II.C.2) even though this change was not induced or controlled by the investigator; if the effects of disease frequency due to changes in exposure can be analyzed, the study is analytic rather than descriptive but it is not experimental (Sheps 1995).

This Section focuses on descriptive and analytic/observational studies because the epidemiologic studies relevant to this thesis have generally been limited to case series, case-control studies and a few cohort studies rather than intervention studies (clinical trials).

B. Descriptive Studies

Descriptive epidemiology describes the general characteristics of the distribution of a disease in relation to who, where and when. "Who" includes demographic factors such as age, gender, race, marital status, occupation as well as life-style related variables such as diet or medication use. "Where" refers to the geographic distribution of the disease such as rural, urban, and variations between and among countries. "When" may refer to a specific time intervals such as seasons or years, or may compare the disease frequency between different time periods such as the present and 100 years ago. Three main types of descriptive studies are the correlational study, case reports/series and cross-sectional surveys (eg. Sheps 1995).

1. Correlational Studies

Correlational or ecological studies are widely used in cancer research because these studies are inexpensive, the data are usually available and the hypotheses they generate could prove useful for future research (Brunette 1996). Correlational studies use data obtained from entire populations to compare outcome (disease) frequencies between different groups within the population during the same time period or, in the same population at different points in time. The whole population is used to correlate an exposure with an outcome but it is not known how many of the exposed individuals had the outcome, or how many of individuals with the outcome had the exposure. That is, it is not known on an individual basis, the relationship between the exposure and the outcome. The data from correlational studies may be used to generate hypothesis that can subsequently be tested in individuals. However, the data from correlational studies cannot be extrapolated to individuals from the population (ecological fallacy) and it is not possible to link an exposure to disease in the same individual (Hennekens and Buring, 1987; Sheps 1995; Brunette 1996).

Data in any epidemiological study is obtained in a sample from a particular source population. Although it is generally assumed that the sample is representative of the source population, it is not known if the findings obtained are generalizable and applicable to other populations, or in other words, if the study has external validity (Hennekens and Buring, 1987; Brunette 1996). For example, correlational studies in India suggested that chilli consumption was linked to submucous fibrosis (Section VI.B.2.b) because this condition is common among Indians and other populations who used chillies to spice their food. Yet in Mexico, South America and other Asian countries where consumption of chillies is also widespread, submucous fibrosis is unknown.

Epidemiological studies generally follow a hierarchy or progression in which correlational studies often provide the first suggestion of a link between a disease and its determinants. Case reports and series (Section II.B.2) may also raise suspicion and together with case-control and cohort studies, generate a hypothesis that can be tested in intervention studies. For example, correlational

studies in South Africa revealed that submucous fibrosis was common among women of Indian origin but rare among blacks, and corresponded with the observation that areca nut chewing was often practiced by Indian women but rarely by South African blacks. Over a 5-year period, case reports and series of submucous fibrosis in a particular Indian community corresponded with the increased popularity of areca nut chewing in that area, and indicated the presence of the habit among patients with submucous fibrosis. These observations led to the formulation of a hypothesis linking areca nut chewing to submucous fibrosis. Case control studies provided estimates of the risks for areca nut chewing and provided evidence for a dose-response relationship. Eventually, the hypothesis linking submucous fibrosis and areca nut chewing was tested in and supported by intervention studies (reviewed by Murti et al., 1995).

2. Case Reports and Series

Chapter 3 of this thesis reviews a case series of 328 SCCs of the tongue. Case reports and series are the most basic type of descriptive study, based on either a single patient or a number of patients, respectively. Cases may be selected for either a disease or exposure of interest and the report should specify which criterion was used. The proposed correlation between the exposure and outcome must be biologically plausible but the correlations obtained from individuals cannot be used to infer the same relationship in a population (atomistic fallacy), (Hennekens and Buring, 1987; Sheps 1995).

Surveillance programs such as communicable disease and cancer registries typically use accumulating case reports to suggest the emergence of new diseases or trends such as epidemics. For example, a cluster of cases of *Pneumocystis carinii* pneumonia in previously healthy homosexual men was the first indication of a previously-unknown disease, subsequently called AIDS (Hennekens and Buring, 1987).

3. Cross-Sectional Surveys

In cross-sectional surveys, the status of individuals with respect to both the exposure and disease are assessed at the same point in time. These surveys are useful for obtaining prevalence data (Section II.D.2.) but the criteria for cases must be very precisely defined in order to distinguish between prevalent (old and new cases) and incident cases (new cases only).

Cross-sectional surveys cannot determine cause or effect because the temporal relationship between exposure and outcome cannot be clearly determined. That is, cross-sectional surveys cannot distinguish whether the exposure preceded the outcome or whether the outcome affected the level of exposure. Nevertheless, the study should be structured so that the exposure does not appear to be contiguous with the outcome (disease) and that the exposure can logically be seen to precede the outcome (Hennekens and Buring, 1987; Sheps 1995).

C. Analytic Observational Studies

Analytic studies include appropriate comparison groups for generating or testing hypotheses. In analytic observational studies, the investigator only observes the natural course of events, noting who is exposed or not exposed and who developed or did not develop the outcome, but the investigator does not manipulate the exposure. Analytic observational studies include the case control and cohort studies (Figure 1.2).

1. Case Control Studies

In case-control studies, investigators look backwards in time to assess the effect of an exposure on a disease that has already occurred (Figure 1.2). A series of patients with the disease of interest and a control or comparison group without the disease are selected for investigation, and their respective exposures are determined retrospectively. Cases and controls must be similar in all respects so that controls could have been cases if they had developed the disease of interest. Case control studies are relatively inexpensive and less time-consuming than cohort studies. Case-

control studies are well-suited for the study of rare diseases or diseases with a long latency, and to investigate a number of risk factors or potential exposures for a single outcome (such as SCC of the tongue). In case control studies both the disease and exposure have already occurred; cause and effect is therefore more difficult to establish and there is increased potential for sampling and measurement/observation bias (Sackett 1979), (Section II.F.3.). In addition, case-control studies provide odds ratios which estimate the relative risks of the exposure for the disease (Section II.E.), (Hennekens and Buring, 1987; Sheps 1995; Brunette 1996).

2. Cohort Studies

In cohort studies, investigators assemble a group of individuals without the disease of interest and classify them on the basis of the presence or absence of exposure to a factor of interest (Figure 1.2). Controls must be comparable to cases in all respects except for exposure to the determinant being investigated. In prospective cohort studies, the exposure has occurred but the outcome has not; therefore the subjects are followed for a specified period of time to determine the development of disease in each exposure group. In retrospective cohort studies, both the exposure and outcome of interest have already occurred at the time the study is started. Cohort studies are well-suited for evaluating rare exposures or multiple outcomes of a single exposure but they are more expensive and time-consuming than case-control studies which cohort studies usually follow. Retrospective cohort studies are susceptible to biases similar to case-control studies. Prospective cohort studies have less potential for selection bias but face increased potential for confounding factors (II.F.2) and loss of subjects to follow-up (Hennekens and Buring, 1987; Sheps 1995; Brunette 1996).

D. Measures of Disease Frequency

Measures of disease frequency are used to quantify the occurrence of disease relative to the size of the source population and to the time period during which the data were collected. However, unless a common time frame and common unit of population are employed, it is not possible to make direct comparisons of disease frequencies between different populations. In addition, the

definition of the disease and the reporting/recording criteria for the disease must be consistent over time and between different populations. For example, throughout the world and even within Canada, there are differences in the criteria for defining cancer cases, and registration and follow-up of cases are not consistent over time or between populations (see below). In the United States, there is no nationwide cancer registry so there is no way of knowing exactly how many new cases of cancer are diagnosed each year, and although the SEER program (Surveillance, Epidemiology and End Results) was instituted in 1973 to collect cancer data, only about 10% of the US population is covered (Wingo et al., 1995). Common problems with collecting cancer data include failure of the reported cases to be histologically verified, failure to specify site and histopathologic type, and failure to report cases altogether (Ostman et al., 1995). Regional differences in early detection activities or availability of overall diagnostic procedures can also affect cancer rates, and may explain why different Canadian provinces report different rates for cancers such as prostate and breast; moreover, increased incidence of prostate or breast cancers may simply reflect improved diagnostic procedures over time (National Cancer Institute of Canada, 1996). Incomplete or delayed reporting of cases to registries or changes in reporting also affect disease frequency; for example, in Manitoba changes in coding have considerably reduced previous artifactual overestimations of female cancers since originally-reported invasive tumours of the cervix and breast were recoded as *in situ* tumours (National Cancer Institute of Canada, 1996).

Differences in the classification of disease prevents the comparison of data from different sources. For example, comparisons of oral/pharyngeal cancer data are thwarted by inconsistent groupings of different cancer sites. That is, a variety of anatomic sites are grouped together although the lesions at these different sites may not reflect the same disease process (eg. Sauter et al., 1992; Greenblatt et al., 1994). Some reports classify sites according to specified revisions of the ICD (International Classification of Diseases; eg. ICD-9) wherein lip is designated as 140, oral tongue and base of tongue are designated as 141, major salivary glands as 142, gum as 143, FOM as 144, other and unspecified parts of the mouth as 145, oropharynx as 146, nasopharynx as 147,

hypopharynx as 148, other and ill-defined sites within the lip, oral cavity and pharynx as 149, larynx as 161, etc. The National Cancer Institute of Canada (1996) collectively groups ICD-9 sites 140-149 together under the heading of "oral" cancer. The BC Cancer Agency et al., (1995-1996) consider the lip, tongue, mouth and pharynx as separate cancer sites in their data analysis, but it is not clear whether base of tongue lesions are included under tongue or pharynx (see Section III). In addition to official registries, different investigators of oral/pharyngeal cancer have also included a variety of site categories in their analyses; for example, Blot et al (1988) and Winn et al., (1991) included ICD-9 sites 141-149 as "oral and pharyngeal" but excluded 142 (salivary glands) and 147 (nasopharynx); Cox et al. (1995) and Ostman et al. (1995) used the term "oral cancer" to include the lip, tongue, salivary glands, mouth and all parts of the pharynx; Boffetta et al. (1992) used the term "oral cancer" to include the oropharynx and oral cavity but it was not clear whether lip and salivary cancers were included; Brugere et al. (1986) included lips, oral cavity, larynx and pharynx but it was unclear whether nasopharynx was also included; Oreggia et al., (1991) limited their analyses to the "tongue" but did not specify whether the base of tongue (BOT) was also included.

An obvious advantage to grouping together all cancers of the oral cavity and/or pharynx is the inclusion of a greater number of cases which enhances the power of statistical analysis. However, this approach precludes the analysis of data by specific sites which may represent site-specific etiologic effects and site-specific mutational differences (eg. Sauter et al., 1992; Greenblatt et al., 1994). That is, carcinogens like tobacco and alcohol may have different effects in different sites, but risks for individual sites can not be determined from pooled data. Moreover, while most reports included men and women, some investigations (eg. Brugere et al., 1986; Macfarlane et al., 1995) were limited to men and excluded women. Overall, general conclusions across studies and between countries over time for trend data of oral/pharyngeal cancer cannot easily be drawn.

1. Ratios, Proportions and Rates

The number of cases of a disease or outcome relative to the population in which they occurred can be described by three types of mathematical relationships: ratios, proportions and rates. Ratios take the general form " a/b " in which " a " and " b " are not necessarily related to one another and no specific relationship between them is implied, such as the number of females to males, or the number of smokers to nonsmokers. Ratios have no units and are a general term for more specific measures such as proportions, percentages and rates. Proportions are a type of ratio with the general form " $a/a+b$ " where observations in the numerator (" a ") are included in the denominator (" $a+b$ "). Ratios of a part to the whole are often expressed as percentages; for example, 66% of all oral/pharyngeal cancers occur in smokers and alcohol drinkers (Blot et al., 1988). A rate is a ratio in which the numerator and denominator are distinctly related to one another and the denominator is a function of time which is reflected in time units (Hennekens and Buring, 1987; Sheps 1995; Brunette 1996). A rate may be a crude rate or a category-specific rate (see II.D.3 below).

The term 'rate' is often used indiscriminantly to refer to rates, proportions or ratios and it is often unclear what measures constitute the numerator and denominator. That is, it is often unclear whether the numbers represent the number of events, or the number of individuals in whom the events occurred. For example, in describing the transformation frequency of premalignant lesions (Section VI.B) to malignancy, some investigators utilized the number of lesions (eg. Mashberg et al., 1973; Cawson and Binnie, 1980) as their measure; some investigators designated the number patients (eg. Einhorn and Wersall, 1967; Banoczy 1977; Murti et al., 1986) as their measure even though more than one lesion occurred in individual patients (eg. Mincer et al., 1972; Silverman et al., 1984, 1985), and some investigators utilized both lesions and patients without clear distinction (eg. Lummerman et al., 1995), (Table 1.11, Section VI). Moreover, the proportion of premalignant lesions that subsequently become malignant is typically referred to as the "transformation rate" (Section VI.B) yet the relevant time intervals are often obscured elsewhere in the article, are not included directly in the rate, and vary from 1 year to over 40 years without

conversion to an annual rate (Table 1.11, Section VI). As a result, the literature is confusing and generally non-comparable.

2. Prevalence and Incidence

Prevalence and incidence are the most commonly used measures of disease frequency in epidemiology.

a. Prevalence

Prevalence is the proportion of individuals in a population which have the disease at either a specific point in time (point prevalence) or during a specified time period (period prevalence). Time units are generally not included in prevalence and therefore prevalence is not a “true rate” (Sheps 1995). Prevalence also provides a crude estimate of the probability or risk that a given individual in the source population will have the disease at a certain time, either calendar time or during their lifetime (Hennekens and Buring, 1987; Sheps 1995)..

$$\text{Prevalence} = \frac{\text{Number of Existing Cases}}{\text{Total Population}}$$

The number of existing cases includes both “old” and “newly-diagnosed” cases of the disease (Hennekens and Buring, 1987; Sheps 1995).

b. Incidence

Incidence is a measure of the natural frequency of disease in a population and it provides an estimate of the probability or absolute risk than an individual will develop the disease during a specified period of time (Sheps 1995). Incidence is a crude rate (see Section II.E. below) and there are two measures of incidence, cumulative incidence (CI) and incidence rate or density (ID).

$$\text{Incidence (CI)} = \frac{\text{Number of New Cases During a Given Period of Time}}{\text{Total Population at Risk}}$$

Cumulative incidence refers to the number of new cases of disease that develop in a population of individuals at risk during a specified time interval. “At risk” refers to individuals who have not yet developed the disease but who could potentially develop the disease. Consequently, individuals who already had or currently have the disease, or individuals who cannot develop the disease should be eliminated from the denominator (Hennekens and Buring, 1987; Sheps 1995). For example, in calculating the incidence of caries, individuals with existing restorations but without present caries should be excluded from the numerator because they represent “old” cases of caries rather than “new” cases; if they were included, the resultant measure would overestimate the true incidence of caries. In like manner, individuals without natural teeth should be excluded from the denominator because they are not at risk for the disease. When persons not at risk are included in the denominator, the resultant measure underestimates the true incidence of disease.

Cumulative incidence assumes all individuals at risk have an equal chance for being diagnosed as a case, and that the entire population at risk at the start of time period was followed for the entire time period. These assumptions are often unrealistic as individuals are lost to follow-up, resulting in variable lengths of observation for the participants. Therefore, incidence rate or incidence density which measures the instantaneous rate of development of disease in a population, is used (Hennekens and Buring, 1987; Sheps 1995).

$$\text{Incidence Rate} = \frac{\text{Number of New Cases of Disease During a Given Time Period}}{\text{Total Person-Time of Observation}}$$

3. Crude, Category-Specific and Adjusted Rates

Rates can be presented for an entire population (crude rate) or for segments or strata of the population based on particular characteristics such as age, gender, etc (category-specific rates).

a. Crude Rates

Crude rates refer to an entire population and they are a summary measure of the total number of cases of the outcome in the population divided by the total number of individuals in that population in a specified time period. For example, in 1990 the number of new cases of oral/pharyngeal cancer in the United States was 10.4 per 10⁵ population, and the mortality rate from oral/pharyngeal cancer was 3.0 per 10⁵ population (Garfinkel 1995a).

Crude rates are easy to calculate and comprehend, and they represent the actual experience of a particular population. Consequently, crude rates are useful for making decisions regarding health resource utilization and public health planning. However, a problem arises in comparing crude rates among different populations because different populations may differ with respect to certain underlying characteristics such as age, gender or race which may confound (Section II.F.2) the outcome. For example, cancer mortality rates rise dramatically with increasing age and a population in which the elderly represent a large segment of the population will have a higher crude cancer mortality rate than other populations in which all age groups are more evenly represented. In order to account for differing distributions of a characteristic between populations being compared, category-specific rates in each population can be compared (Hennekens and Buring, 1987).

b. Category-specific Rates

Category-specific rates are calculated for specific categories of the population based on particular characteristics such as age, gender, or race, etc. By grouping or stratifying the data for the different characteristics, the rates are unconfounded for that characteristic and provide the most detailed information about the pattern of disease in that subgroup. For example, the crude rate of new tongue cancers in BC in 1994 of 3.5 per 10⁵ population can be separated into category-specific rates such as by gender: rate of new tongue cancers for BC males (2.6 per 10⁵ population) and females (0.9 per 10⁵ population). The crude rate can also be categorized by age which

demonstrates that in 1994, the highest rates of new tongue cancers for males and females in BC occurred between ages 60 and 79 years (BC Cancer Agency Annual Report 1995-1996). The category-specific rates can be compared more readily than crude rates to similar data from another time period or another geographic location, but a large number of comparisons are required. Therefore, it would be useful to have a single summary rate for each population that could account for any differences in the structure of the populations (Hennekens and Buring, 1987) and this approach is used in adjusted standardized rates.

c. Adjusted Standardized Rates

Adjusted standardized rates are crude or category specific rates that are adjusted for differences between populations. That is, they are “summary rates that take into account differences with respect to underlying characteristics that differ in distribution among two populations” (Sheps 1995). Once rates have been adjusted for a particular characteristic (eg age), any remaining observed differences between the populations cannot be attributed to confounding by that characteristic. Adjusted standardized rates are typically used to compare morbidity and mortality rates between different geographic regions or between different time periods in relation to a standard or reference population that is typically derived from Census data. However, it is not appropriate to compare age-standardized rates when different reference populations have been used to standardize the rates.

For example, the BC Cancer Agency (1995-1996 Annual Report) used the 1971 Canadian population as the standard for calculating age standardized incidence of cancers in 1994.

Unfortunately, these rates cannot be compared to the National Cancer Institute of Canada age-standardized rates from 1987-1994 or to the 1995/96 rates because different reference populations were used in each instance. The National Cancer Institute of Canada used the World Standard Population to age-standardize cancer incidence and mortality rates from 1987 to 1994, but used the 1991 Canadian population to age-standardize rates for 1995 and 1996. The reason for the change

in reference populations was that the World Standard Population is much younger than the 1991 Canadian population; consequently, the National Cancer Institute rates in 1995 and 1996 are about 30-50% higher than those calculated using the World Standard Population between 1987 and 1994. Hence, the increased rates in 1995/96 do not reflect a sudden increase in the number of cancer cases and deaths; instead, they more closely reflect the actual incidence of cancer per 10⁵ Canadian population which has a much higher proportion of people in the older age groups, in which cancer is much more common (National Cancer Institute of Canada, 1996). Similar discrepancies in reference populations exist in age-standardized cancer mortality rates calculated for the United States. Prior to 1992, US Cancer mortality statistics were standardized to the 1970 US population; starting in 1992, mortality rates were age-adjusted to the WHO standard world population (Wingo et al., 1995).

E. Measures of Association

An epidemiologic hypothesis attempts to link a specific exposure to a disease. The relationship between an exposure and disease is known as an "association" and it refers to the statistical dependence between two variables. Association is also the degree to which the rate of disease in individuals with a specific exposure is either higher or lower than the rate of disease among individuals without that exposure (Hennekens and Buring, 1987). However, the presence of an association does not imply that the observed relationship is one of cause and effect. Judgements of causal association must first determine whether the observed association between an exposure and disease is valid. That is, whether or not the data reflect the true relationship between the exposure and the disease, and this becomes a matter of determining the likelihood that alternative explanations such as chance, bias or confounding (Section II.F) could account for the findings. If these factors are determined to be unlikely explanations for the data, then it can be concluded that a valid statistical association exists between the exposure and the disease (Hennekens and Buring, 1987).

However, the presence of a statistical association (Section II.F) is still not sufficient to establish the observed relationship as one of cause and effect. Causal association must be evaluated with respect to strict criteria (eg. Hennekens and Buring, 1987; Brunette 1996) that include consistency of the findings with other investigations, the strength of the association, biologic gradient (dose-response relationships), temporal sequence of causes and events, biological plausibility, and substantiation by experimental research (Hennekens and Buring, 1987; Sheps 1995). For example, Newcomb and Carbone (1992) reviewed the data for support of a causal association between cigarette smoking and cancer. First, consistency has been demonstrated through numerous studies of different designs in diverse populations over many time periods. Secondly, the strength of the association (odds ratios, relative risks) has been consistently observed to be positive, and thirdly, a dose-response is evident for all cancers with a suspected link to cigarette smoking. That is, gradients of risk are observed with increasing numbers of cigarettes smoked, earlier age at initiation, greater total number of years smoked, degree of inhalation and type of cigarettes smoked. Temporality has been demonstrated by numerous prospective studies which established that smoking exposure occurred prior to the cancer, and by the higher proportion of premalignant changes observed in smokers as compared with nonsmokers. Moreover, risks of most smoking-related cancers diminish after cessation and with increased duration of abstinence. Finally, biological plausibility is supported by the natural history of cancer, and by the biological effects of cigarette smoke demonstrated by molecular biology techniques and experimental animal models. Thus, there is persuasive evidence that cigarette smoking has a causal role in the development of cancer at many sites (reviewed by Newcomb and Carbone, 1992).

The magnitude of the observed association is useful to judge the likelihood that the exposure itself affects the risk of developing the disease and therefore, increases the likelihood of a causal relationship (Hennekens and Buring, 1987). Measures of association compare the frequency of disease between two populations, using an overall summary measure that estimates the association between an exposure of interest and an outcome of interest. Thus, the risk or probability of

developing a disease over a specified period of time, can be compared in relation to the absence or presence of an exposure and the most frequently used measures of association are the relative risk, odds ratios and attributable risk (Table 1.1).

1. Relative Risk

In cohort studies, potential exposures or risk factors are identified at the start of the study, prior to the development of disease. Therefore, incidence data regarding the natural frequency of disease are generated and relative risk (RR) can be calculated by using either cumulative incidence or incidence density. Relative risk is the ratio of the incidence of disease (absolute risk) in the exposed group to the corresponding incidence of disease or absolute risk in the nonexposed group, and it indicates the likelihood of developing the disease in the exposed group relative to the group that was not exposed (Hennekens and Buring, 1987; Greenstein and Lamster, 1995), (Table 1.1).

$$\text{Relative Risk} = \frac{\text{Absolute Risk (Incidence) when the Risk Factor is Present}}{\text{Absolute Risk (Incidence) when the Risk Factor is Absent}}$$

The value of relative risks calculated as the ratio of two absolute risks depends upon the time period over which the risks were calculated because the frequency of disease (incidence) may change depending upon the length of observation; that is, the relative risk after 10 years may differ considerably from that after 1 year. Therefore, it is important to specify the time period on which the calculation of the risk ratio was based (Hennekens and Buring, 1987), (eg. see Table 1.10, Section V).

A relative risk of 1.0 indicates that the incidence of disease in the exposed and nonexposed group are identical and therefore, there is no association between the exposure and the disease. A value less than 1.0 reflects an inverse relationship between the exposure and disease, a decreased risk or protective effect of the exposure. A value greater than 1.0 indicates a positive association, or an

increased risk of disease among those exposed to the factor of interest (Hennekens and Buring, 1987). Some epidemiologists contend that the mere fact that a factor has a risk ratio greater than 1.0 is not in itself a sufficient basis for implicating that factor in the causation of the disease in question. Taubes (1995) and Barnett and Mathisen, (1997) maintain that “unless there is a high degree of biological plausibility for a given factor causing a specific disease, a given risk factor should have a risk ratio of at least 3.0 or 4.0 before it is implicated in the causation of the disease”.

Although relative risk provides an estimate of the strength of the association between an exposure and disease, it is of limited value in predicting risk of impending disease for any individual. For example, a relative risk of 3.9 calculated for individuals who smoke tobacco products (Table 1.10, Brugere et al., 1986) means that these individuals have 3.9 times the risk or are 290 percent (i.e. 3.9 minus the null value of 1.0, times 100; based on Hennekens and Buring, 1987, page 78) more likely to develop oral SCC than nonsmokers. However, the important unanswered question for an individual is, “3.9 times the risk” of what? The information that is needed, is knowledge of the absolute risk or incidence of SCC in nonsmokers. In addition, extrapolating from relative risks calculated in a population to absolute risks for an individual must be done cautiously because it may have different significance, depending upon the circumstances. As Greenstein and Lamster, (1995) illustrate, a relative risk of 10 can indicate risks of 1 per 10^6 in those without the factor : 1 per 10^5 in those with the risk factor. A relative risk of 10 can also indicate a risk of 1 per 10^2 in those without the factor : 1 per 10 in those with the factor; thus, the same relative risks have different absolute risks for the individual (Greenstein and Lamster, 1995).

Only a few studies (eg. Brugere et al., 1986; Murti et al., 1986; Gupta et al., 1989; La Vecchia et al., 1991; Oreggia et al., 1991; Jovanovic et al., 1993a) have reported relative risks rather than odds ratios (see II.E.2. Odds Ratios below) as the measure of association between risk factors and oral/pharyngeal cancer. In one instance (Jovanovic et al., 1993a), the authors clearly stated that odds ratios were used to obtain estimates of the relative risks, and descriptions of methodologies in

the balance of the reports suggest that odds ratios were also used to estimate the relative risks which were reported. For example, La Vecchia et al. (1991) used a case control study to assess the relationship between diet and oral/pharyngeal cancer and clearly, incidence data were not available to calculate RR directly. Unless certain assumptions are made and are clearly stated, odds ratios can not be assumed to be the equivalent measure of association to relative risk (see Section II.E.2 below; Hennekens and Buring, 1987). Gupta et al. (1989) selected a group of 12,212 individuals from a rural region in India who used tobacco. Over 8 years, they performed periodic examinations for the presence of clinically-evident oral precancerous lesions (Section VI) or malignancy, and performed selected biopsies when indicated by clinical suspicion. A control group of nonsmokers was not included and neither the prevalence nor incidence of premalignant lesions or malignancy in nonsmokers was provided, yet relative risks for various oral conditions were reported. The average periods of follow-up varied among the different types of precursor lesions rendering comparisons between relative risks of the different lesions invalid. Moreover, neither p values nor confidence intervals (Section II.F.1) were provided and assessment of significance was limited to a single statement in regards to lichen planus (see also Table 1.10, Section V).

The apparent differences in risk calculations for oral/pharyngeal cancer (Table 1.10, Section V) between different studies may reflect inherent, true differences in the populations that were studied. However, the selection of cases and controls is prone to a multitude of biases (Section II.F.3) which can distort the results. In addition, the definition of exposures and the classification of groups by degree of exposure varies considerably between studies. For example, in assessing the association between alcohol consumption and oral/pharyngeal cancer some investigators (eg. Brugere et al., 1986; La Vecchia et al., 1991; Jovanovic et al., 1993a) used the quantity of pure alcohol consumption as the unit of exposure. La Vecchia et al. (1991) converted each beverage type to a standard measure of ethanol content; thus, 150 ml wine = 330 ml of beer = 30 ml spirits = 12 ml ethanol; consumption was ranked as low (<4 drinks of 12 ml ethanol/day), moderate (4-6

drinks/day) or high (>6 drinks/day) and corresponded to RR of 1.7 (NS-not significant) for moderate consumption and 5.8 for high consumption (La Vecchia et al., 1991). Brugere et al. (1986) considered that 1 glass of any alcoholic beverage contained the same quantity of pure alcohol (i.e. 15 grams (g) of ethanol) so that 1 liter of wine = 80 grams ethanol = 6 glasses; they estimated a RR of 1.0 (NS) for consumption of 0-39 g/day, RR of 2.7 for 40-99 g/day, RR of 13 for 100-159 g/day, and RR of 70 for ≥ 160 g/day. Jovanovic et al. (1993a) assumed that the amount of alcohol per beverage of hard liquor, wine or beer was equivalent to 10 grams of alcohol. Consumption of more than 4 drinks/day was considered heavy and corresponded to OR of 3.3 for SCC of the FOM.

In contrast, Mashberg et al. (1981) used "whiskey equivalents" where one ounce 86-proof whiskey equalled 12 ounces of beer, or four ounces of dry wine with an alcohol content of 11-12%. Consumption of 6-9 "whiskey equivalents"/day corresponded to a RR of 15.2 and heavy consumption of over 10 "whiskey equivalents"/day corresponded to a RR of 10.6 for oral/pharyngeal SCC. Oreggia et al. (1991) used total volumes of consumption as equivalent measures between the different types of alcoholic beverages so that >200 ml/day of wine had a RR of 5.8 and >200 ml hard liquor/day had a RR of 3.3. Blot et al. (1988) used the number of drinks per week as their unit of comparison and for >30 drinks/week, RR for wine was 2.5 (NS), RR for beer was 4.7 and RR for hard liquor was 5.5. Overall, it is difficult to determine to what degree the exposures in the different studies are equivalent and to what extent the risks may be compared.

2. Odds Ratios

In case control studies, participants are selected on the basis of disease which has already occurred, and the size of the diseased and nondiseased groups do not necessarily reflect the natural frequency of disease. Consequently, it is not possible to calculate the rate of development of disease, and incidence data are not available to calculate relative risk. However, the relative risk can be estimated by calculating the ratio of the odds of exposure among the cases to that among the

controls but odds ratios can not be used to predict an individual's chance of developing the disease.

$$\text{Odds Ratios} = \frac{\text{Odds that a Case (Disease Present) is Exposed}}{\text{Odds that a Control (Disease Absent) is Exposed}}$$

In like manner to relative risk, an odds ratio of 1.0 indicates that there is no association between the exposure and the disease; values greater than 1.0 indicate an association between the exposure and the disease.

While odds ratios provide an estimate of relative risk, there are also differences between odds and the probability (risk) that an event will occur. In risk calculations (Table 1.1), the numerator indicates the number of times an event occurred, and the denominator displays the number of times the event could have occurred. In odds, the numerator also includes the number of events that occurred but the denominator indicates the number of times it did not occur. For example, the probability of drawing an ace from a deck of cards is 4/52 or 1/13 but the odds of drawing an ace are 4/(52-4) = 4/48 or 1/12 (Greenstein and Lamster, 1995; Brunette 1996). Although odds are slightly different than probability calculations, odds provide a valid estimate of risk under conditions that prevail in most case-control studies: the disease must have a prevalence of less than 10%, the cases of disease must be newly diagnosed (incident) and old (prevalent) cases must not be included, the selection of cases and controls must not be based on exposure and finally, the ratio of cases:controls must be 1:1 (Hennekens and Buring, 1987; Sheps 1995).

3. Attributable Risk

In contrast to relative risk which is used to determine if a causal effect exists between the exposure and disease, attributable risk (Table 1.1) assumes that a cause-effect relationship exists.

Attributable risk (AR) is also known as the risk difference because it measures the absolute differences in disease frequency between exposed and nonexposed populations and represents the excess risk of disease among the exposed that would remain if risks attributable to all other

competing exposures were removed. Attributable risk is well-suited for public health purposes since AR values greater than '0' represent the number of cases that could be prevented if the exposure was removed. If AR equals '0', there is no difference in disease frequency between exposed and nonexposed groups and therefore, no association between the exposure and disease. AR values less than '0' indicate that the exposure is beneficial (Hennekens and Buring, 1987; Sheps 1995).

F. Chance, Confounding and Bias

For any individual epidemiological study, the observed association between exposure and disease may be valid and reflect the true nature of relationship. It is also possible that the findings have an alternative explanation and result from chance (random errors), bias (systematic errors) or confounding. Chance can occur anytime a sample from a source population is selected and it reflects the "luck of the draw". Bias occurs when a systematic error is made in selecting the sample (selection bias) or in the way information was obtained and reported (observation or measurement bias). Confounding occurs when the effect of some other variable(s) that existed between groups in the sample was not recognized or controlled.

1. Chance

Epidemiological studies assume that evaluation of a sample can be used to draw inferences about the source population but because of chance or random variation from sample to sample, it is unlikely that any two samples from the same total population will be identical. The degree to which chance affects the findings in any particular study is largely determined by the size of the sample. In general, the smaller the sample on which the findings are based, the more variability and less reliability or reproducibility there will be of the findings; conversely, the larger the sample size, the less variability and the more reliable the inference (Hennekens and Buring, 1987; Brunette 1996).

a. p Value

Statistical tests are powerful tools used to quantify the degree to which random errors or chance may account for the results observed in any individual study. Tests of statistical significance report a measure, the “p value”, which is the probability or likelihood that the observed results are due to chance alone. P values and levels of significance are typically used with the statistical test of a hypothesis to arrive at a conclusion such as whether the observed differences between two groups are due to chance or whether the differences are real. By convention, medical studies set the p value or level of significance at 5% (0.05) or 1 in 20 probability, and if p values calculated by the test are less than or equal to 0.05, the results are deemed statistically significant meaning that there is no more than a 5% probability that a result as extreme as that observed was solely due to chance. Mills (1993) explains that “a p value of 0.05 means that there is a 5% chance of concluding that the two groups differ when they actually do not (type error I)” and a “95% probability of correctly concluding that there is no difference when no difference is present”. A statistically significant result does not prove that chance could not have accounted for the findings, only that chance is an unlikely explanation, and it can offer no information about the actual magnitude of the differences between the groups. Moreover, a significant p value cannot assess the adequacy of the study design or rule out the possibility that results may be due to bias or confounding. Conversely, p values greater than 0.05 do not mean that chance was responsible for the findings or that the association cannot be causal; it only means that chance cannot be excluded as a likely explanation (Norman and Streiner, 1986; Hennekens and Buring, 1987; Potter 1994; Brunette 1996).

Unfortunately, many studies use statistical significance or lack thereof as the sole criterion for decision-making, failing to differentiate statistical significance from clinical significance or from biological plausibility (eg. Sackett et al., 1991; Barnett and Mathisen, 1997). It should also be realized that p values are a composite measure that reflect both the sample size and the magnitude of the difference between two groups. If the sample size is too small, even a large effect may not achieve statistical significance and conversely, even a small effect may be statistically significant if

the sample size is sufficiently large (Norman and Streiner, 1986; Hennekens and Buring, 1987; Potter 1994; Brunette 1996). To overcome this problem, a related but more useful measure, the confidence interval estimate, is used.

b. Confidence Interval Estimates

Confidence interval (CI) estimates provide a range of values within which the true magnitude of the effect lies. This range has a designated likelihood or probability (usually 95%) to include the real but unknown mean value. Technically, a 95% CI means that if the same study were repeated 100 times with subjects from the same source population, 95 of the 100 confidence intervals would contain the true value of whatever was being estimated in the study (Mills 1993). Confidence intervals provide all the information of p values in terms of deciding whether an association is statistically significant. The effect of sample size is reflected in the width of the confidence intervals; the narrower the intervals, the lower the variability in estimating the effect which in turn, reflects a larger sample size. In contrast, wider intervals and greater variability reflect smaller samples. If the null value (eg. 1.0 for relative risk and odds ratios) is included in a 95% confidence interval, then the corresponding p value is greater than 0.05 and the association is not statistically significant. In such instances, if the interval is narrow, there is likely no real effect of the exposure whereas a wide interval suggests that the sample size was inadequate and did not have sufficient statistical power to conclude that chance was not a likely explanation for the findings. Thus, the p value and confidence intervals, together, provide the most information about the role of chance (Hennekens and Buring, 1987; Mills 1993; Potter 1994), but they “still ignore the systematic errors, the biases and confounders, that can overwhelm the statistical variation” (Taubes 1995).

In assessing risk factors for oral/pharyngeal cancers, many authors have included p values and confidence interval estimates (eg. Brugere et al., 1986; Boffetta et al., 1992; Franceschi et al., 1992; Oreggia et al., 1991; Barasch et al., 1994; Bundgaard et al., 1994), some included confidence intervals alone (eg. Blot et al., 1988; Winn et al., 1991) and some included neither for

the vast majority of calculations presented (eg. Gupta et al., 1989). To illustrate the usefulness of confidence intervals, the following examples of odds ratios for smoking and oral/pharyngeal cancer in a series of 150 oral SCC are given (Barasch et al., 1994), (see also Table 1.10, Section V). Smoking and SCC of the floor of mouth (FOM) had an OR of 38 (95% CI=4.6-316); whereas smoking and SCC of the tongue had an OR of 1.75 (95% CI=0.63-4.9). These authors correctly concluded that their data showed that smoking was more strongly associated with SCC of the FOM than SSC of the tongue. The CI for the OR of FOM did not include the null value and therefore, the association was statistically significant although the wide CI intervals reflect great variability which is characteristic of a small sample size. The CI of the OR for the tongue included the null value (1.0) and the CI interval was very narrow which together, indicate the lack of statistical significance and the likely absence of any real effect of the exposure. However, the authors (Barasch et al., 1994) advised caution in the interpretation of their data as the study was not a case control (no noncancer control group) and had no information about alcohol consumption which is a confounder for tobacco use (see below).

2. Confounding

Confounding occurs when there is a mixing of the effects between the exposure, the disease, and a third factor that is associated with the exposure and independently affects the development of the disease (Hennekens and Buring, 1987). Thus, the observed relationship between the exposure and the disease can be attributed wholly or in part to the third factor, the confounder which can cause either an increase or decrease of the true association between the exposure and disease. Confounding factors are hidden variables in the population being studied and they can generate an association that may be real but is not what the investigator thinks it is.

The association between the confounder and the disease does not have to be causal; for example, age is always considered to a potential confounder although it can act as a surrogate for other etiologic factors. The confounder must be associated with the disease but independent of its

association with the exposure, and it must be associated with disease among nonexposed individuals. For example, alcohol and smoking are both confounders for oral/pharyngeal cancer. Both are independent risk factors for oral/pharyngeal cancer and their effects are difficult to separate because in the general population, people who drink alcohol also tend to smoke, and people who smoke also tend to drink alcohol. In like manner, most oral/pharyngeal cancer patients have smoked and consumed alcohol (Section V.F.). Consumers of tobacco and/or alcohol typically under-report their habits and they may also use mouthwashes to disguise their habits. Consequently, the results of a study evaluating the association between oral/pharyngeal cancer and the use of alcohol-containing mouthwashes (Winn et al., 1991) was confounded due to the under-ascertained exposure to alcohol and tobacco, and resulted in overestimation of the association (Shapiro et al., 1996).

A confounder cannot be identified by a statistical test of the association between an exposure and disease. Instead, confounders may be identified after re-analysis or stratification of the data for the suspected confounder reveals a change in the measures of association. Ideally, potential confounders should be considered in the design phase of study but they can be corrected for during analysis (Hennekens and Buring, 1987; Sheps 1995; Taubes 1995) as is typically done in oral/pharyngeal cancer studies (eg. Blot et al. 1988; Winn et al., 1991).

3. Bias

Bias is any systematic error that is introduced into the design or conduct of a study. Bias results in incorrect estimates of association between the exposure and disease, and bias is more difficult to evaluate than chance or confounding which can be quantified. Bias can occur during the process of identifying the study population (selection bias) or during the measurement of information about the exposure or the outcome (observation bias). Sackett (1979) has catalogued no less than 56 types of bias and the following is a brief review of common biases that affect epidemiological studies in general and have affected studies of oral/pharyngeal cancer.

a. Selection Biases

Selection bias is due to differential diagnosis, surveillance or referral in a study which results in an observed relationship between the exposure and disease that is different among those who participated in the study and those who would have been eligible but were not selected to participate (Hennekens and Buring, 1987; Sackett et al., 1991). Selection bias is a great concern in case control and retrospective cohort studies because the disease and exposure have already occurred. Selection bias is less likely to occur in prospective cohort studies because the exposure is ascertained before the outcome. All types of epidemiological studies can incur selection bias if the criteria used to define a disease or an exposure differs between studies, rendering the studies non-comparable. This problem may occur with contemporaneous studies or in studies conducted over different points in time. Differential diagnosis, surveillance or referral may occur because the same condition has received different diagnostic labels (diagnostic vogue bias), prevalent cases may not be clearly distinguished from incident cases (prevalent/incident case bias), the common starting point for a disease or an exposure is not identified (starting time bias), or the referral of cases and controls differs (referral filter bias and centripetal bias) (Sackett 1979; Hennekens and Buring, 1987; Sackett et al., 1991; Taubes 1995).

Patients with oral premalignancy or malignancy are typically seen in specialty clinics associated with a dental faculty or a hospital (eg. Silverman and Rozen, 1968). Epidemiological studies which rely on this patient pool suffer from a distorted perception of the disease and its determinants because of centripetal and referral filter biases. Centripetal bias occurs when the reputations of certain clinicians or institutions cause individuals with specific disorders or exposures to gravitate towards them. Referral filter bias occurs when individuals with a specified disease are referred from primary to secondary to tertiary care so that the concentration of rare diseases, rare causes and multiple diagnoses may increase (Sackett 1979). If controls in case-control and cohort studies are also drawn from a clinic or hospital setting, the study is predisposed to Berkson's admission rate bias because the hospitalization or admission rates of exposed and nonexposed cases and controls

will differ, and their relative odds of exposure to the putative cause will be distorted (Sackett 1979). Some case-control studies of oral cancer have used only hospital-based controls (eg. Oreggia et al., 1991; Boffetta et al., 1992; Franceschi et al., 1992) whereas others have used community-based controls (eg. Blot et al., 1988; Winn et al., 1991; Kulasegaram et al., 1995). However, even the “random” selection of controls from the community or population at large is prone to selection bias. For example, the selection of telephone numbers by random digits will omit those individuals without a telephone, who for economic and other factors, may differ significantly from individuals who do have a telephone. Moreover, individuals who are at home to answer the telephone may differ from those individuals who are not at home.

Several examples of differing criteria for diagnosis of disease or assessment of exposure exist in the oral/pharyngeal cancer literature. For example, in studies of submucous fibrosis (Section VI.B.2.b), not all investigations have included the presence of palpable fibrous bands among the diagnostic criteria, nor have the criteria for including areca nut chewing as an exposure been very clear (reviewed by Murti et al., 1995).

In 1978, the World Health Organization (WHO) defined leukoplakia (Section VI.B.a) as a “white patch or plaque that cannot be characterized clinically or pathologically as any other disease”. In essence, any white lesion that could not be identified became “leukoplakia” and such a diagnosis was directly dependent upon the clinical experience of the investigator. Moreover, the term leukoplakia has no histological connotation yet in assessing the prevalence of leukoplakia, some investigators relied solely on clinical presentation (eg. reviews by Pindborg 1980, 1994; Banoczy et al., 1993) whereas others included histopathology for selected cases (eg. Banoczy 1977; Silverman et al., 1968; Gupta et al., 1989; Brown et al., 1993) or routinely for all cases (eg. Silverman et al., 1984; Murti et al., 1986), presumably in attempts to rule out other disease entities in order to leave a “pure” sample of “leukoplakias”. Moreover, in instances where microscopic examinations provided a diagnosis of fungal infection which is a possible etiologic agent (Section

V.F.5), the clinical diagnosis of leukoplakia or erythroleukoplakia was typically retained, in some instances even after successful antifungal therapy (Section V.F.5). However, in many cases of leukoplakia, fungal infection was not assessed nor considered as an etiology in order to rule out a diagnosis of "idiopathic leukoplakia". Therefore, it is not surprising that the use of biased samples, inconsistent criteria, inconsistent diagnostic methods and the questionable reliability among investigators (see below and Section II.G) have resulted in a wide range of prevalence data for leukoplakia and its transformation to malignancy (Section VI.B.1).

Reports of the rate of malignant transformation (Section VI.B.1.c) among patients with oral leukoplakia (including fungal infection) vary from less than 1% over 2 years (Pindborg 1980) to 79% over 8 years (Gupta et al., 1989) and as high as 100% (Cawson 1975). These disparate reports may reflect differences in ethnic origin or risk factors among the different groups of patients investigated. However, the differences could also be due to the inclusion in some studies, of a broader range of benign white lesions of some other type among those classified as leukoplakias, thus giving a lower incidence of malignant transformation than for other groups (Dabelsteen and Mackenzie, 1987); conversely, some studies may have included lesions with a high malignant potential (eg. Gupta et al., 1989), thus raising the incidence of transformation.

It is difficult to reconcile disparate transformation rates over time from the same investigators in the same clinic environment. Silverman and Rozen (1968) reported an incidence of malignant transformation of 6% over 1-11 years of observation, and 16 years later reported an incidence of 18% over a mean observation period of 8.1 years (Silverman et al., 1984). Do these data reflect an altered disease pattern, or the increased clinical experience of the investigators that resulted in the exclusion of benign lesions from the latter group but that would have been included in the first (Dabelsteen and Mackenzie 1980)? Similar conflicts may be involved in the disparate reports of malignant transformation of lichen planus (Section VI.B.2.a). Some studies (eg. Silverman et al., 1985; Murti et al., 1986; Holmstrup et al., 1988) included patients with documented oral lichen

planus; another (Sigurgeirsson and Lindelof, 1991) included patients with cutaneous lichen planus but without any information about oral involvement, yet the authors estimated the risk of oral transformation based on the incidence reported by other investigators (Holmstrup 1992). Meanwhile, Eisenberg and Krutchkoff (1992) argued that true lichen planus, based on precise histological criteria, was less prevalent than commonly accepted and had no inherent malignant predisposition. The confusion may result from a wide variety of clinical lesions that are diagnosed by examiners with varying clinical experience and are included under a single label (eg. leukoplakia or lichen planus), (Ephros and Samit, 1997).

Fortunately, the problems associated with previous definitions and classifications of oral lesions have been recognized and addressed by recent attempts to provide a more uniform clinical staging procedure (Axell et al., 1996). However, other discrepancies persist. For example, in assessing the recurrence of oral dysplasia or malignancy three questions arise: (1) how can the success of treatment of either oral dysplasia or malignancy be compared in different studies (2) is it important to distinguish recurrence of disease (oral dysplasia or malignancy) from second primary events and if so (3) how can they be distinguished? Mincer et al. (1972) evaluated lesions according to the concept of field cancerization (Section V.A.2) and considered the patient's entire oral epithelium as a single site; all lesions that arose subsequent to the initial lesion were considered recurrences or transformations of the first event. Einhorn and Wesall (1967) described tumours as clinically or histologically discrete neoplasms in specific oral sites, and lesions that arose in sites that differed from the first tumour were considered new tumours. Banoczy (1977) failed to define their criteria altogether yet discussed the recurrence and transformation of oral lesions without respect to specific sites. In contrast, Day et al. (1994a, b) defined second cancers using specific criteria that included location, time of detection and histological evaluation in relation to the first lesion.

b. Observation Bias

Observation biases occur when methods of measuring exposures and outcomes, in analyzing or

interpreting the data are consistently dissimilar between the groups under study.

Recall bias is a major concern in case control and retrospective cohort studies where individuals are asked to accurately recall exposure levels to given factors. Individuals with the disease may recall and report past events differently than individuals without the disease, and there may be a tendency to under-report certain behaviours which are considered socially questionable such as excessive alcohol consumption and smoking. Knowledge by the investigator of a subjects' disease (exposure suspicion bias) or exposure (diagnostic suspicion bias) status may influence the intensity and outcome of a search for the exposure or disease, respectively. Subjects may alter their behaviours when they know they are being observed (attention bias), or they may discontinue the study (or die) so that losses to follow-up between exposed and nonexposed groups differ (withdrawal bias), (Sackett 1979; Sacket et al., 1991; Taubes 1995).

Bias can also be introduced during analysis of the data by selecting levels of significance after the statistical tests have been completed (post-hoc significance bias), by deleting outlying data (tidying-up bias), by repeatedly evaluating the accumulating data before completion of the study (repeated peeks bias), degrading or collapsing measurement scales to obscure differences between groups under comparison (scale degradation bias) or to alter interpretation of the data (magnitude bias), confusing statistical significance for biological and clinical significance (significance bias) or equating correlation with causation (correlation bias), (Sackett 1979; Sacket et al., 1991).

A common bias occurs when all possible associations between variables are examined by many independent statistical tests (data dredging bias). In the search for chance associations, the 95% probability of a correct conclusion decreases drastically. Mills (1993) calculated that "by performing just 2 independent tests, the probability that the "significant" differences found by the investigators will reflect true differences is reduced to 90% (0.95×0.95). If 20 tests are performed, the probability is only 36% (0.95^{20}), and 1 of every 20 independent comparisons will

yield a “significant” result” (Mills 1993). Results from such “fishing trips” (eg. Gorsky et al., 1994; Rubright et al., 1996) cannot be used for testing hypothesis but nevertheless, they are useful for generating hypotheses as long as the studies are identified as such and the authors do not disguise the results as conclusions of *a priori* hypotheses testing (Sackett 1979; Sackett et al., 1991; Mills 1993). In fact, chapters 2 and 3 of this thesis utilize multiple independent tests in a search for associations that may generate hypotheses.

G. Measurement Reliability

Measurement reliability refers to the ability to obtain the same measure consistently over sequential measures, and there are several sources of variability that can affect the reliability of a measurement. The first source relates to the normal biological variation inherent in the phenomenon that is being measured, such as hormone levels which may vary with the diurnal or menstrual cycle, or blood pressure which varies throughout the day and under different circumstances. Another source originates from the reliability of the measuring instrument itself. In laboratory-based studies, measurements typically involve the use of instruments such as rulers or weigh scales that are calibrated against recognized, established absolute standards, and observations are performed under well-specified conditions. Usually the results of these measurements are expressed as a standard deviation of the individual values or as confidence intervals around the calculated mean (Brunette 1996).

In epidemiologic studies, investigators may need to make judgements using criteria that are not very specific, or about subject characteristics that are difficult to evaluate. In epidemiologic studies there are no absolute standards and the best that can be done is to determine if the investigators are consistent in their judgements. Comparisons can be made in which the same investigator examines the same subjects two or more times (intra-examiner reliability), or in which different investigators examine the same subjects (inter-examiner reliability). Interobserver variability is minimized when endpoints are well-defined and quantifiable as in measuring height, and is greater when criteria are

vague and subjective, as in diagnosis of leukoplakia or dysplasia. Agreement between observers also tends to improve when result categories are few and straightforward (i.e. dichotomous categories or outcomes), (Hennekens and Buring, 1987).

Inter and intra examiner reliability can be quantitated and one approach is to calculate Pearson correlation coefficients (r).

1. Pearson Correlation Coefficients

Correlation coefficients are a measure of the association or agreement between two sets of data in which perfect agreement or the strongest positive correlation has a value of 1.0, no agreement or relationship is indicated by "0", and perfect disagreement or the strongest negative correlation has a value of -1.0. Correlation coefficients typically overestimate the true reliability and they do not explain how much of the correlation can be explained by variability of the data (Sackett et al., 1991; Brunette 1996).

For example, a patient would reasonably expect that the histological diagnosis of oral dysplasia (Section VI.A) or malignancy (Section VII.A) was reliable (reproducible) and that it represented the truth (was valid). However, in comparing the agreement of six pathologists between their original sign-out diagnosis of oral epithelial dysplasia, and their diagnosis of the same slides made several months, Abbey et al. (1995) reported that the exact agreement ranged from 0.30 to 0.63 with an average of only 0.50.

Another shortcoming of the Pearson correlation coefficient is that it cannot detect situations in which one set of data is systematically different than the others. Using the example of the 6 pathologists above, assume that pathologist A consistently assigned one lesser degree of dysplasia or severity relative to his original sign-out diagnosis, and pathologist B always agreed with his earlier sign-out diagnosis; the Pearson correlation coefficients measuring their respective

agreements between the sign-out and subsequent diagnoses would be the same. This shortcoming can be avoided by using the “intraclass correlation coefficient” which penalizes systematic errors and therefore, would assign a lower score to pathologist A. In addition, intraclass correlation coefficients may be interpreted just like kappa scores which are more commonly used (Sackett et al., 1991).

2. Kappa

A better approach in evaluating reliability is the kappa (κ) statistic because it adjusts for the degree of agreement expected purely by chance.

$$\text{Kappa} = \frac{\text{Observed Agreement} - \text{Chance Agreement}}{1 - \text{Chance Agreement}} = \frac{\text{Actual Agreement beyond Chance}}{\text{Potential Agreement beyond Chance}}$$

For a perfect association, $\kappa = 1.0$ and for no association, $\kappa = 0$. Qualitative terms in relation to kappa values vary (eg. Sackett et al., 1991; Karabulut et al., 1995; Brunette 1996), but Brunette (1996) suggests that κ values below 0.4 indicates “poor” agreement, 0.4 - 0.75 is “fair” and 0.75 - 1.0 is “excellent”.

Epidemiological investigations should include measures of reliability that assess the consistency of evaluations made by the examiners. Ideally, the study should not proceed before the investigators have been trained and calibrated with demonstrated high kappa scores (eg. $\kappa > 0.6$) which are especially crucial when definitive tests or gold standards for assessment are not available (Schechter 1995). For example, by definition a diagnosis of leukoplakia is based upon clinical rather than histological evaluation and although clinical criteria may be defined (eg. Axell et al., 1996), diagnosis is essentially a subjective judgement dependent upon the clinical experience of the investigator (Dabelsteen and Mackenzie, 1987). It is surprising, therefore, that to date no clinical investigations of oral/pharyngeal premalignancy or malignancy have investigated the reliability of

the investigators' clinical diagnoses by reporting kappa scores.

Perhaps in attempts to avoid the unreliability of clinical diagnoses of leukoplakia, some investigators (eg. Silverman et al., 1984) have relied on histopathology for diagnosis of the presence or absence of dysplasia to classify leukoplakia. The classification of disease is traditionally based on pathological anatomy (Wulff 1976) and therefore, the histopathologist's diagnosis is typically regarded as the "gold standard" which is the acknowledged standard for definitive diagnosis (Sackett et al. 1991). However, when pathologists reach a diagnosis, they may be influenced by factors other than the histomorphology of the tissue on the slide. The pathologist's knowledge of the patient's clinical presentation may be considered and incorrectly weighted in reaching a diagnosis, so that the clinical data are "double counted" (Schwartz et al., 1981). For example, a pathologist's knowledge that a biopsy specimen was taken from an area of asymptomatic erythroleukoplakia on the FOM of a heavy smoker and alcohol drinker would raise the suspicion of malignancy (eg. Mashberg 1980; Ephros and Samit, 1997) even before the slide was placed on the microscope stage. In such instances, the dysplasia or carcinoma may be unconsciously graded as more severe than if the clinical information was not available to the pathologist (Schwartz et al., 1981).

The histomorphologic criteria that characterize epithelial dysplasia (Section VI.A) or malignancy (Section VII.A) are well known to pathologists. However, the subjective interpretation of the histological features remains a problem (eg. Pindborg et al., 1985) and the "poor" to "fair" kappa scores of observer variability in the histologic assessment of oral dysplasia may be disconcerting to patients whose biopsy specimens are being examined. In the previous example of the 6 pathologists whose agreement between their original sign-out diagnosis of dysplasia and subsequent re-examinations of the same slides were compared, intraexaminer kappa scores ranged from 0.05 to 0.49 and compared to correlations of 0.30 to 0.63, respectively (Abbey et al., 1995). In the same study, interexaminer kappa scores for the presence or absence of dysplasia ranged

from 0.29 to 0.48 (Abbey et al., 1995). Karabulut et al. (1995) investigated the interobserver variability in grading sections of oral leukoplakia from no dysplasia to carcinoma in situ, and kappa values ranged from 0.27 to 0.45.

In reaching a diagnosis, a pathologist recognizes tissue changes and generally assigns more weight to some histologic features than others (Sections VI.A and VII.A). Attempts have been made to develop grading systems which focus on particular morphologic features of dysplasia or malignancy, and interobserver kappas for different histologic features ranged from 0.30 to 0.42 with the highest kappa measured for the epithelial pattern of invasion (Bryne et al., 1991a; 1991b). When stricter definitions of grading criteria are used, such as restricting assessment and diagnosis to only the most invasive margins of a tumour, an interobserver kappa of 0.63 was reported by Bryne et al. (1992).

Overall, clinicians must appreciate that a pathologist's report rarely reflects only the morphologic findings (Schwartz et al., 1981), and that there are some cases that represent the pathologist's "best guess" so that, in the end, clinical judgement remains the guiding factor in management of the patient (Kaugars 1997). Research continues into the use of biomarkers (Sections II.H. and V.C) which may provide more objective criteria than traditional histomorphology for the diagnosis of dysplasia or malignancy but to date, a valid and reliable diagnostic test has not been identified.

H. Measurement Validity

Measurement validity refers to the truthfulness of the measurement, or in other words, "does it really measure what it claims to measure?" (Brunette 1996). Determining the validity of a measurement requires a comparison to a reference measure or "gold standard" that has been accepted as true. Sensitivity and specificity are two measures of the validity of a measurement; sensitivity is the true-positive rate and specificity is the true-negative rate.

1. Sensitivity and Specificity of Measurement Techniques

Research into the prevention of a disease such as cancer requires well-defined endpoints so that the efficacy and success of a trial can be evaluated. If clinical disease (ie. the incidence of cancer) was used as an endpoint in cancer prevention studies, a large number of subjects and long durations of follow-up would be required (Pillai et al., 1992). However, if biological markers could be used as intermediate endpoints, they could act as surrogates for the disease, reveal responses in a shorter time and require fewer trial subjects to achieve statistical power. That is, biomarkers may represent changes in the continuum of events between the initiation of carcinogenesis and the final expression of clinically-evident disease (Pillai et al., 1992; Greenwald et al., 1995). Biomarkers may be used as a generic term for the target of assays that monitor critical aspects of tumour development, providing a "window into the biology of the epithelium" (Mulshine et al., 1993), (see Sections IV. and V).

When biomarkers are used as potential measures of disease, the techniques for determining the presence of the biomarker must be valid and reliable. That is, the techniques must be sensitive and determine the true level or activity of the biomarker in the tissue or cells, and they must be specific and correctly identify the absence of the biomarker or biomarker activity. For example, the sensitivity of immuno-histochemistry (IHC) as a technique to detect an antigen of interest depends upon the method used to preserve the tissue because different methods may destroy and/or mask the epitopes or antigenic determinants of that antigen, or sterically hinder access of the antibodies. The type and quality of antibodies used will determine how sensitive and specific the antibody-antigen reaction (signal) is, in relation to the background staining (noise), (eg Bacallao et al., 1990; Denk 1987). For example, IHC has been used to identify p53 protein and staining is often considered a surrogate marker for gene p53 mutation (Section V) even in the absence of confirmatory DNA studies. Moreover, the absence of reactivity with p53 antibodies can not exclude genetic alterations since the antibodies may fail to detect truncated p53 protein resulting from frameshift or nonsense mutations (Gopalakrishnan et al., 1997; Scully and Field, 1997). In a

review of 84 studies that utilized both IHC and DNA sequencing, Greenblatt et al. (1994) reported the sensitivity of IHC for detecting p53 protein as only 75% (range 36-100%) and the positive predictive value (Table 1.2) as only 63% (range 8-100%), with considerable variation among tumour types. Consequently, the status of p53 protein as determined by IHC could not be equated with either the wild-type or mutant genotype (Greenblatt et al., 1994).

Polymerase-chain techniques (PCR) are also subject to false-positive and false-negative results. PCR techniques are subject to error rates from $1/10^4$ to more than $1/500$ base pairs, depending upon the reaction conditions, and contamination of the PCR reaction may lead to false-positive or false-negative results (Greenblatt et al., 1994). In DNA studies of the p53 gene, it was established that the gene consists of 11 exons (coding sequences) and exons 2-11 code for protein p53. Most of the mutations in p53 gene occur in exons 5-8 and are single missense base substitutions (substitution of a single nucleotide pair resulting in the substitution of a single amino acid in the original gene product), although allelic loss, insertions, and deletions also occur (Greenblatt et al., 1994; Gopalakrishnan et al., 1997). Unfortunately, the predominance of mutations in exons 5-8 has caused a bias in searching for mutations so that most investigators confined their analysis to exons 5-8, effectively ignoring the remaining exons and consequently underestimating the prevalence of p53 mutations by over 20% in some tumours (Greenblatt et al., 1994).

The recovery of human papillomavirus (HPV) DNA (Section V.F.6) from oral premalignant and malignant lesions also varies with the sensitivity and specificity of the technique. Low-sensitivity techniques such as immunoperoxidase IHC or in situ hybridization can detect over 10 copies of viral DNA per cell, and moderately-sensitive techniques such as the southern blot can detect 1-10 copies of viral DNA per cell (Miller and White, 1996). When low- or moderate-sensitivity techniques are used, the prevalence of HPV in normal oral mucosa is about 7%, and the prevalence in oral SCC is 17% and 25%, respectively. However, with the use of the highly-sensitive PC reactions which can detect less than 1 copy of viral DNA per cell, the prevalence of HPV in normal

mucosa increases to 25% and the prevalence in oral SCC increases to 37% (Miller and White, 1996). Significantly, even the use of a highly-sensitive method such as PCR is not sufficient to yield valid or reliable results if techniques are not standardized. For example, HPV DNA is detected significantly more often in frozen SCC (52%) samples than in paraffin-embedded (22%) tissue. Furthermore, if late region primers are used, PCR will detect only the late region genes which encode for the capsid proteins; significantly, only the early region HPV genes are associated with malignant transformation. Thus, HPV prevalence data should be based on the identification of early region DNA with documentation of viral DNA integration into host cell DNA (Miller and White, 1996; Eversole 1997).

2. Diagnostic Tests

The potential use of biomarkers as measures of oral premalignancy or malignancy has been plagued by the inconsistency of techniques used in different investigations (eg. Greemblatt et al., 1994; see also Section V.C). In addition, many biomarker investigations have not set out to test a stated hypothesis; rather, investigators have scanned a tissue for the presence or activity of a range of suspected biomarkers which may not even be established as significant in the neoplastic process and often, the investigations are driven by the availability of a convenient technique. A major problem in interpreting the data from such studies arises from the paucity of information about the roles of the markers in normal human oral mucosa and of their variations between lining, masticatory and specialized mucosa in different oral sites. Moreover, the role of many biomarkers under inflamed, traumatized or healing conditions is not known, and unless the biomarker can be demonstrated to be absent in situations involving increased cell proliferation and cell movement such as in benign hyperplasia and wound healing, it can not be assumed that the biomarker alterations are specific to neoplastic transformation (reviewed by Johnson et al., 1980). Consequently, the use of biomarkers is hampered by the lack of a marker that is present in all malignant cells but is absent from normal mucosa, although the combined use of two or more markers may be more accurate indicators of precancer or cancer than single markers (Mulshine et

al., 1993; Ogden 1997). In general, biomarkers are tumour-associated rather than tumour-specific, and the only way a biomarker can be evaluated is by means of follow-up studies, yet it is not possible to follow a lesion histologically without altering the lesion (Dabelsteen 1980). Several biomarkers (Section V.C) show potential as diagnostic aids but none are currently used in the routine diagnosis or screening of oral premalignancy or malignancy (eg. Lippman et al., 1990; Pillai et al., 1992; Mulshine et al., 1993). Moreover, a marker that performs well as an indicator of disease progression is not necessarily useful for the early detection of the same disease. That is, a Bayesian approach (eg. Brunette 1996) is relevant to the use of an early-detection tool in monitoring high-risk individuals for detection of second cancers, but the same tool may not be appropriate for screening the population for first cancers of the same type (see below; Schechter and Sheps, 1985; Mulshine et al., 1993; Epstein et al., 1997).

The changes in activity or level of any physiologic, biochemical or molecular marker are typically reflected by continuous measures yet the presence or absence of an abnormality or disease is typically a dichotomous diagnosis (i.e. normal versus abnormal, or health versus disease), although gradations of abnormalities are also used (eg. mild, moderate, severe). Assuming that a biomarker has been associated with an abnormality or disease, different cut-off points in the levels of the biomarker will determine the fraction of true-positive, false positive, true-negative and false-negative results which in turn, produce different estimates of the sensitivity and specificity of the biomarker as a diagnostic test (Table 1.2). When used in relationship to a diagnostic test, sensitivity refers to the probability of a positive test in a patient with the disease. Specificity is the probability of a negative test in a patient without the disease, and significantly, sensitivity and specificity are calculated using the subset of patients from the trial population who have and do not have the disease, respectively. Sensitivity and specificity both range from 0 to 1 and they are converted to a percentage by multiplying by 100 (Sacket et al., 1991; Greenstein and Lamster, 1995; Brunette 1996).

The cut-off point selected to identify disease will dramatically affect the sensitivity and specificity. If a low threshold for disease activity is selected, the sensitivity is usually increased and the specificity is usually decreased. One of the best methods to evaluate the effect of different cut-offs is to use receiver operating characteristic (ROC) analysis. ROC analysis plots the true-positive fraction (sensitivity) as a function of the false-positive fraction ($1.0 - \text{specificity}$), and points along the curve represent different thresholds for the test. Thereby, selection of points towards the left of the curve yield higher specificity and points to the right yield higher sensitivity. ROC analysis also permits the comparison of different tests without any selection of upper or lower reference limits, or any particular sensitivity or specificity. Moreover, ROC curves are independent of the disease prevalence and therefore reflect the true performance of the diagnostic tests (Sackett et al., 1991; Greenstein and Lamster, 1995; Brunette 1996).

In clinical practice, the selection of cut-off points is determined by several factors including mortality and morbidity of the disease, the consequences of over or under treatment, and the cost and time required to perform the diagnostic test. If it is important that all individuals with the disease or its progression are detected, then a low threshold cut-off is selected to provide high sensitivity and high positive predictive values (PTL+, Table 1.2), although they will be associated with an increased number of false-positive results (low specificity and low negative predictive values, Table 1.2). Such an approach would be useful when screening for a serious or life-threatening disease because disease status can be attained by use of confirmation tests (series approach, see below). Confirmation testing is required to ensure that risky or expensive therapy is not mistakenly undertaken and it requires high thresholds to limit the number of false-positives. Therefore, confirmation tests have high specificity and high negative predictive values but lower sensitivity (Greenstein and Lamster, 1995).

Biomarkers (Section V.C) have not been developed for use as routine screening or diagnostic tests of oral/pharyngeal premalignancy or malignancy (eg. Lippman et al., 1990; Pillai et al., 1992;

Mulshine et al., 1993). However, Ogden et al. (1994) described the use of DNA ploidy (Section V.C.8) and keratin analysis (Section III. Table 1.3; Section V.C.7, Table 1.9) of oral exfoliative cytology in the detection of oral cancer. Smears were obtained from 33 biopsy-proven oral cancers and from contralateral normal sites of patients in a Dundee Dental Hospital. DNA profiles had 70% sensitivity, 90% specificity and 90% positive predictive value. Keratin 19 had 90% sensitivity, 50% specificity and 95% positive predictive value; Keratin 8 had 60% sensitivity, 100% specificity and positive predictive value. The combination (in parallel, see below) of an abnormal DNA profile and K19 expression had a positive predictive value of 95% and using these two parameters together, resulted in correct identification of a malignant tumour in 29 of 33 cases (Ogden et al., 1994). Yet, despite the high values of these test characteristics, the use of these methodologies as a screening test of the general population for oral SCC cannot be recommended (see below).

Computer simulated neural networks have also been trained to categorise normal, premalignant and malignant oral smears that were previously classified from a pathologist's report (Brickley et al., 1996). The neural network was able to distinguish between smears obtained from normal mucosa or non-dysplastic lesions, and those collected from dysplastic or malignant lesions with a sensitivity of 76% and specificity of 82%. However, when asked to differentiate non-malignant from potentially malignant lesions the neural network misclassified half of the cases as false positives suggesting (as expected) that a greater difference exists between normal mucosa and mucosa affected by a lesion, than between a benign mucosal lesion and a premalignant one. Nevertheless, the consequences of a false positive diagnosis are merely that the site would be biopsied. The consequences of a false negative diagnosis could be more serious and therefore the authors considered it reassuring that the networks were more sensitive than specific (Brickley et al., 1996).

Theoretically, sensitivity and specificity are considered to be stable properties of a test because they

are apparently not affected by the prevalence of the target disease; however, there is some evidence that sensitivity and specificity do change from one clinical population to another (Hlatky et al., 1984; Hlatky et al., 1987), especially if the stage of the disease varies in different groups of patients (Greenstein and Lamster, 1995). As noted above, sensitivity and specificity are calculated in defined populations in which the disease status of the individuals is known and where only extremes of disease (very sick) and health (very healthy) are represented. However, these circumstances do not represent the true clinical situation in which the diagnostic test is used to determine the disease status in a population comprised of healthy, diseased and equivocal cases. The predictive values (Table 1.2) of a test provide information about how often a test will provide a correct diagnosis in a mixed population, but the predictive values will vary widely as the prevalence of the disease changes (Hennekens and Buring 1987; Sackett et al., 1991; Greenstein and Lamster, 1995).

The rationale for using any diagnostic test is based on the probability that the disease is present prior to the test (the pre-test probability). When considering the use of a test for the general population such as in screening for a disease (see Section II.J below), the prevalence of the disease is used as the pre-test probability. When a test is considered for a specific patient, the clinician estimates the probability about how likely it is that the patient has the disease of interest, or the clinician can assign the disease prevalence as the pre-test probability. The choice of diagnostic test for any particular disease is determined by the power or ability of the test to revise the pre-test probabilities, either upwards to rule-in the disease, or downwards to rule-out the disease. The cut-off probabilities for ruling-in or ruling-out a disease will depend upon the disease and the subsequent courses of action or follow-up that relate to either ruling-in or ruling-out the disease. That is, the consequences of false-positive and false-negative results must be weighed in each case. In addition, if a test is not powerful enough to alter the pre-test probabilities so that a positive or negative test result would alter the pre-test planned course of action, then the test should not be performed (Schechter and Sheps, 1985; Sackett et al., 1991).

For example, the use of toluidine blue (Section VII.B) has been advocated for the detection of oral SCC. The sensitivity of toluidine blue ranges from 93.5% to 97.8% and its specificity ranges from 73.3% to 92.9% (Rosenberg and Cretin, 1989). However, toluidine blue will have different predictive values if it is used as a screening test (Section II.J below) in the general population, or in a tertiary referral centre for oral cancer. The prevalence of SCC in the general population is only 3% (Parker et al., 1996), and therefore, the post test likelihood of a positive toluidine blue test is only 6% (see Schechter and Sheps, 1985 or Epstein et al., 1997 for calculation details). In contrast, the prevalence of SCC either as primary or recurrent disease, is greater (26%, Silverman, 1990; Parker et al., 1996; 33%, Epstein et al., 1997) in a tertiary care centre for oral SCC and consequently, the post test likelihood of a positive test is also greater (51%, Epstein et al., 1997). In a similar manner, if DNA profiles and keratin K19 analyses of oral smears (Ogden et al., 1994) were used as tests for the general population (assuming prevalence of oral SCC is 3%), the post test likelihoods of a positive test would be 18% and 5.3%, respectively. In contrast, if these same measures are used in an oral SCC tertiary care setting where prevalence is assumed to be 33% (Epstein et al., 1997), the post test likelihoods of a positive test would be 76% and 47%, respectively (see Schechter and Sheps, 1985 or Epstein et al., 1997 for calculation details). In the high-prevalence setting, the post-test likelihoods of the tests are considerably higher than the pre-test probabilities, meaning that there is a considerably increased probability that the disease (SCC) is actually present. In contrast, the post-test likelihoods of the same tests in the general population (low prevalence setting) are similar to the pretest probabilities, meaning that there is only a slight increase in the probability that the disease is actually present. Nevertheless, the significance of each positive or negative test must be evaluated on an individual basis by the clinician who must then decide what the subsequent course of action will be (see also Section J below).

Different tests for the same disease can also be used in combination with another, either in series such as confirmation testing, or in parallel, such as in the combined use of DNA profiles and keratins (Ogden et al., 1994). If Tests A and B are used in parallel, then all sites positive for Test

A or Test B are considered positive, and a negative result requires that both Test A and Test B are negative. If tests are used in series, either test A or B can be used first, but a positive result on the first test requires retesting with the other test (Greenstein and Lamster, 1995). In parallel testing, the combined sensitivities are greater than sensitivities of the individual tests but overall specificity is reduced and there is an increased percentage of false-positive results. Thus, parallel testing is more sensitive for detecting disease than series testing, but parallel testing is less efficient at confirming the presence of disease. In contrast, series testing is less sensitive in detecting disease, but series testing has greater specificity and is more efficient at specifically confirming the presence of disease (Sackett et al., 1991; Greenstein and Lamster, 1995).

It is likely that diagnostic tests using biomarkers for premalignancy and malignancy will be developed in the future but their contention that they discriminate between health and disease must be carefully evaluated before histopathological diagnosis is abandoned as the gold standard. Nevertheless, biomarkers may serve as adjuncts to clinical diagnosis and they are potentially useful prognostic indicators in that they may provide information about the patient's clinical course. For example, the histological pattern of tumour invasion (Odell et al., 1994) and mutations in tumour suppressor genes and/or proto-oncogenes have been linked to metastasis and recurrence of oral SCC (see Section V.C). Biomarkers may also provide opportunities for the early detection of disease, before the disease becomes symptomatic or even clinically evident.

J. Early Diagnosis and Survival of Disease

For some diseases, it may be possible to improve the outcome of illness among affected individuals by reducing the severity of the clinical course of the disease or the rate of recurrence. One approach to achieving these objectives is to detect the disease at earlier stages, before the disease becomes symptomatic, and this may be accomplished by screening or case-finding strategies in which a test is applied to persons who are asymptomatic for the disease of interest. The test results are used to classify individuals with respect to their probability of having a

particular disease, and those with a positive test are further evaluated by subsequent tests to determine whether they do have the disease (Hennekens and Buring 1987).

Screening may be voluntary as at health fairs, or it may be compulsory as when pilots submit to periodic electrocardiograms, immigrants to tuberculosis tests, and applicants for life and disability insurance to a combination of disparate tests (Hennekens and Buring 1987). Case finding refers to clinicians who can seek early diagnosis when patients come to them for annual health reviews or unrelated, intercurrent illnesses. For example, most individuals see a dentist or physician once in a while and the clinician can use the opportunity to scan the oral cavity and oropharynx for abnormalities, or measure blood pressures of every adult patient who attends the practice for any reason. In addition, targets for early diagnosis should include risk markers and factors for disease such as family history, alcohol and tobacco use, etc. (Hennekens and Buring 1987).

The assumption underlying screening programs is that early detection prior to the development of symptoms, will lead to more favourable prognosis because treatment begun before the disease becomes clinically manifest, will be more effective than later treatment. The notion of early diagnosis is predicated on an ordered biological progression or 'natural history' of the disease which includes four fundamental time points: biologic onset, clinical onset, the point of usual clinical diagnosis and finally, the outcome which may be recovery, disability or death (Figure 1.3), and the amount of time between each time point will vary with each disease. In addition, there are two important critical points or thresholds: the detection threshold and the therapeutic threshold (Figure 1.3). The location of the detection and therapeutic thresholds in the natural history will vary with each disease and their locations are crucial to the value of early diagnosis. For a disease to be amenable to screening, the disease must have a detectable pre-clinical phase, (i.e. the detection threshold must occur prior to the clinical onset), the preclinical phase must be amenable to treatment, and the treatment must favourably alter the natural history of the disease so that survival, function, quality of life, or all three are improved (Sackett et al., 1991; Schechter 1995).

Patients whose cancers are diagnosed early, have better 5-year survivals than patients who are diagnosed in later, symptomatic stages. However, these observations do not prove the value of early diagnosis because even when cancer therapy is worthless, early diagnosis will always appear to improve survival. This paradox is the result of several biases and a result of how survival is measured. First, individuals who volunteer for screening programs and patients who present for periodic health examinations are generally healthier and they may exhibit exposures or outcomes which differ from those of non-volunteers or “late-comer” patients (volunteer bias, Sackett 1979).

Survival analyses are used to determine the amount of time to an event or health outcome such as death, recurrence of disease, or the occurrence of a sign or symptom. After the clinical diagnosis of cancer, patients are followed over time (eg. 5 years) and each year a certain percentage of patients will die. For example, if 10% of the patients died every year, the 5-year survival would be 50%; if all the patients in this example were diagnosed at age 45, then 50% of them would be alive at age 50. If a screening test was able to detect the cancer a year before patients developed symptoms (i.e. at age 44), and if early treatment was no more effective than at the time of usual clinical diagnosis (age 45), then the survival curve is shifted towards the right because the starting point for the 5-year survival measurement has been shifted one year backwards. As before, only half of the patients will be alive at age 50 but instead of being given an extra year forward of life, they received an extra year backwards of disease. This “zero-time shift” is known as “lead time bias” (Sackett et al., 1991).

Early diagnosis may also result in apparent survival because of “length-time bias” in which slow-growing tumours are preferentially detected. Slow-growing tumours are detectable over a longer time period than fast-growing tumours and consequently, slow-growing tumours are preferentially identified by early-detection programs. Even when therapy is worthless, patients detected through early diagnosis will have a longer survival than those detected at the time of usual clinical diagnosis. In general, patients with long preclinical periods tend to have long clinical durations of

disease, and patients with short preclinical durations tend to have short and rapidly fatal clinical periods (Sackett et al., 1991).

Kowalski et al. (1994) recommended that screening or case-finding strategies be performed by professionals proficient in the diagnosis of oral premalignant and early-stage malignancies. These investigators evaluated the risks of presenting with advanced stage versus early stage (Section VII) oral/pharyngeal SCCs in 336 Brazilian patients, and their study was limited to lesions that could be accessible to self examination by the patients. The main reasons for diagnostic delays were attributed to the patient's ignorance about the disease although income level and educational levels were not associated with stage distribution. Female and older patients sought help more readily than males or younger patients, yet when patients sought medical or dental care for early symptoms, the lesions were frequently misdiagnosed as benign conditions. The majority (58%) of cases were symptomatic for over a month before help was sought and the most common first symptoms were a painful ulcer (63%) in the oropharynx, followed by odynophagia and/or dysphagia (21%). Upon seeking help, there was no delay of referral to a head and neck service for 18% of patients, but almost 12% of cases were delayed in diagnosis and treatment because medical doctors and dentists failed to recognize early lesions. Medical doctors delayed referral for a median of 12 months in 6% cases, and dentists for a median of 6.5 months in 3% of cases; the balance were attributed to delays caused by pharmacists and drug store clerks. Patient and professional delays were not related to the stage of disease but the consequences of advanced stage at diagnosis were considerable increases in treatment costs and longer hospital stays. Kowalski et al. (1994) concluded that early tumours were often asymptomatic and therefore could be detected only during a routine examination of high risk individuals, but the failure of doctors and dentists in recognising early lesions was a major concern.

Cancer survival statistics may be reported as the observed survival rate for a specific patient group. Among any group of patients, some will be lost to follow-up so that there is no information

available concerning their survival. Some patients may die from causes other than the disease of interest, and some may develop diseases other than the disease of interest. As survival analysis calculates the amount of time to an event, that event must be clearly identified. For example, some investigators of oral/pharyngeal cancer have not clearly specified the methods and criteria used for calculating survival (eg. Spiro and Strong, 1974) so that it is unclear if survival refers to survival from death due to any cause (all-cause survival), survival until death from oral/pharyngeal cancer, its treatment or metastasis (cause-specific survival), or survival until local or regional disease recurrence (disease-free survival). In contrast, some investigators (eg. Callery et al., 1984; Franceschi et al., 1993; Zelefsky et al., 1992; Kraus et al., 1993) clearly identified survival criteria as survival to regional and neck failure as well as overall cause-specific survival. Throughout this thesis, survival data are reported using the authors' terms but in general, it was often unclear whether survival referred to overall survival from death due to the oral/pharyngeal cancer.

Cancer survival statistics may also be reported as a five-year relative survival rate which is the ratio of the observed survival rate for the specific patient group to the expected survival rate for persons in the general population who are similar with respect to age, gender, race and calendar year of observation (Wingo et al., 1995). This method has been used to compare differences in survival among different races in the United States (Wingo et al., 1995).

K. Summary

This section has reviewed some basic concepts in epidemiology that should aid the readers of this thesis in their understanding of oral premalignancy (Section VI) and oral SCC (Section VII), and of the results presented in Chapter 3.

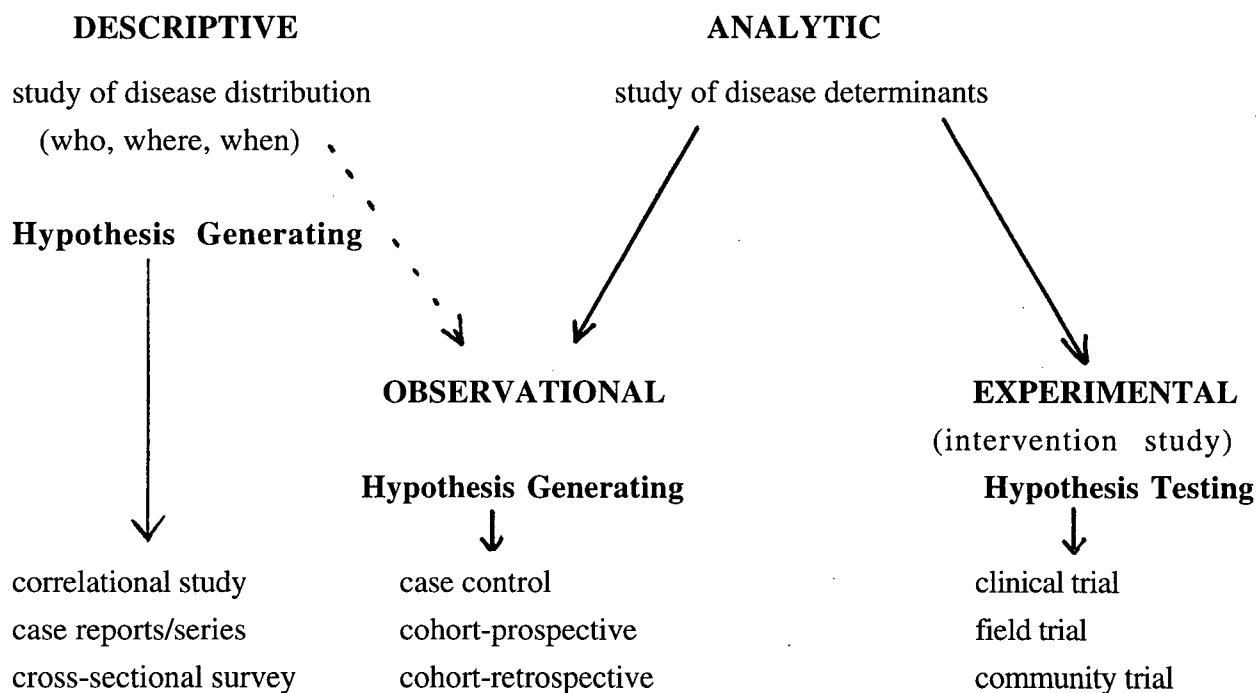
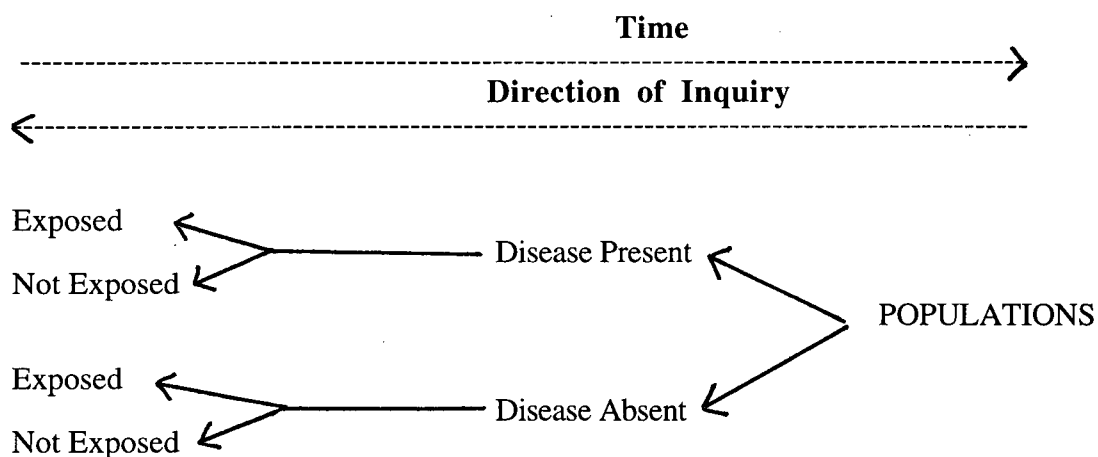
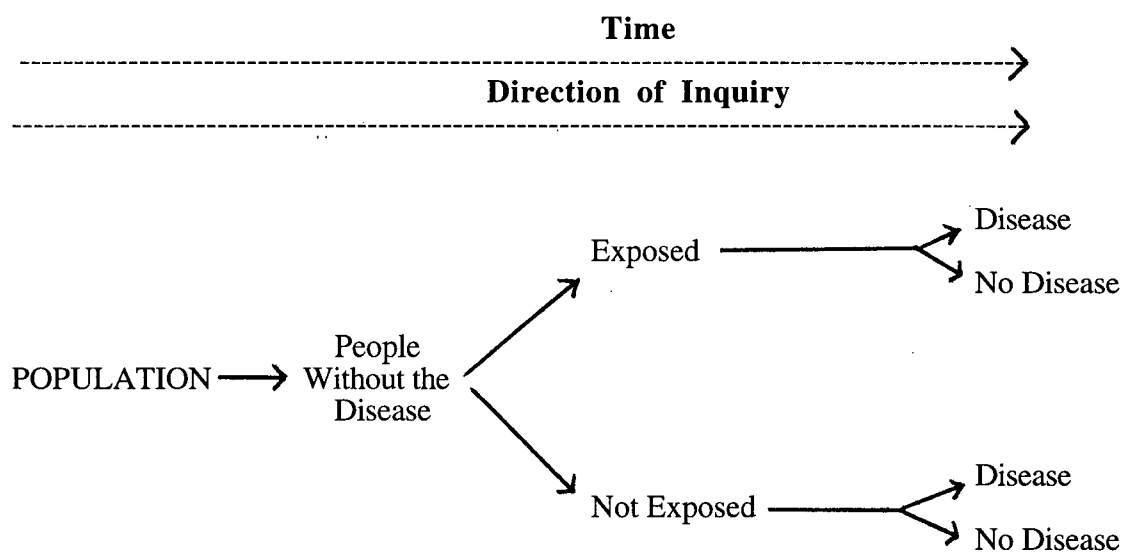


Figure 1.1. Taxonomy of Epidemiologic Studies. Epidemiological studies can be broadly divided into descriptive studies which focus on the distribution of disease, and analytic studies which focus on the determinants of disease. Descriptive studies are always observational studies and they are used to generate *a posteriori* hypotheses. Analytic studies and descriptive studies are inter-related in that analytic studies may be observational and used to generate hypotheses. The degree of intervention of the study variables determines whether an analytic study is observational or experimental. Experimental studies test *a priori* hypotheses and impose strict control over study variables.

(Adapted from Hennekens and Buring, 1987; Sheps 1995)



A. Case Control Study Design



B. Cohort Study Design

Figure 1.2. Designs of Case-Control Study and Cohort Study.

(A). In a case control study, individuals are selected from the population on the basis of the presence (cases) or absence (controls) of the disease of interest. The exposure of cases and controls is determined retrospectively.

(B). In a cohort design, individuals without the disease of interest are selected on basis of the presence (cases) or absence (controls) of exposure to a factor of interest. In a prospective cohort design, the outcome of interest has not yet occurred and the subjects are followed for a specified period of time to determine the development of disease in each exposure group. In a retrospective cohort design, the exposure and the outcome of interest have already occurred at the time the investigator starts the study.

(Sheps 1995)

Study Population	Disease Present	Disease Absent	Totals
Risk Factor Present	a	b	a + b
Risk Factor Absent	<u>c</u>	<u>d</u>	<u>c + d</u>
Totals	a + c	b + d	a + b + c + d

Table 1.1 Contingency Table to Aid in the Calculation of Measures of Association.

Absolute Risks

$$\text{Risk Factor Present} = a/a+b$$

$$\text{Risk Factor Absent} = c/c+d$$

$$\text{Relative Risk} = (a/a+b)/(c/c+d)$$

$$\text{Odds Ratio} = ad/bc$$

$$\text{Attributable Risk} = a/a+b - c/c+d$$

		Gold Standard		
		<u>Disease Present</u>	<u>Disease Absent</u>	Totals
New Diagnostic Test	Disease Present	a	b	a + b
	Disease Absent	<u>c</u>	<u>d</u>	<u>c + d</u>
	Totals	a + c	b + d	a + b + c + d

Table 1.2. Comparison of a New Diagnostic Test to the Gold Standard. The comparison of a new test to criterion standards or the gold standard provides a variety of mathematical probabilities known as test characteristics which aid in the analysis and comparison of different tests.

Accuracy $\frac{a + d}{a + b + c + d}$	the overall agreement between the test and the gold standard test
Sensitivity $\frac{a}{a + c}$	the proportion of diseased people correctly identified by the test (true-positive rate)
Specificity $\frac{d}{b + d}$	the proportion of non-diseased people correctly identified by the test (true-negative rate)
PTL+ $\frac{a}{a + b}$	Post Test Likelihood of a Positive Test (Positive Predictive Value) For a patient with a positive test result, the probability that the disease is actually present
PTL- $\frac{c}{c + d}$	Post Test Likelihood of a Negative Test For a patient with a negative test result, the probability that the disease is actually present . PTL- is not the negative predictive value.
NPV $\frac{d}{c + d}$	Negative Predictive Value For a patient with a negative test result, the probability that the disease is absent
Prevalence $\frac{a + c}{a + b + c + d}$	the overall probability that the disease is present prior to the test; the proportion of patients that have the disease (also known as the pre-test probability)

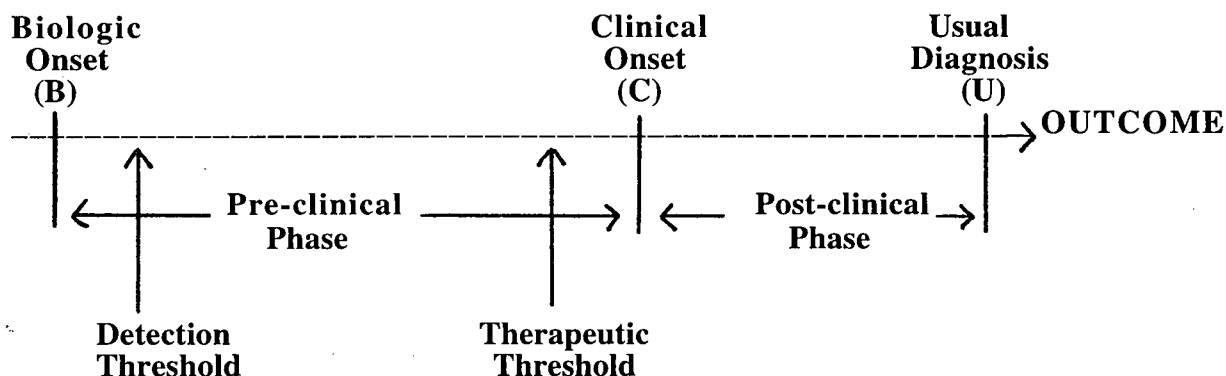


Figure 1.3. The Natural History of a Disease. For a disease to be amenable to screening, the Detection Threshold and Treatment Threshold should occur in the Pre-clinical Phase and preferably, the two thresholds should be separated by a long time interval.

Biologic Onset:	the disease begins with the initial interaction between the patient, the causal factor(s) and the rest of the environment. For example, the interaction between a carcinogen such as nitrosamine in cigarette smoke and basal cells of the oral mucosa may result in point mutations in gene p53.
Clinical Onset:	the first signs are evident but the patient is not aware, is not concerned or denies the situation. For example, the patient may beware of a small, painless white lesion on the tongue
Usual Diagnosis:	in the absence of intervention or spontaneous disappearance, the disease progresses to the point where symptoms appear and the patient seeks clinical help
Outcome:	recovery, disability or death due to the disease
Detection Threshold	although there are no symptoms, the disease mechanisms produce functional or structural changes that could be detected with the appropriate test during screening or case finding. For example, cytologic smears may identify changes in DNA content or mutations in gene p53
Therapeutic Threshold	the last point at which medical intervention has an important effect (usually curative) on altering the natural history of the disease

Adapted from Sackett et al., 1991; Schechter 1995; see also Section V.

III. Development and Anatomy of the Tongue

This Section reviews the embryological development, anatomy and function of the tongue in order to provide an appreciation for the consequences of lingual malignancy and its treatment.

A. Embryological Development

The development of the embryo is divided into 3 main periods: ovum, embryonic and fetal (Sperber 1973). The Period of the Ovum comprises the 7-8 days following conception during which time the ovum implants and the placenta is formed. The Embryonic Period extends from the 8th day to the 8th week and comprises the presomite (8th-20th day), somite (21st - 31st day) and late or post-somite (4th-8th week) periods. The Fetal Period extends from the 3rd month until birth (Sperber 1973).

During the presomite period, the fetal membranes are established and the three primary germ layers, ectoderm, endoderm and mesoderm, are formed. During the late somite period, 5-6 mesodermal swellings known as the branchial arches develop bilaterally on the embryo's ventral aspect, caudal to the head fold of the future mandibulocervical region. Each of the five pairs of branchial arches contains a central cartilage rod that will form the skeleton of the arch, a muscular component, a vascular component, and a nervous element comprised of special visceral motor fibres of one or more cranial nerves that supply the branchial muscle arising from that arch (Sperber 1973). The mesoderm between the branchial arches does not proliferate, leaving the ectoderm (externally) and the endoderm (internally) in contact. The arches are thus separated by ectodermal/endodermal membranes which appear as four branchial grooves on the embryo's exterior and they correspond internally with the five endodermal pharyngeal outpocketings or pouches (Sperber 1973; Romanes 1986). The mucosa of tongue develops from the ventral endoderm lining the anterior internal aspects of the pharyngeal pouches and later, muscle invades these endodermal outpouchings (Atkinson and White 1992).

During the 4th week of development, the tongue rises into the developing mouth as a swelling that develops in two parts from the inner lining of the first four branchial arches. The first part or the anterior two-thirds of the tongue develops from three mesodermal swellings: bilateral swellings on the internal aspect of the 1st branchial arch (mandibular arch) which form the lingual swellings, and a median swelling in the floor of the mouth, the tuberculum impar, which occupies the groove between the mandibular and hyoid (second) arches (Sperber 1973; Scott and Symons, 1974). The lingual swellings and tuberculum impar enlarge and fuse to provide the mucosal covering of the anterior two-thirds of the tongue. Caudal to the tuberculum impar is a blind pit, the foramen caecum which marks the origin of an endodermal duct which migrates to the pharynx to form the thyroid gland (Sperber 1973).

The second part or posterior one-third of the tongue is derived mainly from the third branchial arch which grows forwards, over the hyoid arch on the floor of the mouth (FOM), to join the back of the anterior part of the tongue. The second and third arches elevate into a midventral prominence known as the copula or hypobranchial eminence, from the back part of which develops the epiglottis (Scott and Symons 1974). The mucosa of the second to fourth arches and the copula provide the covering for the posterior third of the tongue (Sperber 1973; Scott and Symons, 1974).

Between birth and adulthood, the tongue normally doubles in length, width and thickness. Failure to achieve normal growth results in microglossia (small tongue), macroglossia (excessively large tongue) or aglossia (failure to develop), and failure of fusion of its components results in a forked bifid or trifid tongue (Sperber 1973; van der Waal and Pindborg 1986).

B. Clinical Anatomy of the Tongue

On the dorsal surface of the tongue, a 'V'-shaped groove known as the sulcus terminalis divides the tongue into an anterior two-thirds and posterior one-third. The apex of the sulcus terminalis points posteriorly and is marked by a pit, the foramen caecum. A row of 7-12 circumvallate

papillae (Section III.C.7) lie immediately anterior to the sulcus terminalis and the median lingual sulcus divides the anterior tongue in half longitudinally (Romanes 1986; Atkinson and White 1992).

The anterior two-thirds of the tongue is also known as the oral, mobile or palatine tongue and it comprises the body and tip of the tongue. The oral tongue is situated within the oral cavity which extends from the vermillion border of the lips to the junction of the hard and soft palate superiorly, the line of circumvallate papillae inferiorly, and laterally, to the palatoglossal arches or anterior pillars of the fauces which comprise the oropharyngeal isthmus. The oral tongue occupies the majority of the FOM where the tongue is separated from the teeth by the lingual sulcus formed by a reflection of the oral mucosa from the alveolar processes onto the ventral surface of the tongue. Posterior to the last molar, the lingual sulcus is filled in by the palatoglossus arch and anteriorly, the sulcus undermines the lateral margins of the tongue to extend beneath its free anterior third. In the anterior ventral midline, a thin crescent of mucosa called the lingual frenum connects the ventral surface of tongue to the FOM. On either side of the lingual frenum the deep lingual veins run beneath the mucous membrane medial to fringes of mucosa called the fimbriated folds. On the FOM, on either side of the frenum, are the sublingual papillae which represent the duct orifices of the submandibular salivary glands. Posterior and lateral to the sublingual papillae are two mucosal elevations, the sublingual folds, which contain the sublingual salivary gland and its numerous ductule openings as well as the duct from the submandibular gland (Romanes 1986; Atkinson and White 1992).

The dorsum of the oral tongue is covered by numerous filiform papillae and scattered fungiform papillae (Section III.C.7). The lateral borders of the oral tongue, just anterior to the palatoglossal arches may contain 5 short, vertical folds of mucous membrane, the foliate papillae (Romanes 1986; Atkinson and White 1992).

The posterior third of the tongue is also known as the pharyngeal tongue or the root or base of the tongue (BOT). It is located in the oropharynx which extends posteriorly from the junction of the hard and soft palate superiorly, the sulcus terminalis inferiorly, the palatoglossal arches laterally, to the level of vallecula and includes the tonsils and posterior oropharyngeal wall. The dorsum of the pharyngeal tongue is smoother and thinner than the dorsum of the oral tongue and does not contain papillae. Instead, the pharyngeal dorsum contains small lymph follicles, the lingual tonsils (Section III.C.5), and multiple mucous-producing minor salivary glands (Section III.C.8). More posteriorly, the lingual mucosa is continuous with that of the vallecula and anterior surface of the epiglottis. The root of the tongue is attached to the hyoid bone and mandible by the hyoglossus and genioglossus muscles (Section III.C.2) respectively, and to the epiglottis by the glossoepiglottic fold (Romanes 1986; van der Waal and Pindborg, 1986; Atkinson and White 1992)

C. Structural Anatomy of the Tongue

1. Mucosa

The mucosa of the tongue consists of a stratified squamous epithelium (Section IV.A. and Section IV.C) and a layer of connective tissue, the lamina propria. Lamina propria is a dense cellular and collagenous tissue of variable thickness that contains collagen and elastic fibres, fibroblasts, blood and lymph vessels and small nerves. On a functional basis, lingual mucosa is divided into a lining mucosa and a gustatory mucosa.

a. Lining Mucosa

Lining mucosa (Sections IV.A. and IV.C.2) covers the ventral tongue and the root of the tongue in areas devoid of lingual tonsils and minor salivary glands. Lining mucosa is designed to permit compression and distention of the tissue, and the epithelium of lining mucosa varies in thickness which generally depends upon the trauma and mechanical loading received. Lining mucosa is generally nonkeratinized (noncornified) but may be slightly parakeratinized (Section IV.C).

Except for the ventral tongue, most areas of the oral cavity that are covered by a lining mucosa also have a submucosa which is a much looser connective tissue underlying the lamina propria and which binds the mucosa to the underlying muscles. The submucosa is not a distinct feature of the dorsal root of the tongue, but it also contains salivary gland acini and ducts, larger nerves and vessels that supply and drain the overlying mucosa (Ross and Reith 1985; Atkinson and White 1992).

b. Gustatory Mucosa

Gustatory mucosa lines the dorsum of the oral tongue. Gustatory mucosa displays the general features of masticatory mucosa (Sections IV.A and IV.C.1) and also contains numerous surface projections or papillae some of which contain taste buds (Section III.C.7). Masticatory mucosa is designed to resist mechanical stress and it is heavily parakeratinized (Section IV.C.1). Many of the papillae are cornified and there are deep interdigitations between the epithelial rete pegs and underlying papillae of the lamina propria. A submucosa is absent as the mucosa is bound directly to the underlying lingual muscles (Section III.C.2), (Ross and Reith 1985; Atkinson and White 1992).

2. Musculature of the Tongue

During the Somite Period, the mesoderm alongside the notochord (primitive vertebral skeleton) divides into a series of 42-44 paired segmental blocks or somites including 4 occipital and 8 cervical somites which are located inferiorly to the ventral surfaces of the pharyngeal pouches, in the floor of the pharynx (Sperber 1973; Romanes 1986). During the 6th and 8th weeks, a strip of muscle from the occipital somites opposite the origin of the hypoglossal nerve, migrates cranially, carrying the hypoglossal nerve along with it to invade the tongue which is still a mucosal swelling on the FOM (Sperber 1973; Scott and Symons 1974; Romanes 1986; Atkinson and White 1992). With the exception of the palatoglossus muscle (Section III.C.2.a.iv) the muscles of the tongue are derived from the occipital somites and thus, their motor innervation is supplied by the hypoglossal

nerve. The palatoglossus muscle fibres and its motor innervation, the pharyngeal plexus, are derived from the third and fourth branchial arches (Sperber 1973).

An incomplete fibrous median raphe or septum divides the tongue musculature longitudinally, into right and left halves. Posteriorly, the septum is attached to the hyoid bone and superiorly, the septum is separated from the dorsal mucous membrane by the superior longitudinal muscle (Romanes 1986). The musculature of each half of the tongue is divided into extrinsic and intrinsic groups.

a. Extrinsic Muscles

The extrinsic muscles originate from outside the tongue and they can move the tongue as well as alter its shape. Four pairs of extrinsic muscles, the genioglossus, styloglossus, hyoglossus and palatoglossus muscles attach the tongue to the mandible, styloid process, hyoid bone and soft palate, respectively (Romanes 1986; Atkinson and White 1992).

(i). Genioglossus

The genioglossus muscle is the largest extrinsic muscle. It originates at the genial tubercles behind the symphysis of the mandible and extends in a fan shape, vertically upwards and backwards into the tongue to the tip (the superior/anterior fibres), the posterior third (the inferior/posterior fibres) and into the dorsum (the middle fibres); the right and left halves of this muscle are in contact in the median plane. Contraction of posterior fibres protrudes the tongue and if only one half of the muscle is active, the tongue deviates to the inactive side. Contraction of the posterior fibres also depresses the tongue in its centre and increases the volume of the tongue, such as in sucking. Contraction of superior and middle fibres depresses the tip of the tongue and retracts it (Romanes 1986; Atkinson and White 1992).

(ii). Styloglossus

This muscle arises from the tip of the styloid process and adjacent part of the stylohyoid ligament. It runs downwards and forwards along the lateral walls of the pharynx to pass between the

superior and middle constrictors of the pharynx to insert into the whole length of the lateral tongue where it mingles with the hyoglossus muscle. Contraction of the styloglossus muscles pulls the posterior-lateral tongue margins backwards and upwards, such as in swallowing (Romanes 1986; Atkinson and White 1992).

(iii). Hyoglossus

The hyoglossus muscle is a flat, quadrilateral muscle arising from the body and superior surface of the greater horn of the hyoid bone. Fibres run upwards to enter the sides of the tongue, lateral to the genioglossus, where they mingle with fibres from the styloglossus. Contraction of the hyoglossus pulls the lateral borders of the tongue downwards and backwards and assists the genioglossus in enlarging the tongue during sucking motions when the hyoid bone is fixed by infrahyoid muscles (Romanes 1986; Atkinson and White 1992).

(iv). Palatoglossus

The palatoglossus muscles contribute to both the tongue and soft palate. The two halves of the muscle meet in the midline where they arise from the undersurface of the palatal aponeurosis and converge on the palatoglossal arch from where they insert from above, into the posterolateral aspect of the tongue to mingle with intrinsic transverse fibres. When the soft palate is fixed by other muscles, contraction of both palatoglossus muscles pulls the posterior third of the tongue upwards and backwards and pulls the palatoglossal arches together to narrow the oropharyngeal isthmus. When the soft palate is not fixed, contraction of the palatoglossus pulls the soft palate downwards towards the dorsum of the tongue to help separate the mouth from the pharynx (Romanes 1986; Atkinson and White 1992).

b. Intrinsic Muscles

The intrinsic muscles lie wholly within the tongue and can only modify the shape of the tongue. Intrinsic muscles are inserted in the deep fibrous connective tissue of the lamina propria of the mucosal covering or in the fibrous midline septa and run in longitudinal, transverse and vertical bundles.

(i). Vertical

The vertical group run from the dorsum inferiorly and laterally to flatten and widen the tongue and roll up the margins.

(ii). Superior Longitudinal

The superior longitudinal group forms a layer on the dorsum; it curls the tip upwards and rolls it posteriorly.

(iii). Inferior Longitudinal

The inferior longitudinal muscles lie lateral to the genioglossus, in the lower part of the tongue to turn the tip downwards and together with the superior muscle, retract and widen the tongue.

(iv). Transverse

The transverse muscle fibres lie inferior to the superior longitudinal muscle and run between the vertical fibres of the genioglossus, hyoglossus and the vertical muscle from the septum to the margins. They narrow the tongue and increase its height (Romanes 1986; Atkinson and White 1992).

3. Innervation of the Tongue

a. Sensory Innervation

The sensory (tactile and gustatory) nerve supply of the mucous membrane of the tongue is explained by the different embryological origins of the tongue which retain their initially-established innervations.

(i). Tactile

The anterior two-thirds of the tongue is supplied by the nerve of the first branchial arch, the lingual branches of the mandibular division of the trigeminal nerve. The posterior one-third of root of the tongue is supplied primarily by the nerve of the third branchial arch, the glossopharyngeal nerve, with contributions from the nerve of the fourth arch, the vagus nerve via the superior laryngeal nerve to a small area adjacent to the epiglottis (Sperber 1973; Scott and Symons, 1974; Romanes 1986; Atkinson and White 1992).

(ii). Gustatory

Taste sensation to the anterior two-thirds of the tongue is supplied by the nerve of the second branchial arch, the facial nerve, via the chorda tympani nerve. Taste sensation to the root of the tongue is supplied by the glossopharyngeal nerve (Sperber 1973; Scott and Symons, 1974; Romanes 1986). Although the circumvallate papillae lie in the anterior two-thirds of the tongue, they are derived embryologically from the same tissues as the posterior third of the tongue and therefore are supplied by the glossopharyngeal nerve (Atkinson and White, 1992).

b. Motor Innervation

The palatoglossus muscle is supplied from the vagus-accessory complex by fibres that reach the pharyngeal plexus through the pharyngeal branch of the vagus nerve (Romanes 1986). The balance of the extrinsic muscles and all of the intrinsic muscles are supplied by the hypoglossal nerve (Romanes 1986; Atkinson and White 1992).

c. Autonomic Innervation

Autonomic nerve fibres follow the course of the lingual blood vessels whose flow they regulate. Autonomic nerves also regulate the secretory activity of minor salivary glands in the tongue and both sympathetic and parasympathetic nerves innervate and stimulate vasodilation of the salivary glands (Ross and Reith 1985).

4. Vasculature of the Tongue

a. Arterial

The main arteries of the tongue are the lingual arteries which spring from the external carotid artery opposite the tip of the greater horn of the hyoid and run anteriorly, underneath the hyoglossus muscle. The lingual artery gives off 3 branches, the suprahyoid branch, the dorsal lingual branches and sublingual branch, before continuing as the deep artery of the tongue.

Deep to the hypoglossal nerve, the lingual artery gives off the suprahyoid branch which runs along the superior border of the hyoid bone, lateral to the hypoglossal muscle. Deep to the hyoglossus muscle, the lingual artery gives off the dorsal lingual artery which branches upwards to supply the tongue musculature, mucosa of the pharyngeal tongue and palatine tonsils. The root of the tongue also receives arterial supply from the tonsillar branch of the facial artery and ascending pharyngeal artery (Romanes 1986; van der Waal and Pindborg, 1986; Atkinson and White 1992)

At the anterior border of the hyoglossus muscle, the sublingual artery arises to run forwards and upwards to supply the sublingual gland and adjacent muscles. The sublingual artery anastomoses, through the mylohyoid muscle, with the submental artery from the external maxillary artery, which occasionally replaces the sublingual artery. The lingual artery continues as the deep artery of the tongue in the middle and anterior tongue, close to the mucosa of the ventral surface. The deep artery is a tortuous vessel permitting for elongation of the tongue and it anastomoses across the midline, with its partner (Romanes 1986; van der Waal and Pindborg, 1986; Atkinson and White 1992).

b. Venous

The arrangement of venous drainage is variable but all veins unite at the posterior border of the hyoglossus muscle to form the lingual veins which follow the artery deep to the hyoglossus to enter the internal jugular vein. The deep lingual vein is the main vein and it originates near the tip and runs backwards on the ventral surface, close to the mucosa, descending along the anterior margin of the hyoglossus. The dorsal lingual veins drain the dorsum and lateral borders before joining the lingual veins (Romanes 1986; van der Waal and Pindborg, 1986).

5. Lymphatic Tissues

In the adult, the sites of the second and third branchial arches are marked by the anterior and posterior faucial pillars, respectively. The ventral aspects of the first and second pharyngeal

pouches are obliterated by growth of the third and fourth branchial arches as they contribute to the tongue, but the dorsal portions persist to develop into the auditory tubes and palatine tonsillar fossae, respectively. During the 3rd to 5th month of development, mesodermal lymphoid tissue invades the palatine, posterior pharyngeal and lingual tonsillar regions to form Waldeyer's ring (Sperber 1973). At birth, the oral mucosa of the posterior third of the tongue becomes pitted by deep crypts which form into rounded elevations, the lingual tonsil, and whose completion is marked by infiltration of lymphocytes (Sperber 1973). The lingual tonsils contain lymph nodules, often with germinal centres (Ross and Reith, 1985).

6. Lymphatic Vessels and Drainage

The regional lymph nodes of the neck are often described by levels because the patterns of metastatic dissemination of epithelial cancers of the upper aerodigestive tract often occur in a sequential fashion to the regional lymph nodes (Shah and Lydiatt, 1995; Shaha and Strong, 1995). The grouping developed at the Memorial Sloan-Kettering Cancer Centre divides the cervical lymph nodes into five levels. The first echelon of nodes or Level I includes nodes of the submandibular triangle, the submental and submandibular nodes. Levels II to IV include nodes in the anterior triangle along the sternocleidomastoid muscle; Level II includes nodes in the upper jugular region such as the jugulodigastric nodes; Level III includes the mid jugular cervical nodes, Level IV includes the low jugular cervical and jugulo-omohyoid nodes. Level V includes nodes in the posterior triangle (Atkinson and White, 1992; Shah and Lydiatt, 1995; Shaha and Strong, 1995).

A number of separate routes drain the lymph capillary plexus of the lingual mucous membrane. The majority of lymph vessels drain along the route of the blood vessels which supply the tongue; some parts drain bilaterally and some areas drain ipsilaterally. The factor limiting the extent of lymphatic drainage is the vertical midline fibrous septum which is impervious to lymph. The septum separates muscles from either side but it does not extend to the tip of the tongue nor to the mucosa covering the tongue. Therefore, ipsi or bilateral drainage depends upon whether lymph

originates from muscle or mucosa (Atkinson and White 1992).

Muscle and mucosa of the tip of the tongue drain bilaterally via the submental nodes into the jugulo-omohyoid nodes on either side. The balance of the mucosa of the oral tongue drains bilaterally to the submandibular nodes into the jugulodigastric nodes, the balance of the musculature of the oral tongue drains unilaterally by the same route. Some lymphatics from the tip and dorsum may bypass the submandibular and submental nodes and drain directly into the deep cervical chain. Both the mucosa and musculature of the posterior third of the tongue drain bilaterally directly into the deep cervical chain or via retropharyngeal nodes (Scott and Symons 1974; Atkinson and White 1992).

7. Papillae and Taste Buds

a. Papillae

The dorsum of the oral tongue contains 4 types of papillae: circumvallate, fungiform, filiform and foliate papillae.

(i). Filiform

At 11 weeks of development, the mucosa of the dorsal oral tongue develops filiform and fungiform papillae. The filiform papillae are the smallest and most numerous papillae and they are evenly distributed over the dorsum. On the body, they are often arranged in rows parallel to the sulcus terminalis; in the tip they run transversely. Filiform papillae are conical projections, facing posteriorly, with a broad base of connective tissue covered by heavily cornified squamous epithelium. Filiform papillae do not contain taste buds and their primary function is to protect the dorsum from frictional and mechanical stresses evoked by speech and mastication (Ross and Reith, 1985; van der Waal and Pindborg, 1986; Atkinson and White, 1992)

(ii). Fungiform

Fungiform papillae are globular mushroom-shaped projections interspersed among the filiforms and they are most numerous on lateral borders and the tip. Fungiform papillae are larger than

filiforms and they are bright red in colour. They consist of a highly vascular connective tissue core covered by a stratified non-cornified squamous epithelium and taste buds are located on their superior surface (Ross and Reith, 1985; van der Waal and Pindborg, 1986; Atkinson and White 1992).

(iii). Foliate

When present and developed, foliate papillae are located on the lateral borders, anterior to the palatoglossal arches at the junction of the oral and pharyngeal tongue. They are red, leaf-like projections covered by cornified epithelium that is interspersed by taste buds (Ross and Reith, 1985; Atkinson and White 1992).

(iv). Circumvallate

Circumvallate papillae develop at 2-5 months in utero. Usually 7-12 large (about 2-3mm in diameter) circumvallate papillae are situated in a row parallel and anterior to the sulcus terminalis. Circumvallate papillae are round structures surrounded by a deep trench or groove whose epithelium contains numerous taste buds. The papillae have a core of connective tissue covered by a lightly cornified squamous epithelium. Small serous glands, the glands of von Ebner, lie beneath the papillae and their secretions flood the base of the trench, dissolving solid matter and enabling it to be tasted (Ross and Reith, 1985; Atkinson and White 1992).

b. Taste Buds

Taste buds develop from epithelial cells starting in the 7th week of development and they appear to be functional, in their adult form, at 13-15 weeks in utero (Sperber 1973). Three types of taste buds can be distinguished histologically (Atkinson and White, 1992) but physiologically, 4 components of taste are identified: sweet, sour, bitter and salt. Rather than individual taste buds responding to only one taste component, it is more likely that individual taste buds, their cells and nerves can respond to different stimuli (Atkinson and White, 1992). Different regions of the tongue demonstrate relative differences in taste sensitivity: the tip is most sensitive to sweet, the lateral margins to sour, the base to bitter (Silloto 1975). Sensitivity to salty sensation is more

widespread but greatest at the tip (Silloto 1975). Taste sensation is conveyed by fibres of the facial (oral tongue) and glossopharyngeal (pharyngeal tongue) nerves which penetrate the basal lamina of the taste bud (Ross and Reith 1985). Prolonged exposure to a pure primary taste stimulus can result in adaptation to that sensation such that insensitivity to some tastes relative to others is produced, and previously neutral substances may then exhibit an apparent taste (Silloto 1975). Afferent taste fibres may also respond to warming or cooling of the tongue; sweet and bitter stimuli appear to be associated with warming temperatures whereas sour and salty stimuli are associated with cooling (Silloto 1975).

8. Lingual Salivary Glands

Minor salivary glands arise from oral ectodermal and endodermal epithelium that remains as discrete acini and ducts scattered throughout the submucosa of mouth, including the tongue. The glands may be serous, mucous or mixed. Serous glands, the von Ebner glands, are located beneath circumvallate papillae where they open into the trough surrounding the papillae. Mucous glands are located in the dorsal surface of the root of the tongue, interspersed amongst the lingual tonsils. Some mucous glands are also located at the tip of the tongue and along the lateral margins. Mixed glands, the glands of Blandin-Nuhn, are located close to the ventral surface, at the tip of the tongue (Scott and Symons, 1974; Ross and Reith 1985; van der Waal and Pindborg, 1986).

D. Functions of the Tongue

The tongue is a mobile, tactile, muscular organ involved with the functions of speech, mastication and taste, swallowing and protective reflexes. These functions are briefly reviewed below but are discussed in depth elsewhere (eg. Matthews 1975; Lavelle 1975; Atkinson and White, 1992).

1. Speech

The production of speech involves a complex coordination of phonation and articulation.

Phonation comprises the regulation of exhaled air flow and the production of sound in the larynx

by the vibration of the vocal cords (Atkinson and White, 1992). The process of articulation modifies the sounds produced by phonation by varying the size of the oral cavity and position of the lips, tongue, palate, jaws and teeth. Although the degree of opening determines the size of the oral cavity, the position and shape of the tongue and lips determine the shape of the oral cavity. The tongue's role in articulation is particularly evident in the production of the consonants d, t, g and k but overall, even small disturbances in tongue function may cause speech abnormalities (Atkinson and White, 1992; van der Waal and Pindborg 1986). Muscle spindles of the lingual muscles provide proprioceptive feedback and positional sense but somatic sensory information is also required for speech as demonstrated by temporarily-altered speech following anaesthesia of the lingual nerve (Atkinson and White, 1992).

2. Mastication

Mastication involves rendering food in the oral cavity into a state suitable for swallowing which occurs only when sensory information from the tongue indicates that the flavour, temperature and texture of the food is acceptable. Mastication involves muscles of mastication, suprahyoid and infrahyoid muscles, muscles of facial expression and extrinsic and intrinsic tongue muscles (Atkinson and White, 1992). The tongue is essential for placing food between the teeth and may also have a direct crushing effect on food by forcing it against the hard palate and pushing it onto the occluding surfaces of the teeth. The tongue also clears food displaced into the vestibules, mixes the food with saliva, forms food into a bolus and moves it posteriorly, into the oropharynx to be swallowed (Atkinson and White, 1992; van der Waal and Pindborg 1986).

3. Taste

During the process of eating and drinking, normal taste sensation is due to the combined stimulation of taste and olfactory receptors, as evidenced by diminished taste sensation during the common cold which eliminates or reduces olfactory input. Taste provides essential information regarding the nature of the substances to be ingested, and tastes considered pleasant or unpleasant

are learnt by experience. Food is either accepted or rejected and reflexes initiated by the olfactory and taste systems either initiate salivation and secretion of gastric juices, or cause avoidance of food and nausea (Silloto 1975).

Unilateral peripheral lesions of the cranial nerves associated with taste, the facial and glossopharyngeal nerves, do not affect overall taste sensitivity. A change in oral taste sensitivity requires a bilateral peripheral lesion of the chorda tympani which would simply result in an increase in the detection thresholds for salt and sweet relative to bitter and sour, but the ability to recognize the four taste qualities would remain (Silloto 1975).

4. Swallowing

Swallowing is primarily a reflex response although the individual is conscious of certain aspects of swallowing. Swallowing is a continuous process which takes about 5 seconds for food to pass from the mouth to the upper oesophagus although oral, pharyngeal and oesophageal phases of swallowing have been described (Atkinson and White, 1992). Voluntary action initiates the swallowing response when the bolus is collected on the tongue, compressed between the tongue and hard palate, and propelled backwards into the pharynx. The tip of the tongue contacts the anterior hard palate, followed by the dorsum contacting the palate in an anterior-posterior direction which obliterates the oral cavity and forces the bolus backwards. Contact of food with the mucosa overlying the posterior oropharyngeal wall or the palatoglossal arches stimulates afferent sensory endings of the glossopharyngeal nerve in the pharyngeal plexus; this initiates a reflex chain of contractions in the pharynx and oesophagus (Atkinson and White, 1992; van der Waal and Pindborg 1986). Respiration is temporarily suspended and positioning of the epiglottis over the larynx prevents aspiration of the bolus.

5. Protective Reflexes

The lower airway and gastrointestinal tract is protected from the entry of foreign bodies by the gag

reflex and from the ingestion of potentially toxic food by the vomiting reflex. The gag reflex occurs when a swallowing reflex is initiated by contact of the oropharynx by an object or material that cannot be swallowed; the mouth is opened and the posterior part of the tongue is elevated in attempts to expel the material. Vomiting occurs when contraction of the anterior abdominal wall forces stomach contents back through the cardiac sphincter and up into the oropharynx and oral cavity (Atkinson and White, 1992).

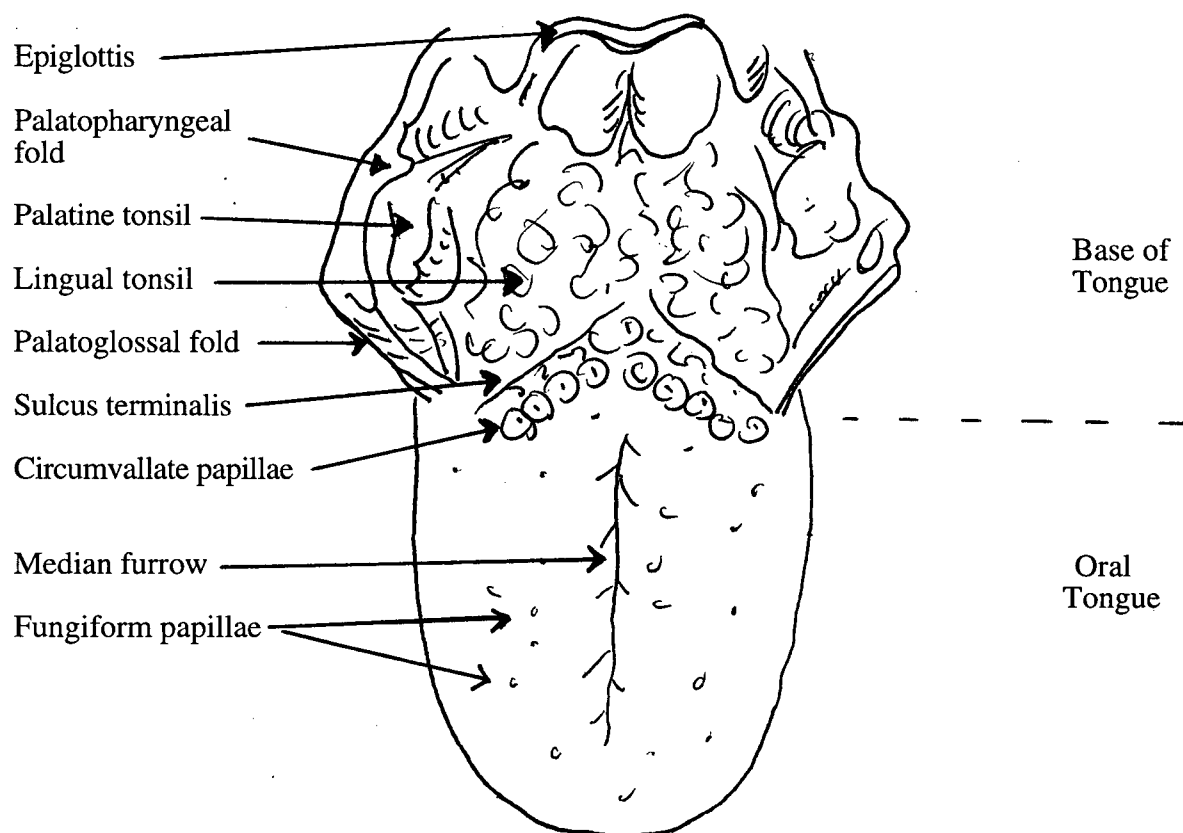


Figure 1.4. The Dorsal Surface of the Tongue.
Adapted from Atkinson and White, 1992, page 329.

IV. Epithelium

This Section reviews the normal organization, function, proliferation and differentiation of oral epithelium because they are relevant to understanding carcinogenesis and the behaviour of malignant epithelium.

A. General Characteristics

An epithelium can be described as a sheet of cells in which there is relatively little intercellular material. The cell sheets are characterized by intimate intercellular contacts (Section IV. B) which couple the cells to one another structurally, electrically, metabolically and mechanically so that the cells, rather than the extracellular matrix (ECM) bear the functional stresses. Epithelium is avascular and therefore dependent upon diffusion of nutrients from the underlying connective tissue from which it is separated by a basement membrane. Epithelial tissues are classified by the shape of the cells (flattened or squamous, cuboidal or columnar) and by the arrangement of the cells into single (simple) or multiple (compound) layers. In compound or multilayered epithelia such as epidermis and oral mucosa, the surface cells are mature, highly differentiated cells that are lost or shed (desquamated) from the surface and they are continuously replaced by the proliferation of less differentiated, more primitive cells that reside in subsurface sites (Scott and Dixon, 1972; Ross and Reith, 1985; Alberts et al., 1989; Atkinson and White, 1992).

Epithelial cells called keratinocytes (Section IV.C) comprise 90% of the cells in oral epithelium; the remaining 10% consist of Langerhans cells (Section IV.B.3) and melanocytes (Lavelle 1976b; Squier 1980). Keratinocytes in the basal layer divide and change their appearance as they differentiate and migrate to the surface where they die (apoptosis, Section IV.D.2.a.iv.) and are shed. In different regions of the oral cavity the rate of cell proliferation, the thickness and number of cell layers, the type of differentiation and nature of the surface layer vary in relation to different functional demands. On a functional basis, oral mucosa is usually divided into

1. masticatory mucosa which is exposed to mechanical compression and friction, and covers the gingiva and hard palate. It is usually a cornified (keratinized) epithelium overlying a dense fibrous connective tissue that is firmly attached to the underlying structures.
2. lining mucosa which flexible and extensible and covers the lips, cheeks, floor of the mouth (FOM), alveolar mucosa, ventral tongue and soft palate. It is usually a noncornified epithelium overlying a loosely-fibrous connective tissue (submucosa) that is flexibly attached to bone or muscle.
3. specialized gustatory mucosa covering the dorsal tongue. Functionally, it is a masticatory mucosa but it contains specialized lingual papilla and taste buds (Squier 1980; Ross and Reith, 1985).

Masticatory and lining epithelium also demonstrate different patterns of interdigitation with the underlying connective tissue. Different ratios of the area of interface to that of the epithelial surface reflect differences in mechanical attachment of the two tissues and in rates of metabolic exchange (Squier 1980). Noncornified lining mucosa is generally thicker than cornified masticatory mucosa but again regional modifications in relation to function exist; for example, noncornified cheek mucosa is thicker than noncornified mucosa lining the FOM. As well, masticatory mucosa exposed to chronic friction will demonstrate increased thickness of the keratin layer (Section IV.C), (Lavelle 1976b). These regional and functional modifications are reflected by differences in mitotic rate and by differences in permeability, both of which may play a role in a tissue's susceptibility to malignant transformation (Section V).

B. Protective Role of Oral Epithelium

The oral epithelium lines the oral cavity and protects the tissues it covers. Oral epithelium not only resists mechanical forces but it restricts the entry of microorganisms and acts as an impermeable

barrier to noxious substances. Many oral diseases are associated with the penetration of antigens and toxins and therefore mechanisms whereby this may be restricted or prevented are essential when considering the initiation of oral mucosal disease or malignancy. The protective functions of oral epithelium are mediated by

1. Desquamation. Desquamation is the normal turnover of tissue whereby the surface layer is continuously shed. This is an important mechanism for elimination of oral microorganisms.
2. Permeability Barriers. Permeability of the oral mucosa is determined by different kinds of macromolecular barriers that include
 - a. the surface layer of adherent mucin derived from salivary glands
 - b. keratin, an insoluble polymer located in the superficial layers of cornified epithelium (Section IV.C)
 - c. the intercellular permeability barrier formed by specialized intercellular junctions, the tight (occluding) junctions, and anchoring junctions (desmosomes and adherens junctions), (Alberts et al., 1989). Tight junctions form independent, impermeable barriers of contact between cell membranes of adjacent cells, effectively sealing adjacent cells together, obliterating the intercellular space and blocking diffusion. Anchoring junctions rely on transmembrane proteins called cadherins, and anchoring junctions connect the cytoskeleton of one cell to adjacent cells via desmosomes and adherens junctions. Adherens junctions are intercellular connection sites for actin filaments of adjacent cells. Desmosomes are intercellular connection sites where bundles of intermediate filaments called tonofibrils (Section IV.C) from one cell are connected to tonofibrils in neighbouring cells within and between cell layers so that the filaments form a continuous network throughout the epithelium (Alberts et al., 1989). Desmosomes and tonofibrils are essential for maintaining the integrity of the epithelium and for disseminating locally-applied forces. Their

importance is illustrated by serious skin conditions such as pemphigus in which antibodies against desmosomal linker proteins disrupt the desmosomes (Alberts et al., 1989), and epidermolysis bullosa simplex in which mutations of tonofibrils (keratins) K5 and K14 cause fragility of the basal keratinocytes (reviewed by McLean and Lane 1995).

Adjacent epithelial cells also communicate with one another via gap junctions which are 3-nm-wide gaps between the plasma membranes of adjacent cells. Gap junctions allow coupled cells to share small molecules such as inorganic ions, sugars, amino acids, nucleotides and vitamins which pass freely from one cell to another (Alberts et al., 1989; Atkinson and White, 1992).

d. the barrier formed by the basement membrane which consists of two layers, the basal lamina produced by epithelial cells and the lamina reticularis produced by connective tissue cells. Basal lamina also controls the orientation, intracellular organization, attachment and migration of basal epithelial cells which are attached to the basal lamina by hemidesmosomes and focal contacts. Hemidesmosomes represent the termination site of intermediate filaments; focal contacts are connection sites mediated by integrins which are transmembrane receptors linking actin filaments to components of the ECM.

(reviewed by Lavelle 1976b and Squier 1980)

3. Cellular defense mechanisms comprising Langerhans (dendritic) cells (eg. Daniels 1987) and polymorphonuclear leukocytes.

1. Desquamation

The continual physical shedding of the surface epithelial layer is an important mechanism for removing adherent bacteria which are lost along with the desquamated cells. In order to maintain homeostasis, the cell loss at the surface must be balanced by the rate of mitosis and the rate of differentiation and migration of cells to the surface (transit time). In general, the turnover of oral mucosa is faster than skin but slower than intestinal mucosa. Within the oral mucosa, noncornified

regions turn over faster than cornified regions; for example, the turnover time for human oral lining mucosa is 5-16 days compared to 28-40 days for human gingiva (Squier 1987). An example of the faulty control of basal cell proliferation and surface desquamation is evident in psoriasis which is a common benign skin disorder characterized by erythematous, scaly plaques. In psoriasis the rate of basal cell proliferation is greatly increased and the cell turnover rate is up to eight times greater than normal. Hyperproliferation of the epidermis results in epithelial hyperplasia but cells are shed from the surface before they have had adequate time to fully cornify, generally within as little as a week after their formation in the basal layer (Laskaris 1988; Alberts et al., 1989; Regezi and Sciubba, 1989).

Mitotic activity in gingival epithelium appears to increase with age and it has been suggested that this change reflects diminishing control of cell proliferation and might relate to the higher incidence of cancer in the elderly (Lavelle 1976b). Uncontrolled proliferation results in neoplasia which can be either benign or malignant (Sections V, VII) and the complex mechanisms controlling cell proliferation are reviewed in Section IV.D.

2. Permeability Barriers

Saliva has a definite protective role in the oral cavity (reviewed by MacFarlane 1976) but its role as a solvent may also facilitate penetration of substances that are dissolved in saliva. Moreover, the dissolved substances are maintained in solution at the surface of the mucosa and some oral locations also serve as "puddling spots" (Moore and Catlin, 1967) which are continuously immersed in saliva. Significantly, 80% of oral malignancies occur in a horseshoe-shaped area that corresponds to "saliva reservoirs" but which comprises only 20% of the surface area of the entire oral mucosa (Moore and Catlin, 1967). For example, pipe smokers characteristically develop a symmetrical leukoplakia of the palate with inflammation and swelling of the minor salivary glands (nicotinic stomatitis); however, the palate is a rare site for carcinoma yet when a pipe smoker develops oral cancer, it is commonly in the retromolar region and FOM (Cawson 1975). Perhaps

the leukoplakia results from the physical effects of heat from the pipe; the malignant change may be the result of carcinogens dissolved in saliva, draining and accumulating in the FOM (Cawson 1975; Squier 1980).

The most superficial barrier to diffusion is provided by salivary mucins which cover the surface of the oral epithelium. Mucins provide not only a permeability barrier, but they protect against desiccation of the epithelium and offer lubrication against surface abrasion. Mucins also play an important role in regulating microbial clearance and adherence in the oral cavity by aggregating organisms, and by trapping or concentrating protective molecules such as salivary IgA and lysozyme on the tissue surface (reviewed by Levine et al., 1987).

Toxins and antigens produced by oral microorganisms, and carcinogens are potentially harmful if they are able to penetrate and cross the oral epithelium. Substances can penetrate the epithelium by traversing through the epithelial cells by endocytosis which includes pinocytosis (intake of fluids) and phagocytosis (intake of solid particles). In oral mucosa, cells of the stratum basale and spinosum (Section IV.C) are capable of endocytosis but this does not appear to be a likely transport mechanism across the entire epithelium (Squier and Johnson, 1975). In some epithelia, molecules and ions are transported by active transport but this has not been demonstrated in oral epithelium. Molecules can also diffuse across cell membranes through either a lipid phase or along aqueous channels but this would be an inefficient method in a complex stratified epithelium such as oral mucosa (Squier and Johnson, 1975).

Although oral epithelial cells are closely apposed, tight junctions are not common in oral epithelium and therefore they are not the major intercellular barrier (Squier and Johnson, 1975). Hence, the intercellular space is sufficient to permit diffusion of some molecules and ions, and this appears to be the main mechanism of penetration through oral epithelium (Lavelle 1976b; Squier and Johnson, 1975; Squier 1987). The rate of diffusion and permeability of a substance are affected

by a number of factors including temperature, pH, molecular weight, interaction between the solvent and solute, concentration of the substance, duration of contact between the substance and the mucosa, thickness of the mucosa, and integrity of the permeability barriers (Lavelle 1976b; Squier 1980). In general, molecules penetrate more readily than ions, small molecules more readily than large molecules, and gases and volatile substances the most readily of all (Squier and Johnson, 1975). Substances that are lipid-soluble move more rapidly across mucosa than water-soluble ones, and substances with permeability in both penetrate most rapidly (Squier 1980).

The skin is less permeable to water than any oral region. Within the oral cavity, the gingiva is the least permeable and most similar in permeability to epidermis, followed by the buccal mucosa and then the FOM which is most permeable (Squier and Hall, 1985). Keratin (Section IV.C) offers the major resistance to diffusion of water and both polar and nonpolar substances, and in terms of barrier function, parakeratinized epithelium is similar to orthokeratinized epithelium (Section IV.C). Polar molecules and electrolytes are also limited by the presence of lipid-containing membrane-coating granules which are evident throughout the superficial cell layers of cornified and noncornified regions. However, differences in the lipid content of the surface layers of cornified and noncornified epithelia may account for differences in their respective permeabilities. Consequently, cornified epithelium is impermeable to water and polar compounds; noncornified epithelium is less effective in resisting penetration of water and polar compounds but may be capable of resisting penetration of larger molecules such as proteins (Squier and Johnson, 1975; Squier 1987).

Significantly, many oral lesions such as lichen planus and other premalignant conditions (Section VI) as well as SCC, are most frequently found in noncornified lining regions but not all noncornified areas are equally susceptible (Squier 1987). That is, the FOM is a 'high risk' site for SCC but buccal mucosa is not, yet both sites are lined by noncornified epithelium. The thickness of the buccal mucosa is typically thicker than FOM mucosa, suggesting that thickened epithelium is

less permeable and hence less susceptible (Lavelle 1976b, Squier 1987). However, increased thickness of epithelium does not appear to automatically confer improved barrier function and in fact, may reduce it. For example, hyperplastic and hyperkeratotic oral epithelia do not demonstrate improved barrier function, a finding that is consistent with measurements of increased permeability in hyperkeratotic palmar and plantar epidermis as compared to thin skin (Squier 1987). In fact, increased thickness of epithelium is often associated with less complete maturation of the surface layers and therefore barrier functions are reduced (Squier and Johnson, 1975).

If noxious substances breach the superficial permeability barrier and the epithelium, further penetration of some substances may be limited by the basal lamina at the epithelial-connective tissue interface. The basal lamina is a differentially-permeable membrane that regulates metabolic exchange between the epithelium and connective tissue and allows passage of selected molecules and certain migratory cells such as polymorphonuclear leucocytes. Basal lamina also restricts penetration of many substances including endotoxin and immune complexes (Squier 1987). If the basal lamina is breached, the connective tissue of oral mucosa is not an effective barrier against penetration of polar substances although it may limit diffusion of macromolecules and non-polar substances (Squier and Johnson, 1975).

3. Cellular Defence Mechanisms

The oral mucosa also contains immunocompetent antigen-presenting cells, the Langerhans cells (reviewed by Daniels 1987) which may have a role in induction of contact hypersensitivity or tolerance. The density of oral Langerhans cells displays regional variations in that the frequency of Langerhans cells varies inversely with the degree of cornification (Daniels 1984). In noncornified mucosa, Langerhans cells are located in deep, suprabasal epithelium approximately parallel to the basement membrane and their number is similar to the number of Langerhans cells in epidermis. In cornified mucosa of the hard palate and gingiva, Langerhans cells are located in midepithelium, parallel to the surface and their numbers are much lower, including irregular sites without

Langerhans cells. On the dorsal tongue, Langerhans cells are absent from the interpapillary epithelium but are abundant at the tips and one side of the filiform papillae (Daniels 1984).

Erosion, ulceration, and mucosal atrophy, possibly in relation to age, result in the reduction or loss of the superficial epithelial permeability barrier; hence, permeability of the epithelium is increased, favouring the penetration of antigenic substances (Squier 1987). Antigenic material that penetrates into the epithelium may be trapped by antibodies as immune complexes within or beneath the epithelium. Inflammatory cells accumulate beneath the epithelium and elaborate proteolytic enzymes which damage the basal lamina, threatening the permeability barrier to other molecules. Neutrophils also infiltrate the epithelium, secrete proteolytic enzymes and thus reduce intercellular permeability barriers. Inflammation often produces hyperplastic changes characterized by acanthosis (thickening of the stratum spinosum) and hyperkeratosis due to accelerated cell division and accelerated passage of cells to the surface. Consequently, there is insufficient time for normal barrier layers to be formed and permeability is further increased (Squier and Johnson, 1975; Squier 1980). The effects of erosion and inflammation upon permeability are illustrated by denture-loaded palatal mucosa in which inflammation and erosion cause permeability to water to increase to twice that of normal noncornified buccal mucosa (Riber and Kaaber, 1978).

C. Differentiation of Oral Epithelium

Differentiation of basal epithelial cells is a complex process associated with the structural organization of the stratified squamous epithelium. It involves an ordered sequence of defined morphological changes accompanied by the sequential expression and modification of specific differentiation products. This Section reviews the biochemical and morphological changes that reflect the differentiation of basal cells as they migrate from the basal layer to the superficial layers of the mucosa. Discussion also includes differences in cornified and noncornified mucosa, differences in keratin expression and a brief review of vitamin A and connective tissue influences on oral epithelial differentiation.

In the literature, the use of the term “keratin” may be confusing because it is used to describe a class of intermediate filaments (Section IV.C.3) that are unique to epithelial cells (eg. Goldman and Steinert, 1990; Morgan and Su, 1994) as well as the tough, insoluble cross-linked keratin polymer formed by the accumulation of intermediate filaments in a filaggrin matrix and located in the superficial layers of ortho and parakeratinized epithelium (Ross and Reith, 1985).

Keratinocytes are epithelial cells that produce keratin. Whereas all keratinocytes produce a keratin filament, not all keratinocytes (i.e. keratinocytes from noncornified epithelia) produce keratin-polymer in their surface layer (eg. Atkinson and White 1992). The term ‘keratinized’ is synonymous with the term ‘cornified’ and throughout this thesis, the term ‘cornified’ will be used to describe epithelium which contains the keratin polymer in its superficial layer. The term ‘noncornified’ is used to describe epithelium which does not contain the keratin polymer in its superficial layer.

1. Cornified Masticatory Epithelium

Keratinization is the process of differentiation whereby viable keratinocytes transform or terminally differentiate into dead surface cells that are packed with keratin polymer (Atkinson and White 1992). Keratinization is an orderly, dynamic process of cellular proliferation and differentiation that is reflected by the structural and morphological organization of the cornified masticatory epithelium into four layers: stratum basale or germinativum, spinosum, granulosum and corneum. As cells migrate from the basal layer to the surface, they demonstrate an increasing accumulation of different intracellular proteins (eg. cytokeratin filaments, involucrin, filaggrin), an increasing number of desmosomes and an increased thickness of their cell membranes (eg. Ross and Reith, 1985; Atkinson and White, 1992). Differentiation is also reflected by the expression different keratin proteins which is discussed in Section IV.C.3.

a. Stratum Basale

In masticatory mucosa subjected to high mechanical loads, the junction between the basal epithelial layer and the connective tissue is highly interdigitated due to the interlocking arrangement of connective tissue papillae and epithelial rete ridges. Typically, the rete ridges are almost parallel-sided and they taper downwards to blunt ends (Kramer 1980). The basal layer is attached to the basal lamina and consists of a single layer of cuboidal or columnar cells. The basal cells are progenitor or stem cells whose continual division replenishes and compensates for the cells shed from the surface of the epithelium (Ross and Reith, 1985; Atkinson and White, 1992).

Basal cells express major histocompatibility class I (MHC) antigens (HLA-A, B, C) which are retained throughout their differentiation (reviewed by Eversole 1993). Basal cells also express MHC class II antigens (HLA-DR, DP, DQ) (reviewed by Eversole 1993), and undifferentiated keratins K5/K14 (Table 1.3), (eg. Darmon 1991); however, neither the class II antigens nor the primary keratins are expressed in the suprabasilar layers.

b. Stratum Spinosum

As soon as the basal cells detach from the basement membrane, phenotypic changes occur. The cells produce involucrin, a protein that forms a cross-linked, thickened layer on the cytoplasmic surface of the cell membrane. A new set of keratin filaments (K1, K10; Table 1.3) are produced and the keratin filaments aggregate into bundles called tonofibrils which form an intracellular network that attaches to the plasma membrane at desmosomes (Ross and Reith, 1985; Darmon 1991). In this layer, the number of desmosomes increases and the cells are slightly separated from each other, assuming an irregular polyhedral shape with long “spiny” cytoplasmic processes by which the cells attach to each other via desmosomes. The spinous layer may be several layers thick and the cells become increasingly flattened as they move farther away from the basal layer (Atkinson and White, 1992). Occasionally, the basal and spinous layers are classified together as the Malpighian layer (eg. Leeson and Leeson, 1970) which is collectively responsible for

proliferation and the initiation of keratinization.

c. Stratum Granulosum

Desmosomes are structurally best-organized in the suprabasilar layers up to the granular layer where they begin to lose their organization. The granular layer consists of several layers of flattened cells whose long axis is parallel to the surface. The thickness of the plasma membrane increases and the cells synthesize filaggrin which accumulates in the cytoplasm as globular masses called keratohyaline granules. In the superficial granular cell layers, the number and size of the keratohyaline granules increases, and the cytoplasm also contains lipid-containing membrane-coating granules (Ross and Reith, 1985; Darmon 1991; Atkinson and White, 1992).

d. Stratum Corneum

(i). Orthokeratin

In orthokeratinized epithelium, there is an abrupt change in appearance between the viable cells of the granular layer and the dead, cornified scale-like cells or squames of the corneal layer which contain the keratin polymer. Desmosomes have disappeared and the cells have a thickened, reinforced plasma membrane due to the tough, cross-linked layer containing involucrin. The cells are devoid of a nucleus and organelles but instead, they are packed with cytokeratin filaments which have aggregated in a matrix of filaggrin released by the keratohyaline granules. As well, the lipid content of the membrane-coating granules is excreted and the protective impermeable cornified stratum corneum is formed (Squier 1980; Ross and Reith, 1985; Darmon 1991; Atkinson and White, 1992).

(ii). Parakeratin.

In parakeratinized epithelium, cells retain their nucleus and some degenerate organelles. However, the cytoplasm is packed with keratin filaments embedded in filaggrin (Atkinson and White, 1992).

2. Noncornified Lining Epithelium

Noncornified epithelium is relatively distensible, adjusting to the movements of the underlying muscles, and is generally thicker than cornified epithelium. Rete pegs of lining mucosa are fewer in number and are rounder and shorter than in masticatory mucosa. Lining mucosa consists of four layers: stratum basale, spinosum, intermedium and superficiale. Keratin filaments (K13, K4; Table 1.3) are expressed by lining epithelium but they are arranged into a loose network rather than into bundles of tonofibrils as in cornified mucosa. The frequency and size of desmosomes in all strata are less than in cornified mucosa. As well, the stratum intermedium lacks keratohyaline granules and the stratum superficiale is generally not cornified although it may be parakeratinized in some locations. The surface cells in lining mucosa are less flattened with highly folded cell walls, enabling the mucosa to elongate in response to tensile forces. Glycogen may be present in the superficial epithelium and some of the organelles including the nucleus, persist to the surface (Lavelle 1976b; Squier 1980; Ross and Reith, 1985; Atkinson and White, 1992).

3. Keratins as Markers of Differentiation

Keratins are a class of intermediate filaments that are unique to epithelial cells. Intermediate filaments comprise part of the cytoskeleton and they are polymers of intracellular proteins organized to provide the principal structural support and stabilization for the epithelium. There are at least 30 distinct keratin types which consist of two classes: acidic (type I) and neutral/basic (type II). Equal numbers of type I and II keratin subunits are combined to form heterodimers called keratin or cytokeratin filaments (Table 1.3). Keratins are encoded by a large family of homologous genes and from the time a new keratinocyte in the basal layer is transformed into a superficial cell of either the stratum corneum or superficiale, it expresses a succession of different genes from its cytokeratin gene repertoire (Moll et al., 1982; Cooper et al., 1985; Darnell et al., 1986; Morgan and Su, 1994). Thus, epithelial cells express multiple keratins, usually in consistent pairs, and the expression of keratins is correlated to the degree of epithelial differentiation, making keratins the best marker of epithelial differentiation (Moll 1987).

Stratified epithelia demonstrate primary keratin markers K5/K14 in their basal compartment and secondary differentiation-specific keratins that are unique to the suprabasilar compartments of cornified and noncornified epithelium (Table 1.3). In cornified oral epithelia, suprabasilar layers are characterized by K1/K10 and K6/K16, the latter pair indicating a high cell turnover rate. In noncornified oral epithelia, the suprabasilar layers of are characterised by K4/K13 as well as K6/K16. In addition, K19 is distributed throughout all layers of noncornified oral epithelium but predominately in the basal layer (Morgan and Su, 1994; Kautsky et al., 1995; Su et al., 1996). Furthermore, as part of a normal variation, the suprabasilar layers of small cell groups in an epithelium may display keratins that are uncharacteristic of that epithelium. That is, lining mucosa of the cheek may display small foci of K1/K10 (markers of cornification) and attached gingiva or hard palate may display foci of K4/K13 (noncornification markers), (Morgan and Su, 1994).

The lateral and ventral surfaces of the tongue are lined by noncornified epithelium and thus demonstrate K19; K4/K13; K6/K16. The dorsal surface of the tongue demonstrates mixed patterns of cornification. The filiform and fungiform papillae demonstrate markers of cornified epithelium but the inter-papillary epithelium expresses noncornified markers. The taste buds (associated with some types of papillae) demonstrate K19 and simple epithelium markers K8/K18 (Morgan and Su, 1994).

The profile of keratins expressed between and within different epithelia is a sensitive indicator of epithelial differentiation. However, keratins have a long half-life of at least 4 days (Denk et al., 1987) and therefore, their detection may mask actual changes in keratin gene activity. In fact, keratin mRNA may be present in tissues in which keratin protein is either not detected or would not be expected (Su et al., 1996). Ideally, both keratin protein and keratin mRNA should be assessed but there are limitations in sensitivity of both immunohistochemistry (eg. Bacallao et al., 1990; Denk 1987; Moll 1987) and in situ hybridization (eg. Terenghi and Fallon, 1990; Su et al., 1996) techniques, respectively (see also Section II.H.1). Close correlation in location between mRNA

and its protein indicates that control of gene expression is regulated at the transcriptional level. This transcriptional control is evident in oral epithelium for K14 (primary keratin of stratified epithelium) and noncornified oral epithelium for K19. In contrast, cornified epithelium expresses K19 mRNA but not its protein, indicating that K19 expression in these cells is controlled post-transcriptionally (Su et al., 1996).

4. Influence of Retinoids on Epithelial Differentiation

The differentiation of keratinocytes is due to many changes in gene expression, some of which are related to the control of cell division and others to the expression of the squamous differentiated phenotype, including keratin markers. The proliferation and differentiation of keratinocytes is mediated locally by vitamin A (retinol) and its derivatives (retinoids) and a greatly over-simplified summary of a complex topic is presented below (see also Table 1.4), (eg. Darmon 1991; Pfahl 1994; Love and Gudas, 1994; Gudas et al., 1994; Jetten et al., 1994).

Retinol is oxidized to retinoic acid (RA) and its isomers which are the biologically-active forms of vitamin A in epithelia. In the adult, retinoids promote the normal differentiation of epithelial cells, and its effects on the synthesis of differentiation markers by keratinocytes occur at the transcriptional level and are mediated through two types of nuclear receptors (RAR for RA; RXR for other retinoids) and their isoforms (α , β , γ), (Darmon 1991; Love and Gudas, 1994) which differ in their abilities to bind RA and retinoids (Xu et al., 1994). RARs belong to a large family of DNA-binding regulatory proteins that bind to promoter regions of specific genes and thereby modulate the expression of those genes. In order for a keratinocyte to respond to RA, the keratinocyte must contain nuclear RA receptors (RARs), and the genes that are expressed during differentiation must contain promoter regions that contain RA-responsive elements (RAREs) able to bind the RA receptors (Darmon 1991). Examples of genes that contain RAREs include genes for laminin, growth hormone, the receptor for epidermal growth factor, and keratin K14 (Darmon 1991). RARs may also facilitate cross-talk between different hormone signalling pathways,

suggesting a hormone-like control of cell differentiation by RA (van Poppel 1993; Pfahl 1994; Love and Gudas, 1994; Gudas et al., 1994; Jetten et al., 1994). Furthermore, two sets of cellular RA-binding proteins (CRABPs) sequester RA in the cytoplasm. CRABPs maintain intracellular RA concentrations for the appropriate differential regulation of gene transcription, possibly by preventing the interaction of RA with its nuclear receptors (Love and Gudas, 1994; Kautsky et al., 1995).

Retinoids are multifunctional agents and similar to growth factors (Section IV.D.2.b.iii), their ability to elicit specific cellular responses depends upon the biological content or nature of the local ECM (Nathan and Sporn, 1991). Since RA controls the expression of a large variety of genes including genes encoding growth factors and their receptors, cellular enzymes and structural proteins including keratin, altered levels in any of these products can drastically alter the differentiation state of a particular cell. For example, retinoids control genes encoding gap junctional proteins; enhanced gap-junctional communication suppresses cell growth and suppresses malignant transformation, whereas inhibition of communication enhances these processes. Retinoids are also involved in restructuring of the ECM, including glycoproteins, metalloproteinases and their inhibitor, TIMP. Consequently, retinoids influence cell membrane permeability and cell-cell interactions including communication and adhesion not only amongst epithelial cells but also between the epithelium and connective tissue. In turn, the ability of retinoids to elicit specific cellular responses may rely on the nature of the underlying connective tissue (van Poppel 1993; Love and Gudas, 1994; Gudas et al., 1994).

Terminal differentiation of oral epithelium may be influenced by at least two different RA-associated regulatory mechanisms. One is via a direct, RA-concentration-dependent mechanism that controls intrinsic properties of the epithelium itself. For example, under experimental conditions, low concentrations of RA decrease expression of K13 and K19, markers of noncornified oral epithelium, but increase the expression of profilaggrin and K1, markers of

cornification (Table 1.4). High concentrations of RA have the opposite effect; i.e. increase markers of noncornification and decrease markers of cornification (Kautsky et al., 1995) including the formation of involucrin involved in formation of the crosslinked cell membrane (Jetten et al., 1994). The clinical application of topical retinoids to the epidermis decreases the number of tonofilaments and desmosomal attachments. As a result, intercellular spaces are widened and cohesiveness of the stratum corneum is decreased which causes increased fragility of the upper epidermis. In addition, the function of the permeability barrier is impaired; transepidermal water loss is increased and the percutaneous absorption of topical agents is enhanced which can be either beneficial or potentially toxic (Peck and DiGiovanna, 1994).

In essence, RA suppresses expression of the squamous (cornified) differentiated phenotype (Darmon 1991), and the maintenance of the noncornified state of squamous cells may depend on the continuous presence of retinol (Xu et al., 1994). Significantly, keratinocytes derived from either cornified or noncornified oral mucosa respond similarly to RA regulation of cornification, including K1, K13 and filaggrin expression. However, the expression of K19 in these two keratinocyte types differs with RA concentration and is due to the differential expression of RAR isoforms in the two cell types. RAR isoforms have differential control of cytokeratin and filaggrin expression and RAR γ in particular, may control expression of K13, K1 and profilaggrin. RAR α and RAR γ mRNAs are equally expressed in both cornified and noncornified cell types and their levels are only minimally affected by RA. In contrast, RAR β is expressed predominantly in noncornified cell types and is linked to expression of K19. Moreover, elevated concentrations of RA induce expression of both RAR β and K19 mRNA levels (Crowe et al., 1991; Hu et al., 1991; Kautsky et al., 1995).

A second level of differentiation control may be exerted by the subepithelial connective tissue but, the mesenchymal influences on epithelial differentiation vary depending upon the body site and the particular epithelium and connective tissue involved (eg. Mackenzie and Hill, 1984). That is,

expression of pre-existing patterns of keratin expression, basal proliferation and cell turnover may be maintained following recombination of the epithelium with a connective tissue from a site not normally associated with such patterns; in that instance the connective tissue permitted the epithelial phenotype, or alternatively, the epithelium was not capable of responding to the connective tissue signals (Hill and Mackenzie 1989). In contrast, some epithelia acquire keratin patterns and proliferative rates corresponding to those of the epithelium normally associated with the connective tissue, indicating directive or inducing influences from the connective tissue to an epithelium that was capable of responding (Mackenzie and Hill, 1984; Hill and Mackenzie 1989). Oral lining epithelium cornifies in response to inductive signals from masticatory subepithelial connective tissue and this behaviour forms the basis for grafting masticatory mucosa to sites of lining mucosa along cervical tooth margins (gingival grafting), (Karring et al., 1972, 1975). However, in contrast to subepithelial connective tissue, deep connective tissue does not have the full potential to induce cornification, and epithelium grafted onto deep connective tissue expresses a hybrid mixture of cornified and noncornified keratin markers (Ouhayoun et al., 1988).

Depending upon the differentiation marker measured, there may be a continuous cross-talk between the oral fibroblasts and oral keratinocytes which leads to a terminal differentiation of the epithelial cells. For example, K19 expression may be intrinsically predetermined whereas K1, K13 and profilaggrin expression may be receptive to extrinsic influences (Kautsky et al., 1995). *In vitro* studies (Kautsky et al., 1995) have demonstrated that subepithelial fibroblasts can modulate the sensitivity of keratinocytes to RA and thereby influence differentiation of oral keratinocytes. Fibroblasts from cornified oral mucosa can inhibit the RA response of oral keratinocytes, resulting in increased expression of markers of cornification (profilaggrin, K1). In contrast, fibroblasts from noncornified oral mucosa potentiate the RA response of oral keratinocytes as indicated by their expression of K13. Oral fibroblasts appear to influence the apparent RA exposure of the keratinocytes independently of the actual RA concentration, and fibroblasts may elaborate diffusible factors which indirectly regulate the ability of CRABPs to

sequester RA in the epithelial cytoplasm (Boukamp et al., 1990; Kautsky et al. 1995).

Thus, the regulation of keratinocyte growth and differentiation is controlled by intrinsic epithelial programs but it is also dependent upon underlying fibroblasts and the ECM. Components of the ECM bind growth factors thus affording the ECM a key regulatory mechanism in tissue organization. The turnover rate of ECM and basement membrane is carefully controlled by mechanisms which regulate the levels of metalloproteinases (eg. collagenase), levels of TIMP and the synthesis of new ECM components (Ansari and Hall 1992). RA also plays an important role in turnover of ECM as RA stimulates laminin production but inhibits the secretion of collagenase by keratinocytes (Table 1.4). RA influences fibroblasts and can either stimulate or inhibit synthesis of type I collagen and fibronectin by fibroblasts depending upon their source and the concentration of RA. In fibroblasts, RA regulates the expression of metalloproteinases and TIMP in an inverse manner by repressing the expression of metalloproteinases and favouring expression of TIMP (Gudas et al., 1994). These latter effects may be mediated by direct control of RA at the transcriptional level, or they may be mediated indirectly via the induction of a growth factor, transforming growth factor β (TGF β) which has an effect similar to RA on ECM turnover (Gudas et al., 1994).

Mechanisms whereby retinoids affect communication between cells and regulate turnover of the ECM including neovascularization, are essential to maintaining tissue homeostasis as well as physiological repair of tissue injury. They are also essential features in carcinogenesis which is discussed in Section V.

D. Epithelial Proliferation

In normal tissue, the number of cells with a particular phenotype is strictly regulated through control of stem cell proliferation, cell differentiation and appropriate spatial organization balanced by the loss of cells to terminal differentiation and natural cell death (apoptosis, IV.D.2.a.iv). In

stratified squamous epithelium, proliferation limited to basal stem cells, is necessary to maintain homeostasis of the epithelium because the surface layers are terminally differentiated and incapable of dividing. The surface cells are continuously shed and this cell loss must be balanced by continual production of new cells from the basal layer.

It is essential that each tissue maintains a size appropriate for the body's needs and under normal conditions, cells divide only when instructed to do so by other cells in their vicinity. Appropriate control of cell reproduction is carefully regulated by complex pathways that converge on the cell cycle (Figures 1.5, 1.6).

1. Cell Cycle

The cell cycle (Figure 1.5) is a repetitive sequence of interphase, mitosis and return to interphase. It consists of 4 phases: Gap 1 (G1), S (synthesis), Gap 2 (G2) and M (mitosis). Together G1, S and G2 represent interphase and M represents mitosis. During Gap 1 phase, the cell increases in size and performs structural and synthetic functions in preparation for duplicating its DNA. The length of G1 phase is variable and its length determines the length of the cell cycle. In contrast, the length of time spent in phases S, G2 and M is constant and irrespective of the rate of cell production. The synthesis (S) phase requires about 6 hours during which time the DNA is duplicated in the nucleus. During Gap 2 phase, the components required for mitosis are assembled, requiring 2-4 hours. During the one-hour long M (mitosis) phase, the nuclear membrane breaks down, mitotic spindles form and the duplicated chromosomes are evenly separated into two newly formed daughter cells. Immediately after M phase, the daughters may rejoin the cell cycle in G1 and pass rapidly through to M phase again. Cells with a long life span typically enter a G0 phase which is a nonproliferative phase in which they perform their normal functions for variable lengths of time (days or years) but are able to return to G1 as required (eg. Alberts et al., 1989; Atkinson and White, 1992).

2. Control of Cell Cycle

The cell cycle is controlled by a system of checks and balances that safeguards against disordered growth. This system includes a network of intrinsic cellular mechanisms and extrinsic factors including “social” contacts between cells, cells and the ECM, and growth factors. A brief review is provided below and in Figure 1.6, Table 1.5 and Table 1.6 because alterations in these control mechanisms distinguish normal cells from their malignant counterparts.

a. Intrinsic Mechanisms

(i). Restriction Point

Two classes of genes control the cell cycle: proto-oncogenes which promote cell division (Table 1.5) and tumour suppressor genes such as p53 and Rb (retinoblastoma) which suppress proliferation (Table 1.6). Proto-oncogenes are part of the normal vertebrate cell genome and they correspond to their counterpart oncogenes which are mutated tumour-promoting genes. Proto-oncogenes encode for growth factors such epidermal growth factor (EGF), growth factor receptors (R) such EGFR which is coded by gene c-erbB, cytoplasmic signalling proteins such as the ras family proteins, and nuclear DNA-binding transcription-activator proteins such as c-myc (reviewed by Hollywood and Lemoine, 1992, Weinberg 1996).

Simplistically, the control of cell division can be depicted as a switch whereby, depending upon the signals received by the cell, the signal for division can be switched from “off” to “on” (Weinberg 1996). A crucial switch or control point in the cell cycle occurs late in G1 at the Restriction Point which, once passed, commits cells to move into S, G2 and M phases. The Restriction Point is controlled by a protein, pRb, which is a product of tumour suppressor gene Rb. pRb is a powerful growth inhibitor and acts like a “master brake”. When pRb is in its unphosphorylated form it sequesters the transcription factors necessary for proliferation and consequently, it acts like a “brake” actively inhibiting cell division. However, two cytoplasmic proteins, cyclins (cyclin D initially) and cyclin-dependent kinases (CDKs), can form a complex which phosphorylates pRb,

thereby releasing the “brake” and the sequestered transcription factors, allowing the cell to pass the restriction point. The levels of cyclins are strictly controlled. There are at least 8 distinct cyclin genes (eg. Bcl-1 for cyclin D) but cyclin and CDK levels are also controlled by other proteins and their genes such as p15, p16 and p21 which, in turn, are controlled by tumour suppressor gene, gene p53, and its protein p53 (Alberts et al., 1989; Atkinson and White 1992; Weinberg 1996).

(ii). p53 Gene and Protein

p53 is an important tumour suppressor gene that is essential for normal cell growth. p53 has been described as a “molecular policeman”, monitoring the integrity of the genome. Its protein product, wild-type p53 protein is normally present at very low levels in all normal cells and tissues. p53 protein has a role in regulating the transcription of genes that suppress cell proliferation and effect passage from late G1 to S phase. p53 also functions as a G1 checkpoint control because when cellular DNA is damaged, p53 protein levels significantly increase and arrest the cell cycle in G1 so that DNA can be repaired prior to cell division. However, if DNA repair fails, then p53 may trigger the programmed death of the cell called apoptosis (Raybaud-Diogene et al., 1996; Weinberg 1996). p53 protein has the ability to adopt two different conformations and it is therefore is a multifunctional protein. As noted above, the wild-type protein has a suppressor function for cell proliferation. In contrast, its mutant form has a promoting effect and therefore has been the focus of investigation in carcinogenesis, particularly of head and neck SCCs (Section V; eg. Warnakulasuriya and Johnson, 1994; Raybaud-Diogene et al., 1996; Weinberg 1996).

(iii). myc Genes

The myc genes code for transcription factors that activate other growth-promoting genes. Therefore, myc genes have important roles in the maintenance of the proliferative state and the blockade of differentiation pathways. In particular, the c-myc gene controls exit from the cell cycle to the G0 resting state, and the level of c-myc determines whether a cell will continue or cease proliferation. Transient repression of c-myc results in rapid disappearance of its protein which

signals the cell to enter G0; conversely, sustained levels of c-myc and its protein force continued cycles of proliferation (Hollywood and Lemoine, 1992).

(iv). Apoptosis

Apoptosis is the programmed death of a cell and is best demonstrated by the terminal differentiation process of keratinocytes. Apoptosis also acts as a back-up system to destroy cells whose essential components such as DNA are irreparable or, cells whose control systems become deregulated by an oncogene or by the disabling of a suppressor gene. Although apoptosis is undesirable for the affected cell, it is beneficial for the tissue and body as a whole. Apoptosis is a metabolically-active process, often associated by the active synthesis of new proteins (Ansari and Hall, 1992). Under normal circumstances, apoptosis is triggered by p53 protein. A second gene, bcl-2, codes for an intracellular membrane protein which modulates apoptosis by inhibiting the effects of p53, and thereby protects the cell by preventing the triggering of apoptosis too easily (Duke et al., 1996; Atula et al., 1996).

(v). Telomeres

The number of divisions that a stem cell and its daughters undergoes is tightly constrained and at some point, further cell division is no longer possible and the cell becomes senescent. The intracellular defense mechanism guarding against runaway proliferation may rely on telomeres which “count” and limit the total number of cell divisions. Telomeres have been compared to the plastic tip on the ends of shoelaces (Greider and Blackburn, 1996) in that they are a cap of special DNA sequences at the end of each chromosome. With each cell division, a small number of telomere sequences are lost, shortening the telomere, and when the telomere shrinks below a threshold length, the cells become senescent and attempts at further cell division result in cell death (Greider and Blackburn, 1996; Weinberg 1996).

Abnormalities of cellular ‘counting mechanisms’ may underlie diseases such as psoriasis in which

there are increased numbers of divisions (Ansari and Hall 1992). Tumours also demonstrate uncontrolled proliferation and in some types, the tumour cells are able to repair or replace their telomeres by activating a gene that encodes for the enzyme telomerase. This enzyme is absent in most normal cells but is present in most tumour cells. Telomerase maintains the telomeres, enabling the tumour cells to divide endlessly and allowing the tumour to increase in size. Unfortunately, it also permits the pre- or already-cancerous cells to acquire additional mutations that may further increase their ability to proliferate, invade and metastasize (Greider and Blackburn, 1996).

b. Extrinsic Mechanisms

External or environmental regulation is crucial to the control of cell division and includes cell contact with its extracellular matrix (anchorage dependence), contact with neighbouring cells (contact inhibition) and the influence of growth factors (reviewed by Ansari and Hall, 1992 and Templeton and Weinberg, 1995).

(i). Regulation by Cell-Matrix Interactions

In order to divide, healthy cells require contact with a substrate and this phenomenon is called anchorage dependence (eg. Folkman and Moscona, 1978). Cell contact with the substratum maintains the organization and cohesiveness of a tissue, and normal cells that become detached from their surroundings cannot proliferate. Different cell types have different requirements for the amount or size of substratum necessary to promote cell growth (Maroudas 1973a, b), but anchorage is required in order for cyclin levels to rise to levels sufficient for the cell to pass through the restriction point in G1 of the Cell Cycle. Once the restriction point is passed, contact with the ECM is no longer required, and cells typically lose contact and their ability to divide (reviewed by Ruoslahti 1996).

Anchorage dependence is mediated by integrins which couple contacts with the ECM to the

organization of the cell's cytoskeleton which, in turn, is linked to signal transduction ("outside-in" signals), (Hynes 1992; Haas and Plow, 1994). Integrins are transmembrane cell receptors, each type specific for a component of the ECM such as fibronectin, laminin or different types of collagen, but the affinity of an integrin for its ligand can also be influenced by cytoplasmic events ("inside-out" signals), (Hynes 1992; Horwitz and Thiery, 1994; Horwitz 1997). Interactions between the ECM and its integrin function like a "molecular address system" (Ruoslahti 1996) by directing and maintaining cells in their proper place in a tissue and in the body, and integrins may also play an important role in metastasis which is reviewed in Section V.

Division of basal stem cells is usually asymmetric (eg. Alberts et al., 1989) because only one of the daughters remains a stem cell and the other daughter loses contact with the basal lamina, is forced to differentiate and ultimately die (apoptosis). Early in the differentiation of keratinocytes, there is a decreased expression of genes encoding certain integrins and ECM proteins (eg. fibronectin, laminin) and this down-regulation may be involved in the migration of cells from the basal to suprabasal layers. Significantly, this early stage of differentiation is not sensitive to retinoids (Jetten et al., 1994) which are involved in epithelial/connective tissue interactions across the basement membrane by influencing ECM synthesis, metalloproteinases and TIMP.

(ii). Regulation by Cell-Cell Interactions

The division of a basal stem cell can be asymmetric or it can result in two identical daughter stem cells by simple duplication of the stem cell. The number of stem cells and their growth pattern is strictly controlled and the fate of the daughters can be affected by the relationships of the stem cell to the other cells in the tissue. For example, denudation of the epithelium from the connective tissue stimulates undamaged adjacent epithelial cells from the stratum basale to migrate from the wound margins into the wound. While some basal cells are migrating, other basal cells a short distance back from the leading edge undergo simple duplication to restore the stem cell population. Once cells from different margins make contact across the wound, they stop their migration and

this behaviour is called contact inhibition of movement. Once a confluent sheet of cells has been re-established, asymmetric division restores the differentiated suprabasal layers. However, simple duplication also ceases, a phenomenon known as density-dependent inhibition of cell division or contact inhibition of cell division (eg. Odland and Ross, 1968; Lavelle 1975; Alberts et al. 1989; Atkinson and White, 1992).

Cell proliferation is also regulated by the differentiated cell population to which the dividing cells belong. For example, the rate of production of keratinocytes is regulated by the thickness of the epithelium in that the presence of the outer differentiated layers appears to exert an inhibitory influence upon stem cells in the basal layer. If the normal epithelial thickness is reduced by stripping away just the surface differentiated layers, the rate of mitosis in the basal layers is increased; once the normal thickness is restored, the rate declines to normal, suggesting that there are signalling pathways from the superficial layers to the basal stem cells (Alberts et al., 1989; Atkinson and White, 1992).

(iii). Regulation by Growth Factors

Growth factors act as local chemical regulators, either stimulating or inhibiting cell division and differentiation, and most cell types probably depend upon a specific combination of growth factors rather than a single factor. Growth factors are present in blood and extracellular fluid and they are also bound to components of the ECM where they play a key regulatory role in tissue organization (Alberts et al., 1989).

The binding of a growth factor to its specific receptor at the cell's surface triggers a cascade of biochemical changes called secondary-messenger systems. These systems relay information from the cell's surface to its nucleus where the response, appropriate to the surface signal, is generated (Alberts et al., 1989). Some of the internal signalling pathways switched on by growth factors may also be linked to activation of molecules that reside with integrins in focal adhesions,

suggesting that ECM molecules and growth factors may sometimes modulate one another's signals through convergent or intersecting pathways (Horwitz 1997).

The main effect of growth factors occurs at the transition of cells from G0 to G1; progression through the remainder of the cell cycle is largely independent of extracellular influences. Most growth factors possess both stimulatory and inhibitory growth properties, depending upon the cell type. Moreover, the same cell type may respond in different ways depending on the presence or absence of other growth factors (Nathan and Sporn, 1991).

One group of growth factors with stimulatory growth effects for epithelial cells includes epidermal growth factor (EGF) which is produced by subepithelial fibroblasts (Alberts et al., 1989), and transforming growth factor α (TGF α) which is normally produced by epithelial cells (Kannan et al., 1996). EGF and TGF α interact with the same receptor, the epidermal growth factor receptor (EGFR), which is encoded by proto-oncogene c-erbB. Under normal conditions, TGF α is seen in the basal and suprabasal epithelial layers, and EGFR is found in high concentrations in proliferating basal keratinocytes. Binding of growth factors to EGFR results in signal transduction via second messenger systems and results in subsequent cell division (Alberts et al., 1989; Kannan et al., 1996).

In contrast, transforming growth factor β (TGF β) inhibits the growth of most epithelial cells. TGF β inhibits the transcription of c-myc; as a result, c-myc protein levels are reduced and the cell is unable to proceed to G1 (Hollywood and Lemoine, 1992). As well, TGF β regulates the activity of gene p15 whose protein product inhibits the activity of cyclin-dependent kinases (Weinberg 1996). TGF β also regulates the expression of receptors for EGF, promotes synthesis of collagens and TIMP but depresses synthesis of metalloproteinases (Ansari and Hall, 1992); these latter actions of TGF β are similar to those of retinoids but it is unclear whether the similarity is due to converging signalling pathways, or whether the action of retinoids are mediated via synthesis and

secretion of TGF β (Gudas et al., 1994).

(iv). Regulation by Subepithelial Connective Tissue

Connective tissue fibroblasts elaborate EGF which stimulates keratinocyte proliferation, and the interaction between the connective tissue and epithelium in relation to differentiation (keratin expression) and turnover of the ECM has already been discussed (Section IV.C.4). Further support for the role of connective tissue in regulating epithelial differentiation and proliferation stems from alterations observed in the subepithelial connective tissue in association with premalignancy and carcinoma discussed in Section V.

<u>A. Type of Keratin and Distribution</u>		<u>Class of Keratin Filaments</u>	
		<u>Type I</u> (acidic)	<u>Type II</u> (basic/neutral)
<u>Primary Keratins - Simple</u>		K18	K8
<u>Primary Keratins - Stratified</u>			
	Basal cells	K14	K5
<u>Differentiation-Specific</u>			
	Simple Epithelium	K19	K7
	Stratified Epithelium		
	Cornified - suprabasal	K10	K1
	Noncornified basal	K19	
	Noncornified suprabasal	K13	K4
<u>Fast Turnover-Stratified</u>			
	Cornified - suprabasal	K16	K6
	Noncornified - suprabasal	K16	K6
<u>B. Type of Epithelium</u>		<u>Keratin Markers</u>	
All Stratified Epithelia	K5, K14	primary markers in basal compartments	
Cornified Oral Epithelium	basal layer	K5, K14	
	suprabasal:	K1, K10 differentiation markers K6, K16 fast turnover markers	
Noncornified Oral Epithelium	basal layer	K5, K14 and K19	
	suprabasal	K4, K13 differentiation markers K6, K16 fast turnover markers	

Table 1.3. Distribution and Classes of Keratin Filaments in Oral Mucosa.

(A). Type of keratin (simple, differentiation, or fast turnover) and distribution in simple or stratified epithelia (cornified or noncornified, basal or suprabasal), and class of keratin (type I or Type II).

(B). Type of epithelium (cornified or noncornified) and keratin markers in basal or suprabasal layers. Adapted from Morgan and Su, 1994.

A. *in vitro* Effects of Different Retinoid Acid Concentrations

Low [RA]

- decreased expression of markers of non cornification (K13, K19)
- increased expression of markers of cornification (profilaggrin, K1)

High [RA]

- increased expression of markers of noncornification
- decreased expression of markers of cornification

B. *in vivo* Effects of Retinoic Acid

Suppresses

- expression of cornified phenotype by decreasing
 - expression of involucrin
 - number of tonofilaments
 - number of desmosomes
- expression/secretion of collagenase by keratinocytes
- expression/secretion of metalloproteinases (eg. collagenase) by fibroblasts
- production of type I collagen and fibronectin by fibroblasts

Enhances

- gap junctional proteins and intercellular communication which suppress cell growth
- expression of TGF β which also suppresses growth (Table 1.6)
- expression of receptor for epidermal growth factor (EGFR)
- expression/secretion of laminin by keratinocytes
- expression/secretion of TIMP by fibroblasts
- production of type I collagen and fibronectin by fibroblasts

Table 1.4. Summary of Some Effects of Retinoic Acid on Oral Epithelium.

- A. Some *in vitro* effects of high and low concentrations of retinoic acid.
 B. Some *in vivo* effects of Retinoic Acid.

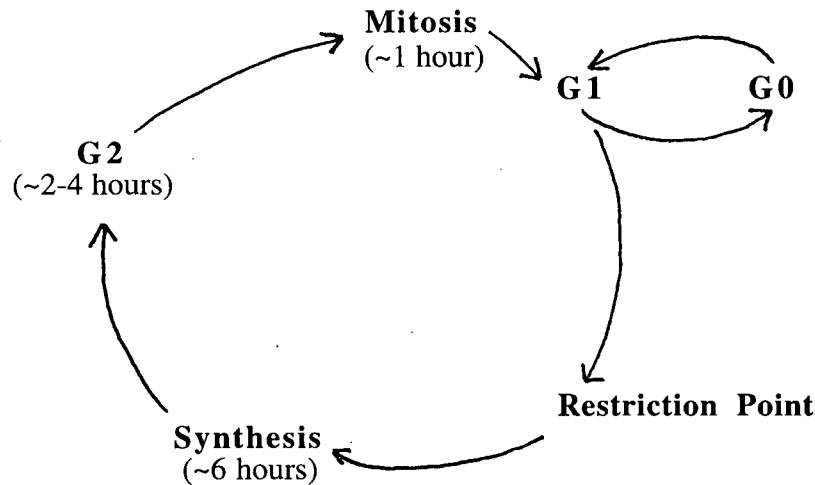


Figure 1.5. The Cell Cycle.

The Cell Cycle consists of mitosis (cell division) and interphase which includes G1 (Gap 1), S (synthesis) and G2 (gap 2) phases. The Cell Cycle begins with G1.

- G1 -is of variable length and its length determines the length of the Cell Cycle
 -cell enlarges and synthesizes proteins in preparation for DNA duplication and cell division
 -includes the Restriction Point which is a control point that once passed, commits the cell to completion of the cell cycle
- S -DNA duplication in the nucleus
- G2 -cell prepares for division
- M -the nuclear membrane breaks down, mitotic spindles form and duplicated chromosomes are separated (nuclear division)
 -two new daughter cells are formed (cytoplasmic division)
- G0 -shortly after completion of M phase, cells may re-enter G1 or exit the Cell Cycle to G0 which is a nonproliferative phase of variable length

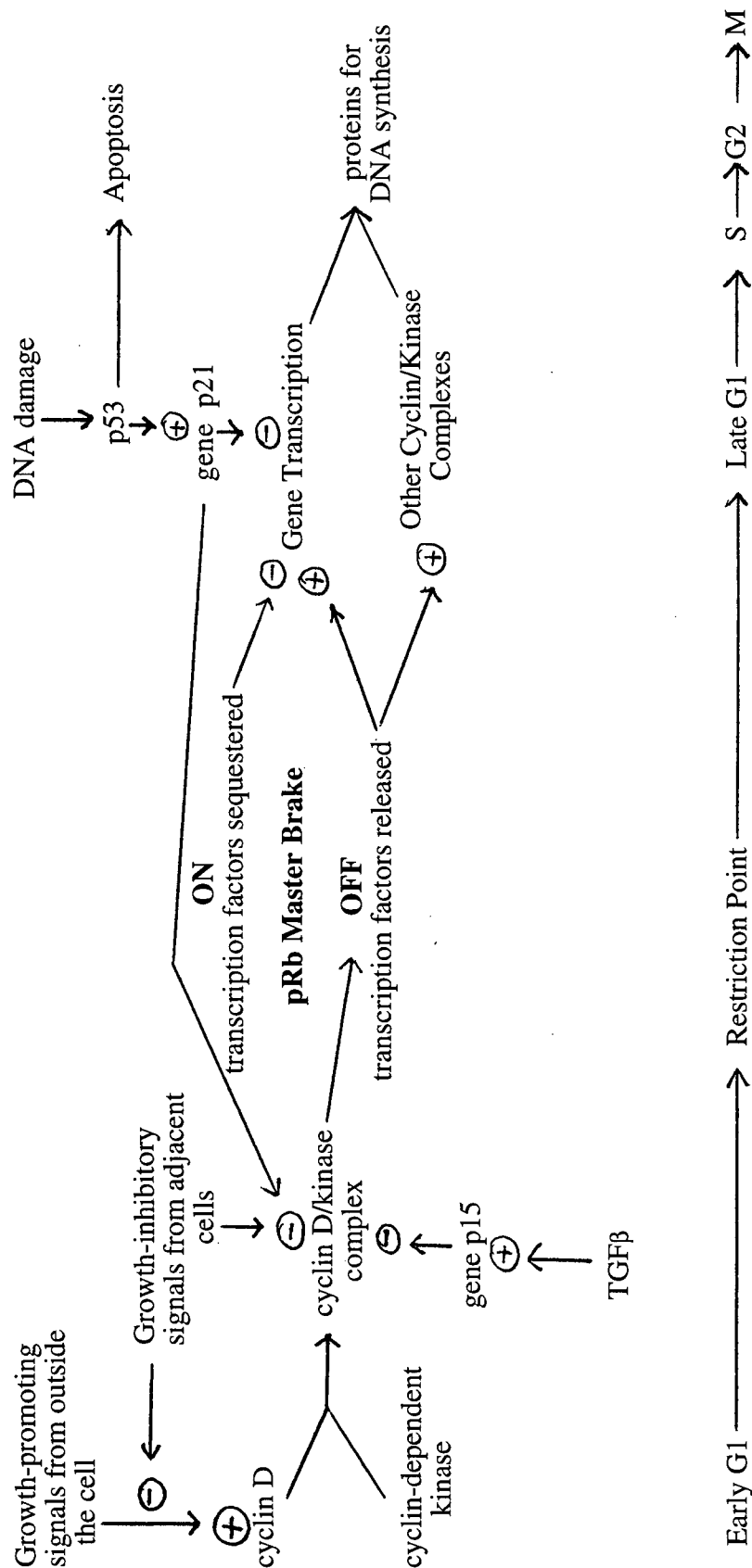


Figure 1.6. Control of the Cell Cycle. Progression through the Cell Cycle is driven by rising levels of cyclin proteins and the first cyclin is cyclin D. In late G1, the cell must commit itself to cell division by passing through the Restriction Point. In order to pass through the Restriction Point and enter S phase, the “master brake” protein pRb must be switched from ON to OFF. When pRb lacks phosphates, it actively blocks the Cell Cycle (brake “on”) by sequestering transcription factors. However, as levels of cyclin D rise in response to growth-promoting signals, they combine with and activate cyclin-dependent kinases; the resultant complex phosphorylates pRb, thereby releasing the pRb “brake”. Transcription factors are released by pRb, freeing them to act on genes which code for proteins required for continued progression through the Cell Cycle. However, there are additional checks in the Cell Cycle, including p53 which regulates the transcription of genes that effect passage from late G1 to S phase. P53 can also arrest the Cell Cycle in G1 to permit repair of damaged DNA or failing repair, can trigger cell death (apoptosis). Adapted from Weinberg 1996, and Scully and Field 1997.

Proto-oncogenes

- Bcl-1 gene codes for cyclin D which is involved in the phosphorylation of protein Rb which is required for passage through the Restriction Point
- Bcl-2 gene codes for intracellular membrane protein that modulates apoptosis and prevents triggering of apoptosis too easily
- c-erb B gene codes for receptor for epidermal growth factor (EGFR)
- myc genes code for transcription factors that activate other growth-promoting genes
 - high levels of c-myc protein sustain cell cycle; low levels signal cell to enter G0
- ras family of genes codes for proteins which are involved in coupling growth factor receptors to effector proteins in the cell

Cell-matrix Interactions

- anchorage dependence ensures adequate cyclin levels for passage through Restriction Point

Growth Factors

- EGF produced by subepithelial fibroblasts
 - TGF α produced by epithelial cells
- } both interact with EGFR which results in cell division of basal cells

Table 1.5. Some Factors that Enhance Proliferation of Epithelial Cells.

Tumour suppressor genes

- Rb: -Rb protein acts as “master brake” controlling the Restriction Point by sequestering the transcription factors necessary for progression through the Cell Cycle
-must be phosphorylated by cyclin/kinase complex to release transcription factors and permit entry to Cell Cycle
- p53: -protein p53 regulates transcription of genes that suppress cell proliferation and allow passage from G1 to S phase
-arrests cell in G1 to permit repair of damaged DNA
-triggers apoptosis
- genes p15, p16, p21:
-the protein products of these genes inhibit cyclin-dependent kinases which are required for passage through G1
-these genes are controlled by p53 protein
-gene p15 activity is also increased by TGF β which has a suppressive effect on proliferation

Telomeres

- shrinkage below threshold level result in cell senescence

Contact inhibition of cell division

- simple cell duplication ceases once a confluent sheet of cells is re-established after wounding of the basal layer of epithelium

Growth Factors

- TGF β -inhibits transcription of c-myc so that cells are unable to proceed to G1
-regulates expression of EGFR
-increases activity of gene p15 whose protein inhibits cyclin-dependent kinase
-promotes synthesis of collagen and TIMP
-depresses syntheses of metalloproteinases

Retinoic Acid Receptors

- expression of different RA nuclear receptor isoforms can modulate effects of retinoic acid

Table 1.6. Some Factors that Suppress Proliferation of Epithelial Cells.

V. Carcinogenesis

Neoplasia occurs due to changes in gene expression and gene control which allows the altered cells to proliferate faster than cells in the surrounding tissue from which they arose, and thereby produce a mass of abnormal new tissue called a neoplasm. Depending upon their destructive effects, neoplasms are classified as either benign or malignant. Benign neoplasms do not metastasise. They are typically slow growing and non invasive although ameloblastomas (Regezi and Sciubba, 1989) are an exception. Benign tumours are sharply demarcated from adjacent normal tissue and cells comprising the benign neoplasm are usually indistinguishable from those of their tissue of origin. Benign neoplasms typically cause damage by the pressure they exert on the adjacent tissues and by occluding local blood supply (Atkinson and White, 1992).

Malignant neoplasms are characterized by unregulated cell division and by abnormal cell differentiation. The appearance of malignant cells usually differs significantly from that of their normal counterparts in that they may be poorly differentiated (anaplastic) and so irregular in shape and size (pleomorphic) that it is difficult to recognize their tissue of origin. Transformed cells have escaped the controls that regulate orderly tissue growth and differentiation. Malignant cells lose their normal intercellular contacts such as desmosomes, and gap junctions are reduced or absent; their content of actin filaments is increased as is their secretion of proteolytic enzymes. Malignant cells no longer exhibit contact inhibition of movement or contact inhibition of cell division; they are anchorage independent and proliferate largely independent of control by external growth factors. Their high rate of proliferation increases demand for blood-borne nutrients and rapidly growing, poorly vascularized neoplasms may suffer necrosis of their central portions. Consequently, most tumours larger than several millimeters in diameter, produce angiogenic growth factors to promote neovascularization (reviewed by Templeton and Weinberg, 1995). Neovascularization is closely correlated to metastasis (Section V.E.) in which the transformed cells breach their basement membrane, penetrate the endothelium and enter the blood or lymph circulation where they must survive and evade immune surveillance. The malignant cells must also escape from the circulation

and finally implant and proliferate in foreign tissue to form secondary neoplasms (reviewed by Templeton and Weinberg, 1995 and Ruoslahti 1996).

A. Models of Transformation

Several mechanisms for malignant transformation are possible and significantly, more than one mechanism may be induced by the same carcinogenic agent (reviewed by Eversole 1993).

1. Monoclonal Transformation

Haematopoietic tumours and possibly some oral tumours exhibit evidence of clonality in which all of the malignant cells are descendents of the single mutant cell. For oral/pharyngeal SCC, the model of monoclonal transformation predicts that a single keratinocyte is transformed to a malignant phenotype and subsequent simple duplication expands the malignant clone (Eversole 1993).

2. Polyclonal Transformation and Field Cancerization

If several different basal keratinocytes are transformed either simultaneously or metachronously, their transformation results in the expansion of many different clones that may be anatomically widespread. Independent foci of malignant clones may progress to independent primary tumours or, several foci may eventually coalesce to produce a single large tumour (Eversole 1993).

Slaughter et al. (1953) applied the term "field cancerization" to findings of two or more independent oral and upper respiratory SSCs. They reported the incidence of field cancerization as 11.2 % based on 43 of 88 patients who presented with two separate primary tumours of the same anatomical area in the oral cavity (Slaughter et al., 1953).

Histologic premalignant changes (eg. Ogden et al., 1991) and biomarkers of malignancy (eg. Regezi et al., 1995; Yan et al., 1996; Scully and Field, 1997) have also been detected in the oral mucosa of patients with malignancies at a distant oral site, suggesting that oral cancer exerts a

regional effect upon normal oral mucosa. That is, a whole tissue region repeatedly exposed to carcinogens (eg. alcohol, tobacco) is at increased risk for developing multiple foci of malignant change (Slaughter et al., 1953). Field cancerization may also explain the local recurrence of oral cancer whereby pre-existing multicenters of premalignancy or malignancy persist at varying distances outside the area of treatment for the first premalignancy (eg. Mincer et al., 1972) or first primary SCC (Slaughter et al., 1953).

3. Contiguous Transformation

Hypothetically, an infectious transforming agent such as a virus could be transmitted laterally or horizontally, from one basal cell to the next, so that contiguous cells become transformed. This mechanism could offer another explanation for the occurrence of anatomically diffuse longitudinal spans of malignancy (reviewed by Eversole 1993).

B. Tumour Initiation, Promotion and Progression

The transformation of cells from normalcy to full malignancy is a long, multistep process in which cells pass through a series of stages including initiation, promotion and progression. As a result, tumours are a heterogeneous, evolving population of cells; subpopulations of cells that have a selective growth advantage expand, acquire additional mutations, and ultimately dominate the tumour cell population (eg. Alberts et al., 1989; van Popple 1993; Vogelstein and Kinzler, 1993).

Carcinogenesis is initiated with a heritable, irreversible genetic change in the cell's DNA sequence (Alberts et al., 1989; van Popple 1993; Vogelstein and Kinzler, 1993), so that the expression or function of genes or their products, or both, is altered (Scully and Field, 1997). Tumour initiators include chemical, viral or physical carcinogens. Chemical carcinogens typically cause simple local changes in the nucleotide sequence, and viruses introduce foreign DNA. Ionizing radiation acts through the production of reactive hydroxyl radicals (in aqueous media) which attack the ring structure of guanine in DNA, resulting in chromosome breaks and translocations (Alberts et al.,

1989; Lawley 1994), (see also Section VII.E.2.a).

Specific chemicals are designated as genotoxic when they or their metabolites react with DNA to induce mutations, or to generate reactive oxygen or hydroxyl radicals which interfere with DNA repair. Usually, the altered or damaged DNA is repaired; however, if the rate of cell division is rapid, such as in growing organisms, the chances for successful repair are decreased (Alberts et al., 1989; Lawley 1994). In addition, there may be an impaired ability to repair DNA damage, which in some cases occurs as an inherited trait (Scully and Field, 1997). The altered DNA undergoes further changes during replication so that proto-oncogenes and suppressor genes may be affected. A proto-oncogene may be converted to an oncogene by several mechanisms but typically, only at a limited number of sites within the gene. The gene may be altered by a simple point mutation (change in a single nucleotide pair such as AT to CG), insertion or deletion of nucleotide pairs, chromosome rearrangement or translocation, or by insertion of additional, foreign genetic material such as from a virus (Alberts et al., 1989; Weinburger and Williams, 1995). If these gene changes occur in an area coding for a protein, the activity of the protein may be affected (eg. p53 protein); if these changes occur in control regions, the gene may be simply overexpressed (increased transcription), (Alberts et al., 1989). The gene may also be amplified due to anomalies during chromosome replication which result in multiple copies of the same gene (Alberts et al., 1989). Particular genes are affected by specific abnormalities in response to particular carcinogens. For example, members of the myc gene family are typically over-expressed or amplified (Weinburger and Williams, 1995; Scully and Field, 1997). The ras gene can be altered in only 3 of its 188 codons (the triplet of nucleotides that specifies one amino acid), and in specific types of skin tumours, an 'A' to 'T' point-mutation occurs (Greenblatt et al., 1994; Weinburger and Williams, 1995). For tumour suppressor gene p53, mutations (predominantly base substitutions) in 95 of its 353 codons can cause inactivation; tumour suppressor gene Rb is usually affected by deletions or mutations and its inactivation requires involvement of both alleles (Lawley 1994; Weinburger and Williams, 1995).

The altered genotype is usually not fully expressed unless a tumour promoter is present. Tumour promotion is considered reversible and involves an epigenetic change which is a change in the pattern of gene expression without a change in the DNA sequence. In order to express their cancer-associated genes, cells with abnormal DNA must divide and develop, which will determine the rate of cancer expression and the cancer risk. Promoters alter the balance between the growth of normal cells and initiated cells by providing a growth stimulus to cells with the altered genotype, favouring their clonal expansion and development (Lawley 1994; Weisburger and Williams, 1995). As the numbers of initiated cells increases, the probability of additional mutagenic events which may confer malignancy also increases because rates of mutation appear to be higher for already-initiated cells. For example, cells with inactivated p53 genes are genetically less stable and accumulate mutations and chromosomal rearrangements faster, leading to rapid selection of malignant clones (Lawley 1994).

Promoters exert their effects through a variety of mechanisms that includes disturbing gap junctions and intercellular communication, and enhancing intracellular signalling pathways for growth stimuli. To be effective, promoters must be present at high levels for a long time but fortunately, their effects are usually tissue specific, reversible, and not permanent (Alberts et al., 1989; reviewed by van Popple 1993; Weinburger and Williams, 1995).

A single somatic mutation is insufficient to cause cancer. An estimated three to seven independent genetic and epigenetic changes are required before slightly abnormal cells evolve into the invasive end-stage cancer cell (Vogelstein and Kinzler, 1993). For those cancers that have a discernible etiology, there is almost always a long delay between the causal event (initiation) and the onset of clinical disease. During this period of tumour progression, the prospective cancer cell(s) that are already favoured for clonal expansion, acquire additional mutations that may further enhance their ability to proliferate. Eventually a cell may accumulate sufficient genetic mutations to render it malignant (Alberts et al., 1989). Some genetic changes may be primary or essential to the

malignant process, whereas others may appear during tumour progression and be secondary (Scully and Field, 1997). Some sites on chromosomes are particularly liable to mutations and are termed “fragile” sites; these fragile sites are increased in frequency in individuals who smoke or chew tobacco (Scully and Field, 1997).

C. Molecular Basis and Biomarkers for Squamous Cell Carcinoma of the Head and Neck

Neoplastic cell populations are characterized by relatively uncontrolled proliferation that is the result of specific gene mutations, amplifications or losses of one or more of the cell cycle genes. For example, persistent mitogenic signalling results from the activation of several proto-oncogenes and overproduction of growth factors and their receptors (eg. Ras, c-myc, EGF/c-erbB; see Tables 1.5, 1.6 in Section IV.); a cell may also become less responsive to negative growth factors (eg. TGF β) with the loss of tumour suppressor genes (eg. p53, Rb). Not all combinations of oncogenes will transform cells; if both oncogenes act through the same pathway, the cell may maintain adequate control of the cell cycle. There are also synergistic oncogene combinations such as c-myc and bcl-2 which commonly occur in lymphoma. Overexpression of c-myc is associated with neoplastic growth but under conditions of poor vascularity which reduce available nutrients and growth factors, cells overexpressing c-myc undergo apoptosis. If these cells also overexpress bcl-2, they are rescued from apoptosis due to its effects on p53 (Vogelstein and Kinzler, 1993), (Table 1.5 Section IV).

A mutation that creates an oncogene may have an effect on cell behaviour even in the presence of a normal copy of the corresponding proto-oncogene but typically, an oncogene exerts its cancer-provoking effect only if function of tumour suppressor genes is already perturbed (Alberts et al., 1989). In most epithelial cells, neoplastic pathways are guarded by suppressor genes rather than oncogenes (Vogelstein and Kinzler, 1993) so that cancer may be explained in terms of the loss of a tumour suppressor gene rather than acquisition of an oncogene. Normal DNA has two copies or

alleles of every sequence, but specific regions in a chromosome may be lost or deleted, resulting in allelic loss (Scully and Field, 1997). Tumour suppressor genes usually lose their function upon loss of both wild-type alleles; usually one allele is lost, followed by a missense mutation in the other (substitution of a single nucleotide pair), (Vogelstein and Kinzler, 1993). For example, in the hereditary form of retinoblastoma, multiple independent tumours affect both eyes. These children have a predisposition for the disease because one copy of their Rb gene is abnormal; a single somatic mutation in the remaining normal gene of even one cell is sufficient to initiate a cancer. In contrast, the nonhereditary form of retinoblastoma involves a single tumour in only one eye. The condition is very rare because two somatic mutations must occur in a single retinal cell in order to destroy both copies of the Rb gene (Alberts et al., 1989). Mutations in tumour suppressor gene p53 may operate by a different mechanism which is discussed below (Section V.C.1.).

DNA is a receptor for carcinogens and specific covalent chemical bonds or products called adducts identify the interaction between the carcinogen and DNA. Adducts can be identified and quantified by special physical/chemical analyses, and different carcinogens produce a "fingerprint" that is linked to the mutational activity of that agent (Lawley 1994; Greenblatt et al., 1994). Adducts may be the first stage in cancer initiation, causing DNA polymerase to misread the base pairing due to altered hydrogen bonding properties of a base that contains an adduct. Adducts may also render the bases unreadable and "stall" the replication process (Greenblatt et al., 1994). The study of adducts, specific gene mutations and other biomarkers (see below) in the population forms the basis of molecular epidemiology (Perera 1993) in which standard epidemiological principles of investigational design and statistical analysis are applied to detection of adducts and biomarkers (Section II), (Greenblatt et al., 1994). The expectation is that adducts and biomarkers can be incorporated into cohort and case-control studies to identify causal relationships between exposure to a specific agent and the increased risk of malignancy. Ultimately, certain adducts and biomarkers may replace clinical disease as an endpoint, and thereby provide an earlier outcome so that those individuals at highest potential risk can be identified for intervention and follow-up

(Perera 1993).

Abnormal karyotypes (chromosomes) are seen in 50-96% of oral/pharyngeal SCCs (Burkhardt 1996) and although all chromosomes may be involved, the most frequently altered chromosomes encode for some of the well-known oncogenes and tumour suppressor genes (Table 1.7), (reviewed by Burkhardt 1996; Scholes and Field, 1996; Scully and Field, 1997). Chromosomal alterations include the loss or gain of whole chromosomes, rearrangements of chromosomes such as inversions or translocations, deletions or amplifications (Scully and Field, 1997). Whereas a consistent chromosome abnormality common to all SCCs of the head and neck has not been identified, genetic aberrations involve, in order of frequency, chromosomes 3, 9, 11, 13 and 17 (Table 1.7; Scully and Field 1997). Allelic losses on chromosomes 3, 9 and 17 are seen as early events in SCC of the head and neck; but losses may be seen on additional chromosomes, consistent with a generalised increased rate of DNA replication errors and defective repair during tumour progression. Significantly, it is the accumulation of genetic changes and not the sequence of genetic changes that determines progression to malignancy (Scully and Field, 1997).

If alterations in specific genes and in the protein products they control reflect a different stage or mechanism in carcinogenesis, then changes in gene/protein expression and function may serve as biological markers of malignancy that ultimately replace clinical malignancy as an endpoint in diagnosis (Perera 1993; Slootweg 1996). However, the mere association of a biomarker with malignancy cannot establish whether the altered biomarker is the cause or result of the malignant transformation (see also Sections II.E and II.H). To serve as a reliable diagnostic tool, a biomarker should (1) be present in cells that do not demonstrate conventional histological signs of malignancy but are already at risk for transformation as shown by the subsequent development of dysplasia or carcinoma and (2) be useful in identifying dysplasia at risk for progressing to invasive SCC (Slootweg 1996).

A brief overview of potential biomarkers in association with oral/pharyngeal premalignancy and/or oral/pharyngeal SCC is presented below and in Table 1.8 (see also Tables 1.3-1.6 in Section IV). Unfortunately, much of the literature is contradictory and confusing due to the lack of uniformity in the techniques used to assess the changes in either the gene or protein biomarker (Greenblatt et al., 1994; Slootweg 1996; Dowell and Ogden 1996; Raybaud-Diogene et al., 1996). For example, the diversity of immunohistochemical (IHC) techniques, in epitope specificities of the antibodies employed, in antigen retrieval, and counting methods has failed to establish whether or not the overexpression of p53 protein is associated with increased cell proliferation, aneuploidy, tumour grade or stage, metastasis, response to chemo or radiotherapy, recurrence, or survival time (reviewed by Greenblatt et al., 1994; Slootweg 1996; Raybaud-Diogene et al., 1996). Until a standardized quantitative analysis of gene/protein expression and function can be linked to histomorphology, the use of biomarkers for diagnostic and prognostic assessment of dysplasia or SCC is debatable (Slootweg 1996; Dowell and Ogden 1996).

1. Tumour Suppressor Gene p53 and Protein p53

a. p53 Protein

Normally, p53 protein functions as a checkpoint control in G1, preventing duplication of damaged DNA either by facilitating its repair by blocking entry to S phase, or initiating apoptosis. The normal function of p53 protein can be abolished with loss of one p53 allele and a mutation in the second allele. However, the mutant protein from one mutated allele, despite retention of the second wild-type allele, can block function of the wild-type protein and may be actively involved in cell transformation (Raybaud et al., 1996). Moreover, wild type protein has a short half-life of only 20-30 minutes whereas the mutant conformationally-altered protein has a prolonged half-life up to 24 hours (Hollywood and Lemoine, 1992), allowing accumulation to levels detectable by IHC. Since wild-type protein levels are not usually detectable, the detection of p53 protein is assumed to indicate overexpression and is used as a surrogate marker for the presence of a p53 gene mutation or deletion (eg. Sauter et al., 1992; Greenblatt et al., 1994; Dowell and Ogden,

1996). However, the possibility that p53 expression is a result rather than the direct cause of cell proliferation has not been established (Warnakulasuriya and Johnson, 1994).

Cells expressing mutant p53 protein are not blocked at G1/S phase of the cell cycle and cannot undergo DNA repair or enter apoptosis. Consequently, the mutant p53 protein can function as an oncogene promoting cell proliferation and conferring a growth advantage to p53-positive neoplasms (Gopalakrishnan et al., 1997), but tumour growth could also be the result of lengthened survival by the inhibition of apoptosis of transformed cells, rather than a stimulation of cellular proliferation (Riva et al., 1995).

p53 protein has been reported in 11% to 80% of oral SCCs; it has also been identified in adjacent areas of dysplastic and normal epithelium (eg. Regezi et al, 1995; Yan et al., 1996), in lichen planus and in chronic inflammation (reviewed by Slootweg 1996; Raybaud et al., 1996; and Warnakulasuriya and Johnson, 1996). In specific regard to SCC of the tongue, overexpression of protein p53 has been associated with improved survival, including stage IV tumours (Sauter et al., 1992) but has failed in predicting angiogenesis or lymph node metastasis (Leedy et al., 1994).

b. p53 Gene

Mutations in p53 gene have been described as an early event in SCCs of the head and neck (Greenblatt et al., 1994; Scully and Field, 1997). p53 gene mutations have been found in histologically normal epithelium at significant distances from the primary tumour, and they may be detected early during transition from normal to dysplastic epithelium (Greenblatt et al., 1994; Raybaud et al., 1996; Slootweg 1996). By sequencing p53 gene, 27% to 60% of oral dysplastic lesions demonstrate p53 mutations which precede histologic changes and are proportional to the change of dysplasia (reviewed by Scully and Field, 1997). As compared to the frequency of p53 mutations observed in noninvasive lesions (19% reported by Boyle et al., 1993), there is also an increased frequency of mutations in invasive carcinomas (43%, Boyle et al., 1993). Moreover, a

majority (64%) of the mutations were changes associated with carcinogens from cigarette smoke (guanine adducts), (Boyle et al., 1993). Several studies demonstrated an increased frequency of gene p53 mutations in smokers as compared to nonsmokers with head and neck SCC and premalignant lesions (reviewed by Slootweg 1996), and in one study p53 mutations were observed only in premalignant lesions of tobacco smokers (Lazarus et al., 1995). In smokers the p53 mutations are widespread throughout the gene whereas in non-smokers with similar clinical diagnoses, the spectrum of mutations are limited to sites in the gene that were characteristically seen with spontaneous mutations (Slootweg 1996). For users of betel quid, the data regarding gene p53 mutations are contradictory and may reflect different chewing habits (reviewed by Slootweg 1996). The prevalence of p53 mutations also varies geographically: only 7% of oral carcinomas from the Indian subcontinent demonstrate p53 mutations compared to 47% to 81% of oral carcinomas from Europe and USA. Geographic differences are also seen in ras mutations (see No. 3 below) and may also be attributed to oral carcinomas with different etiologies (reviewed by Paterson et al., 1996). The association of human papillomavirus (HPV) with alterations in p53 gene is also controversial and although HPV -16 and HPV-18 appear to be risk factors (Section V.F.6) associated with oral SCC, their mode of operation is unclear. Possible mechanisms include the binding and degradation of p53 protein by HPV proteins and the co-expression of HPV-DNA and mutant p53 (reviewed by Slootweg 1996).

p53 mutations have been equivocally associated with angiogenesis (Leedy et al., 1994; Slootweg 1996) and although mutations in p53 gene can occur at different sites within the gene, these sites are maintained during tumour progression and metastasis (Burns et al., 1994; Sakata 1996; reviewed by Slootweg 1996; Raybaud et al., 1996). Consequently, the use of p53-mutant specific probes may be a useful tool in detecting tumour cells that are not visualised by conventional histopathology, and may offer a method for distinguishing multiple primary tumours from recurrent or metastatic tumours (Greenblatt et al., 1994; Slootweg 1996).

The subdivision of head and neck tumours by anatomic region of origin demonstrates differing patterns of p53 mutations among the various primary mucosal sites (Greenblatt et al., 1994). In laryngeal and pharyngeal primary SCCS, the mutation prevalence is low (34%) and the spectrum of mutations resembles that of lung cancer (predominantly G:C to T:A transitions). In contrast, primary tumours of the oral cavity have a high prevalence of mutations (81%) and a different spectrum of mutations (G:C to A:T, and A:T to G:C transitions as well as deletions and insertions), (Greenblatt et al., 1994). Greenblatt et al. (1994) concluded that the grouping together of anatomic sites (eg. as oral/pharyngeal or head/neck cancers) when addressing carcinogenesis and therapy, ignored mutational differences which could reflect differences in carcinogen exposure, variation in carcinogen activation, or variation in DNA repair between distinct mucosal sites.

In specific regard to SCC of the tongue, genetic changes in p53 gene have been correlated to tumour size and histological differentiation (Atula et al., 1996), and metastasis to lymph nodes (Burns et al., 1994).

2. c-erbB Gene, Epidermal Growth Factor Receptor and its Growth Factors

Both TGF α and EGF are growth factors promoting epithelial proliferation (Table 1.5, Section IV) and they compete for binding to EGFR (eg. Scully and Field, 1997). Levels of EGF are often increased in premalignant oral lesions and malignant oral SCC (Kannan et al., 1996). TGF α is usually expressed in low levels in the normal adult but may be increased in SCC (Sauter et al., 1992). In malignant tissues, TGF α appeared increased relative to levels of EGF (Kannan et al., 1996), but TGF α levels were either not significantly related to (Sauter et al., 1992) or correlated inversely (Scully and Field, 1997) with EGFR expression. TGF α has been implicated in abnormal cell growth and induction of anchorage independence (Kannan et al., 1996). TGF α produced by an epithelial cell may act in autocrine fashion by acting on autologous receptors as well as in paracrine fashion by acting on receptors of adjacent cells. Thus, these growth loops may increase proliferation and simultaneously provide a stimulus for progression to malignancy (Kannan et al.,

1996).

The receptor for EGF (EGFR) is often overexpressed in basal cells as well as other cell layers with a tendency for greater EGFR expression in poorly differentiated carcinomas, and strong EGFR expression in oral SCC has also been associated with a short survival time (reviewed by Burkhardt 1996; Warnakulasuriya and Johnson, 1996). Increased EGFR levels do not necessarily correlate to amplification of the c-erbB gene although a progressive increase in c-erbB expression is noted in normal, hyperplastic, dysplastic and malignant human oral mucosa, particularly at the time of early invasion, with increased tumour burden, and with more aggressive tumour behaviour (reviewed by Burkhardt 1996; Warnakulasuriya and Johnson, 1996). However, expression of EGFR and c-erbB in precancerous oral lesions and oral SCC are too inconsistent (Burkhardt 1996; Scully and Field, 1997) to be utilized as standard diagnostic procedures, and correlations with prognosis are not established (Burkhardt 1996).

In specific regard to SCC of the tongue, Sauter et al. (1992), reported that EGFR was overexpressed in 60% of SCCs of the BOT (20 patients) but no statistically significant trends were observed in relation to survival. In addition, 35% of the BOT SCCs demonstrated overexpression of TGF α ; and overexpression of TGF α in patients with stage IV tumours may be related to a poorer prognosis ($p=0.1$, Sauter et al., 1992).

3. ras Oncogene Family

Three closely-related genes of the ras family encode proteins which are involved in coupling growth factor receptors to effector proteins in the cell. Mutant ras protein is involved in prolonging the signal for cell proliferation and its occurrence is often paralleled by EGFR expression (Alberts et al., 1989; Burkhardt 1996). Several studies have demonstrated geographical differences in ras gene changes in oral SCC which may reflect differences in etiology (Burkhardt 1996; Scully and Field, 1997). For example, ras amplification was demonstrated in 100% of SCCs associated with

tobacco chewing in India, 18% of SCCs from betel quid chewers in Taiwan and in 66% of buccal mucosa SCC in elderly Japanese. In the United States, ras changes were observed in 32-68% of primary oral carcinomas and were associated with increased tumour size and later stages of disease (reviewed by Burkhardt 1996). Oral carcinomas from India and South East Asia are generally characterized by the absence of p53 mutations and the involvement of ras oncogenes including ras mutations (35% frequency), loss of ras heterozygosity (30%), ras amplification (28%) as well as myc amplification (29%); in contrast, ras and myc changes are uncommon in the West (reviewed Paterson et al., 1996).

Overall, overexpression of ras proteins may be important in the early stages of malignancy but the relationship of ras mutation to etiology and stage of disease is not established (reviewed by Burkhardt 1996).

4. myc Oncogene Family

The myc family of genes encodes for transcription factors that activate other growth-promoting genes, and in particular, high levels of c-myc protein maintain cell proliferation. c-myc protein has been identified in early oral dysplasias with increased prevalence as the degree of atypia increased (Eversole 1993), and most studies support the overexpression of the c-myc gene in oral carcinomas (Burkhardt 1996; Scully and Field, 1997). Amplification of c-myc may indicate an increased metastatic potential and poor prognosis but further studies are required for establishing a prognostic link (reviewed by Burkhardt 1996; Warnakulasuriya and Johnson, 1996).

5. Bcl-1 and -2 Oncogenes

Bcl-1 codes for cyclin D1, a stimulatory component of the cell cycle. The amplification of Bcl-1 was demonstrated in 35% -48% of head and neck SCCs, and was seen more often in poorly differentiated, aggressive tumours associated with a poor prognosis (reviewed by Burkhardt 1996; Warnakulasuriya and Johnson, 1996).

Bcl-2 inhibits apoptosis; its protein product is not evident in basal cells of normal oral mucosa but it may be expressed in hyperplastic lesions like leukoplakia where it delays terminal differentiation in epithelial cells with subsequent hyperkeratosis (reviewed by Burkhardt 1996). In regards to SCC of the tongue, bcl-2 expression was detected in only 16% of samples. No expression was found in tissue of non-smokers but there was a correlation between smoking and bcl-2 expression which seemed to predict more aggressive tumour behaviour and a poorer prognosis (Atula et al., 1996).

6. TGF β Growth Factor and Attachment Receptors

TGF β is produced by both normal and transformed keratinocytes. TGF β is involved in the regulation of ECM proteins and enzymes that modify the matrix by promoting synthesis of collagens and TIMP but depressing synthesis of metalloproteinases (Table 1.6, Section IV). TGF β also has an inhibitory effect on keratinocyte proliferation by regulating the expression of receptors for EGF. Malignant epithelial cells may become refractory to inhibition by TGF β (Burkhardt 1996); loss of this negative regulation may be linked to the invasion of stroma by the epithelium due to elaboration of metalloproteinases which degrade collagen in the basement membranes, and by increased keratinocyte proliferation (Gudas et al., 1994). Invasion does not always connote metastatic potential but alterations in integrin expression by transformed cells have been linked to a subset of head and neck carcinomas with a high risk for recurrence (Eversole 1993). The loss or reduction in cadherin expression has also been correlated to detachment and separation of cells from the primary tumour and to cell invasion and metastasis in SCC of the head and neck (reviewed by Kinsella et al., 1994).

7. Retinoic Acid Receptors and Keratin Markers

Retinoids may also be involved in the invasion process because during the progression of cells from premalignant to malignant, the expression of different RARs may change (reviewed by Love and Gudas, 1994), (Tables 1.4 and 1.6 in Section IV). For example, relative to normal control

mucosa and adjacent normal and hyperplastic tissues, only minor changes were detected in the expression RAR- γ mRNA and RAR- α mRNA in head and neck carcinomas (Lotan 1994; Xu et al., 1994). In contrast, the expression of RAR- β mRNA was reduced or lost in carcinomas and dysplastic tissues (Lotan 1994; Xu et al., 1994). Moreover, the decreased expression of RAR- β in premalignant lesions (eg. leukoplakia) in patients without SCC suggested that decreased RAR- β expression may be an early event in head and neck carcinogenesis and may indicate a high risk for tumour development (Xu et al., 1994). Significantly, treatment with retinoic acid can increase expression of RAR- β which has been correlated with clinical response (Lotan 1994).

In normal keratinocytes, RAR- β is linked to the expression of K19 and several investigations (eg. Lindberg and Rheinwald, 1989; Hu et al., 1991; Heyden et al., 1992) have suggested that K19 as well other keratins (Section IV., Table 3) would be useful indicators of oral premalignancy and SCC. However, reports (reviewed by Morgan and Su, 1994; Su et al., 1996) are equivocal and findings are summarized in Table 1.9A, B.

In general, the potential value of keratins for diagnosing early tumours is limited by the observation that there is no keratin marker that is present in all malignant lesions, but is not present in normal oral mucosa (Ogden 1997). Nevertheless, Ogden et al. (1994) used keratin profiles in combination with DNA profiles (see 8. DNA Content below) of exfoliative cytology as a potential screening test for the early detection of oral SCC in high-risk communities. In combination with an abnormal DNA content, K8 and K19 were strongly associated with malignancy (Ogden et al., 1994), (see also Section II.H.2). To date, the most widely used application of keratin markers in the diagnostic field has been with anaplastic tumours where they may aid in clarifying the tissue of origin. As noted previously, keratins represent only two of six major classes of intermediate filaments. Fortunately, intermediate filaments are a constant feature of all carcinomas, irrespective of their degree of differentiation, and they are usually sufficiently conserved in anaplasia. Consequently, intermediate filaments serve as tissue-specific markers in distinguishing epithelial

tumours from nonepithelial tumours, and in distinguishing different types of epithelial tumours (Moll 1987, Morgan and Su, 1994).

8. DNA Content

Normal germ cells are haploid and contain 23 chromosomes; somatic cells are diploid and contain 23 pairs of chromosomes. In contrast, malignant cells are often aneuploid because they have an abnormal number of chromosomes that is not a multiple of 23 (see also Burkhardt 1996; Scully and Field, 1997). DNA content can be quantitated by flow cytometry in which a fluorochrome, bound stoichiometrically to DNA, emits light directly proportional to the DNA content. Flow cytometry is often used in conjunction with other markers such as c-myc expression, p53 protein, EGFR, keratins (Ogden et al., 1994), etc. but the use of flow cytometry as a prognostic indicator is not established (reviewed by Warnakulasuriya and Johnson, 1996).

Some studies of SCC of the tongue (Saito et al., 1994; Baretton et al., 1995) reported no relationship between ploidy, prognosis, survival or recurrence of tongue SCC. Other studies of tongue and larynx SCC reported moderate correlation between ploidy and tumour grade, stage and survival where diploid tumours were well-differentiated, were a lower grade and had a better prognosis than aneuploid, poorly differentiated higher-grade tumours (Gandour-Edwards et al., 1994). Flow cytometry also measures the fraction of cells in the S-phase; in SCC of the tongue, no correlation between percent S-phase cells and histological grade was found but a high percent S-phase fraction was associated with increased clinical stage and nodal involvement (Monasebian et al., 1994). Aneuploidy rates of 42% (Saito et al., 1994) and 50% (Baretton et al., 1995) were reported for SCC of the tongue, with tongue carcinomas being diploid more often than other oral SCCs (Baretton et al., 1995). There was also a higher incidence of cervical lymph node metastasis in nondiploid cases than in the diploid cases (Saito et al., 1994; Baretton et al., 1995; King et al., 1995).

During carcinogenic damage to DNA, chromosome and DNA-containing chromatin fragments called micronuclei may be created in proliferating cells. Many abnormally-proliferating cells contain extranuclear micronuclei that can be quantitated to provide information on tissue- or organ-specific abnormalities (Lippman et al., 1990; Pillai et al., 1992). For example, in the aerodigestive tract, micronuclei are formed in the basal cells that migrate to the surface where they are exfoliated. Hence, micronuclei can be analyzed in samples obtained noninvasively such as from mucosal brushings. The presence and frequency of micronuclei can quantitatively reflect ongoing DNA damage. A high frequency of micronuclei has correlated well with cancer risk in high-risk individuals such as smokers and patients with premalignant lesions, but correlated inconsistently with clinical response to chemopreventive agents (Chapter 5). Consequently, the use of micronuclei as an intermediate endpoint marker of carcinogenesis was considered premature (Lippman et al., 1990; Pillai et al., 1992).

D. Tumour Kinetics

Malignancy is associated with the loss or abnormal expression of normal differentiation pathways or the expression of new differentiation pathways. In addition, the genetic material of malignant cells is more unstable and demonstrates increased mutation rates as compared to normal cells. As a tumour progresses, mutations accumulate and the blocks in differentiation may become progressively more complete resulting in both genotypic and phenotypic heterogeneities (eg. Alberts et al., 1989). Characteristically, the tumour becomes less differentiated or more anaplastic and more difficult to treat. In addition, there may be important differences in cell proliferation throughout a tumour and between primary tumours and metastatic growths (Ansari and Hall, 1992).

Malignant tumours are not masses of rapidly dividing cells; instead, only some cells in the tumour will be cycling in the cell cycle (Section IV.D.2; Figure 1.5), and the majority will be in G₀, incapable of dividing (sterile), or dead. In most instances, there are no consistent, significant

differences in the durations of the S, G2 and M phases of the cell cycle between normal and malignant cells that could be exploited therapeutically (Fleming et al., 1995). The most variable and longest phase is G1 which can range from 2-3 hours to several days, and the time between mitoses (intermitotic time) for most normal human cells is 1-2 days compared to the intermitotic time of 2-3 days for most malignant cells. The proportion of cells in the cell cycle and synthesizing DNA at a particular time can be determined by exposing the cells to an isotope (autoradiography) and calculating the labelling index (LI); most solid tumours have a LI of 1% - 8% whereas epithelium of the normal gut has a LI of 16%. If the duration of the S phase and intermitotic time of the tumour cells are also known, then the fraction of proliferating cells in the tumour or its growth fraction can be determined (Fleming et al., 1995).

During the early preclinical phases of a tumour, the rate at which cells are produced and the rate at which cells are lost from the tumour are proportional to the number of cells in the tumour at a given time. Cell production exceeds the rate of cell loss and for a large part of its life, a tumour grows exponentially so that by the time it is detected clinically, the tumour has already doubled in size about 30 times and has a mass of 10^9 cells or about 1 gram (Gregory 1992; Fleming et al., 1995). In general, the more anaplastic the tumour, the higher the growth fraction but at the clinical stage, many tumours have a low growth fraction because the growth rate has begun to decline exponentially, eventually resulting in a maximum volume of tumour. The exact mechanisms underlying this growth pattern are unclear but as the tumour increases in size, a high percentage of the daughter cells die, probably because of inherent genetic instabilities of the transformed cells, ischemic necrosis due to tumour growth outstripping the vascular supply, decreased availability of nutrients and hormones, and accumulation of toxic metabolites (Gregory 1992; Fleming et al., 1995).

The growth pattern of a tumour throughout its life span can be described by a mathematical model and plotted as an asymmetric sigmoidal curve call the Gompertzian growth curve (Gregory 1992;

Fleming et al., 1995). By the time tumours are detected clinically, they are 'high' on the Gompertzian curve where the growth fractions are low. The rationale for treatment of malignancies by radio or chemotherapy relies on Gompertzian growth curves because these therapies target primarily cycling cells, and the responsiveness of a tumour to treatment is dependent on the point in its growth curve at which therapy is initiated. Thus, the aim of initial or inductive therapy is to increase tumour susceptibility to therapy by increasing its growth fraction, i.e. moving the tumour to a lower point on the Gompertzian curve. Surgical debulking or initial radio or chemo therapy will reduce the number of cells in the tumour; consequently, cells previously in G0 will re-enter the cell cycle so the growth fraction and growth rate of the tumour are increased which increases the tumour's susceptibility to therapy (Gregory 1992; Fleming et al., 1995), (see also Section VII.E).

1. Implications for Radio and Chemo Therapy

The guiding principles of cancer therapy are based on the observation that a given dose or course of chemo or radio therapy will kill a constant fraction or proportion of the cell population, rather than a constant number of cells. Hence, multiple courses of therapy are needed to eradicate the tumour because tumour cell regrowth occurs between each treatment dose, and small changes in treatment dose translate into large changes in cell survival. To increase the cell kill at a given dose, the duration of therapy exposure must be prolonged to allow more cells to enter the susceptible phase of the cell cycle (characteristically the S or M phase; see Section VII.E). Ideally, the intensity, frequency and duration of radio or chemo therapy should be matched to the tumour's growth rate and to the point reached in the tumour's growth curve (Gregory 1992; Fleming et al., 1995). As early tumours are generally rapidly-growing and demonstrate an exponential growth pattern, cancer therapy produces a greater fractional cell-kill to fast-growing tumours than to slow-growing tumours (Gregory 1992). Thus, the more rapid the growth rate, the more intensive, frequent and short-lived should be the therapy. In addition, for rapidly-growing tumours, therapy should be initiated as soon as possible because even a short delay could permit a large increase in

tumour size with a severe reduction in chances of a cure. In general, large tumours are slowly growing and therefore less responsive to therapy. For such tumours, initial therapy may be aimed at eliminating the dividing cells which may be only a small fraction of the tumour. If the tumour is reduced to a smaller size, the tumour is moved to a lower point on the Gompertzian growth curve which may result in a smaller, faster-growing tumour that offers new possibilities for treatment and cure (Gregory 1992).

It was initially assumed that radiation therapy and many chemotherapies killed malignant cells directly by disrupting their DNA but in fact, DNA is damaged to a relatively minor extent such that the DNA could be easily repaired (Weinberg 1996). It was also assumed that since p53 was involved in the cellular response to DNA damage, switching off DNA replication and allowing extra time to repair, that loss of p53 function in malignant cells was responsible for the relative success of conventional radio and chemotherapies. That is, tumour cells with altered or absent p53 were expected to be more susceptible to the killing effects of DNA-damaging agents because their genome was unstable, they had a reduced G1 delay, and they failed to repair DNA damage before attempting to replicate through the damage (Murnane and Schwartz, 1993; Lawley 1994).

Recently, it has been understood that radiation exposure causes wild-type p53 protein to be stabilised, leading to elevated p53 protein levels and associated increases in transcription of p53-responsive genes (including apoptosis-inducing genes) which results in the induction of growth arrest and apoptosis (Lane 1993; Wilson et al., 1995). In addition, cells with DNA damage as a result of irradiation mistakenly perceive that the inflicted damage to their DNA cannot be repaired easily, and therefore cells with normal p53 undergo apoptosis. These discoveries imply that cancer cells with p53 mutations may be able to evade therapy-induced apoptosis, and may be far less responsive to radiation treatment than cells homozygous for normal p53 (Greenblatt et al., 1994; Weinberg 1996). In fact, "gene dosage" (homo or heterozygosity for mutated or absent p53) was clearly related to resistance to radiation-induced apoptosis in *in vitro* (thymocyte, Clarke et al.,

1993; Lowe et al., 1993) and *in vivo* (mouse) models (reviewed by Lane 1993). However, two separate studies used IHC to determine p53 protein levels of human head and neck cancers but neither demonstrated a correlation with tumour response to chemotherapy (Riva et al., 1995), radiotherapy (Riva et al., 1995; Wilson et al., 1995), or to long term survival (Riva et al., 1995). Although the G1 checkpoint control by p53 may play a minor role in determining radiation sensitivity of head and neck cancers (Murnane and Schwartz, 1993; Wilson et al., 1995), the effectiveness of existing radiation and chemotherapy treatments may nevertheless be improved if therapies could restore a cell's capacity for apoptosis (Weinberg 1996).

Cancer therapies are directed against cycling cells but radio or chemotherapy can not selectively target tumour cells with the exclusion of normal cells that are also proliferating. Moreover, there may be no intrinsic differences in the radio or chemosensitivity of normal and malignant cells (Gregory 1992; Fleming et al., 1995); consequently, the initial direct cytotoxic effects of therapy are manifested by rapidly proliferating cells such as normal oral lining epithelium. Proliferation rates in some oral lining tissues may be 1.5 to 5 times greater than in masticatory mucosa (Section IV.B) and these differences are reflected clinically in the rapid appearance of therapy-induced mucositis of noncornified mucosa. Mucositis represents ulceration or breakdown of the epithelium which occurs because cell division in the damaged proliferative basal compartment can no longer match or replace desquamation at the surface. The basement membrane region may also break down and blisters may form so that the complete thickness of epithelium is lost. There is also an acute vascular response and edema due to damage sustained by the endothelium, and damage to salivary glands reduces salivary volumes and its protective functions (Baker 1982; Squier 1990). At a minimum, mucositis is painful and diminishes the quality of life, but it may compromise the patient's nutritional status, may lead to interruptions in therapy, and may allow pathogenic organisms to gain entry and thereby facilitate secondary systemic infections, particularly in individuals treated with systemic chemotherapy which also causes myelosuppression (Baker 1982; Squier 1990; reviewed by Epstein 1992 and Epstein 1994). Radiotherapy also causes late tissues

changes which are discussed in Section VII.E.2.c.

E. Invasion and Metastasis

In SCC, attention is conventionally focused on malignant transformation of the keratinocyte which results in its uncontrolled clonal expansion, penetration of the basement membrane, invasion of the underlying connective tissue and finally, metastasis. Although overt histological and biomarker changes are manifest in epithelial cells, changes may also occur in the subepithelial connective tissue. Smith (1980) argued that changes in the epithelium developed due to altered influences of the subepithelial connective tissue upon the epithelium. For example, if carcinogens exerted their effect primarily upon the connective tissue, then connective tissue influences on the epithelium may be disturbed in such a way as to support malignant change. Conversely, even if malignant characteristics developed in the epithelium, invasion may be resisted by the connective tissue influences (Smith 1980). Some support for stromal interaction in development of oral carcinoma is seen in submucous fibrosis (Section VI.B.2.b.) in which an atrophic, dysplastic epithelium accompanies fibroelastic changes in the connective tissue that include increased collagen production, decreased collagen breakdown, a reduced number of fibroblasts with an altered phenotype and a variable chronic inflammatory cell infiltrate. In addition, the possible role of subepithelial fibroblasts in the modulation of epithelial differentiation and proliferation has already been discussed in Section IV.

Tumour growth, invasion and metastasis involves changes in both the epithelium and connective tissue. During carcinogenesis, malignant cells produce proteolytic enzymes that degrade components of the ECM as they penetrate across the basement membrane, through connective tissue and between healthy cells. Oral SCCs have demonstrated increased collagenase production as collagen in basement membranes is degraded ahead of infiltrating tumour cells (Johnson et al., 1980). Ultrastructurally, gaps are seen in the basal lamina with basal cell pseudopodia extending into the connective tissue through the gaps. The collagen content of the underlying connective

tissue is reduced and appears related to an increased susceptibility to tumour development (Smith 1980).

Whereas invasion of the connective tissue by transformed epithelial cells defines the progression to invasive SCC; invasion does not necessarily connote metastatic potential as evidenced by the behaviour of most ameloblastomas of the jaws and basal cell carcinoma of the skin (Eversole 1993). Metastasis requires the invasion of lymphatics and/or vascular channels and depends upon events that are separate from invasion of subepithelial connective tissue. Integrins and cadherins are involved in normal cell:cell adhesion, and integrins are also involved in cell:matrix adhesion and cell motility. Many transformed cells lose some or all of their inter-cellular cadherins, display an altered array of integrins and secrete ECM molecules which facilitate motility. For example, in a subset of head and neck carcinomas with high risk for recurrence, expression of integrin $\alpha_6\beta_4$ is increased and associated with cells that are highly invasive, and expression of integrin α_3 is associated with increased motility (reviewed by Eversole 1993). Moreover, for tumour cells to cross a basal lamina and invade or metastasise, they must express integrins for laminin in order to adhere to the lamina, and they must also secrete collagenase type IV to digest the lamina (Alberts et al., 1989).

Transformed cells have become anchorage independent so that their cyclin/kinase complex remains continually active, permitting continual passage through the restriction point. These cells have also escaped the normal "social" controls exerted by the adjacent cells; therefore, once they breach the basement membrane, they are motile within the connective tissue. Malignant cells can readily penetrate the thin walls of lymphatic vessels which carry the cells downstream to lodge in one or more local lymph nodes. Lymphatic capillaries are more permeable than blood capillaries because unlike the typical blood capillaries, lymph capillaries lack a basal lamina and are essentially tubes of endothelium (Ross and Reith, 1985). The lack of a basal lamina can be correlated to the extreme permeability of lymph capillaries although as lymph vessels become larger, their walls thicken due

to connective tissue and smooth muscle bundles (Ross and Reith, 1985). Lymphatic capillaries are very numerous under the epithelium of the skin and mucous membranes, including the oral cavity and pharynx (Ross and Reith, 1985). Thus, the rich lymphatics of the oral/pharyngeal region (Atkinson and White, 1992; see also Section III.C.6), and the increased permeability of lymph capillaries relative to blood capillaries (Ross and Reith, 1985) may account for the high proportion of metastases to the cervical lymph nodes (Section VII.B). However, the malignant cells also encounter the basement membranes of small blood vessels and if they can penetrate this barrier as well as the endothelial cells lining the blood vessel, they are free in the blood circulation.

Expansion of the tumour mass also relies on the tumour's ability to stimulate angiogenesis which it requires for nutritional support, dissemination of waste products and as a pathway for metastasis. In turn, the new capillary endothelium stimulates tumour cells to produce growth factors which further promote angiogenesis. The newly proliferating capillaries may also be more permeable and hence more likely than mature vessels to be penetrated by tumour cells (reviewed by Hirshberg and Buchner, 1995; Ruoslahti 1996). Once within the circulation, tumour cells must escape the immune system but often, the cells are trapped in the first capillary bed they encounter. Because all organs, other than the intestines, send their blood first to the lungs, the lungs are the most common site of metastasis, followed by the liver. However, some tumours demonstrate a striking preference for unexpected sites that cannot be explained by the pattern of circulation alone, and their metastasis may be mediated by integrins that are expressed by the tumour for specific ECM components (reviewed by Hirshberg and Buchner, 1995; Ruoslahti 1996; Horwitz 1997). For example, metastasis to the oral region is uncommon, but about 30% of oral metastases are the first sign of a metastatic process (Hirshberg and Buchner, 1995). The breast is the most common primary source of tumour metastasising to the jawbones and in particular, the posterior mandible. In contrast, the lung is the most common primary source for metastases to the oral soft tissues, particularly the attached gingiva, followed by the mobile tongue and then the base and/or the posterior border (reviewed by Hirshberg and Buchner, 1995).

After reaching the target organ, the tumour cells extravasate from the capillaries and depend upon local growth factors for further proliferation (reviewed by Hirshberg and Buchner, 1995; Ruoslahti 1996). After a period of growth, a tumour mass may remain dormant and remain clinically undetectable for months or years. During dormancy, cell proliferation is balanced by cell death. Inhibitors of angiogenesis may control metastatic growth but the dormant tumours nevertheless, pose a continuous risk of recurrence and metastasis (reviewed by Hirshberg and Buchner, 1995).

F. Risk Factors Associated with Oral/Pharyngeal Squamous Cell Carcinoma.

At most, only 5% of all cancers can be traced directly to environmental or occupational exposure; throughout the world, lifestyle and related behaviours are much more likely to cause or promote cancer development (Weinburger and Williams, 1995). These conclusions have been reached on the basis of research into organ-specific cancers in relationship to environmental conditions associated with their development. In most cancers including oral/pharyngeal SCC, the associated etiologic factors are not single chemicals, but rather, complex lifestyle-related mixtures. Lifestyle refers to self-imposed habits such as tobacco smoking or other tobacco uses, alcohol use, areca nut habits and dietary patterns (Weinburger and Williams, 1995)

Chemical carcinogens may be genotoxic because they react with DNA and cause heritable mutations, and most human carcinogens are genotoxic. Carcinogens may also be epigenetic because their mechanisms of actions do not involve DNA; instead, they may be cytotoxic, cause chronic tissue injury, hormonal imbalances or immunological effects, or they may act as promoters (Weinburger and Williams, 1995). Table 1.10 lists the various risk factors and the relative risks (RR) or odds ratios (OR) associated with oral SCC ; a brief discussion is presented below and in Chapter 1, Section VI.

It should be recognized that in calculating risks, many studies have grouped together all cancers of the oral cavity and/or pharynx (eg. Brugere et al., 1986; Blot et al., 1988; Winn et al., 1991; see

also Section II.D). An obvious advantage of this approach is the inclusion of a greater number of cases. However, this approach precludes the estimation of relative risks by site and obscures the effect that well-defined carcinogens like tobacco and alcohol may have in different sites (eg. Oreggia et al., 1991; Greenblatt et al., 1994). The apparent differences in risk calculations probably reflect inherent differences in the populations studied, but the selection of cases and controls is subject to a multitude of biases, as is defining and ascertaining exposure to the risk factors of interest (see Sections II. D and F).

1. Tobacco

For oral premalignant lesions as well as oral/pharyngeal SSC, tobacco use is regarded as the predominant etiologic factor (Eversole 1993). Tobacco has been used in different forms throughout the world for centuries. Chewing tobacco and areca nut (Section VI.B.2.b.) are used in India and Southeast Asia by both males and females; smokeless tobacco (ST) is also popular with males in Scandinavia and with young males in the United States and Europe. Smoking of tobacco increased significantly with the introduction of commercially-manufactured cigarettes at the turn of the century; men became heavy users during World War I and women during World War II (Eversole 1993; Weinburger and Williams, 1995). Not surprisingly, the prevalence of oral SCC has increased among males born after 1920; the rate in women has tripled since the 1930's and for young American males, the rate has increased four-fold since 1960 (Eversole 1993), (see also Section VII.D).

Use of black tobacco carries a greater risk for oropharyngeal, laryngeal, lung and bladder cancer than blond flue-cured tobacco. Black tobacco not only contains a higher concentration of carcinogens than blond tobacco but because of its higher alkalinity, black tobacco is less easily inhaled and therefore it remains in contact with the oral mucosa for a longer time (Oreggia et al., 1991). Tobacco and tobacco smoke contain a number of powerful genotoxic carcinogens such as nitrosamines, polycyclic aromatic hydrocarbons and heterocyclic amines that interact with DNA

(Weinburger and Williams, 1995). Nitrosamines are produced from nicotine during the curing process of tobacco, during the combustion of tobacco, and *in vivo* from the reaction of nitric oxides with nicotine and related alkaloids (Weinburger and Williams, 1995). There are wide differences between individuals in their ability to metabolize nitrosamines to damaging intermediates and individuals who most effectively activate these carcinogens, may be at higher risk (Hecht et al., 1994).

Tobacco and its smoke also contain phenols and terpens which are not genotoxic, but they may act as cocarcinogens and promoters, activities that are highly dose dependent and reversible to some extent. Thus, their effects account for the long latency associated with smoking, the steep increase in disease risk with consumption above 20 cigarettes/day, and the decreased risk of disease upon cessation of tobacco use (Spitz 1994; Weinburger and Williams, 1995). Tobacco smoke also contains chemicals that induce enzymes; as a result, the metabolism of hormones and substances such as vitamins are increased so that smokers may have increased requirements for vitamins and other essential nutrients (Weinburger and Williams, 1995). Smoking also appears to have immunosuppressive effects in that smokers have lower levels of NK (natural killer) cell activity in their peripheral circulation than nonsmokers, and experimental exposure of animals to cigarette smoke reduces cell-mediated immunity which is associated with accelerated tumour progression (Browman et al., 1993). Furthermore, cigarette smoking increases the carboxyhemoglobin content of blood, causing a leftwards shift of the hemoglobin-oxygen dissociation curve and a relative hypoxia of the tissues. Tissue hypoxia may have a general compromising effect on tissue resistance and healing, and during radiotherapy of the tumour, smoking-induced hypoxia may interfere with the oxygen-dependent effects of radiation (Browman et al., 1993), (see also Section VII.E.2.a).

a. Smoking tobacco

Tobacco smoking is the major cause of cancers of the tongue, salivary gland, mouth and pharynx,

and tobacco use is directly attributable for about 92% of the oral cavity tumours in men and 61% of these tumours in women (Newcomb and Carbone, 1992).

As shown in Table 1.10, the risks of oral/pharyngeal cancer increase with the number of cigarettes smoked per day and the duration of smoking but show little relationship to age started smoking; risks decline following cessation of smoking (Blot et al., 1988; Bundgaard et al., 1994). After adjusting for age, duration, amount smoked and alcohol use, males who smoked only filter cigarettes experienced 50% of the risk of smokers of only nonfilter cigarettes; mixed filter and nonfilter smokers experienced 80% of the risk compared to pure nonfilter smokers (Blot et al., 1988). Unfortunately, a similar trend was not observed for females; filter and mixed smokers experienced 120% and 90%, respectively, the risk of nonfilter smokers (Blot et al., 1988). Smokers of cigars and pipes tend to inhale less than to cigarette smokers but their overall risk estimates approximate those of cigarette smokers for buccal SCCs but not for pharyngeal malignancies (Spitz 1994).

Risk assessments by anatomic site and tobacco smoking vary. In a large study of 1114 drinking and smoking patients with SCC of the oral cavity and/or pharynx, and 1268 population-based controls, Blot et al. (1988) found that among males, risk trends with smoking were weaker for tongue cancer (OR range 0.8-3.2) than other oral sites (OR range 1.2-5.2) or the pharynx (OR range 1.5-5.8). Pipe and cigar smoking were more closely associated with cancers of the FOM and buccal mucosa than either tongue or pharyngeal cancer. Among females, the effects of smoking were stronger for pharyngeal cancer (OR range 1.6-36.7) than oral cancer (OR range 0.8-9.7) or tongue cancer (OR range 1.3-8.1), (Blot et al., 1988). In a study of 57 males with lingual SCC and 353 controls, Oreggia et al. (1991) examined the possible risk determinants for SCC of the tongue alone, excluding other sites of the oral cavity. Current smokers had a RR of 29.4 and former smokers had a RR of 11.8 compared with nonsmokers (RR 1.0). The type of tobacco smoked was a strong determinant of risk; exclusive users of black tobacco had a RR of 4.0

compared with a RR of 1.8 (not significant, NS) for smokers of mixed black and blond tobacco, and a RR of 1.0 for exclusive smokers of blond tobacco (Oreggia et al., 1991).

In a case-control study of northern Italian males, 102 with tongue cancer, 104 with cancer of the mouth (FOM, gingiva, retromolar trigone), and 726 healthy controls, differences between patients with cancer of the tongue and those with cancer of other oral sites, were found in the type of tobacco smoked (Franceschi et al., 1992). The adjusted odds ratio for cancer of the tongue were lower for pipe/cigar smokers (OR 3.4; NS) than for current cigarette smokers, but for cancer of the mouth, the odds ratios were higher for cigar/pipe smokers (OR 21.9) than for current cigarette smokers (OR 11.8). However, for both tongue and mouth cancers, smoking-related risks increased markedly with increased amount and duration of smoking, but smokers of high tar cigarettes had a 10-fold increased risk of tongue cancer and a 14-fold increased risk for cancer of the mouth, as compared to nonsmokers (Franceschi et al., 1992). In a case series of 150 American patients with oral SCC, 97% patients with SCC of the FOM were smokers compared to 64% of tongue-cancer patients and 50% of gingival-cancer patients (Barasch et al., 1994). The odds of smoking among persons with FOM cancer were 38 times the odds of smoking among persons with gingival cancer and 21 times the odds of smoking among persons with lingual cancer (Barasch et al., 1994).

In addition to malignancy, tobacco smoking is also a risk factor for oral premalignancy (Section VI). The risk (OR) of a having a dysplastic lesion for smokers compared with nonsmokers or exsmokers for over 10 years, was calculated at 7.0 with a dose-response relationship for tobacco dependent upon the level of cigarette consumption. Moderate smoking (<20 cig/day) produced an OR of 3.7 and heavy smoking (≥ 20 cig/day) an OR of 13.8 (Kulasegaram et al., 1995).

Theoretically, marijuana is carcinogenic because its smoke is quantitatively similar to cigarette smoke but marijuana smoking results in a greater tar burden to the respiratory tract, especially

because of the rapid, deep inhalation used in smoking marijuana (reviewed by Spitz 1994). The potential carcinogenic effects of marijuana smoking are difficult to separate from tobacco smoking and alcohol because most abusers of marijuana also consume tobacco and alcohol (confounding, Section II. F.2), (Spitz 1994).

b. Smokeless Tobacco

With the exception of ST use among teenage males and professional baseball players, ST is not as popular as other tobacco products. The prevalence of ST use is only 6.1% of American males and of these, only 3.6% limit their tobacco use to ST and do not use other tobacco products (Spitz 1994). ST or snuff is carcinogenic but some forms of ST have low correlations with oral malignancy. In a recent review of the literature, Eversole (1993) reported that leukoplakia was found in 13% - 78% of ST users and most lesions correlated to the site of tobacco placement, usually the mucobuccal fold. The prevalence of leukoplakia was directly related to the duration and frequency of use and type of ST, snuff being more common than chewing tobacco (reviewed by Eversole 1993).

Snuff-related lesions appeared to show significant dysplastic changes less commonly than other forms of leukoplakia and erythroplakia; carcinomas arising from snuff lesions tended to be well-differentiated, slow growing and with late metastasis (Mincer et al., 1972). Overall, the incidence of carcinoma among ST users is not high, particularly among those using snuff; only 1.4% of SCCs were related to ST use and only 7.7% of verrucous carcinoma patients were ST users (reviewed by Eversole 1993).

The use of tobacco in conjunction with areca nut is reviewed in Section VI.B.2.b.

2. Alcohol

a. Alcoholic Beverages

Alcohol is considered a promoter and a possible cocarcinogen of tobacco because it increases the amounts of reactive carcinogenic metabolites from procarcinogens in tobacco by acting as a solvent for tobacco and other carcinogens (Winn et al., 1991; Newcomb and Carbone, 1992; Weinburger and Williams, 1995), and enhancing their penetration by increasing the permeability of the oral mucosa to tobacco-associated nitrosamines and polycyclic hydrocarbons (reviewed by Eversole 1993). Chronic alcohol use also induces microsomal enzymes that enhance the metabolic activation of tobacco and other carcinogens, and through this mechanism, may affect tobacco carcinogenesis at distant organs (Newcomb and Carbone, 1992). Alcohol may suppress the efficiency of DNA repair after exposure to nitrosamine compounds (Shaha and Strong, 1995), and alcoholic beverages themselves, especially hard liquors, also contain congeners and carcinogenic contaminants (Weinburger and Williams, 1995). Moreover, chronic alcohol use is often associated with nutritional deficiencies which may contribute independently to oral carcinogenesis (see below), (Blot et al., 1988).

Risks associated with alcohol use increase with total alcohol consumption and the smoking-adjusted excess risk associated with high alcohol consumption among men and women is approximately 9-fold (Blot et al., 1988). The effects of alcohol are interrelated with smoking (see Section 3 below) but there is also a deleterious effect of heavy alcohol consumption among nonsmokers, indicating that tobacco is not a requisite cofactor for alcohol-related cancer (Blot et al., 1988). For example, in nonsmokers, a metabolite of alcohol, acetaldehyde, can induce cancer of the oesophagus and this risk is higher in heavy drinkers of hard liquors (Weinburger and Williams, 1995). Overall, the risks of alcohol consumption are greatest for hard liquor and beer drinkers, and least for wine drinkers, suggesting that ingredients other than alcohol are involved as beer and hard liquor contain nitrosamines and polycyclic hydrocarbons (Blot et al., 1988; Weinburger and Williams, 1995).

In a large study in the United States (Blot et al., 1988), the majority of patients consumed beer and hard liquor as compared to wine; there was a small decrease in risk among light and moderate wine drinkers but risks rose sharply beyond 2 drinks/day (Blot et al., 1988). In contrast, in a large study in France (Brugere et al., 1986) 92% of patients consumed wine although only 30% drank wine only; overall, average alcohol consumption exceeded amounts observed in other countries and was reflected by the large relative risk (Table 1.10; RR range 2.7-70.3). In a case-control study in Northern Italy (Franceschi et al., 1992), consumption of wine was considerably greater than consumption of beer or hard liquor and this trend was reflected in the risks for alcohol consumption and oral cancer: wine had a higher risk (OR range 1.0-6.8) than either beer (OR 1.1-NS) or hard liquor (OR 0.5-NS).

Risk assessments by anatomic site and alcohol consumption also vary. After correction for tobacco smoking, risk trends with drinking for females were greatest for pharynx cancer (OR range 1.0-15.0), followed by tongue (OR range 1.0-11.0) and other oral sites (OR range 1.0 - 6.6), (Blot et al., 1988). In contrast, risks for males were greatest for cancer of other oral sites (OR range 1.0 - 12.3), followed by pharynx (OR range 1.0 - 10.4) and tongue (OR range 1.0-6.0), (Blot et al., 1988). Similar risks for tongue cancer and alcohol consumption up to 200 ml/day were observed in Uruguay with RR ranging 1.5-6.8; consumption above 200 ml/day resulted in RR of 11.6 (Oreggia et al., 1991). In northern Italy, the overall risks for tongue and oral cancer were similar (OR 3.4 and 3.3 respectively) for total alcohol consumption which was predominantly wine consumption (Franceschi et al., 1992).

The relationship between alcohol consumption and oral dysplasia is not as strong as between alcohol consumption and oral cancer, as Kulasegaram et al.(1995) were not able to demonstrate an increased risk of oral dysplasia among self-reported consumers of alcohol. Nevertheless, alcohol consumption was greater among cases than controls and the proportion of hard liquor drinkers amounted to 33% of cases compared to 13% of controls (Kulasegaram et al., 1995).

b. Alcohol-containing Mouthwashes

Common brands of mouthwash contain from 1% - 30% alcohol along with flavouring and sweetening. Oral swishing with a mouthwash containing 25% ethanol may provide a local alcohol exposure to the oral mucosa that is similar to drinking a 50% ethanol beverage (Winn et al., 1991). However, Shapiro et al. (1996) argued that the ingestion of alcohol had many effects, other than topical ones, that appeared to play a role in alcohol-associated carcinogenesis. Moreover, the quantity of alcohol ingested from mouthwash use would be exceedingly low and in addition, there were major differences in other ingredients between alcoholic beverages and mouthwashes (Shapiro et al., 1996).

Several studies have evaluated the association between mouthwash use and oral/pharyngeal cancer and results were inconsistent (reviewed by Shapiro et al., 1996). Winn et al. (1991) reported that risks for oropharyngeal cancer and mouthwash use were elevated only for mouthwashes with an alcohol content of 25% or higher. Risks tended to increase with early age at start of use and with increased duration of use. Adjusted risks for site and mouthwash use were similar for tongue (OR range 1.2-1.4), other oral sites (OR range 1.2-2.0) and pharynx (OR range 1.4-1.5), (Winn et al., 1991). Significantly, mouthwash use was reported more often among smokers than nonsmokers; among denture wearers versus those without dentures, and among individuals with periodontal disease than those without (Winn et al., 1991). Mouthwash may be used to mask the use of tobacco and alcohol and typically, these habits are under-reported by study participants and under-ascertained by study investigators (Section II.F). Consequently, confounding by tobacco and alcohol use results in spuriously elevated odds ratios for mouthwash use and oral/pharyngeal cancer (Shapiro et al., 1996).

3. Tobacco Smoking and Alcohol

It is difficult to separate the effects of tobacco smoking and alcohol because nearly all oral/pharyngeal cancer patients have been tobacco smokers and consumers of alcohol (eg. Trieger

et al., 1958; Brugere et al., 1986; Blot et al., 1988; Oreggia et al., 1991; Boffetta et al., 1992; Bundgaard et al., 1994). Increasing risks with smoking are observed across all levels of alcohol consumption, and increasing risks with alcohol consumption are seen across all levels of tobacco smoking; in combination, heavy smoking and heavy drinking result in very large increases in oral/pharyngeal cancer risk suggesting that the combined effect of tobacco and alcohol is greater than the sum of the two factors (Spitz 1994). The combined effect of alcohol and tobacco may increase the risk of oral cancer by as much as 50% to 100% over the rates observed in nondrinking smokers or nonsmoking drinkers (Newcomb and Carbone, 1992). Estimates of the population-attributable risk of oral/pharyngeal risk due to smoking and/or drinking (rather than due to each separately) was calculated at 80% for males and 61% for females; overall, 66% of oropharyngeal tumours could be attributed to heavy consumption: smoking ≥ 2 packs cigarettes/day for ≥ 20 years and/or drinking ≥ 30 alcoholic beverages/week (Blot et al., 1988).

Significantly, the consumption of alcohol and tobacco is closely associated not only with the development of oral cancer, but also with the course of the disease in that these lifestyle habits are associated with a poor prognosis (Bundgaard et al., 1994), (see also Section VII). By itself, tobacco use has a significant prognostic impact, for example a patient with a stage I tumour and tobacco habit of 45 gram/day has the same prognosis as a non-smoker with Stage II disease (Bundgaard et al., 1994). Patients who continue their lifestyle habits of smoking and drinking have a higher mortality from intercurrent diseases than non-users, a higher frequency of second primary tumours, and a higher mortality from primary tumours and/or second primaries (Johnston and Ballantyne, 1977; Bundgaard et al., 1994). Patients who continue to smoke during radiotherapy have a longer duration of mucositis (Rugg et al., 1990) and more severe side-effects, leading to more frequent interruptions of treatment and thus compromising effectiveness of treatment. Compared to nonsmokers or patients who do not smoke during therapy, smokers have lower rates of response and survival (Johnston and Ballantyne, 1977; Browman et al., 1993).

In a study of American veterans (181 cases, 497 controls), Mashberg et al. (1981) reported the synergistic effects of alcohol and tobacco smoking, and also proposed an independent role for alcohol in oral SCC. The cancer risk for alcohol was more dose-related than for cigarettes so that for an individual who smoked and drank alcohol, doubling the alcohol consumption lead to much greater risk of oral SCC than doubling cigarette smoking. In a case-case study of 690 patients with SCC of the lip and oral cavity, Jovanovic et al. (1993a) calculated the risks between smoking, alcohol consumption and developing SCC at various oral subsites, relative to SCC of the tongue. The risks observed by anatomical site were more pronounced for tobacco smoking than for use of alcohol. For heavy drinkers, the greatest risk was observed for SCC of the FOM (OR 3.3). For heavy smokers (>20 cig/day), the risk for developing SCC of the FOM was 7.5 times higher than SCC of the tongue (OR 1.0, reference), which in turn, was about 1.5 times higher than OR for SCC of the cheek (OR 0.67, NS).

In a case control study of 359 males with 424 oral cancer lesions, and 2280 controls, the relationship between tobacco smoking, alcohol consumption and the site of SCC were investigated by Boffetta et al. (1992). The lesions were located in FOM (43%), oral tongue (14%), anterior tonsillar pillar (12%), and soft palate (10%); lingual aspect of retromolar trigone, alveolar ridge, buccal mucosa and hard palate together accounted for only 6% of the total cases. Odds ratios for alcohol drinking for cancers from the FOM (OR range 1.0 - 12.1) and oral tongue (OR range 1.0 - 10.9) were greater than OR for tobacco smoking for the same sites (FOM range 1.0 - 2.5; oral tongue 1.0 - 2.1), suggesting that cancers of FOM and oral tongue were more strongly associated with alcohol than with tobacco exposure. In contrast, tobacco seemed to play an important carcinogenic role in the etiology of cancers of the soft palate as compared to alcohol which had a weaker association for soft palate cancer than for cancers in other sites (Boffetta et al., 1992). These results supported the hypothesis of Bofetta et al. (1992) that tobacco and alcohol exerted their carcinogenic effect via a contact mechanism: tobacco smoking was more strongly associated with lesions in sites heavily exposed to inhaled smoke; alcohol consumption had a stronger effect

on structures belonging to the “food channel” and “reservoir” sites (Moore and Catlin, 1967). However, Eversole (1993) suggested that although most oral SCCs occurred on the FOM and lateral/ventral tongue, these locations did not receive the highest concentrations of consumed alcohol. Unfortunately, those sites that did receive high concentrations of alcohol were not specified by Eversole (1993).

4. Diet, Oral Health, Dental Restorations and Socioeconomic Class

Free radicals and reactive oxygen molecules are normal metabolic byproducts and they are also derived from tobacco smoke; they react with membrane lipids, denature proteins and attack nucleic acids (reviewed by Winn 1995). Some vitamins such as vitamin A and retinoids, beta-carotene, vitamin C and E may have protective effects on carcinogenesis because they are anti-oxidants and scavengers of free-radicals. Retinoids are also essential for normal epithelial differentiation and their deficiencies can lead to squamoid metaplasia. Vitamin C may also inhibit formation of nitrosamines, may detoxify carcinogens and may enhance immune responses of phagocytes. Vitamin E may block nitrosamine formation and promote cell repair and immunity (reviewed by Winn 1995).

The relationship of fruits and vegetables or their antioxidant roles in the prevention of cancer is complicated because vitamins, minerals, antioxidants, fibre, etc., tend to be jointly present in many foods. Hence, it is difficult to distinguish the active agents and to determine how much daily intake is required to produce a cancer-protective effect (Garfinkel 1995b). Overall, the data relating to the role of diet and oral/pharyngeal SCC is confusing and conflicting. In some studies, protective effects have been attributed to niacin, thiamin and vitamins A, C, E, fibre, fruits and vegetables; other studies found no benefit, and still others attributed increased risk to fats, riboflavin and retinol (McLaughlin et al., 1988; La Vecchia et al., 1991; Gridley et al., 1992; reviewed by Eversole 1993 and Winn 1995). However, diet may also act as a surrogate for a generally-healthy lifestyle in that individuals who consume fresh fruits and vegetables also tend to

exercise, avoid tobacco and excessive alcohol consumption, and overall, tend to be thinner, better educated and Caucasian, which may explain why a poor diet is consistently correlated to socioeconomic status (eg. La Vecchia et al., 1991; Gridley et al., 1992; reviewed by Winn 1995).

Poor oral hygiene, poor dental health, and denture trauma have been associated with an increased risk of oral SCC. Chronic irritation from poorly-fitting dentures, from sharp edges of carious, broken teeth or restorations is not considered an initiator of oral cancer; rather, chronic irritation and poor oral hygiene may modify or hasten the progress of a cancer that is started from another cause (Regezi and Sciubba, 1989). The incidence of poor oral hygiene and poor dentition is so high that is impossible to prove a causal relationship to oral cancer. In addition, the association between oral cancer and poor dentition or edentulism may be related to inadequate surveillance for oral cancer. Ismail et al. (1987) reported that only 12% of edentulous adults in the general American population aged 65 to 74 years had a dental visit within the last year compared to 59% of dentate adults aged 65 to 74 years. Rubright et al. (1996) reported that edentulous oral cancer patients had not visited a dentist for an average of 11.6 years before the discovery of the cancer, compared to dentate oral cancer patients who had their last dental visit 4.8 years before diagnosis of the cancer.

Smoking, drinking, oral health status and nutritional status are all strongly correlated to social status and occupation which in turn, are correlated to race (Day et al., 1993). In most studies, the overwhelming confounding effect of other-risk factors such as tobacco and alcohol use have not been eliminated but once these factors as well as socioeconomic status are controlled, no strong association between tooth loss, denture wear, socioeconomic status or race and oral SCC remains, and the risks of oral and pharyngeal cancers are directly attributable to smoking and drinking (Maier et al., 1993; Pukkala et al., 1994; reviewed by Winn et al., 1991; and Ma et al., 1995).

Once tobacco and alcohol use are considered, there is also no significant association between the

presence of dental restorations and tongue carcinoma (Ma et al., 1995). However, in a small subset of patients with asymmetrical restorations, SCC of the tongue was more prevalent on the side adjacent to a restoration, as opposed to the contralateral side without the restoration (Ma et al., 1995). The relationship between good oral hygiene, an extensively-restored dentition and oral cancer has not been considered but ironically, a common ingredient of toothpaste, sodium lauryl sulfate, increases the permeability of oral mucosa (reviewed by Eversole 1993). Perhaps the concurrent use of toothpaste, mouthwash containing alcohol, and other dental factors requires further investigation.

5. Candida

The epithelium of leukoplakias, particularly nonhomogeneous forms (Section VI.B.1) is often invaded by yeasts and certain strains of *Candida albicans* may have a causal role in the development of oral SCC. Results vary, but Banoczy (1977) reported *Candida* infection in 63% of oral SCCs as compared to only 14% of leukoplakias. In a review by Cawson and Binnie (1980), up to 61% of erythroleukoplakias showed Candidal invasion and of these, 71% were associated with dysplasia. By comparison, 3% of homogeneous leukoplakias were invaded by *Candida* and 0% - 40% showed dysplasia, but 67% of leukoplakias with dysplasia were invaded by *Candida* (reviewed by Cawson and Binnie, 1980). For leukoplakias with Candidal invasion, 30% transformed to malignancy compared to only 10% of non-Candidal leukoplakias (Cawson and Binnie, 1980). Significantly, antifungal therapy can transform a nodular, erythroleukoplakia which has a high risk of malignant transformation, into a homogeneous leukoplakia which has a much lower rate of transformation (Pindborg 1980; van der Waal et al., 1986).

It is not known whether yeasts are etiologic factors in the development of leukoplakias or just act as secondary invaders (van der Waal et al., 1986). Because yeasts are intraepithelial parasites, their presence may also affect the behaviour and susceptibility of the epithelium to carcinogens (Cawson and Binnie, 1980). Moreover, iron deficiency has been associated with chronic Candidal

infection (reviewed by Scully et al., 1993) and also has associations with premalignancy (Plummer-Vinson or Paterson-Kelly Syndrome; Chapter 1, Section VI.B.2.d.).

Some yeast strains catalyze the formation of nitrosamines from precursor nitrites, amines and amides which are introduced into the oral cavity through dietary intake and presumably dissolved in saliva. When yeasts isolated from oral leukoplakias and erythroleukoplakias were examined for their nitrosation ability, there was no association between nitrosation rate and advanced dysplastic histological changes (Krogh et al., 1987). However, nodular erythroleukoplakias demonstrating moderate dysplasia or carcinoma-in-situ were exclusively associated with yeasts with high-nitrosation ability and the majority were confined to the FOM. The yeasts were present in the superficial epithelial cell layers as branching hyphae which also extended to the deeper cell layers. The hyphal tube system may provide a transportation system which channels nitrosamine precursors, dissolved in saliva at the epithelial surface, to the deeper layers where nitrosamine is produced and initiates malignant transformation (Krogh et al., 1987).

6. Viruses

In oral SCC, current evidence favours the role of human papillomavirus (HPV) rather than Epstein-Barr virus (EBV) or herpes simplex virus (HSV), (see Table 1.10).

a. Epstein-Barr Virus

In addition to environmental factors such as nitrosamines from smoked fish and genetic factors, EBV DNA has been identified in undifferentiated nasopharyngeal carcinoma and high antibody titers against certain EBV antigens may be predictive of future development of nasopharyngeal carcinoma. EBV is strongly linked to hairy leukoplakia but for oral SCC, the association between EBV DNA or EBV antigens is not well established and reports are contradictory; the current assessment is that EBV is not related to the development of oral SCC (reviewed by Scully 1996).

b. Herpes Simplex Virus

In vitro studies support the view that under specific circumstances, HSV may be oncogenic (reviewed by Scully 1996). For example, HSV may act synergistically with tobacco-specific nitrosamines in transforming cells. In tobacco smokers with oral SCC, antibodies to HSV are increased; smoking and alcohol use may both suppress activity of NK cells which are involved in the control of HSV. Recovery of HSV DNA and antigens in oral SCC has been inconsistent but overall, suggests an association of HSV with oral SCC (Table 1.10). Since carcinogenesis is a multi-step process rather than a single-event, it is possible that HSV acts synergistically with HPV in oral carcinogenesis (reviewed by Scully 1996).

c. Human Papillomavirus

Over 75 different types of HPV have been isolated with different types exhibiting variations in tissue tropism and malignant potential. For example, HPV DNA is detected in up to 90% of cervical carcinomas but the rate of detection of HPV DNA in oral SCC is much lower and the HPV subtypes differ between the two sites (Miller and White, 1996; see also Section II.H.1). HPV DNA has also been recovered from normal oral mucosa, oral benign lesions (eg. oral squamous papilloma, verruca vulgaris, condyloma acuminatum, focal epithelial hyperplasia), premalignant lesions (leukoplakia) and oral verrucous carcinoma. When HPV DNA is recovered from benign oral or cervical lesions, it exists as free episomes. In cervical carcinomas, the HPV DNA is integrated within the genome of the host cell but in oral carcinomas, HPV is seldom integrated (reviewed by Scully 1996; Miller and White, 1996).

The transforming ability of HPV is linked to its early genes, E5, E6 and E7, but transformation also appears to require additional factors such as co-infection with HSV or cytomegalovirus, exposure to tobacco and alcohol, hormones, etc. The oncoprotein encoded by HPV gene E5 can modulate the activity of EGFR. The oncoprotein encoded by HPV gene E6 binds to and interferes with p53 protein, and oncoprotein E7 binds to and interferes with protein product of suppressor

gene Rb. The significance of E6-p53 and E7-pRb interactions is reflected by the malignant potential of the HPV type. For oral SCC, HPV types -16 and -18 are likely to be the most carcinogenic because their E6 oncoproteins can associate with p53 whereas E6 proteins from "low-risk" HPV-6 or -11 can not. In addition, E7 oncoproteins from HPV-16 and -18 bind pRb more strongly than does E7 from benign types HPV-6 and -11 (reviewed by Palefsky et al., 1995; and Scully 1996). Some studies have demonstrated HPV E6 or E7 gene expression in oral SCC, and they support the hypothesis that a critical process in squamous cell carcinogenesis involves inactivation of the normal function of p53 or pRb (reviewed by Palefsky et al., 1995; Scully 1996; Scully and Field, 1997).

DNA from several types of HPV, usually HPV-2, -6, -11, -16 and -18, have been identified in premalignant conditions such as lichen planus, premalignant lesions (Section VI) and carcinoma; however, recovery of HPV DNA from normal mucosa casts doubt on a causal relationship (Ostwald et al., 1994; Palefsky et al., 1995; reviewed by Scully 1996; Miller and White, 1996). Depending upon the sensitivity of the detection method used (Section II.H.1), the rate of HPV detection in oral SCC ranges from 0% to 94%; with the use of in situ hybridisation and PCR, HPV-11, -16 or -18 DNA sequences are detected in 10% - 60% of oral SCCs (Nielsen et al., 1996; reviewed by Scully 1996).

For example, in a series of 22 oral precancerous lesions (Section VI.B.1) and 51 oral SCCs, 16% of lesions contained HPV DNA as detected by in situ hybridization: 12% were carcinomas and 29% were dysplasias (Syrjanen et al., 1988). The most frequent sites of HPV DNA-positive lesions were on the palate (57%), followed by FOM (25%), tongue and gingiva (12%), but the most common sites for HPV-infected dysplasias were found on the tongue, followed by FOM and palate. "High-risk" HPV types -16 and -18 were found in 9 cases consisting of 6 SCCs and 3 dysplasias (Syrjanen et al., 1988).

In a series of 49 patients with oral premalignant lesions and using a variety of detection assays, HPV was detected in 41% of the lesions, and HPV was not detected in the control sample of 20 individuals with normal mucosa (Nielsen et al., 1996). Of the HPV-positive lesions, 62% were verrucous leukoplakias, 50% were erythroplakias, 46% were homogeneous leukoplakias, 33% were erythroleukoplakias and 12% were nodular leukoplakias (Nielsen et al., 1996). The majority of HPV-positive lesions were located on the buccal mucosa, usually as verrucous leukoplakia with no or slight dysplasia, or as erythroleukoplakia with moderate dysplasia. In contrast, the majority of HPV-negative lesions were located in the sublingual region, usually as erythroleukoplakia and nodular leukoplakia with slight to moderate dysplasia (Nielsen et al., 1996), findings that contradict those of Syrjanen et al. (1988), (see above). In the Nielsen et al. (1996) study, 25% of the HPV-positive lesions were positive for HPV-16, a "high-risk" type of HPV, and the remainder were positive for HPV-6, -11, 18, -31 and -33. During the follow-up period of 4-12 years, 3/40 patients developed SCC and all 3 were positive for HPV and all 3 were smokers (Nielsen et al., 1996). In oral SCC, HPV may reflect a focal infection as the frequency of HPV detection in non-neoplastic mucosa of patients with oral SCC decreased with increasing distance from the tumour (Ostwald et al., 1994).

In a review of the literature up to September 1995, Miller and White (1996) determined that the prevalence of HPV increased with dysplasia. HPV was detected twice as often in oral SCC as in normal mucosa, and HPV was detected 11 times more often in malignant tissue than distant oral mucosa. The HPV genotype identified in primary oral SCC was maintained in 76% of nodal metastases and there was an apparent correlation between recovery of high-risk HPV-16 and -18, and aggressiveness of the tumour. The tongue was the most common site (32%) of oral SCC, and the most common site of HPV-positive SCC (29%). 87% of SCC cases were associated with a history of tobacco and alcohol and of these, 50% were also associated with HPV. In 7.3% of SCC cases, no known risk factors other than HPV infection were identified, and in 5.3% of cases, no risk factors were identified. These findings suggested that HPV rarely acts alone in oral SCC

and that other factors (eg. alcohol, tobacco) play a role. HPV may be only transiently present ("hit and run" phenomenon) but even intermediate presence may be sufficient to initiate chromosomal damage if the early genes (E6 and E7) can inactivate p53 and Rb proteins. Subsequent analytic procedures such as PCR would fail to detect HPV genes in oral SCC yet a viral contribution to SCC could nevertheless have been made (Miller and White, 1996).

Odds ratios linking HPV to oral SCC range from 2.8-154, depending upon the type and HPV DNA detection method used (Table 1.10; International Agency for Research in Cancer Monographs 1995).

7. Genetic Factors

Heritable differences in susceptibility to cancer can be identified at almost every phase of carcinogenesis. For example, there are differences between individuals with respect to their sensitivity to mutagens, in their ability to metabolize carcinogens, in the stability of DNA and their ability to repair DNA, and in the expression of proto-oncogenes and tumour suppressor genes (reviewed by Spitz 1994; Scully and Field, 1997). In a large case-control study in four areas of the United States, Day et al. (1993) reported that although a history of cancer in a parent or sibling was unrelated to oral cancer risk in either Blacks or Caucasians, there was a significantly increased risk among Blacks who had a brother with cancer (OR 7.4; 95% CI 1.8-3.1), but there was little or no evidence of an excess risk among Caucasians with a similar family history (OR 1.1; 95% CI 0.7-1.6; Table 1.10).

Gorsky et al. (1994) investigated the relationship between ethnic origin and 342 cases of oral cancer in the Israeli Jewish population between 1970 to 1980. Almost 85% of patients were over the age of 45 years and SCC was the leading malignancy, affecting the tongue in 38% of cases and the lip in 27%. In relating malignancy to ethnic origin, 72% of cases were Ashkenazi Jews, 15% were Sephardi Jews, and 13% represented the Eastern ethnic group. By comparing oral cancer

prevalence in each ethnic group to the fraction that each ethnic group represented in the total Israeli population, the authors reported the “risk” of the Ashkenazi ethnic group developing oral cancer to be almost three times higher than for the Sephardi group, and two times higher than the Eastern ethnic group. Unfortunately, multiple statistical comparisons were made and risk factors such as alcohol use or tobacco use were not reported; nevertheless, the authors concluded that heredity played a role in the development of oral cancer (Gorsky et al., 1994).

Chromosome	Alteration in Carcinogenesis
Chromosome 3	<ul style="list-style-type: none"> -allelic loss associated with oral premalignant and malignant lesions -allelic loss reported in 47% - 81% of head and neck tumours -allelic loss was strongly associated with prognosis -74% of allelic losses in head and neck carcinomas were at site of gene for retinoic acid receptor -may also contain some tumour suppressor genes
Chromosome 7	<ul style="list-style-type: none"> -aberrations associated with premalignant and malignant lesions -contains gene for EGF receptor (EGFR) -both overexpression and decreased expression of EGFR reported in oral dysplasia, carcinoma and normal tumour-adjacent epithelium -overexpression of EGFR appears to correlate with tumour size and stage
Chromosome 8	<ul style="list-style-type: none"> -contains gene for c-myc which is amplified in 7% of head/neck carcinomas -amplification and overexpression associated with oral premalignancy and carcinomas -amplification of c-myc gene correlated with tumour size, differentiation and nodal metastases
Chromosome 9	<ul style="list-style-type: none"> -loss of allele reported in 72% of head/neck SCCs; also noted in severe dysplasia and carcinoma in situ -contains p15, p16 genes (inhibitors of cyclin-dependent kinases)
Chromosome 11	<ul style="list-style-type: none"> -contains genes for H-ras; mutations rare in Western world (~5%) -contains genes for bcl-1 which is amplified in 58% head/neck SCCs, mainly in advanced tumours with metastases
Chromosome 13	<ul style="list-style-type: none"> -contains Rb gene -Rb gene may be mutated in oral SCC but loss of Rb gene is uncommon in head/neck SCCs
Chromosome 16	<ul style="list-style-type: none"> -contains gene for E-cadherin (epithelial cell-cell adhesion protein) -a minority of head/neck cancers demonstrate loss of allele of this chromosome
Chromosome 17	<ul style="list-style-type: none"> -contains gene p53 -loss of allele for p53 seen in up to 55% studies of head/neck SCCs -p53 mutations reported in 27% - 60% of oral dysplastic lesions -p53 changes seen as early change in oral carcinogenesis -also contains gene c-erb B

Table 1.7. Chromosomal Alterations in Squamous Cell Carcinoma of the Head and Neck.

Chromosomes and associated genes associated with SCC of the head and neck. In order of frequency, genetic aberrations involve chromosomes 3, 9, 11, 13, and 17. The changes involving chromosomes 3, 9 and 17 appear early in carcinogenesis, but it is the accumulation of genetic changes rather than the sequence of events, that appears to determine progression to malignancy. Summarized from Scully and Field, 1997.

Biomarker**Possible Role in Carginogenesis**

p53	<ul style="list-style-type: none"> -protein p53 normally regulates transcription factors, controls passage from G1 to S phase; arrests cells in G1 for DNA repair or apoptosis -cells expressing mutated protein are not blocked at G1/S phase, cannot undergo DNA repair or enter apoptosis and their proliferation is promoted
c-erb B	<ul style="list-style-type: none"> -codes for EGFR -overexpression of cerb-B gene results in overexpression of EGFR which results in increased intracellular signalling by EGF and TGFα for proliferation
TGF α	<ul style="list-style-type: none"> -normally produced by epithelial cells as growth stimulus -overexpression linked to anchorage independence and increased proliferation
EGF	<ul style="list-style-type: none"> -normally produced by subepithelial fibroblasts but overexpression of its receptor (EGFR) by transformed cells may lead to increased cell proliferation
ras family	<ul style="list-style-type: none"> -codes for proteins involved in coupling growth factor receptors such as EGFR to effector proteins -amplification of ras genes prolong signals for cell proliferation
myc family	<ul style="list-style-type: none"> -codes for transcription factors that activate other growth-promoting genes and sustains the Cell Cycle -overexpression of myc genes favors proliferation
Bcl-1	<ul style="list-style-type: none"> -its protein product is cyclin D required for passage through Restriction Point -gene amplification results in increased levels of cyclin D and hence, increased proliferation
Bcl-2	<ul style="list-style-type: none"> -its protein product normally modulates apoptosis -amplification of Bcl-2 gene is expected to inhibit apoptosis and may also be involved in delay of terminal differentiation
TGF β	<ul style="list-style-type: none"> -normally inhibits epithelial proliferation by regulating the expression of EGFR, and by upregulating gene p15 which inhibits cyclin-dependent kinases -malignant cells may be refractory to inhibitory effects of TGFβ

cadherins	<ul style="list-style-type: none">-cadherins are involved in intercellular adhesion of epithelial cells-loss of chromosome 16 allele may result in loss or reduction of cadherins which has been related to cell separation, cell detachment and metastasis
integrins	<ul style="list-style-type: none">-integrins are ECM receptors and link the ECM and the cytoskeleton-alterations in integrin expression linked to anchorage independence and metastasis
retinoic acid receptors	<ul style="list-style-type: none">-allelic loss of chromosome 3 results in loss of gene for RA receptor-retinoids are required for normal cell growth and differentiation-in particular, loss of RARβ associated with development of malignancy

Table 1.8. Biomarkers and Possible Roles in Carcinogenesis.

Keratins	Altered Expression or Localization
K5, K14	-expression may extend beyond the basal layer.
K1, K10 K4, K13	-reduced expression of these keratins may be related to the degree of dysplasia with the greatest loss observed where dysplasia is most severe. -these keratins may be co-expressed such as when dysplastic epithelium in normally noncornified sites undergo cornification. This behavior may be observed in dyskeratosis congenita, a premalignant condition (Section VI.B.2.e.).
K6, K16	-increased expression of may be observed in dysplastic epithelium and in clinical leukoplakia (Section VI.B.1.a.) regardless of dysplasia.
K19	-the suprabasal localization of in relation to dysplasia is controversial because significant K19 is seen in leukoplakia irrespective of dysplasia, in inflamed gingiva and in HPV-infected oral epithelium.
K7, K8, K18	-expanded expression of these simple keratins may be correlated with severity of dysplasia.

Table 1.9A. Keratin Markers in Oral Epithelial Dysplasia
Summarized from Morgan and Su, 1994; Su et al., 1996.

Keratins**Altered Expression or Localization**

K1, K10
K4, K13

- general reduction in expression of these secondary keratins
- in well-differentiated SCCs they may be localized to prickle cells and cells at the center of tumor islands
- tumour cells may express both sets of differentiation keratins (cornified K1, K10 and noncornified K4, K13 concurrently)

K7, K8, K18

- these simple keratins may be expressed ectopically and inversely with the degree of differentiation
- strongest expression is seen in the most poorly differentiated tumors which also have a worse prognosis
- stronger expression of simple keratins is seen in lymph node metastases from oral SCCs than in primary SCCs.

K19

- Levels of K19 mRNA and protein expression vary markedly in amount and distribution
- K19 protein levels may be related to whether the tumor arose from an originally cornified or noncornified site
- the persistence of K19 in the suprabasal cells of noncornified epithelium may be due to their delayed commitment to terminal differentiation and may indicate retention of proliferative (stem cell) potential (Lindberg and Rheinwald, 1989).

K5, K14

- expression of primary keratins is reduced or lost.
- well- and moderately differentiated SCCs express both K14 mRNA and protein at reduced levels, transcriptional control is maintained
- in poorly differentiated SCCs, K14 mRNA is present but protein is not

Table 1.9B. Keratin Markers in Oral Squamous Cell Carcinoma.
Summarized from Morgan and Su, 1994; Su et al., 1996

<u>Risk Factors</u>	<u>Odds Ratios</u>			<u>Relative Risks</u>
	<u>Males</u>	<u>Females</u>	<u>Combined</u>	
Smoking				
never	1.0* ¹	1.0* ¹	1.0* ⁹	
cigar or pipe	1.9 ¹		21.9 ⁹	
exsmoker			3.6* ⁹	
current cig. smoker			11.8 ⁹	
cigarettes ¹				
1-19/day ¹	1.2*	1.8		
20-39/day ¹	2.1	3.6		
30/day ⁸			1.26	
40+/day ¹	2.8	6.2		
cigarettes ⁹				
≤14/day			4.5	
15-24/day			11.0	
≥25/day			9.6	
Years of smoking ¹				
1-19	0.8*	1.0*		
20-39	1.9	2.9		
40+	3.6	5.0		
Years of smoking ⁹				
≤29			3.5*	
30-39			11.0	
≥40			14.3	
Age started smoking ¹				
<17	2.1	2.9		
17-24	1.8	3.1		
>25	1.8*	2.8		
Age started smoking ⁹				
≤19			11.0	
>20			6.5	
Tar Yield⁹				
low (<22mg)			7.1	
high (≥22mg)			14.4	
Tobacco Consumption⁶ (cigarettes, pipe and cigar combined)				3.9-15.4

<u>Risk Factors</u>	<u>Odds Ratios</u>			<u>Relative Risks</u>
	<u>Males</u>	<u>Females</u>	<u>Combined</u>	
Alcohol				
all types ¹				
<1/wk	1.0*	1.0*		
1-4	1.2*	1.2*		
5-14	1.7*	1.3*		
15-29	3.3	2.3		
>30	8.8	9.1		
all types ⁶				2.7-70.3
all types ⁹				
≤19/wk			1.0*	
20-34			1.1*	
35-59			2.1*	
≥60			3.0*	
hard liquor	2.6-5.5 ¹	4.9-7.8 ¹	0.5-1.0* ⁹	
(≥15 drinks/wk) ¹			(≥7 drinks/wk)	
beer	1.7-4.7 ¹	2.9-18.0 ¹	1.0* ⁹	
(≥5 drinks/wk) ¹			(≥14 drinks/wk)	
wine	2.5* ¹	1.6* ¹	3.6-6.8 ⁹	
(>30 drinks/wk) ¹			(≥56 drinks/wk)	
Smoking and Alcohol¹				
	1.5-37.7	5.1-107.9		
Mouthwash				
high alcohol ²	1.6	1.9		
low alcohol ²	0.7*	0.8*		
Edentulousness				
			1.7* ³	
			0.7-1.5* ²	
Periodontal Disease²				
			0.6-1.6*	
Bleeding Gums²				
			0.8-1.0*	
Human Papillomavirus⁴				
Types 16, 18			6.2*-154	
Types 6, 11			2.8*-3.1*	
Herpes Simplex Virus ¹³				
Types 1 or 2			0.8-1.8*	

<u>Risk Factors</u>	<u>Odds Ratios</u>			<u>Relative Risks</u>
	<u>Males</u>	<u>Females</u>	<u>Combined</u>	
Precursor Lesions				
leukoplakia ²	12.7	4.3		26
homogeneous leukoplakia ⁵				3243
nodular ⁵				44
ulcerated ⁵				16* ⁵
lichen planus				3.3* ¹¹
red areas/ulcers ⁵				303
oral sores ²		0.8-1.2*		
cold sores ²		0.9-1.1*		
submucous fibrosis ⁵				397
Genetic Factors				
in Blacks, brother with oral cancer ³			7.4	
Protective Factors				
Cessation of smoking ¹				
<10 years	1.1*	1.8*		
>20 years	0.7*	0.4*		
Fruit containing vitamins				
A, C, and E ⁷	0.2.-0.7*	0.5-0.8*		
fresh fruits ⁹			0.5-1.0*	
green vegetables ⁹			0.4-1.0*	
carrots ⁹			0.4-1.0*	
vitamin E ¹²			0.4-0.6	
Low vegetable (≤2/wk)				
and				12.0 ¹⁰
High cig/day (21+/day)				

Table 1.10. Risk Factors, Odds Ratios and Relative Risks Associated with Oral and Pharyngeal Cancer. Odds Ratio and Relative Risk are measures of the association between exposure to the risk factor and the disease, oropharyngeal cancer. A value of 1.0 indicates there is no association between the exposure and disease. Values greater than 1.0 indicate an increased risk; values less than 1.0 indicate a decreased risk or a protective effect. However, the mere fact that an OR or RR exceeds 1.0 is not in itself, a sufficient basis for implicating that factor. In fact, unless there is a strong biological plausibility for its causing a specific disease, a given factor should have an OR or RR of at least 3.0 or 4.0 before implicating that factor as a cause of the disease (Taubes 1995; Barnett and Mathisen, 1997); see also Chapter 1, Section II.E.). Asterisks (*) indicate that the RR or OR was not statistically significant as determined by P values or confidence intervals.

1. **Blot et al., 1988:** case-control study in the United States; 1114 cases with oropharyngeal SCC, 1268 population-based controls
2. **Winn et al., 1991:** case-control study in the United States; 866 cases with oropharyngeal SCC, 1249 community controls
3. **Day et al., 1993:** case-control study in the United States; 1065 cases with oropharyngeal SCC; 1182 population-based controls
4. **International Agency for Research in Cancer Monographs 1995.** Studies in Cancer in Humans; review of 12 studies relating HPV-DNA to oropharyngeal and respiratory tract cancers. Controls for cases were typically obtained from normal patients undergoing biopsies or scrapes from matched sites for reasons other than cancer. Detection techniques included PCR, southern and dot blots, in situ hybridization (Section II.)
5. **Gupta et al., 1989:** cohort of 12,212 tobacco users in India followed from 1977/78-1986 for development of oral lesions and malignancy; mean periods of observation differ for the various lesions and oral conditions
6. **Brugere et al., 1986:** case-control study in Paris, France, 2540 consecutive cases of males with cancer of the larynx, pharynx and mouth; results obtained from National Survey on Health and Medical Care were used as comparison group.
7. **Winn 1995:** review paper
8. **Bundgaard et al., 1994:** case series in Denmark of 161 consecutive patients with oral SCC
9. **Franceschi et al., 1992:** case-control study in Italy, 104 male cases with oral SCC, 726 hospital-based controls
10. **Oreggia et al., 1991:** case-control study in Uruguay, 57 male cases with incident SCC of the tongue, 353 hospital-based controls.
11. **Murti et al., 1986:** cohort of 722 individuals with lichen planus in India, followed over 10 years with a mean observation period of 5.1 years
12. **Gridley et al., 1992:** case-control study in four areas of the United States, 1103 cases with oropharyngeal cancers and 1268 population-based controls
13. **Maden et al., 1992:** case-control study in Washinton state, USA, 131 cases of males with oral SCC, 136 matched male population controls

VI. Oral Premalignancy

“Premalignant” or “precancerous” are terms used retrospectively to describe lesions which subsequently become malignant. They are used retrospectively because there are no consistent clinical or histopathological criteria which reliably predict malignant transformation (Atkinson and White 1992) although certain histopathological (Section VI.A.) and clinical (Section VI.B.) features are associated with an increased risk or rate of development of SCC, and they are used in the assessment of the prognosis. In order to determine rates of malignant transformation in association with these criteria, follow-up of suspected premalignant lesions, both clinically and histologically, is required and such studies have formed the basis for classification of certain clinical lesions and conditions as premalignant. Unfortunately, different investigators have employed different criteria for assessing the lesions clinically and for grading epithelial dysplasia histologically; consequently, the data may not be very reliable (Pindborg et al., 1985); (see also Section II.G.2).

A. Histopathology

Histopathological diagnosis frequently represents the final diagnosis or the “Gold Standard” because, traditionally, classification of disease is pathoanatomically oriented (Wulff 1976). Premalignancy and malignancy can be diagnosed only at the histological level and diagnosis depends wholly on the recognition and evaluation of epithelial changes. However, in reaching a diagnosis the pathologist typically considers several clinical factors such as the location of the lesion, its clinical appearance and suspected etiology (Schwartz et al., 1981) which are also important factors in the assessment of the prognosis (Shafer 1980; Karabulut et al., 1995).

Designating lesions as either benign or premalignant is predicated on the belief that well-defined prognostic characteristics actually exist in a lesion at any time and furthermore, that these characteristics can be identified by the morphological appearance of the cells and tissues examined with the light microscope. These assumptions also imply that there is a continuum ranging from

normal epithelium or simple, benign hyperkeratosis at one end of the spectrum to invasive SCC at the other (van der Waal et al., 1986; Leong et al., 1995). This concept further assumes that the progression of malignant transformation through increasingly severe dysplastic changes to carcinoma in situ, and ultimately to invasive carcinoma follows a regular pattern in all individuals and therefore, a lesion may be assessed at any point along the continuum and its course can be predicted (van der Waal et al., 1986); unfortunately, data to support this model are incomplete (Karabulut et al., 1995).

The histologic diagnosis of epithelial dysplasia is based on the presence of certain tissue changes that are also characteristics associated with carcinomas (Dabelsteen 1980). Despite the generally good agreement on the light microscopic features that characterize epithelial dysplasia (see below), there is still insufficient knowledge of which histological criteria are the most important for the diagnosis, the grade, and in predicting transformation (Karabulut et al, 1995). Moreover, proper interpretation of what is seen and quantitation of those observations is difficult and highly subjective (Pindborg et al., 1985; also Section II.G.2). For example, the thinner the epithelium, the more difficult it is to evaluate disturbances in stratification which is an important feature in dysplasia. Also, the superficial dysplastic epithelium of many nodular leukoplakias is infected with *Candida* and hence, some of these "dysplasias" are reversible with anti-fungal therapy (Pindborg et al., 1985). Overall, subjective interpretation is most pronounced in the late stages of metaplasia and dysplasia, a time when the correct diagnosis is most important (Kramer 1980a) as errors can have serious therapeutic consequences for the patient. The grading of oral dysplasia according to severity does not provide a reliable guide to the likelihood of malignant change (Kulasegaram et al., 1995), but nevertheless, the presence of epithelial dysplasia seems to be the most important indicator of malignant potential, and the grade of dysplasia is related to the likelihood of malignant transformation (Banoczy and Csiba, 1976; WHO 1978; van der Waal et al., 1986).

Generally accepted components of epithelial dysplasia include drop-shaped rete pegs, disturbed polarity of the basal cells, hyperplasia of the basal layer, crowding, irregular stratification and maturation, variations in cell shape and size (pleomorphism), prominent enlarged nucleoli, nuclear hyperchromatism, increased nucleus:cytoplasm ratio, abnormal and/or increased numbers of mitosis in the basal compartment as well as mitotic figures in the suprabasal layers, reduced cellular cohesion and intra-epithelial cornification (WHO 1978; reviewed by Kramer 1980a; van der Waal et al., 1986).

No single feature of epithelial dysplasia is an absolute discriminator that can separate a lesion that is precancerous from one that is not (Kramer 1980a). Computer analysis of histological data on leukoplakias that proceeded to carcinoma and those that did not, determined that the eight most important histological features, in order, that distinguished the two groups of lesions were abnormal mitoses in the stratum spinosum, disturbed polarity of basal cells, abnormal mitoses in the stratum basale, nuclear hyperchromatism, Russell bodies in the lamina propria, enlarged nucleoli, pleomorphism and intraepithelial keratinization (Kramer 1980b). However, analysis did not assess those histological features that reduced the overall assessment of the seriousness of the lesion and significantly, there is not a perfect correlation between the degree of epithelial dysplasia and the likelihood of invasive change. Similar degrees of epithelial dysplasia may carry different risks for transformation depending, for example, on location (eg. high risk sites like FOM) and clinical appearance (eg. erythroleukoplakia versus homogeneous leukoplakia); (reviewed by Kramer 1980b). Smith and Pindborg (1969) recommended standardization of epithelial dysplasia by photographic standards that were combined with a weighting system for different histologic changes but their approach was too cumbersome for routine use (Pindborg et al., 1985).

More recently, Lumerman et al. (1995), reviewed histopathological features associated with transformation of clinically premalignant lesions to carcinoma, and their "minimal" criteria included basal cell hyperplasia, nuclear enlargement and hyperchromaticity, and drop-shaped rete ridges.

They also graded the severity of dysplasia as “mild” when “minimal” alterations were confined to the lower third of the epithelium; “moderate” when dysplastic features were evident in up to two-thirds the thickness of the epithelium; “severe” when dysplastic cells filled more than two-thirds but less than the entire thickness of the epithelium; “carcinoma in situ” when the entire thickness of the epithelium contained less differentiated basaloid or squamous cells with enlarged hyperchromatic nuclei and atypical mitotic figures but without invasion of the submucosa (Lumerman et al., 1995). Also included was the category “verrucous hyperplasia with dysplasia” which included epithelium exhibiting surface thickening with surface papillations, hyperkeratosis and parakeratin plugging, and occasional dysplastic cells confined to the lower third of the epithelium. However, it is critical to note that the authors (Lumerman et al., 1995) reported an interobserver agreement of only 54% between two experienced pathologists independently grading the specimens with these criteria, thus confirming the subjective interpretation of many criteria (Pindborg et al., 1985).

Although examination of putative premalignant lesions has concentrated on the histologic features of the epithelium, changes in the underlying connective tissue, particularly the inflammatory response, may also be important and inflammation may distort the interpretation of epithelial dysplasia. As noted above, Kramer (eg. 1980b) noted that the apparent presence of Russell bodies among the cellular infiltrate was important in discriminating malignant change. Erythroplakias that on microscopy present as carcinoma in situ or early invasive carcinoma often demonstrate a submucosal vascular reaction and round cell infiltrate, which appear to be an immune response to the developing lesion (Mashberg and Samit, 1995). Some premalignant conditions, submucous fibrosis, lichen planus and Plummer-Vinson syndrome are associated with subepithelial infiltrates yet another premalignant condition, dyskeratosis congenita, is characterized by pancytopenia. The immune system may influence the development of malignancy but it is not known whether the lesion develops because of a lack of recognition mechanisms, or because of a failure in immune response. Immune activity generally diminishes with increasing age and as oral cancer is an age-

related disease, it has been suggested that the increasing incidence of cancer with age is related to impaired immune response (reviewed by Leong et al., 1995). Moreover, iron deficiency (as in Plummer-Vinson syndrome) and several potential carcinogenic factors such as smoking and alcohol can reduce cell-mediated activity. However, there is no evidence that patients with a primary immunodeficiency have an increased incidence of oral SCC, and the most common intraoral malignancies in AIDS are Kaposi's sarcoma and lymphoma (reviewed by Leong et al., 1995).

The presence of dysplasia must be regarded as an indication that there is a significant risk of malignant change although there is no way of predicting such an outcome because not all lesions diagnosed as dysplastic progress to an invasive carcinoma if left untreated (Dabelsteen 1980; Leong et al., 1995). For example, following a diagnosis of dysplasia, Mincer et al. (1972) reported that 22% of patients were stable over 8 years but 11% of patients developed carcinoma in up to 7 years of observation; 11% of lesions disappeared or decreased in size but 11% of lesions increased in size or severity of dysplasia throughout 8 years of follow-up. Lumerman et al. (1995) reported that 16% of patients diagnosed with oral dysplasia developed carcinoma within 7-78 months, within a mean transformation time of 33.6 months. Moreover, two of these 7 patients developed carcinomas in a site distant from the original dysplasias, supporting the hypothesis of "field cancerization" (Slaughter et al., 1953). Silverman et al., (1996) reported that 36.4% of patients with oral dysplasia developed carcinomas over a mean time of 8.1 years of follow-up. As well, 15.7% of patients with clinically suspicious lesions but without histologic dysplasia developed carcinoma within a similar observation period (Silverman et al., 1996). Lumerman et al. (1995) concluded that 13.8% of oral dysplasias transform to invasive SCC within a follow-up period of up to 20 years. These results illustrate the problems of follow-up without treatment or excision (see VI.B.1.d.below) of the epithelial dysplasia: malignant change can occur relatively suddenly and the tumour may progress rapidly between periods of observation; in addition, there is the problem of patients being lost to follow-up, and the differences in the treatment of the initial

lesions in retrospective studies (Section VI.B.1.d).

To date, a reliable and valid substitute for the traditional histopathological evaluation of oral lesions does not exist (Karabulut et al., 1995) but this deficiency has prompted the investigation of biological markers that may characterize and predict malignant transformation (eg. Greenblatt et al., 1994; Scully and Field, 1997). Investigations generally include biomarkers that are associated with malignancy and these have already been reviewed in Chapter 1, Section V.

B. Clinical Features

1. Premalignant Lesions

“Premalignant lesion” implies an identifiable change that precedes the development of cancer at the same site after a latent period (Cawson 1975). In 1973, the World Health Organization defined a premalignant or precancerous lesion as “a morphologically altered tissue in which cancer is more likely to occur than in its apparently normal counterpart” (Pindborg 1980); this definition was reaffirmed in 1994 by the International Collaborative Group on Oral White Lesions (Axell et al., 1996).

Very little information exists about the natural history of premalignant dysplastic lesions. For example, their incidence is unknown because unlike SCC, premalignant lesions are not registered and it would be difficult to establish nonsubjective clinical and histological criteria for registration purposes (Cawson 1975). Even if the progression or regression of dysplasia in premalignant lesions could be traced by means of sequential biopsies, only part of the lesion is sampled by biopsy, in which case it offers no information about the remainder of the lesion. Moreover, there is always uncertainty as to whether the most informative area, i.e., the area at greatest risk, has been selected for biopsy, as well as the variability inherent in histological diagnosis. Alternatively, if the entire lesion is excised, its natural development has been precluded (Cawson 1975; Ephros and Samit, 1997). Although many studies have discussed malignant transformation (i.e. the

development of a frank cancer), times of follow-up have varied. For example, premalignant lesions “may precede the appearance of cancer by months or years, or they may be present together with the carcinoma when the patient is first seen” (WHO 1978) whereas Gupta et al. (1989) specified that the criterion included development of a “cancer on a lesion preexisting for one or more years”. Reports of the simultaneous occurrence of a premalignant lesion and SCC ranges from 11%-60% in 10 studies (reviewed by Pindborg 1980) and in India, almost 80% of oral SCCs are preceded by premalignant lesions (Gupta et al., 1989). Given the limitations of clinical and histological assessment and follow-up, Ephros and Samit (1997) suggested that valid determinations of progression may not be possible and that conclusions about the progression (or regression) of dysplasias were of questionable validity.

Oral precancerous lesions comprise (a) leukoplakias which include (i) homogeneous and (ii) non-homogeneous leukoplakias and (b) erythroplakias (Axel et al., 1996).

a. Leukoplakia

“Oral leukoplakia is a predominantly white lesion of the oral mucosa that cannot be characterized as any other definable lesion; some oral leukoplakias will transform into cancer” (Axel et al., 1996). Implicit in this definition is the condition that the white lesion is adherent to the oral mucosa and cannot be scraped off (Pindborg 1980). Depending upon the diagnostic investigation, the diagnosis of leukoplakia is either definitive or provisional. A provisional diagnosis of oral leukoplakia is made when a lesion cannot be clearly diagnosed as any other disease of the oral mucosa with a white appearance (Axel et al., 1996). A definitive diagnosis is made as a result of the identification, and if possible, elimination of suspected etiological factors (Axell et al., 1996). If lesions do not regress within 2-4 weeks following attempts to eliminate the suspected etiological factors, then a biopsy should be taken. White lesions for which a local cause other than the use of tobacco can be identified include frictional lesions, lesions associated with dental restorations and those associated with cheek biting; consequently, these lesions are listed according to etiology and

are not included as leukoplakias. White lesions of unknown etiology are classified as idiopathic leukoplakias and those associated with, or thought to result from, the use of tobacco or chemical products are tobacco- or chemical-associated (Axell et al., 1996). Ironically, white lesions associated with pipe smoking (nicotinic stomatitis), reverse smoking (palatal keratosis) or "snuff-dipper's lesion" are not traditionally described as tobacco-associated leukoplakias although they are at least partly white and are associated with tobacco use (Axell et al., 1996).

Leukoplakia is used only as a clinical term without implication to histological features. The histological features accompanying oral leukoplakia may include various types and degrees of hyperkeratosis; epithelial dysplasia may or may not be present and its severity may vary. Oral leukoplakias that demonstrate epithelial dysplasia have an increased risk of malignancy; however, malignancy can also occur within oral leukoplakias for which earlier biopsies have not demonstrated dysplasia. In those instances where carcinoma-in-situ or carcinoma is diagnosed histologically, the histological diagnosis replaces the provisional diagnosis of leukoplakia (Axell et al., 1996). Premalignant lesions may also be classified and staged according to whether their diagnosis is provisional or definitive, the size of the lesion, its homogeneity and pathological features (Axell et al., 1996), and use of such a system would improve standardization and facilitate comparison of data derived from different sources.

(i). Homogeneous Leukoplakias.

Homogeneous leukoplakia is a predominantly white lesion of uniform, flat, thin appearance that may exhibit shallow cracks and has a smooth, wrinkled or corrugated surface with a consistent texture throughout. In general, homogeneous leukoplakias have a low risk of malignant transformation (Axell et al., 1996).

(ii). Nonhomogeneous Leukoplakias

Nonhomogeneous leukoplakias may be predominantly white, or both white and red (erythroleukoplakia). They may be irregularly flat, nodular or exophytic. Nodular lesions usually

have slightly raised, rounded, red and/or white excrescences. Exophytic lesions have irregular blunt or sharp projections. Nonhomogeneous lesions demonstrate an increased risk of malignant transformation (Axell et al., 1996).

b. Erythroplakia

Erythroplakia is used analogously to leukoplakia and it is a predominantly red lesion of the oral mucosa that cannot be characterized as any other definable lesion (Axel et al., 1996).

Erythroplakias are diagnosed either provisionally or diagnostically and some will transform into cancer.

c. Epidemiology of Premalignant Oral Lesions

It is generally not known if the same diagnostic criteria have been applied in the many surveys of oral premalignant lesions and therefore it is not known what the true prevalence of these lesions and their rates of transformation are (Pindborg 1980; Section II.D).

The prevalence of leukoplakia varies geographically and typically ranges from 0.4%-17% but prevalences up to 54% have been reported in Eastern Europe and may reflect differences in tobacco habits (van der Waal et al., 1986). Leukoplakia is most prevalent among men in most countries except India where certain tobacco habits are practiced by women; the sex ratio of males:females for leukoplakia ranged from 3:1 to 6:1 (van der Waal et al., 1986). Homogeneous leukoplakias are more prevalent than non-homogeneous, and tobacco-associated leukoplakias are more common than idiopathic forms (Pindborg 1994). 60% - 90% of leukoplakias involved the commissures and buccal mucosa, followed by the lip (3.7%); alveolar ridge (3.0%), tongue (1.4%), FOM (1.3%), the vestibular mucosa (1.1%) and palate (0.9%), (reviewed by van der Waal et al., 1986).

The incidence of leukoplakia has been reported in several studies in India and the overall annual incidence of leukoplakia ranged from 1.1 to 26.2 per 10³ population. The annual incidence ranged

from 0.6 to 5.8 per 10³ population among those who did not use tobacco, and from 19 to 56 per 10³ population depending upon the tobacco-use pattern (smokeless tobacco alone or with betel quid or tobacco smoking), (reviewed by Kleinman et al., 1993).

In most surveys, leukoplakias of the tongue constituted only a small percentage of the total number of leukoplakias. In a large Hungarian survey of 7820 elderly subjects, the prevalence of tongue lesions was only 18.5% and of these, leukoplakia represented only 0.47% (Banoczy et al., 1993). However, in a study in Burma, lingual leukoplakia represented 12.6% of all leukoplakias and in Germany, lingual leukoplakia accounted for 20% (reviewed by van der Waal and Pindborg, 1986). No explanations for the large difference between the Burmese and German results compared to other studies were offered, but perhaps some investigators included lesions such as lingual lichen planus, chronic hyperplastic candidiasis or "galvanic" lesions that were not included by others (van der Waal and Pindborg, 1986). Most of the lingual leukoplakias are idiopathic, homogeneous patches located on the lateral borders, followed by the ventral surface and lastly, the dorsal surface which is a rare location (van der Waal and Pindborg, 1986). Leukoplakias on the ventral surface are more often associated with tobacco use, and leukoplakias on the dorsal surface may be associated with tertiary syphilis (Section VI.B.2.c.) or chronic hyperplastic candidiasis of which 13%-15% of cases are found on the tongue (van der Waal and Pindborg, 1986). Histologically, the majority of lingual leukoplakias demonstrate either hyperorthokeratosis or hyperparakeratosis but lingual leukoplakias appear to have a high frequency of epithelial dysplasia and transformation, compared to leukoplakias in other areas (van der Waal and Pindborg, 1986).

The simultaneous occurrence of oral leukoplakia and oral cancer ranged from 11-60% in a series of 10 studies (reviewed by Pindborg 1980). In reviewing the literature, Cawson (1975) cited transformation rates (see also Section II.D.1) of oral leukoplakia ranging from 30% - 100%, and rates of malignant change differ over variable time periods (Table 1.11). In general, the incidence of carcinoma increases linearly with age and the longer the duration of the lesion, the greater the

chance for transformation. Cawson (1975) concluded the incidence of transformation was about twice as great after 10 years as after 5 years and it was assumed that approximately 5% of all leukoplakias with an initially non-carcinomatous histology would transform into SCC in an average of 5 years (van der Waal et al., 1986). Overall, 90-95% of oral leukoplakias remain benign, yet patients with leukoplakias have a likelihood of developing oral cancer which is 50-100 times greater than the rest of the population (Cawson 1975). Several studies (eg. Banoczy 1977; Silverman et al., 1984; Lumerman et al., 1995) have indicated that transformation rates of leukoplakias located on the tongue exceed rates in other oral sites and therefore the tongue should be considered a risk area (see below) for leukoplakia (van der Waal and Pindborg, 1986).

Silverman et al. (1996) reported that 91% of patients with dysplastic leukoplakias also had a red component associated with their leukoplakia. Erythroplakias are typically described as bright, fiery red, well-defined areas with an irregular outline and with a velvety texture. Some lesions may have a nodular or granular texture and include white areas (van der Waal and Pindborg, 1986), and therefore would probably be classified as nonhomogeneous leukoplakias or erythroleukoplakias (Axell et al., 1996). Red lesions may be level or depressed in relation to the surrounding mucosa and many contain islands of entrapped normal mucosa, suggesting coalescence of several malignant sites (Mashberg 1980) or "field cancerization" (Slaughter et al., 1953). Histologically, erythroplakia is associated with marked epithelial atrophy and a variable degree of epithelial dysplasia (WHO 1978). The erythroplastic character of the lesions may be related to submucosal vascular proliferation and inflammatory infiltrate (Mashberg and Samit, 1995). Erythroplasias and mixed red/white lesions have an appreciably higher potential for malignant change than homogeneous white lesions; the results vary but Pindborg (1980) reported that 90% of erythroplakias showed carcinoma, carcinoma-in-situ or severe epithelial dysplasia; Mashberg (1980) reported that 80% of erythroplasias were carcinoma-in-situ and significantly, only 31% of red or predominantly red lesions were benign. Therefore, persistent red lesions with or without a white component should be regarded as carcinoma in situ until proven otherwise, especially in

patients at high risk (smokers and drinkers), (Mashberg 1980; Mashberg and Samit, 1995; Ephros and Samit, 1997).

Erythroleukoplakias have an unpredictable behaviour; Mincer et al. (1972) reported that over 8 years, 22% of erythroleukoplakias remained static, 7% regressed spontaneously, 11% increased in size and degree of atypia, and a further 11% transformed to malignancy. Gupta et al (1989) reported that over 8 years of observation, 16.2% of nodular leukoplakias transformed and represented 74% of all new SCCs in that time period. Erythroplakias were more commonly associated with asymptomatic carcinomas than leukoplakias (Mashberg 1980); in a study of asymptomatic early carcinoma, 98% were erythroplasias and only 5% were white with or without a red component (reviewed by Cawson 1975). In contrast, most asymptomatic leukoplakias were benign and only 2-8% were diagnosed as carcinoma or carcinoma-in-situ (Mashberg 1980).

Erythroplakia is rare in comparison to leukoplakia (Pindborg 1980; van der Waal and Pindborg, 1986). Erythroplasias occur equally in males and females and few (19%) occur in the 5th decade or younger. By contrast, 39% of leukoplakias occur in patients in the 5th decade or younger (reviewed by Shafer 1980). There also regional differences in oral location between erythroplakia and leukoplakia. The mandibular gingiva, alveolar ridge and mucobuccal fold constitute the most common site for erythroplakia in females; in males, erythroplakia is most common on the FOM with a rate of occurrence nearly twice that of any other single oral site and followed by the retromolar pad (Shafer 1980). By comparison, FOM and retromolar pad are uncommon sites for leukoplakia (reviewed by Shafer 1980; van der Waal et al., 1986) although in the cases reported by Lumerman et al. (1995), 51.7% of all oral leukoplakias with dysplasia were located in the FOM or on the tongue.

There may be "high" and "low" risk sites for premalignant lesions, similar to carcinoma which develops with higher frequency in some areas of the oral cavity as compared to others; 75% of oral

cancers develop in a region that comprises only 20% of the whole area of the oral mucosa (Moore and Catlin, 1967). The high risk sites for carcinoma include the FOM, ventrolateral tongue and soft palate complex which comprises the lingual aspect of the retromolar trigone, anterior tonsillar pillars and posterior buccal mucosa (Moore and Catlin, 1967). For premalignant lesions with either severe dysplasia or carcinoma-in-site, the “high” risk sites include the FOM with 28% of cases, tongue with 24% of cases and lips with 16% of cases (Shafer 1980).

In a review of 240 cases of oral SCC, 97.5% of the SCCs were located in high risk sites and 64.4% of the SCCs were red lesions, 73.3% were predominantly red with white components; 36.4% were predominantly white with red components; and 7.5% were white only (Mashberg 1980). In a study of 158 early, asymptomatic oral SCCs, 16% were located on the oral tongue and of these, 96% appeared clinically as erythroleukoplakias (Mashberg et al., 1973). In relating histologic grade to location and clinical appearance of the lesions, the most severe dysplasias and SCC were observed on the tongue and were “erosive leukoplakias” (Banoczy and Csiba, 1976). In a study of 670 oral leukoplakias, lingual leukoplakias accounted for only 8% of the total but, they demonstrated the highest transformation rate for all oral sites, accounting for 38% of all oral SCCs originating from leukoplakias (Banoczy 1977). In a sample of 45 patients with malignant transformation of leukoplakias, the tongue represented 29%, the highest number of malignant transformations within the oral cavity (Silverman et al., 1984). In a review of 308 cases, dysplasias most often involved the FOM (26.5%) and the tongue (25.2%), and 73.1% of oral dysplasias were leukoplakias (Lumerman et al., 1995). Gupta et al. (1989) calculated relative risks (RR) of malignant transformation based on clinical appearance: erythroleukoplakia had a RR of 3243; red areas/ulcers a RR of 303; ulcerated leukoplakia a RR of 44, and homogeneous leukoplakia of 26 (see Section V, Table 1.10).

d. Management of Premalignant Oral Lesions

Cawson (1975) advocated excision of dysplastic lesions because there was the chance that the

procedure could be curative and at least, the whole of the lesion was accessible for histological evaluation, thus ensuring that severe foci of dysplasia or carcinoma had not been missed in the initial biopsy. Unfortunately, the excision of some lesions, especially large lesions, carry risks and morbidity which much must be weighed against the possible benefits of the excisional procedure. Moreover, surgical excision is not necessarily curative and strict follow-up at intervals of not longer than three months are required (Cawson 1975). Banoczy (1977) reported that of 68 dysplastic lesions, 13.2% (9 cases) transformed to malignancy; 8 out of these 9 cases originated from the non-surgically-treated group but only 1 originated from the surgically-treated group. Mincer et al. (1972) reported that following the excision of 20 dysplastic lesions, 35% recurred within 2 years after excision and usually within 1 year. In addition, in 3 of the 20 patients treated with excision of dysplastic lesions, recurrence was as carcinoma and these patients were included in the overall transformation rate of 11% over 8 years. Of the dysplastic lesions left untreated, 11% were totally or partially reversed without surgery and another 11% increased in size or severity of dysplasia (Mincer et al., 1972). Einhorn and Wersall (1967) noted that twice as many white lesions transformed after surgical excision (4.6%) as compared to those white lesions that were left untreated (2.5%) although, the outcome may reflect the selection of the worst cases for excision. Nevertheless, Lumerman et al. (1995) and Silverman et al. (1996) recommended the surgical removal of premalignant lesions with epithelial dysplasia, regardless of degree. In the series of 240 patients with oral epithelial dysplasia followed by Lumerman et al. (1995) over 20 years, only 6.2% of patients who had excision of the dysplasia subsequently developed invasive SCC; 81.6% were cured by the excision of the dysplasia; 12.3% had a recurrence of dysplasia. In contrast, only 17.6% of patients with dysplasia and who received no treatment, improved without treatment, but 15.4% of patients who received no treatment for their dysplasia developed invasive SCC (Lumerman et al., 1995).

The systemic use of vitamin A analogues in chemoprevention of malignancy is still under investigation, controversial and not without side-effects (reviewed by Eversole 1993; Greenwald et

al., 1995; see also Chapter 5), and the use of retinoids applied topically to premalignant lesions still requires the appropriate clinical trials. Unfortunately, clinical practice has not advanced significantly from 1975 when Cawson observed that "even when a lesion is regarded as premalignant, there is no means of management that has been shown with any degree of certainty to reduce the risk of malignant change" (Cawson 1975; see also Chapter 5).

However, management of dysplastic lesions must include the removal of all irritants or potential etiologies in order to determine the possibility of reversibility (Banoczy 1977; van der Waal et al., 1986). Interventions such as patient education about tobacco habits have also demonstrated success. For example, tobacco chewers and smokers were selected from three rural Indian areas (Gupta et al., 1986; reviewed by Kleinman et al., 1993). Participants were interviewed for their tobacco habits and examined for oral changes, in particular leukoplakia, at baseline and annually over 10 years with a follow-up rate of 97%. At baseline, tobacco habits, prevalence of leukoplakia, and incidence rates of oral cancer (16-21 per 10⁵ population) were similar in the three communities. In some districts, individuals (the intervention cohort) were counselled personally and via mass media about their tobacco habits; in contrast, individuals in the control cohort did not receive any educational intervention. The intervention cohort demonstrated a substantial decrease in tobacco habits as compared to the control cohort and significantly, the incidence rate of leukoplakia in the intervention cohort decreased. For example, in one district the five-year age-adjusted incidence rates of leukoplakia among men in the intervention cohort was 11.4 per 10³ population as compared to 47.8 per 10³ population prior to the intervention, representing a 24% decrease in incidence rate. Among women, the five-year age-adjusted incidence rates of leukoplakia in the intervention cohort decreased by 18%, from 33.0 to 5.8 per 10³ population. Over 10 years, new cases of oral cancer and precancer developed exclusively among tobacco chewers and smokers, and all new oral cancers developed among individuals in whom oral precancerous lesions had been diagnosed previously (Gupta et al., 1986). As a high percentage (79%, Gupta et al., 1989) of SCCs in India are preceded by premalignant lesions, the study

provided evidence for the effectiveness of intervention of tobacco habits for primary prevention of oral cancer (van der Waal et al., 1986; Kleinman et al., 1993).

2. Premalignant Conditions

A premalignant or precancerous condition is "a generalized state associated with a significantly increased risk of cancer" (WHO 1978; Axell et al., 1996). Premalignant conditions include lichen planus, submucous fibrosis, syphilis, Plummer-Vinson syndrome (van der Waal and Pindborg, 1986; Regezi and Sciubba 1989) and dyskeratosis congenita (Laskaris 1988; Eversole 1993).

a. Lichen Planus

Lichen planus is a mucocutaneous disease affecting 0.02% to 1.9% of the population (van der Waal and Pindborg, 1986; Eversole 1993), generally in middle age with an equal sex distribution (van der Waal and Pindborg, 1986), a 60% prevalence in females (Eversole 1993) or a female predominance of 2:1 (Silverman 1991). Cutaneous lesions usually resolve within 2 years and have no reported malignant potential (Williams 1996). Of patients with cutaneous lesions, 9% to 60% also have oral lesions (van der Waal and Pindborg 1986). Of patients with oral lesions, 25%-30% have cutaneous lesions (van der Waal and Pindborg, 1986). About 25% of patients have only oral lesions which may reflect the more prolonged clinical course of oral lichen planus (Williams 1996) and only 2% (Silverman 1991) to 17% (Eversole 1993) show spontaneous remission.

There are 6 clinically recognizable forms of oral lichen planus: reticular, papular, plaque-like, atrophic, erosive or ulcerative, and bullous. The reticular pattern is the most common type, affecting over 90% of patients. However, several forms may exist concurrently with involvement of more than one site. Intra-orally, the buccal mucosa is the most common site, bilateral and multiple oral lesions occur frequently, and pain is the usual complaint for which patients seek consultation (Silverman 1991). Erosive lesions are the most persistent form and erosive lichen planus of the gingiva results in desquamative gingivitis which has a clinical appearance similar to

mucous membrane pemphigoid (reviewed by Williams 1996). Atrophic and erosive forms are more common in older individuals and the plaque form is more commonly observed in patients who use tobacco (Eversole 1993).

The relative frequency of tongue lesions of lichen planus varies from 17% to 77% (reviewed by van der Waal and Pindborg, 1986). On the dorsal tongue, the presence of papillae may alter the appearance of the various types of lichen planus as compared to other areas of the oral mucosa. For example, the typical pattern of Wickham's striae is rare on the dorsum but is common on the lateral borders and may surround the fungiform papillae. Early lingual lesions of lichen planus may develop at the middle of the dorsum but usually they are located at the junction of the oral and pharyngeal tongue where they present as an atrophic depapillated area that may be ulcerated and surrounded by a white zone containing Wickham's striae (van der Waal and Pindborg, 1986). The plaque type of lichen planus is the most common type on the dorsal tongue; the erosive form is most common on the dorsum and lateral borders (van der Waal and Pindborg, 1986).

Similar to leukoplakia, lichen planus may not be a singular disease. Instead, lichen planus may represent a variety of conditions that can be grouped together under a set of clinical and histological parameters. For example, some oral mucosal lesions do not precisely conform to the clinical descriptions of lichen planus and are referred to as "lichenoid reactions". However, histologically they may demonstrate a T-cell mediated immunologic response which is common feature in all such lesions (Eversole 1993; Porter et al., 1997). Lichen planus is an immunologically-mediated disease but it is not a classic auto-immune disease characterized by circulating auto-antibodies. A lichen planus specific-antigen has not been consistently identified, but the clinical features of lichen planus are very similar to graft-versus-host disease, thereby supporting the view that lichen planus results from a cell-mediated response against an intra-epithelial antigen (Eversole 1993; Williams 1996). It is possible that lichen planus/lichenoid reactions are really a delayed hypersensitivity reaction to a variety of antigens including microbes, medications, foodstuffs, metals such amalgam

and mercury, tumour antigens and autoantigens. As more antigens are identified in lichenoid reactions, the prevalence of idiopathic lichen planus may decrease (Eversole 1993).

Histological features of lichen planus typically include epithelial atrophy with hyperkeratosis, loss of the basal cell layer, civatte bodies (degenerated keratinocytes) within the epithelium, and a dense subepithelial lymphocytic infiltrate. The basement membrane interface is indistinct and rete ridges have a "saw-tooth" configuration (reviewed by Eisenberg and Krutchkoff, 1992). An increased number of activated Langerhans cells are clustered within the epithelium and are associated with the accumulation of cytotoxic/suppressor T lymphocytes in the lower epithelial layers and with helper/inducer T lymphocytes in the lamina propria. Both types of lymphocytes are memory T cells which indicates that their response is to a previously-encountered or persistent antigen. In lichen planus, suprabasilar keratinocytes express HLA-DR, possibly in response to signals from the infiltrating T cells, and suggests that they now share features with antigen-presenting cells so that antigens permeating the oral mucosa may be processed within the cytoplasm of these keratinocytes, followed by presentation of cell surface antigens directly to CD4 (helper) T cells. In lichen planus, basilar and suprabasilar keratinocytes also express an intercellular adhesion molecule (ICAM-1) which permits keratinocytes to bind to and immobilize activated lymphocytes within the epithelium. Moreover, antigenically-activated keratinocytes secrete a variety of chemoattractants which generate a diffusion gradient for leukocyte migration, and the altered keratinocytes appear to have a high affinity for activated cytotoxic CD8 lymphocytes which may mediate damage of the keratinocytes (reviewed by Eversole 1993; Williams 1996; Porter et al., 1997).

The potential for malignant transformation of lichen planus is controversial and it remains unclear as to whether the lesions themselves are precancerous or whether the disease renders the mucosa more susceptible to carcinogens (Williams 1996). The confusion about transformation exists because documentation has generally been inadequate to prove that lichen planus was present at the outset; that is, diagnosis was limited to clinical impression and excluded histological assessment.

Moreover, in rare instances when biopsy was included, epithelial dysplasia was not recognized in lesions that were otherwise lichenoid from a histologic standpoint (reviewed by Eisenberg and Krutchkoff, 1992). Some authors (eg. Eisenberg 1992; Eisenberg and Krutchkoff, 1992) maintain that lichen planus is a benign condition but that "lichenoid dysplasia" is a true premalignant lesion. The term "lichenoid dysplasia" is used to describe a red and white lesion that clinically, resembles both erosive lichen planus and leukoplakia. Histologically, it displays lichenoid features such as a subepithelial infiltrate but significantly, it also displays two or more features of epithelial dysplasia (Lovas et al., 1989; Eisenberg and Krutchkoff, 1992). Therefore, clinical lesions that only resemble lichen planus may actually represent a form of dysplasia that could be permitted to evolve into SCC and hence, support the malignant transformation of lichen planus (Eisenberg 1992; Eisenberg and Krutchkoff, 1992).

In contrast, Eversole (1992) suggested that dysplastic features observed in conjunction with lichenoid features both histologically and clinically, would be expected if lichen planus was indeed a premalignant lesion with transformation potential. Among studies with documented cases of classical lichen planus progressing to SCC, the transformation rate varied: 0.12% in a mean time of 3.4 years after the onset of lichen planus (Silverman et al., 1985); 0.4% over a mean observation period of 5.1 years (Murti et al., 1986); 0.4% to 12.3% observed over 1-26 years (Holmstrup et al., 1988); and 0% over 8 years (Brown et al., 1993) for an overall transformation rate estimated at 0.5% to 2% over a 5-7 year period (Eversole 1993).

The relative risk of a lichen planus developing into SCC was estimated at 16 (Gupta et al., 1989); the relative risk of lichen planus in males transforming to SCC was calculated at 5.9 (Sigurgeirsson and Lindelof, 1992), and the relative risk of lichen planus as compared to a tobacco user developing SCC was estimated at 3.3 (Murti et al., 1986), (see also Section V, Table 1.10).

In a review of 101 cases of malignant transformation of lichen planus, the most common location

was the buccal mucosa, followed by the tongue (Mashberg et al., 1973). In a review of 46 transformed cases, 39% were located on the tongue, and in yet a third review of 40 transformed cases, 28% involved the lateral border of the tongue, 20% the dorsum and 13% the ventral surface (reviewed by van der Waal and Pindborg, 1986).

b. Submucous Fibrosis

Submucous fibrosis is an insidious, chronic irreversible disease affecting any part of the oral cavity and sometimes the pharynx and oesophagus as well. It may be associated or preceded by vesicle formation and is characterized by stiffness of the oral mucosa which causes trismus and difficulty in eating and talking. Burning of the oral mucosa, especially with eating spicy food, ulcerations and recurrent stomatitis are common symptoms, and the loss of pigmentation in a marbled pattern and associated leukoplakia of the affected mucosa are frequent early signs. Later, palpation reveals symmetrical fibrous inelastic bands of the lips and buccal mucosa, palate and faucial pillars. The uvula may be destroyed and tongue movement may become impaired due to fibrosis. The dorsal tongue papillae may atrophy with development of leukoplakic or erythroplakic patches (van der Waal and Pindborg, 1986; Laskaris 1988; Regezi and Sciubba 1989; Eversole 1993).

Submucous fibrosis is reported primarily among East Indians from southeast Asia or India but cases are also reported in Africa, the United Kingdom, Fiji Islands and Thailand (Reichert 1995). The prevalence ranges between 0.3% - 1.2% in India and 0.5 - 3.4% among Indians in South Africa (van der Waal and Pindborg, 1986; Eversole 1993). Those affected are typically between the ages of 20 and 40 years old but the condition is also seen in younger and older individuals (Regezi and Sciubba, 1989). Submucous fibrosis is associated with the use of chili and tobacco as well as various vitamin deficiencies, but the strongest association is with the habitual use of the areca nut. The nut may be chewed by itself or be used in various preparations such as mawa, pan masala or betel quid which combines the betel leaf, areca nut, slaked lime and may or may not contain tobacco (Reichert 1995; reviewed by Murti et al., 1995). The relative risks for submucous

fibrosis associated with all forms of chewing the areca nut range from 94-780; areca nut chewing alone showed the highest RR of 154; RR for chewing pan with tobacco was 64, and RR for chewing pan without tobacco was 32. The attributable risk (AR) for chewing areca nut products, as compared to no chewing was 98% (reviewed by Murti et al., 1995).

The prevalence and pattern of submucous fibrosis varies, possibly in relation to the arecoline alkaloid content of the areca nut and the pattern of areca nut use. For example, in districts where individuals chew the nut without tobacco, submucous fibrosis affects primarily the posterior buccal mucosa, soft palate, uvula and retromolar area. These individuals swallow the juice which exposes the posterior sites to higher concentrations of areca nut carcinogens than the anterior aspects of the oral mucosa. In contrast, individuals who chew areca nut as an ingredient of betel quid with tobacco demonstrate more generalized submucous fibrosis involving the tongue, FOM and hard palate. These individuals hold the quid and its juice for a longer time, spitting out only when they become bland; this habit results in a more generalized contact of the quid with the oral mucosa and hence there is a more generalized condition (Murti et al., 1995).

The etiology of submucous fibrosis may include hypersensitivity to dietary constituents such as chili. Histologically, there is always a subepithelial inflammatory reaction that is eventually followed by the lamina propria changing into a fibro-elastic, acellular tissue. The epithelium atrophies which may increase its permeability and hence susceptibility to carcinogens because variable degrees of dysplastic change are observed. The areca nut contains alkaloids that yield powerful carcinogenic nitrosamines, and components of the areca nut cause fibroblasts to alter their phenotype and increase collagen synthesis by 170% as compared to controls. Some areca nut components form cross-links between the collagen peptides, inhibiting collagen degradation by collagenase. The inflammatory infiltrate further stimulates fibroblast proliferation and collagen synthesis; the type I collagen fibers become densely packed, type III collagen is increased around blood vessel walls and collagenase activity is significantly decreased in the submucosa as

compared to normal mucosa of control specimens (reviewed by Eversole 1993; Murti et al., 1995).

Submucous fibrosis is an oral precancerous condition as epithelial dysplasia is observed in 14% of all cases (van der Waal and Pindborg, 1986; Laskaris 1988; Eversole 1993). Transformation rates vary from 2% per year to 4.5% over 8 years and 7.6% over 10 years (Gupta et al., 1989); the relative risk for the malignant transformation of submucous fibrosis was calculated as 397 (Gupta et al., 1989). Invasive SCC is found in 5 - 6% of submucous fibrosis cases without clinical signs of carcinoma (Laskaris 1988), and in India, 40 - 50% of oral cancer coexists with submucous fibrosis (van der Waal and Pindborg, 1986; Laskaris 1988). In a study of 40 oral cancer patients with submucous fibrosis, 40 had malignant lesions of the buccal mucosa and 32 had lesions of the tongue which also displayed marked atrophy of the papillae (van der Waal and Pindborg, 1986).

c. Tertiary Syphilis

Historically, *Treponema pallidum* was implicated with lingual carcinomas. *T. Pallidum* causes an interstitial glossitis with endarteritis which leads to atrophy of the overlying epithelium and papillae. The only relationship between syphilis and carcinoma may be due to the atrophic glossitis which renders the tongue surface more susceptible to the action of carcinogens and development of leukoplakia and carcinoma (van der Waal and Pindborg, 1986; Laskaris 1988). Ironically, the arsenical compounds previously used to treat syphilis are carcinogenic and have also been implicated in tongue carcinomas (Regezi and Sciubba, 1989).

Due to early diagnosis and successful treatment, tertiary syphilis has become so rare that the true relationship between *T. pallidum* and carcinoma is difficult to study. Thoma's 1941 textbook of oral diseases lists the tongue as the most common oral site of leukoplakia and states that 27% of patients with oral leukoplakia also had a positive Wassermann reaction (cited in Shafer 1980). In 1942, a study by Nielsen (1942) of 108 cases of tongue cancer in Denmark, reported a frequency of syphilis of 19%. In an American study of 180 patients with SCC of the tongue, only 20

(18.5%) had positive findings for syphilis and 19 of these 20 had carcinoma of the oral tongue, suggesting that syphilis was not a significant etiology in malignant lesions of the posterior third and base of tongue (Triegeer et al. 1958). In 1977, a follow-up study (Banoczy 1977) of 680 patients with leukoplakia showed that 40 patients developed carcinoma and among those, 10% had syphilis; among those that did not develop carcinoma during the time of investigation, the frequency of syphilis was 2.5% (reviewed by van der Waal and Pindborg, 1986).

d. Plummer-Vinson Syndrome

Plummer-Vinson syndrome, also known as sideropenic (anemic) dysphagia or Patterson-Kelly syndrome, is the only convincing nutritional deficiency that has been associated with malignancy (Regezi and Sciubba, 1989). This syndrome involves predominantly females between the 4th and 6th decades and is characterized by severe iron-deficiency anemia (hypochromic, microcytic) and dysphagia due to the formation of post-cricoid webs in the oesophagus (van der Waal and Pindborg 1986; Laskaris 1988). Webbing is due to atrophic degeneration and keratinization of the oesophageal epithelium with inflammatory cell infiltration (Eversole 1993). It is unclear whether the webbing is caused by the iron deficiency or some other factor(s) as not all patients with webs have anemia, and not all patients with anemia have sideropenic dysphagia (Eversole 1993).

Oral lesions may include angular cheilitis, pale and atrophic oral mucosa, and a smooth atrophic, depapillated, red tongue is seen in about 60% of cases (Eversole 1993). Xerostomia is also common, and leukoplakia and multiple oral carcinomas may develop (van der Waal and Pindborg 1986; Laskaris 1988; Regezi and Sciubba, 1989; Eversole 1993).

e. Dyskeratosis Congenita

Dyskeratosis congenita is a rare form of ectodermal dysplasia probably inherited as a recessive autosomal and X-linked trait, and it is the only genokeratosis that shows a predisposition for malignant transformation (Cannell 1971; Ogden et al., 1988). Dyskeratosis congenita is

characterized by mental handicap, nail dystrophy, hyperhidrosis, telangiectasia, skin hyperpigmentation and skin atrophy, usually on the face, neck and chest, with dermal bullae and blepharitis. There is a pancytopenia, aplastic anemia and the T cell CD4:CD8 ratio is severely depressed (Laskaris 1988; Eversole 1993).

Oral manifestations usually precede dermal signs (Cannell 1971) and include recurrent bullae, mainly on the lateral tongue and buccal mucosa that leave raw ulcerated surfaces. Repeated episodes may result in atrophy of the oral mucosa. Most patients between 10 and 27 years develop a severe erosive form of leukoplakia with a transformation rate to malignancy of 34%, often with multiple primaries and frequent recurrence (Cannell 1971; Ogden et al., 1988). However, most deaths are not due to oral malignancy but rather to secondary infection as result of pancytopenia (Ogden et al., 1988).

Transformation Rate (%)	Time Period of Observation	Authors
2.4	10 years	}Einhorn and Wersell, 1967
4	20 years	
6	1-11 years	Silverman and Rozen, 1968
13.2	mean 6.3 years	Banoczy and Csiba, 1976
6	30 years	Banoczy 1977
0.13	2 years	}Pindborg 1980
0.8-9.9	mean 21.3 years	
17.5	mean 8.1 years	Silverman et al., 1984
79	8 years	Gupta et al., 1989
16	mean 34 months	Lummerman et al., 1995

Table 1.11. Examples of Transformation Rates of Oral Leukoplakia to Malignancy.

The comparison of rates between different studies is complicated by the inconsistent designation of measures for the numerator and denominator. That is, it is often unclear whether the numbers represent the number of events or the number of individuals in whom the events occurred. Some investigators utilized the number of lesions (eg. Gupta et al., 1989) as their measure; some investigators designated the number patients (eg. Einhorn and Wersall, 1967; Banoczy 1977) as their measure even though more than one lesion occurred in individual patients (eg. Silverman et al., 1984), and some investigators utilized both lesions and patients without clear distinction (eg. Lummerman et al., 1995). Moreover, the relevant time intervals were not included directly in the rate, and range from 1 year to over 40 years without conversion to an annual rate (see also Chapter 1, Section II.D).

VII. Oral Squamous Cell Carcinoma

The prevalence and incidence rate of oral and pharyngeal cancers vary widely around the world. In Europe and North America, cancers of the head and neck constitute approximately 3-4% of all cancers (Garfinkel 1995a; Shaha and Strong, 1995), and cancers of oral origin represent about 1% of all cancers (Paterson et al., 1996). The majority of oral cancers occur in males with a sharp rise in incidence after 40 to 50 years of age. There is a relatively loose association with oral premalignant lesions and the development of SCC which is commonly found in a horseshoe-shaped trough formed by the ventrolateral borders of the tongue, FOM and soft palate complex (soft palate proper, lingual aspect of the retromolar trigone, and anterior tonsillar pillars), (Mashberg and Samit, 1995). Most oral cancers are SCCs that are well-differentiated and produce keratin. The oral tumours are typically endophytic and deeply penetrating but localised lesions have a more favourable prognosis than extensive (metastatic) ones (Paterson et al., 1996). Survival rates are generally better in women than men but overall, there has been little change in North American 5-year survival rates for oral cancer since 1950 (reviewed by Eversole 1993, and Paterson et al., 1996).

In India and Southeast Asia where the use of chews containing tobacco and areca nut are common, 40% of all cancers are oral in origin and the peak age of oral cancer is at least one decade earlier than for oral cancers in the West (Paterson et al., 1996). The highest rates of oral cancer among women occur in India so that the sexes are affected equally. Most oral cancers in India and Southeast Asia are preceded by a premalignant lesion such as leukoplakia or erythroplakia, and tumours are usually exophytic and very large at presentation (reviewed by Eversole 1993, Blot et al., 1994; Paterson et al., 1996).

The etiology of oral cancer predominantly involves the use of tobacco and alcohol, frequently in combination. The differences in prevalence and incidence rates, and clinical presentation of oral cancer in different parts of the world may reflect different etiologies or different forms of exposure

to the etiological agents as well as inherent differences in the study populations. In addition, there are differences in the collection of data about oral cancer and therefore, general conclusions across studies and between countries over time for trend data cannot easily be drawn (see Section II). This Section includes a review of oral SCC in general but concentrates on European and North American reports and SCC of the tongue.

A. Histopathology

The histologic features of invasive squamous cell carcinoma include all of the features of epithelial dysplasia (Section VI.A) and the disruption of the basement membrane with extension of the dysplastic or malignant cells into the underlying connective tissue. The WHO describes oral SCC as a tumour consisting of strands, nests, or columns of malignant epithelial cells that infiltrate subepithelially, and the tumour cells may resemble any or all of the layers of stratified squamous epithelium (reviewed by van der Waal and Pindborg, 1986). Many attempts have been made to quantitatively grade histologic malignancy so that cytomorphology may be correlated to clinical staging and prognosis, similar to the TNM staging system (see Section VII.C. and Table 1.12).

For any histologic grading system to be effective, the optical magnification and number of fields examined should be standardized. In addition, a three-dimensional picture of the tumour is required and this can only be provided by serial sectioning, yet the direction of sectioning will influence the interpretation and pattern of invasion (Anneroth et al., 1987). Some features of malignancy such as the degree of differentiation and pleomorphism, the number of mitotic figures and the pattern of local invasion have been inconsistently related to the incidence of metastasis and to survival rates. Moreover, histologic characteristics often vary within an individual SCC and typically, the pathologist is unable to adequately assess all parameters that may be included in a malignancy grading system. As a result, the malignancy grade of biopsy tissue tends to be lower than the grade of the definitive surgical specimen from the same patient (reviewed by Anneroth et al., 1987).

The grading system developed by Jakobsson et al. (1973) included an analysis of the carcinoma cell population for keratinization patterns, nuclear aberrations and number of mitoses, as well as an evaluation of the tumour-host relationship which included the mode and stage of invasion, vascular invasion and the degree of lymphoplasmocytic infiltration (reviewed by Anneroth et al., 1987). For example, grade I had a well-defined borderline, grade 2 had a less-marked borderline with invasion of a few cords of cells, grade 3 had groups of cells invading in a nodular pattern into the connective tissue, but without a distinct borderline, and grade 4 had diffuse, massive invasion. Application of the Jakobsson grading system to glottic carcinoma demonstrated significant correlation between malignancy grading and recurrence as well as survival rates, and in predicting 5-year results with or without recurrence, the histologic grading of malignancy was found to be a better predictor than the TNM classification (reviewed by Anneroth et al., 1987). Yamamoto et al. (1972) applied the Jakobsson system retrospectively to 102 treated cases of oral SCC and concluded that the more invasive the tumour cells were to the host, the more frequent metastasis formation was. Cases with grades 1-3 modes of invasion showed a low frequency of metastasis (13.9%) while grade 4 modes of invasion showed a high frequency of metastasis (66.7%). Moreover, there was clear histologic similarity of the mode of invasion between primary and metastatic lesions in 60% of cases (Yamamoto et al., 1973).

Anneroth et al. (1987) based their grading system on the hypothesis that invasive cells may have characteristics that differ from those of cells in other areas of the tumour, and they advocated that grading be restricted to the least differentiated tumour at the deepest invasive front of oral carcinomas. Their system also recognized the influence of such factors as the direction of the sectioning and the degree of inflammatory response in adjacent areas, and considered the depth of invasion, distinguishing between carcinoma in situ, direct invasion of the lamina propria only, or invasion below lamina propria adjacent to or into muscles, salivary glands or periosteum. When only invasive margins rather than the entire epithelial layers were graded, higher interobserver kappa scores (Bryne et al., 1991a) were reported as well as increased correlations between invasive front

grading and tumour aggressiveness (Bryne et al., 1992), and metastasis and tumour recurrence (Odell et al., 1994).

De Araujo et al. (1997) used the Anneroth system (Anneroth et al., 1987) in combination with IHC for p53 protein in the investigation of 40 cases of oral SCC. The expression of p53 protein was detected in only 62.5% of the carcinomas studied and the protein was not related to either the tumour architecture (pattern of invasion, $r=0.149$) or the host response (inflammatory response, $r=0.026$). However, accumulation of p53 protein was related to the histological grade of malignancy ($r=0.456$), degree of keratinization ($r=0.317$), nuclear polymorphism ($r=0.339$), and the number of mitosis ($r=0.626$), (De Araujo et al., 1997).

The thickness of primary oral tumours has also been correlated to the incidence of nodal metastases with histologically thin tumours demonstrating improved prognosis (reviewed by Mashberg and Samit, 1995; Shah and Lydiatt, 1995). Fakih et al. (1989) related tumour depth of oral tongue SCCs to the development of metastatic neck nodes. Seventy-eight percent of patients with tumour depths of less than 4 mm did not develop neck nodes as compared to only 24% of patients with a tumour depth greater than 4 mm ($p<0.01$). Moreover, when overall survival was compared, 81% of patients with shallow tumours (<4 mm) survived as compared to only 43% of patients with a tumour depth greater than 4 mm ($p<0.01$).

Fukano et al. (1997) reviewed 34 primary tongue carcinomas. In univariate analysis, statistically significant predictors of regional metastasis were invasion mode ($p=0.0019$) and depth of invasion ($p=0.0003$). Cervical lymph node metastasis was found starting at a depth exceeding 3 mm but increased markedly when invasion depth exceeded 5 mm. The overall metastatic rate was 35.3% (12/34), but in the group in which tumour depth exceeded 5 mm, the metastatic rate was 64.7%. In contrast, the metastatic rate was only 5.9% when the tumour depth was less than 5 mm (Fukano et al., 1997).

Spiro et al., (1986) reviewed 105 cases of SCCs confined to the tongue and FOM, and with clinically-negative (N0) necks. For lesions ≤ 2 mm deep, 13% of patients had lymph node metastasis and 3% died from the cancer. For tumours with a depth of 3 - 8 mm, 46% of patients had lymph node metastasis and 17% died from the cancer; for tumours ≥ 9 mm, 65% of patients had lymph node metastasis and 35% died from the cancer. Although the difference in survival for tumours 3-8 mm thick and those that exceeded 9 mm thick was not significant ($p=0.10$), multivariate analysis revealed that tumour thickness ($p=0.03$) had a greater impact on survival than TNM stage of disease ($p=0.05$); and analysis of the factors influencing local recurrence, metastasis or both, indicated that tumour thickness was the only significant ($p=0$) variable. However, the authors concluded that if tumour thickness was to be used as a guide to treatment, accurate depth measurements would probably require excision of the primary tumour, rather than an incisional biopsy (Spiro et al., 1986).

In contrast, Morton et al. (1994) found no association between tumour thickness in 26 cases of early tongue SCC (TNM stage I and II) and nodal metastases, disease recurrence or survival, and reported that neural infiltration was the only factor to approach statistical significance ($p=0.05$) in association with nodal metastases. However, perineural spread is not a common feature of lingual SCC, in contrast to lymphatic and vascular spread (van der Waal and Pindborg, 1986). Most SCCs of the tongue are moderately- or well-differentiated and many cases demonstrate dense infiltrates of plasma cells and lymphocytes which correlated to improved prognosis in some reports (reviewed by van der Waal and Pindborg, 1986). Lesions with the least inflammatory response demonstrated the highest incidence of subsequent metastases, suggesting a relationship between cancer progression and immune competence (Mashberg and Samit, 1995).

The subjective interpretation of histological features (reviewed in Sections II.G. and VI.A) may limit the clinical value of histologic grading systems or classifications and to date, clinical TNM (Table 1.12) classification is the only system in general use for predicting the clinical outcome of

oral SCC (Bryne et al., 1991a).

B. Clinical Features

Oral squamous cell carcinomas are well-suited to early diagnosis because typical sites of involvement are easily accessible to direct examination. However, the early diagnosis of asymptomatic oral SCCs requires a high index of clinical suspicion because the clinical appearances of the lesions are highly variable and changes in appearance of the oral tissues associated with malignancy can be very subtle and deceptively innocuous (Mashberg and Samit, 1995; Leong et al., 1995). Early lesions are often missed because of a failure by clinicians to concentrate efforts on individuals at highest risk (tobacco smokers, alcohol drinkers), and failure to focus attention on intraoral sites of highest risk. In addition, there may be an excessive emphasis on leukoplakia as a precursor lesion and a concomitant lack of appreciation of erythroplasia as an early clinical manifestation of SCC (Mashberg and Samit, 1995). Symptomatic lesions are more readily diagnosed because a patient's complaints of pain, bleeding, ulceration, a mass, otalgia and/or dysphagia and odynophagia will usually direct the clinician to the primary lesion (Mashberg and Samit, 1995).

The use of diagnostic aids such as toluidine blue may raise the clinician's suspicion of malignancy and aid in selection of sites for biopsy (Mashberg and Samit, 1995; Epstein et al., 1997).

Toluidine blue is a basic thiazine metachromic dye with affinity for nucleic acids, and excepting the dorsum of the tongue, the gingival crevices and other sites with sloughed keratin, retention of the dark blue stain by oral tissues signifies a positive result. Penetration of the dye through several epithelial cell layers may be facilitated by the increased intercellular spaces between transformed cells (Mashberg and Samit, 1995); also, the dye may be taken up by cells manifesting increased DNA synthesis, such as dysplastic cells, malignant cells or cells involved in wound healing (Section IV.D.2.b). Hence, sites of inflammation or ulceration may yield false-positive results, but false-negative results are also possible. Therefore, toluidine blue application is not a substitute

for a thorough patient history and a thorough clinical examination comprising visual and digital components. Ultimately, clinical suspicion must always mandate biopsy regardless of staining outcomes (Mashberg and Samit, 1995; Epstein et al., 1997).

There are no specific aspects of SCC of the tongue that distinguish it from other oral carcinomas. In the early stages, lingual SCCs are typically asymptomatic because the intrinsic muscles provide little restriction to tumour growth and invasion and therefore, lesions may reach considerable size before producing symptoms (Krupala and Gianoli, 1993). Lingual SCCs may present as erythroplakias, leukoplakias, erythroleukoplakias, papillomatous or verrucous growths, as a lump or fissuring, ulceration, or as an exophytic lesion. Typically, the patient is aware of a painless indurated or non-healing ulcerative mass. As the tumour enlarges, infiltration and necrosis of adjacent structures occurs, sometimes accompanied by infection, so that local pain and tenderness results. As well, branches of the lingual and glossopharyngeal nerves may become infiltrated with tumour and so that the patient experiences ipsilateral referred otalgia or jaw pain, and pain or difficulty with swallowing and speech. Tumour involvement of the base of tongue (BOT) often remains asymptomatic until the tumour reaches considerable size. Unless specifically searched for, BOT lesions often escape detection even by experienced clinicians who seek explanations for a persistent sore throat, sometimes in the form ofodynophagia or glossopharyngeal neuralgia, or referred otalgia (Spiro and Strong, 1974; reviewed by van der Waal and Pindborg, 1986; Regezi and Sciubba, 1989; Krupala and Gianoli, 1993; Leong et al., 1995). Tumour extension from the oral tongue to the FOM or alveolar ridge, or from the BOT to the hyoid bone may cause fixation and tethering of the tongue with loss of tongue mobility and deviation of the tongue to the affected side on protrusion. Tumour extension from the BOT to the pterygoid muscles may also produce trismus (Krupala and Gianoli, 1993).

In a review of 1554 American patients with tongue cancer treated between 1939 and 1953, the majority of tumours (80%) were 2 cm or larger in diameter and 20% were in excess of 5 cm

(Frazell and Lucas, 1962). The predominant symptom reported by these patients was a visible, palpable lingual mass (62%) followed by localized pain (33%), lump in the neck (13%), and dysphagia (9.5%). The predominant symptoms varied according to the portion of the tongue that was involved. For lesions involving the oral tongue, the majority of patients were aware of a visible palpable mass (77%), followed by localized pain (29%), neck lump (6.4%) and dysphagia (3.5%). For lesions involving the BOT, most patients experienced localized pain (46%), followed by neck lump (31%), dysphagia (27%) and palpable lingual mass (15%), (Frazell and Lucas, 1962). This pattern of symptoms probably reflected the advanced size of the tumours as a review of 297 patients (Franceschi et al., 1995) with less advanced tongue tumours (71% stage I or II; 21% stage III, 8% stage IV; 60% located in the middle third of the tongue) reported the most common presenting symptom and sign as localized pain (45%) and the presence of an ulcer (50%). In a recent review of the literature, Krupala and Gianoli (1993) reported that overall, 30% of patients with lingual SCC have pain as the initial symptom, 6% report a neck mass and 5% report dysphagia.

About one-third of lingual SCCs occur in the posterior third or BOT which is a difficult site to visualize; consequently, lesions are more advanced and often metastatic at the time of their discovery which is reflected by the poorer prognosis for BOT than for oral tongue lesions (Spiro and Strong, 1974; Regezi and Sciubba, 1989). In addition, extension of tumour into contiguous tissues occur with much greater frequency in BOT lesions (72%), as compared to lesions of the oral tongue (25%), (Spiro and Strong, 1974). Tumours of the oral tongue rarely occur at the tip or on the dorsum of the tongue, but approximately 55% (Krupala and Gianolo, 1993) to 70% (Bomford et al., 1993) of oral tongue SCCs occur on the lateral border at the junction between the middle and posterior thirds of the oral tongue. Tumours of the posterior lateral tongue borders may also extend laterally onto the FOM or extend posteriorly through the deep musculature to the tongue base and anterior tonsillar pillar (Krupala and Gianolo, 1993).

Metastatic spread of cancer of the tongue is facilitated by the rich lymphatic network of the tongue (Section III.B.6) and the tendency for lymphatic metastasis increases with the size of the primary tumour. For tongue cancers of all sizes, approximately 50% of cases present with lymph node involvement (van der Waal and Pindborg, 1986), and approximately 30% of oral tongue cancers present with ipsilateral cervical metastasis (Bomford et al., 1993; Krupala and Gianolo, 1993), typically involving the submandibular or jugulodigastric groups at the angle of the mandible (Leong et al., 1995). For lingual cancers less than 2 cm in size, 15% of cases present with nodal metastasis (van der Waal and Pindborg, 1986), and in these early carcinomas of the oral tongue, 98.5% of all metastasis occur in the supraomohyoid region (Krupala and Gianolo, 1993). For oral tongue tumours larger than 2 cm or deeply invasive lesions, the incidence of occult (hidden) metastasis to the regional nodes approaches 25% to 40% (Krupala and Gianoli, 1993). Since lymphatic drainage from some lingual sites crosses the midline, contralateral nodes may be involved even though the tumour appears clinically to be confined to one side of the tongue. For SCC of the BOT, 60% or more of cases have ipsilateral nodal disease at time of presentation, and up to 20% have bilateral involvement of the subdigastric and midjugular groups (Leong et al., 1995). If the capsule of the lymph node is breached by the tumour, the whole mass becomes fixed to the overlying soft tissue or skin and may involve the cranial nerves and result in paralysis (Krupala and Gianoli, 1993; Leong et al., 1995).

Examination of the head and neck includes assessment for the presence or absence of tenderness, the number, size, location, texture and mobility of lymph nodes. Nodes that are soft, tender and freely mobile suggest an inflammatory reaction but nodes that are hard or firm, nontender and not freely mobile are more consistent with tumour involvement. As lymphatic drainage of the upper aerodigestive tract occurs in a relatively predictable fashion to the regional nodes, the identification of a metastatic lymph node in the neck may draw attention to a primary tumour that is not readily visible, or to the potential primary site of an occult primary. Examination for cervical adenopathy (enlargement) is also essential prior to biopsy of any oral/pharyngeal lesion because lymphoid

hyperplasia related to the biopsy can not be readily differentiated clinically from tumour involvement (Mashberg and Samit, 1995; Shah and Lydiatt, 1995). That is, unless the status of the neck nodes is established prior to biopsy, the tumour may receive an advanced staging due to the presence of cervical lymphadenopathy (Section VII.C. below and Table 1.12, Mashberg and Samit, 1995; Shah and Lydiatt, 1995).

False-positive (i.e. clinically positive/histologically negative) findings of neck nodes may subject the patient to unnecessary and more extensive surgery or radiotherapy, but false-negative (i.e. clinically negative/histologically positive or occult nodes) findings may condemn a patient to inadequate treatment. Unfortunately, clinical palpation of cervical nodes fails to detect nodal metastasis in over 30% of cases (Bryne et al., 1991a; Mukherji et al., 1996) and the incidence of both false positive and false negative neck nodes is approximately 20% (Ali et al., 1985). A normal-sized lymph node in an adult neck may range from 2 mm to 2 cm, and the palpability of a lymph node depends on its location, consistency, size, and the type of neck (Ali et al., 1985). In the hands of an experienced examiner and in a neck of average size, the lower limit of palpability is about 0.5 cm in a superficial area such as the submental and submandibular areas, and 1.0 cm in a deeper site (Ali et al., 1985). Thus, not all palpable nodes will be enlarged, and not all palpable nodes contain metastatic deposits (false positives).

In a review of 266 neck dissections of 255 patients treated for head and neck SCCs, Ali et al. (1985) found no relationship between the T-stage (Table 1.12) of the primary tumour and the incidence of false-positive or false-negative neck nodes. However, the incidence of false-positive neck nodes decreased progressively with an increase in the histologic grade of the primary tumour so that there was a direct relationship between the histologic grade and the accuracy of the clinically-positive neck nodes. The overall accuracy of clinical examination for metastatic nodes (as compared to histological examination following neck dissection) was 80%; sensitivity was 91.3%, specificity was 59.6%, and PTL+ was 80.5% (Ali et al., 1985). The rate of false-positive

and false-negative neck nodes varied in relation to location of the primary tumour. False-positive rates ranged from 25% for supraglottic lesions, 16.7% for oral cavity lesions, to 13.0% for oropharynx lesions. False-negative rates ranged from 44.4% for oropharynx lesions, 22.8% for oral cavity lesions to 5.0% for supraglottic lesions (Ali et al., 1985); other investigators have reported false negative rates for primary lesions of tonsil at 36% and BOT primary lesions at 54.5% (reviewed by Ali et al., 1985).

Franceschi et al., (1993) reviewed nodal involvement in 297 patients treated for SCC of the oral tongue. The distribution of nodal metastasis involved nodes in level I (19%), level II (33%), level III (14%), level IV (11%) and level V (1%). There was an overall false-positive rate of nodal involvement of 26% and a false-negative rate of 41%, but the association between these rates and location of the metastatic nodes was not described.

1. Adjunctive Diagnostic Investigations

When distant metastases from the tongue and other head and neck carcinomas do occur, the lungs are the most common sites, followed by the liver, mediastinal lymph nodes and the brain (van der Waal and Pindborg, 1986; Shaha and Strong, 1995). The incidence of distant metastases varies with location of the primary tumour and is related to advanced stage of the disease and bulky nodal disease; cancers of the nasopharynx and hypopharynx have the highest incidence of distant metastases (Shaha and Strong, 1995).

Unless a patient is symptomatic, an extensive metastatic work-up for distant dissemination is seldom indicated for patients with primary carcinomas of the head and neck, and in most instances, a chest radiograph and routine blood chemistries for liver function are adequate (Shah and Lydiatt, 1995). Nevertheless, in 15% of patients with an oral primary cancer, adjunctive examinations of the head and neck by laryngoscopy, oesophagoscopy and a chest film will identify a second primary (Krupala and Gianoli, 1993). When an early oral primary tumour is associated with

lymph node or distant bone or organ metastases, a second, more advanced primary upper aerodigestive or lung tumour may be responsible for the metastases, and the risk of multiple primaries is increased in patients with primary pharyngeal carcinomas or in patients presenting with carcinoma metastatic to cervical lymph nodes with an occult primary (Shah and Lydiatt, 1995). Widely disseminated disease arising from a head and neck tumour is more typically a post-mortem finding, and in most head and neck cancers, death is the result of residual or recurrent disease at the primary or cervical site that often occurs before distant metastasis become clinically significant (Mashberg and Samit, 1995).

Diagnostic procedures such as computed tomography (CT) scans or magnetic resonance (MR) imaging are included with investigations of head and neck tumours to assess the deep extent of disease to aid in staging of the tumour, and to rule out additional primary tumours. CT scans are often useful in assessing bony erosion and in the evaluation of cervical adenopathy, and MR imaging permits evaluation of possible extrinsic muscle, nerve or vascular involvement (Krupala and Gianoli, 1993). Lymphatic, vascular and perineural invasion are all associated with an overall reduction in survival rates from SCC of the head and neck. Vascular invasion by SCCs is associated with an increased likelihood of cervical metastasis, and local and nodal recurrence; the presence of perineural invasion is associated with a reduced likelihood of obtaining tumour-free margins at surgery and therefore, a reduced a reduced probability of local control. CT is superior to clinical examination in detection of cervical metastasis, but CT is still associated with a false-negative rate of 15% for detecting nodal metastasis (reviewed by Mukherji et al., 1996).

Mukherji et al. (1996) investigated the ability of CT to predict perineural or vascular invasion by SCCs that arose in the oral cavity and BOT by comparing preoperative CT scans to histopathologic findings of tissues obtained from gross resections of 48 primary tumours. CT scans were reviewed by two independent radiologists with good interobserver agreement ($\kappa = 0.76$). The overall sensitivity of CT (as compared to histopathology) for detecting either perineural or vascular

invasion was 88% (95% CI = .73, .99); specificity was 83% (.67, .99); PTL+ was 85% (.71, .94) and NPV was 84% (.69, .99). There was good association between CT findings of vascular or perineural invasion and ultimate histopathologic stage of the tumour ($p < 0.001$). Of the 26 tumours with positive CT findings of invasion, 85% were stage III or IV (Table 1.12), and 15% were stage II; none of the tumours with positive CT findings of invasion was stage I. Patients with perineural or vascular invasion had a statistically significant ($p < .02$) higher probability of nodal involvement, and tumours with mean diameters of at least 2 cm on CT scans were significantly ($p < .001$) more likely to have perineural or vascular invasion compared with tumours with mean diameters of less than 2 cm (Mukherji et al., 1996).

Crecco et al. (1994) investigated the accuracy of presurgical MR imaging in determining the T stages of 52 SCCs of the oral tongue, BOT or FOM as compared to the clinical data for 6 of the cases and pathological data from resected tumours of 46 of the cases. The test characteristics of MR imaging varied with the location of the tumour. For example, in evaluating the relationship of the tumour with the midline, MR had an accuracy of 98%, a sensitivity of 95%, and a specificity of 100%; for evaluating infiltration of the alveolar ridge, accuracy of MR was 83%, sensitivity 60% and specificity 92%. For evaluating the relationship between tumours of the BOT and oropharyngeal structures, accuracy was 90%, sensitivity was 86% and specificity was 100%. The authors concluded that MR offered high contrast between tumours and fat or muscles, and even if MR could not demonstrate the tumour in all instances, it could exclude infiltration into the muscle bundles or deep fascial planes. Thus, the authors concluded that any T2 or larger SCC of the tongue or FOM could be staged objectively by MR (Crecco et al., 1994).

C. Classification and Staging of Oral/Pharyngeal Cancers

From a clinical point of view, the purpose of disease classification is to separate patients into groups with an assumed similar clinical presentation and assumed similar clinical course of disease (Wulff 1976). Consequently, results from one patient to another, or from one treatment center to

another can be compared so that treatment outcomes and survival can be correlated with the extent of disease initially present, and clinicians can offer meaningful prognostic information to the patient and plan treatment accordingly.

The TNM system (Table 1.12) is a classification system that is used to stage or assess the extent to which cancer has progressed in an individual patient before the start of therapy. It is critical that the tumour stage is accurate as staging provides the basis for prognosis, selection of treatment and subsequent reporting of results. TNM classification of oral and pharyngeal tumours is based on visual and tactile clinical observations as well as radiographic examinations, and the most accurate staging occurs when clinical assessment is matched by radiographic evaluation of the deep-tissue extent of the tumour (Crecco et al., 1994). Nevertheless, the overall agreement between different examiners for TNM classification is only 70% - 80% (Bryne et al., 1991a).

Stage I and II tumours are generally considered to be early cancers with a more favourable prognosis than advanced stage III and IV tumours (Shaha and Strong, 1995). However, wide variation can exist among identically-staged tumours at the same primary site. For example, a 1.8 cm indurated, palpable painful ulcer in the FOM, and an asymptomatic, nonpalpable, 0.6 cm area of erythroplasia in the FOM would both be designated as T1 lesions, yet their respective prognoses could be quite different. Consequently, Mashberg and Samit (1995) suggested that symptomatology and palpability of the lesions should be considered when assessing prognosis, and they advocated the incorporation of these factors into the staging classification.

The incidence of regional nodal metastases (N category) is influenced by the tumour (T) size. Oral T1 lesions, although small, may show regional nodal involvement in 10 - 20% of cases; T2 in lesions in 25 - 30%, and T3 to T4 tumours in 50 - 75% of cases. For patients with T1 lesions, the 3-year survival rate is 70 - 85%, while patients with T2 lesions have 50 - 60% survival, and those with T3 and T4 lesions have only a 20 - 30% survival rate (reviewed by Epstein 1994; Krupala and

Gianoli, 1993). Without lymph node metastases, overall 3-year survival is 50 - 60% (Epstein 1994) but the presence of nodal metastases reduces survival to 15 - 30% (Krupala and Gianoli, 1993).

D. Epidemiology of Oral/Pharyngeal Squamous Cell Carcinoma

1. Overview

Although the diagnosis of head and neck cancer is relatively easy, only about one third of patients present at an early stage (I or II) of the disease, and approximately half the patients present with locally advanced disease, either at the primary site or with cervical lymph node metastasis (Shaha and Strong, 1995). Despite the efforts of Cancer Societies to educate the public about the warning signs of head and neck cancer such as dysphagia, chronic ulcer and neck lumps, two of every three American patients with head and neck cancer present with the hallmark signs and advanced stages of disease (Shaha and Strong, 1995). In a recent survey of 53 American patients with oral cancer (Rubright et al., 1996), 87% of patients denied knowledge of the warning signs of oral cancer, and only 4% (2/53) patients admitted to performing self-oral examinations specifically to screen for oral cancer, prior to finding their oral cancer. Over half the patients (58%) presenting with advanced-stage disease felt no pain in association with the tumour but for patients with symptoms, there was a mean delay of 5.4 months between the onset of symptoms in the oral cavity and the time of staging. Delay in diagnosis was shortest for cancers located on the tongue and greatest for cancers in the FOM, and there was a significant, inverse relationship between the time since the last dental visit and late-stage disease. Dentists discovered 32% of the oral tumours and 59% of these were localized; physicians discovered 38% of the oral tumours and only 33% were localized (Rubright et al., 1996). Among Brazilian oral cancer patients, 58% were symptomatic for over a month before they sought professional help, and misdiagnosis by primary health workers occurred in 12% of cases causing median delays in diagnosis of 6.5 months by dentists and 12 months by physicians (Kowalski et al., 1994). Overall, conditions that discourage detection of oral tumours include lack of symptoms, location hidden from view, lack of self-oral examinations, ignorance of the warning

signs of oral signs, and lack of patient and/or professional surveillance (Rubright et al., 1996).

Death from cancer in the head and neck is typically due to erosion of major blood vessels, erosion of the cranial base, cachexia, and secondary infection of the respiratory tract. The initial treatment of a primary tumour is critical because there is rarely a second opportunity for a cure (Epstein 1994), and more patients with cancer of the oral cavity die from recurrent or uncontrolled neck disease than from localized disease (Teichgraeber and Clairmont, 1984). In patients cured of their primary tumour, 20 - 25% will develop second primaries in the upper aerodigestive tract. For patients who continue to smoke, this risk is up to six-fold compared to those discontinued smoking; and only after 5 years of smoking cessation does this risk begin to decrease toward the population norm (Krupala and Gianoli, 1993; see also Section VII.F).

In the United States between 1981 and 1986, the 5-year survival of oral and pharyngeal cancer was 52% for males and 56% for females. A review of 5-year survival rates in 6 countries (Norway, Finland, Switzerland, France, South Australia and Italy) revealed an overall male survival rate of 34.7%, but included rates as low as 20% for oropharynx cancers and as high as 58% for FOM cancers in Norway. For females, the overall survival rate was 47% and included rates as low as 34% for oral and pharyngeal sites in Switzerland, and as high as 61% and 62% for FOM cancers in South Australia and Norway, respectively (Boffetta et al., 1994).

Between 1975-1979 and 1987-1991, age-adjusted incidence rates for all cancers in the United States increased by 18.6 % among males and 12.4% among females, largely as a result of rising rates for prostate cancer among men, and for breast and lung cancers among women. The total cancer incidence also rose at all ages but different tumours were responsible for the increases at different ages. Mortality rates for all cancers combined rose less steeply, 3% and 6% among men and women, respectively, and were driven mostly by continuing increases in lung cancer mortality. Death rates for the majority of cancers either remained steady or declined, and mortality rates for all

cancers combined declined among male and females under 55 years of age, and increased only among older persons (Devesa et al., 1995).

In Europe and North America, cancers of the head and neck constitute approximately 3-4% of all cancers (Garfinkel 1995a; Shaha and Strong, 1995), and cancers of the oral cavity constitute about 1% of all cancers (Paterson et al., 1996). Incidence rates of oral/pharyngeal cancer are about 2.5 times as high in males as in females and tumours typically occur in the fifth and sixth decades of life (Shaha and Strong, 1995). Deaths from oral/pharyngeal cancers represent approximately 2% of all cancer-related deaths (Garfinkel 1995a).

Between 1975-1979 to 1987-1991, incidence rates of cancers of the mouth and pharynx (excluding lips, salivary glands and nasopharynx) among white females were unchanged but mortality rates decreased by 13% (Devesa et al., 1995). In this same time period, the overall incidence rate of oral/pharyngeal cancer for white males decreased by 1.7% and mortality rates decreased by 22% (Devesa et al., 1995). Since 1970, the largest number of oral SCCs occurred in American patients a decade earlier than in patients whose conditions were diagnosed before 1970; that is, before 1970, the largest number of patients with oral SCCs were in the seventh decade of life, whereas after 1970, the largest number of patients were in the sixth decade of life (Krolls and Hoffman, 1976). Among black men, the incidence of upper aerodigestive tract cancers is almost double that of white men for those younger than 65 years of age (Spitz 1994). The oral cancer mortality rate in black men has also doubled, surpassing that of white men in the 1960's before peaking in the early 1980's (Spitz 1994; Blot et al., 1994). Geographical variations in the rates of oral/pharyngeal cancers show increased rates among males in the northern USA, particularly in large urban centers. Among females, mortality between 1950 and 1989 was highest in the southern rural areas where smokeless tobacco use was commonplace, but more recently, new high-rate areas for women have appeared along the Pacific and Florida coasts, resembling the pattern of lung cancer among women (Blot et al., 1994).

Cancer of the tongue represents approximately 25% to 40% of oral cancers (Regezi and Scuibba, 1989; Spitz 1994), and registries reporting the highest rates of tongue cancer also report high rates of oral cancer (Macfarlane et al., 1992). Despite the overall recent declines in mortality from oral and pharyngeal cancer among white American males, an increase in tongue cancer rates at young ages as well as a rising death rate from tongue cancer among white American males below the age of 40 is apparent although concomitant increases in rates for other oral sites have not been observed (Blot et al., 1994). However, Devesa et al. (1995) reported that between 1975-1979 and 1987-1991, the incidence of oral/pharyngeal cancers in white males between the ages of 15 and 34 years increased by 267%. In the western world, 98% of oral/pharyngeal cancer patients are over 40 years of age (Ostman et al., 1995), and in the United States, 1% to 3% of cases of oral/pharyngeal SCC now occur in patients 40 years of age and younger (Burzynski et al., 1992).

McGregor et al. (1983) reviewed 27 BCCA cases of oral tongue cancer in patients under 40 years. The majority of lesions were T1N0 (15/27), no specific etiologic factors were obvious but in patients under 30 years of age, there was a female preponderance (3:1). Treatment consisted of surgical excision (15 patients), brachytherapy (6 patients) and/or external beam radiotherapy (2 patients) and with follow-up ranging 2-7 years, the absolute disease-free survival rate was 80% (McGregor et al., 1983). Sarkaria and Harari (1994) reviewed the literature for 152 world-wide cases of oral tongue cancers in patients less than 40 years, and also included 6 cases of tongue cancer in patients under 40 years treated at the University of Wisconsin Hospital. The 158 cases were predominantly early stage disease (64% stage I and II), yet the cases were characterized by a high rate (57%) of local/regional failure and disease-specific mortality (47%). These results contrasted with several reports such as Spiro and Strong (1971) who reported 76% local/regional control and 62% 5-year survival in older patient cohorts with T1-T3, N0 oral tongue lesions, and Sarkaria and Harari (1994) concluded that compared to older patients, patients under 40 years have a relatively brief or nonexistent history of tobacco and alcohol exposure, yet SCC in younger patients appeared to be a more aggressive disease that required aggressive combined therapeutic

approaches.

Burzynski et al. (1992) reviewed 1387 head and neck SCCs; 28 cancers occurred in patients aged 40 years of age or younger, 18 were male, 5 were female, and age at diagnosis ranged from 21 to 40 years. The majority of SCCs occurred in the oral cavity (39%) and oropharynx (48%), and 30% occurred on the tongue (8.6% oral tongue, 22% BOT). Current or former tobacco use with an average of 25 pack years was admitted by 91% of patients, alcohol intake was admitted by 70%, and both tobacco and alcohol use occurred in 30%. A similar trend in tongue and oral cancer was reported in the Netherlands where patients with oral cancer and under 40 years of age were mostly males, and SCC of the tongue was the most common site (Jovanovic et al., 1993b).

In Sweden between 1960 and 1989, there was a statistically significant ($p < 0.01$) increase in the age-standardized incidence rate for malignant tongue tumours in males, while the corresponding figures for females remained constant; for malignant FOM tumours, there was a statistically significant ($p < 0.01$) increase in incidence for both men and women (Ostman et al., 1995). In Sweden, the male:female ratios for cancer of the tongue changed from 1.3:1 (1960-1969) to 1.9:1 (1980-1989); and for tumours in the FOM from 3:1 (1960-1969) to 3.6:1 (1980-1989); the trend towards the more accentuated male dominance was attributed to changes in tobacco and drinking habits (Ostman et al., 1995). In Scotland the incidence of tongue and mouth cancer among men and women has been rising since 1960, and in young adult males, the incidence had trebled at both sites since 1970 (Macfarlane et al., 1992; Llewelyn and Mitchell, 1994).

The reason for the increasing incidence of tongue cancers is unclear. In most western countries, the smoking of tobacco (mainly cigarettes) increased from early in the 1900's to reach a peak in the 1960's and then declined (Fiore 1992). Among regular smokers, about 90% start to smoke by the age of 20 years and whereas smoking initiation declined among young males between 1965 and 1987, there was a slower decline in the rate of smoking initiation among women. In the early

1960's, about 41% of all American adults smoked whereas in 1989, approximately 28% of American adults smoked (Fiore 1992). However, the decline in cigarette smoking has not been equal among all segments of the American population as blacks, women and young people lag behind other groups in rates of smoking decline. Consequently, there has been a gradual convergence in smoking prevalence between the sexes over the past 25 years (Fiore 1992).

The use of smokeless tobacco decreased in the early 1900's to a prevalence of 5% or less in the United States (Fiore 1992; Blot et al., 1994), but due to the recent popularity of smokeless tobacco among young American males between the ages of 8 and 18, prevalence may reach 30% (Macfarlane et al., 1992) and may be related to increased rates of lingual cancers. However, smokeless tobacco is more typically associated with malignancies of the cheek and gingiva rather than the tongue (Macfarlane et al., 1992; Blot et al., 1994), and in Scotland, the use of smokeless tobacco is not widespread yet lingual cancers are increasing (Macfarlane et al., 1992).

Trends of increasing tongue and oral/pharyngeal cancers appear to be more closely correlated with trends of rising alcohol consumption in developed countries than with patterns of tobacco consumption (Blot et al., 1994). For Scottish patients with cancer of the tongue, the incidence of smoking was less than in oral cancer patients as a whole, but the incidence of alcohol intake was higher than for oral cancer patients as a whole (Llewelyn and Mitchell, 1994). In the United Kingdom, average alcohol consumption between 1960 and 1985 increased by 40% (Macfarlane et al., 1992), and in Denmark, increased alcohol intake preceded by 5-10 years a parallel rise in oral and pharyngeal cancers (Blot et al., 1994). An exception is France, where per capita alcohol intake decreased by 24% between 1960 to 1985 and mortality from oral and pharyngeal cancer among French men declined in the 1980's (Blot et al., 1994).

In the United States, the estimated total number of cancers in 1997 is 1,382,400 and of these, 6400 (4.6%) will involve the tongue and 11,000 (7.9%) will involve other oral sites. The estimated total

number of cancer deaths in 1997 is 560,000 and of these 1,820 (3.2%) deaths are estimated to occur from cancer of the tongue and 2,500 (4.5%) from cancer involving other oral sites (Parker et al., 1997).

2. Cancer Statistics for Canada

Since 1984, the average annual percent changes in incidence and mortality rates for all cancer sites among Canadian women have remained relatively stable as they have changed less than about 2% per year. Among Canadian men, overall incidence rates have increased due to the sharp increase in prostate cancer, but male mortality rates appear to have peaked and are declining due to decreased rates in mortality from lung and colorectal cancers. For oral/pharyngeal cancer among Canadian men and women, there has been less than 2% change per year in either incidence and mortality rates since 1984 (National Cancer Institute of Canada 1996).

In 1991, a total of 109,442 new cases of cancers were diagnosed in Canada; 3,017 cases occurred in the oral cavity and pharynx, and included 516 cancers of the tongue (0.47% of all cancers; 17% of oral/pharyngeal cancers) of which 377 (73%) occurred in males and 139 (27%) occurred in females (male:female ratio = 2.7:1), (Table 1.13). In 1993, cancer deaths from oral and pharyngeal cancer represented 1.7% of total cancer deaths; deaths from cancer of the tongue, represented 0.43% of the total deaths and 24.5% of oral/pharyngeal cancer deaths (National Cancer Institute of Canada 1996; Table 1.13).

For 1996, the estimated number of new cases of oral/pharyngeal cancer was 3090, representing 2.4% of the total number of estimated new cases; 71% (2,200 cases) were estimated to occur in males and 29% (890 cases) were estimated to occur in females (male:female = 2.5:1), (Table 1.13). The vast majority of new oral/pharyngeal cancer cases were expected in Ontario (44% or 1350 cases), followed by Quebec (20% or 700 cases) and BC (12.3% or 380 cases). The age-standardized incidence rate (Section II.D) for oral/pharyngeal cancer in Canadian males in 1996

was estimated at 15 per 10⁵ population (Table 1.13), and included rates as low as 10 per 10⁵ population in New Brunswick, and as high as 37 per 10⁵ population in Newfoundland; the estimated incidence rate for B.C. males was estimated at 13 per 10⁵ population. For Canadian females, the 1996 age-standardized incidence rate for oral/pharyngeal cancers was estimated at 5 per 10⁵ population (Table 1.13), and included rates as low as 3 per 10⁵ population in Saskatchewan, and as high as 6 per 10⁵ population in Nova Scotia, Ontario and Manitoba. The estimated 1996 age-standardized incidence rate for oral/pharyngeal cancers in females in British Columbia was 5 per 10⁵ population (National Cancer Institute of Canada 1996), .

The lifetime probability of a Canadian male developing a cancer is 41.6%, and the inverse of that probability ($1.00/0.416$) indicates that 1 in 2.4 Canadian men will develop a cancer at some point in their lives (Table 1.13). Canadian women have a lifetime probability of 37.1% of developing cancer, or 1 in 2.7 females will develop cancer at some point in their lives (Table 1.13). Approximately 1 in 3.7 men, and 1 in 4.5 women will die of cancer (Table 1.13). For oral/pharyngeal cancer, 1 in 57.1 Canadian men will develop oral/pharyngeal cancer at some point in their lives, and 1 in 169.5 will die of oral/pharyngeal cancer (Table 1.13). The probability of Canadian men developing oral/pharyngeal cancer increases with age; there is a 0.2% probability by age 50, a 1.0% probability by age 70, and 1.7% probability by age 90 (National Cancer Institute of Canada 1996).

For 1996, the estimated number of deaths from oral/pharyngeal cancer was 1,070 or 1.7% of the total estimated deaths; 72% (770 deaths) were estimated to occur in males and 28% (300 deaths) were estimated to occur in females (Table 1.13). The majority of deaths from oral/pharyngeal cancer were expected in Ontario (34% or 360 deaths), Quebec (33% or 350 deaths), and BC (12.6% or 135 deaths). For 1996, the estimated age-standardized mortality rate for oral/pharyngeal cancer was 5 per 10⁵ Canadian male population (Table 1.13); the highest rates of 8 per 10⁵ male population were expected in Quebec and Prince Edward Island, and the lowest rate of

3 per 10⁵ male population was expected in Saskatchewan; the estimated rate in B.C. was 5 per 10⁵ male population (National Cancer Institute of Canada 1996). For 1996, the estimated age-standardized mortality rate for oral/pharyngeal cancer among women was 2 per 10⁵ population (Table 1.13); the estimated rate in B.C. was 2 per 10⁵ female population (National Cancer Institute of Canada 1996).

The death:cases ratio is a crude measure of disease severity and represents the number of deaths divided by the number of new cases. Ratio values of 0.30 or less indicate a very good prognosis for the disease; ratios greater between 0.30 and less than 0.50 indicate a fairly good prognosis for the disease, and ratios greater than 0.60 indicate a poor prognosis for the disease. The overall ratio of Canadian cancer deaths to new cases of cancer estimated for 1996 was 0.48 and the ratios were generally similar for males and females within each site of cancer. The 1996 deaths to cases ratio estimated for oral/pharyngeal cancer was 0.35, signifying a fairly good prognosis (National Cancer Institute of Canada 1996; Table 1.13).

3. Cancer Statistics for British Columbia

From 1984 to 1992, the number of new cases of all cancers in BC increased by an average of nearly 4% a year, reflecting the increasing incidence of lung and prostate cancers as well as population growth and aging of the population. In 1994, a total of 14,971 new cases of cancer were diagnosed (Table 1.14), 8055 in men and 6916 in women. Of these, 125 cases were designated as mouth (excluding lip) cancers (68 male, 57 female; male:female = 1.19:1), 60 cases as tongue cancers (45 male, 15 female; male:female = 3:1), and 124 cases as pharynx cancers (88 male, 36 female; male:female = 2.4:1), (Table 1.14). Among males in 1994, 1 tongue cancer occurred at less than 20 years of age, 5 cases occurred between 20-39 years, 15 cases between 40-59 years, 21 cases between 60-79 years, and 3 cases at 80+ years. Among females, the majority of tongue cancers occurred between 40-59 years (7 cases) and 60-79 years (6 cases), (British Columbia Cancer Agency et al., Annual Report 1995-1996).

Between 1990 and 1994, the age-standardized incidence rate of tongue cancer among males averaged 2.1 per 10⁵ BC population, and among females averaged 1.0 per 10⁵ BC population (Table 1.14). For men and women, the age-standardized rates for tongue cancer were lower than rates for mouth or pharynx cancer (Table 1.14). The number of new cases estimated for 1996 in BC included 81 tongue cancers, 153 mouth cancers and 136 pharynx cancers out of a total of 16,858 new cancer cases (Table 1.14; British Columbia Cancer Agency et al., Annual Report 1995-1996).

In 1994, deaths due to all cancers totalled 6945 and included 36 (0.52%) deaths due to tongue cancer, 37 due to mouth cancer, and 63 due to pharynx cancer (Table 1.14). In 1994, the total number of all cancer deaths per 10⁵ BC population (crude mortality rate) was 215.4 for males and 187.9 for females and included crude rates of 1.3 and 0.8, respectively for males and females, for tongue cancer. Crude mortality rates for tongue cancer were highest for males aged 60-79 years (6.2 per 10⁵) and females aged 80+ years (6.1 per 10⁵). Between 1990 and 1994, age-standardized mortality rates for tongue and mouth cancers were similar but lower than rates for pharynx cancers (Table 1.14). The estimated number of deaths from all cancers in BC in 1996 totalled 7444 and included 32 deaths from tongue cancer, 35 from mouth cancer, and 66 from pharynx cancer (Table 1.14; British Columbia Cancer Agency et al., Annual Report 1995-1996).

E. Treatment Modalities for Oral/Pharyngeal Cancer

The primary objective in the treatment of any malignancy is to cure the patient of the cancer but in addition, efforts should be made to minimize sequelae of treatment and to prevent a second primary cancer. In addition, preserving form and function are especially important in management of head and neck tumours and if preservation is not possible, then restoration of form and function to retain a good quality of life are essential (Shah and Lydiatt, 1995). Therapies for tumours of the head and neck typically include aggressive surgical resection, local irradiation, regional irradiation or both, and all of these therapies are often associated with severe deformities, impaired speech and

swallowing, osteoradionecrosis, mucositis, impaired nutrition and disturbed body image. Yet despite these aggressive and deforming interventions, local and regional recurrences are common and less than 25% of head and neck tumour patients remain alive five or more years after diagnosis (Aisner et al., 1994).

The choice of treatment for head and neck cancer is influenced by the site and stage of the primary tumour, the history of any previous treatment, histology of the primary tumour as well as the experience and treatment preferences of the treating physician. Patient factors such as age, medical history, tolerance and compliance with treatment will also influence the choice of treatment, and in some countries, the patient's socioeconomic status is a major determinant of the treatment modality.

For tumours involving the tongue, treatment depends on the T stage, whether the lesion involves the oral tongue or the BOT, upon proximity or involvement of the mandible and the incidence of micrometastasis or gross metastasis to the neck. The following discussion reviews the principles of surgical resection, radiotherapy, and chemotherapy and their sequelae, followed by treatment approaches typically used in management of lingual SCCs.

1. Surgical Resection

The use of surgery in treatment of oral cancer depends on the size of the tumour, its location in general and in particular, its relation to the mandible or maxilla. Details of the various surgical modalities used in treatment of oral/pharyngeal cancer in general and tongue cancer in particular, are beyond the scope of this thesis and therefore, surgical treatment of the tongue is discussed in very general terms only.

Tumours that are accessible, localized to the oral tongue without crossing the midline, and without evidence of metastasis, may be treated by partial glossectomy or hemiglossectomy in which the

lingual artery and hypoglossal nerve of the opposite side are preserved. When tumours extend more than 1 cm across the midline, the opposite lingual artery must also be sacrificed, resulting in a functional total glossectomy (Crecco et al., 1994). Whenever possible, surgical access is gained through the mouth, but tumours that are located more posteriorly or involve the adjacent mandible, require access via cheek and lip flaps, and may require excision of a portion of the mandible (Spiro and Strong, 1974). Tumours that are located at the BOT or extend to the BOT, the tonsillar bed or hyoid bone, would require a total glossectomy (Crecco et al., 1994), and possibly a total laryngectomy as well as a radical neck dissection (Spiro and Strong, 1974). Various types of flaps may be used for reconstruction after surgery of the tongue, but these procedures are beyond the scope of this thesis.

More patients with cancer of the oral cavity die from recurrent or uncontrolled neck disease rather than from local disease (Shaha and Strong, 1995). As there is a high incidence of regional metastasis from oral and pharyngeal cancers, surgical and/or radiation therapy are often recommended as elective treatment of the clinically-negative N0 neck, as well as for management of N+ disease. Both radical and modified neck dissections have been used but the radical neck dissection is generally recognized as standard treatment of the N+ neck (Shaha and Strong, 1995; see also Section VII.E.2.e. and Section VII. E.4). Radical neck surgery removes all lymph nodes in levels I through V, removes the sternocleidomastoid (SCM) muscle, internal jugular vein and spinal accessory nerve with subsequent loss of shoulder function (Shaha and Strong, 1995). The modified neck dissection technique preserves the accessory nerve, the internal jugular vein and SCM muscle (Teichgraeber and Clairmont, 1984). Bilateral simultaneous radical neck dissection is associated with a 17% mortality rate (Shaha and Strong, 1995) and therefore, radical neck dissections are staged, or a radical neck is performed on one side with a modified neck dissection on the other side. Neck dissections can also be selective and limited as in supraomohyoid dissections where the lymph nodes at levels I, II and III only are removed (Shaha and Strong, 1995).

In a clinical trial assessing the benefits of elective neck dissections, Fakih et al. (1989) randomized 95 Indian patients with T1 or T2 N0 SCCs of the oral tongue to either hemiglossectomy alone or hemiglossectomy with elective ipsilateral radical neck dissection. Although the results are impaired by the short follow-up time (minimum of 12 months, median 20 months), 57% of patients treated with glossectomy alone developed metastatic ipsilateral neck nodes within a mean time of 6 months. Among patients treated with hemiglossectomy and radical neck dissections, 33% demonstrated histologically-positive nodes at their neck dissection. Among patients with histologically-negative nodes at their neck dissection, 20% subsequently developed contralateral nodes within 4 months of the initial treatment. Disease-free survival at 12 months was 52% in the hemiglossectomy group, and 63% in the hemiglossectomy/radical neck dissection group, but the differences were not statistically significant.

Spiro et al. (1986) reviewed the recommended surgical protocols for cancer of the oral tongue, and the consensus was to surgically treat the neck in all undifferentiated or poorly differentiated T1 lesions, in all T2 or larger lesions, and in all lesions with poor surgical margins (Spiro et al., 1986) but comments regarding nodal status were not included. Teichgraeber and Clairmont (1984) advocated ipsilateral elective irradiation or modified neck dissection for poorly differentiated T1 lesions and all T2 or greater lesions; bilateral neck dissection or irradiation was recommended for midline tongue lesions T2N0 or greater. Franceschi et al. (1993) advocated surgical extirpation of early tongue cancers but were unable to demonstrate a survival advantage for elective neck dissection in early tongue cancers. Nevertheless, they concluded that some type of elective lymphadenectomy in the N0 neck was reasonable, and that supraomohyoid dissection was adequate for removing nodes at highest risk in early tongue cancer. For advanced (stage III and IV) lesions, Franceschi et al. (1993) preferred a combination of resection and postoperative radiation. Shaha and Lydiatt (1995) reported that for primary tumours in the oral cavity and a N0 neck, a supraomohyoid neck dissection was an appropriate elective neck dissection, but midline lesions required bilateral elective limited neck dissections. Shaha and Strong (1995) preferred

surgery such as the supraomohyoid dissection over radiation therapy for N0 necks (see also Section VII.E.4).

2. Radiotherapy

a. Mechanisms of Action

The principles of radiation physics and radiation biology are briefly reviewed to provide an appreciation of the effects of ionizing radiation on tumours as well as normal tissues which suffer acute and chronic sequelae from radiotherapy.

(i). Radiation Physics

At the center of an atom is the nucleus which contains most of the atom's mass, and around the nucleus, electrons move in a few specific orbits or shells. The simplest atom is hydrogen which consists of one positively-charged particle, the proton, and one negatively-charged electron. The nucleus of all atoms except hydrogen also contains neutrons whose numbers almost equal the number of protons which is represented by the atomic number (Z) of an element. Complete atoms contain the same number of electrons and protons so the over-all charge is balanced, but the number of neutrons in an atom can vary to produce different isotopes which have similar chemical properties but weigh different amounts. Some isotopes are stable but as there a limited number of stable arrangements of protons and neutrons in the nucleus, isotopes of some elements are unstable. In order to achieve a more stable nuclear configuration, unstable nuclei will spontaneously emit any surplus energy as electromagnetic radiation called gamma radiation. Gamma rays are identical in nature to X-rays except that gamma ray are spontaneously emitted from certain radioactive isotopes (eg. uranium-235, cobalt-60, gold 198), (Bomford 1993).

Electron shells are associated with a specific energy or binding energy which is equivalent to the amount of energy necessary to remove that electron from its location. Binding energies increase as the shell's distance from the nucleus decreases so the shell closest to the nucleus has the greatest

binding energy. The transfer of an electron from one shell to another is accompanied by either the absorption or emission of energy equal to the difference in the binding energy between the shells. The process whereby an electron is moved from one orbit to a more distant orbit is known as excitation, and the process whereby an electron is completely removed from an atom is known as ionization. An ionized or excited atom can resume its stable state by attracting another electron into the vacant space in the orbit concerned (Bomford 1993). The movement of an electron through an atom's shell structure from an outer to an inner shell is accompanied by the emission of electromagnetic radiation which may be visible as in fluorescence, or invisible as in ionizing or X-radiation. The emitted X-radiation is of a wavelength that is peculiar or characteristic to the specific shells involved and is useful in the identification of elements as each has its own spectrum of characteristic radiation (Price 1988).

Electromagnetic radiation from a point source emanates isotropically (i.e. equally in all directions), and the intensity of the radiation is inversely proportional to the square of the distance from the source (the inverse square law). Electromagnetic radiation travels in straight lines and is usually regarded as a wave, but it may be better understood as particles or packets of energy called photons or quanta (Price 1988). The electromagnetic spectrum includes a range of wavelengths and as the wavelength decreases, the energy of the photons increases. In order of decreasing wavelengths and increasing energy, the electromagnetic spectrum includes radio waves, television waves, radar waves, infrared or radiant heat rays, visible light, ultraviolet rays, X-rays and gamma rays (ionizing radiation). Hence, X-rays and gamma rays (ionizing radiation) have the shortest wavelengths but the highest energies. Ionizing radiation covers a much larger section of the electromagnetic spectrum than does visible light, but just as visible light of different wavelengths has different properties (such as the colours produced by a prism), ionizing radiations of different wavelengths also differ and the shorter the wavelength, the more penetrating the x-ray beam (Bomford 1993; Fleming et al., 1995).

Diagnostic X-ray tubes consist of an evacuated glass tube containing an anode and a cathode which contains a filament (eg. tungsten) that can be electrically heated to produce a stream of electrons. The electrons are focused towards a target (eg. tungsten) on the anode, and when the electrons strike the anode, most of the electron energy is converted to heat although some is given off as X-radiation (Price 1988). In the production of therapeutic radiation by a linear accelerator, electrons produced by the cathode are accelerated by radiowaves, and the electrons can be used directly or they can be directed towards a target at the anode (Rubin and Doku, 1976; Bomford 1993). At the target, the impinging electrons interact with target electrons in several ways, but the majority of X-ray emissions occur as a result of impinging electrons that interact with several target electrons. With each collision, the impinging electron loses part of its energy to cause excitation or ionization of the target electrons. If target electrons from an inner shell are displaced, the resultant orbital vacancy in the target atom can be filled by electrons from an outer shell within the same atom and this process is accompanied by the emission of photons which have a range of energies known as the continuous spectrum of X-rays (Rubin and Doku, 1976; Price 1988; Bomford 1993). The quantity and quality of the X-rays produced at the target can be controlled so that ionizing radiations of different wavelengths and energies can be produced. If the current passed through the filament of the cathode is increased, the rate of electron production is increased which in turn, increases the quantity of X-rays produced. If the potential difference (kVp) across the tube is increased, the velocity of the impinging electrons is increased so that X-rays of shorter wavelength and higher energy are produced (Price 1988); the accelerating voltage (kVp) determines the minimum wavelength in the spectrum but all the longer wavelengths will be present (Bomford 1993). The penetrating ability of an X-ray beam as it emerges from the X-ray head is referred to as the quality of the beam and is described in terms of the kVp. The extent to which the X-ray spectrum subsequently interacts with tissue depends on the wavelengths contained in the X-ray spectrum and characteristics of the tissue (Bomford 1993).

(ii). Radiation Biology

The same tissues will differentially absorb photons of different energies, and the dominant reaction between the tissue and ionizing radiation varies with the energy of the radiation in use. When shorter wavelength, more energetic radiation beams such as those commonly used in therapeutic radiotherapy are used to irradiate materials such as tissue, the dominant interactions occur via the Compton effect. In the Compton effect, an incident photon interacts with a loosely-bound orbital electron of a tissue atom. The photon gives up some of its energy to the electron which is known as the recoil or Compton electron, and the photon continues with reduced energy on a different path (scattering). The recoil electron may interact directly with an atom in a cell and approximately one-third of the cellular damage is due to this process (Fleming 1995). The remaining two-thirds of the cellular damage is due to indirect action in which the recoil electron interacts with a loosely-bound orbital electron of water molecules in the cells (water constitutes about 70% of most cells), (Fleming 1995), producing free radical ions (H_2O^+ and a free electron). The H_2O^+ is unstable and quickly forms a hydrogen ion (H^+) and hydroxyl ion (OH^*) which has an unpaired electron; these ions are highly reactive with DNA and cause chromosome breaks (Bomford 1993).

In contrast, when lower energy X-ray photons are used as in diagnostic radiography, the dominant interaction is via the photoelectric effect. In the photoelectric effect, an incident photon has an energy that is just greater than the binding energy of a inner shell electron. Collision between the incident photon and the inner-shell electron results in a complete transfer of the photon's energy to the electron which is known as the photoelectron. There is no scattered photon as its energy has been transferred to the photoelectron which escapes with energy equal to that of the incident photon minus the binding energy required to release it from orbit. The vacant space left in the shell is filled by an electron from an outer shell and is accompanied by production of characteristic X-rays (Price 1988; Bomford 1993).

X-ray photons of the same energy are differentially absorbed in different tissues and the intensity

of the X-ray beam is reduced or attenuated to varying extents depending upon the atomic number, density and thickness of the tissue. Materials that have a higher atomic number (Z) have a greater number of atomic particles and since X-ray photons interact with atomic particles, such materials will absorb and scatter more radiation. Density or specific gravity is related to the atomic mass and the number of atoms in a given volume. Materials with a high density have a greater number of atomic particles per unit volume and thus, such materials cause greater attenuation. The number of atomic particles also varies with the thickness of the material so that an object of triple thickness will have three times the number of atomic particles and therefore, cause greater attenuation (Price 1988). The photoelectric effect (diagnostic radiographs) is highly dependent upon the atomic number (Z) in a Z^3 relationship (Rubin and Doku, 1976). The atomic numbers of bone (13.8), muscle (7.4) and adipose tissue (5.9) differ so that bone has an anatomic number that is about twice that of fat and muscle. Consequently, bone will absorb about 8 times ($Z^3 = 2^3$) more energy than soft tissue and therefore bone and soft tissue appear quite different on a diagnostic X-ray film (Rubin and Doku, 1976; Fleming et al., 1995).

In the Compton effect, the high energy of an incident photon is shared between the recoil electron and the scattered photon of lower energy. The probability of Compton interactions depends primarily on the electron density of the target tissue rather than the atomic number (Fleming 1995). Bone, muscle and adipose tissue have near-equal electron densities (Bomford 1993) and therefore, they absorb about the same amount of radiation energy, gram for gram, due to the Compton effect (Fleming et al., 1995). However, bone is nearly twice as dense as soft tissue and therefore bone overall, will absorb more radiation than soft tissue. Moreover, bone also produces more scatter of radiation than soft tissue and therefore, soft tissue close to bone and soft tissue elements actually living inside bone, always receive a higher radiation dose than they would if the bone was replaced by soft tissue (Bomford 1993).

In the Compton effect, the recoil electron is ejected in a forwards direction and with increasing

kinetic energy, and even though its range is extremely small, there is a build-up of electrons immediately below the surface of the irradiated tissue. The dose distribution within an irradiated tissue is known as the isodose chart and each line on the chart represents points of equal dose (similar to height contours on a geographic map). The distribution of dose along the central axis of an X-ray beam will decrease with increasing tissue depth due to the inverse square law, and attenuation throughout the successive layers of tissue which depends on the nature of the tissue, the quality of the X-ray beam, as well as the size and shape of the radiation beam (Bomford 1993) (see also VII.E.2.d below).

The amount of energy actually deposited in a small mass is called the absorbed dose. The standard unit for reporting dose is the gray (Gy) which is one joule per kilogram. Previously, the term 'rad' was used as the standard reporting dose; 1 rad equals 0.01 Gy or 1 centigray (cGy), (Fleming et al., 1995). However, very little energy is absorbed by the tissues from a given dose of ionizing radiation as the energy is used to break chemical bonds and produce biological damage (Bomford 1993). Ionizing radiation can be administered in one of two ways. When the radiation source is at a distance (generally 80 to 100 cm) from the target it is called teletherapy. When the distance between the source and the target is short, the term brachytherapy is used. One application of brachytherapy is interstitial therapy whereby small sealed gamma ray sources such as grains of gold-198 are implanted directly into the tissues (Bomford 1993).

The interaction of X-rays or gamma rays with a cell initiates a chain of molecular events that results in delay and inhibition of cell division and ultimately in cell death. Damage to cell membranes and microtubules may be supplementary killing mechanisms but lethal injury is believed to result from damage to the cell's DNA (Fleming et al., 1995). Thus, the specific target of radiation damage is DNA, and single or double strand breaks may occur. Double strand breaks are more difficult to repair than single strand breaks but repair of double strands may result in chromosomal rearrangements (mutations). The interaction between DNA and X-rays or gamma rays depends

upon the quality of the radiation, and conventional radical (curative intent) radiotherapy usually delivers on the order of 2 Gy daily, each dose reducing the surviving tumour population by about 50%. Cells are most sensitive to the effects of ionizing radiation in M phase and most resistant in late S phase, and variation in cell survival between these two phases may be fivefold (Bomford 1993). If there is appreciable length to the G1 phase, there is also a resistant period in early G1, followed by a sensitive phase in late G1. Finally, G2 is another sensitive phase and its sensitivity may approach the sensitivity of M phase. Throughout the cell cycle, radiosensitivity may be related to oxygen (see below), or sulfhydryl compounds which scavenge free radicals and are therefore natural radioprotectors. The intracellular levels of sulfhydryl are highest during S phase and lowest near mitosis, correlating with radiation resistance and sensitivity, respectively (Fleming et al., 1995).

Cells also vary in their intrinsic radiosensitivity; for example, oral mucosa is a very sensitive early-responding tissue and connective tissue is a relatively radioresistant late-responding tissue. Tumour cells may also be radiosensitive or radioresistant but unfortunately, there is no significant correlation between the responsiveness of a tumour to ionizing radiation and eradication of the tumour. That is, a radiosensitive tumour may be incurable, and a radioresistant tumour may well be curable. The overall response of a tumour also depends on the programmed lifetime of the cells within the tumour, the proliferation kinetics of the tumour cells (Section V.D) and the rate of removal of dead cells from the tumour (Fleming et al., 1995).

If the total dose of radiation is divided into a number of separate doses or fractions (fractionation), small differences in cell survival for a given dose can be amplified and the differences in response between rapidly dividing tumours (with a higher growth fraction) and late-responding normal tissues can be exploited (Bomford 1993). However, there may also be differences in survival for the same cell lines irradiated under different conditions because cell survival is influenced by the 4 R's of radiation biology: repair, reassortment, repopulation, and reoxygenation.

1. Repair

Ionizing radiation may not damage a critical site within a cell and therefore the cell is unaffected. Radiation damage may be lethal if damage occurs at a critical site causing the cell to die during one of its subsequent divisions. Damage may also be sublethal if it is insufficient to kill the cell and if the damage may be repaired, given enough time, energy and nutrients. The degree of recovery from sublethal damage depends directly upon the degree of oxygenation (see below) but the capacity for repair also varies among normal tissues and tumours. Rapidly-responding tissues such as oral noncornified mucosa often have incomplete repair, partly because of continued pressure to proliferate rather than to repair. Slowly-responding tissues such as connective tissue have a greater repair capacity than tumours, and if the interval between multiple small treatment fractions is at least 6 hours, then slowly-responding tissues can be spared (Bomford 1993; Fleming et al., 1995).

2. Reassortment

The redistribution or reassortment of cells in phases of the cell cycle does not significantly affect late-responding tissues, but it is a major factor in the response of acutely-responding normal tissues and tumours. Once the radiosensitive cells have been killed and removed, the residual cells are temporarily synchronous in the radioresistant phases of the cell cycle. However, the typical 24-hour interval between treatment fractions allows cells to progress towards a more radiosensitive phase (eg. M phase), and although the intermitotic times vary widely in most tumours, the radiosensitivity of the asynchronous tumour cells after 24 hours still exceeds that of the tumour population immediately after the radiosensitive population has been removed (Bomford 1993).

3. Repopulation

During a course of fractionated radiotherapy, normal and tumour cells continue to undergo cell division. In response to damage, normal tissue may recruit inactive stem cells or cells in G₀, as well as shorten the duration of the cell cycle. In this instance, repopulation is beneficial because it reduces overall injury to the tissue and enables acutely-responding tissues to tolerate much larger doses delivered as multiple fractions than would be the case with an equivalent single fraction.

Overall, repopulation is greater in acutely-responding tissues than in tumours but a similar therapeutic differential does not exist between tumours and late-responding tissues (Bomford 1993; Fleming et al., 1995).

In tumours with a high growth fraction, repopulation between fractions may outstrip tumouricidal effects of radiation and in some tumours, radiation may actually accelerate repopulation, perhaps the result of a better vascular supply as the tumour shrinks. In cancers of the head and neck, repopulation accelerates about 28 days after the start of fractionated radiotherapy (Fleming et al., 1995); hence, radiotherapy should be delivered over as short as time as possible without delays during treatment. Conversely, with a prolonged fractionated regime, later fractions will be less effective and may allow significant accelerated tumour repopulation (Bomford 1993; Fleming et al., 1995).

4. Reoxygenation

Oxygen probably aids in the production of cell damage by combining with an unpaired electron of a radiation-induced free radical and yielding a non-repairable peroxide in the target atom (Fleming et al., 1995). Tumours may become hypoxic if the increased demand for nutrients to sustain tumour growth cannot be met by their vascular supply. About 15 to 20% of tumours contain hypoxic cells and even a small population of hypoxic cells can affect the success of radiotherapy because hypoxic cells are 2-3 times more radioresistant than well-oxygenated cells (Bomford 1993). However, as the radiosensitive population is removed by successive fractions of radiation, the hypoxic cells become reoxygenated as they are closer to the vascular supply. The amount of reoxygenation between fractions varies between tumours and may be influenced by the fraction intervals (Bomford 1993).

Efforts to overcome tumour hypoxia include maintenance of normal patient haemoglobin concentrations throughout therapy and may include blood transfusions. The oxygenation of an entire patient can be increased by placing the patient in a hyperbaric oxygen tank, increasing the

pressure to three times atmospheric pressure and then delivering radiotherapy. Clinical trials comparing radiotherapy of advanced head and neck cancer in hyperbaric oxygen or air demonstrated improvement in local control and survival for treatment under hyperbaric oxygen. However, this treatment approach has not been widely adopted because of the modest improvements, technical difficulties for patients and staff, and increased costs (Bomford 1993).

In addition to oxygen, a number of chemical compounds can increase the radiosensitivity of tumours because, like oxygen, these radiosensitising compounds are electron-affinic. The most extensively-investigated compounds are nitroimidazoles; within this group, misonidazole demonstrated *in vitro* success but unfortunately, no useful clinical gain was demonstrated and none of the nitroimidazoles can be recommended for routine clinical use (Bomford 1993).

b. Clinical Considerations

Tumour control is directly related to total dose of radiation. For subclinical disease ($<10^6$ cells per cm^3), 4500 to 5000 cGY are adequate to control disease in over 90% of patients (Fleming et al., 1995). The consideration of dose, time and number of treatment are based on the 4 R's of radiobiology and basically, fractionation spares normal tissues by facilitating repair of sublethal damage and repopulation between fractions, and increases tumour damage by facilitating reoxygenation of hypoxic cells and reassortment of cells into more radiosensitive phases. The biological effect of radiation on normal tissues and tumours depends not only on the total radiation dose, but also on the number of fractions per day, the dose per fraction and the total treatment time, and all of these parameters must be presented when reporting results of radiation therapy or comparing different studies of radiation effect (Fleming et al., 1995).

The curative or palliative intent of radiotherapy must be defined prior to the initiation of treatment. In a curative approach, a patient has a probability of surviving after adequate therapy whereas in palliative therapy, there is no hope for cure or extended survival. In curative therapy, significant

side effects may be acceptable although undesirable; in a palliative approach, side effects are less acceptable as the intent of therapy is to improve the patient's quality of life by relieving or preventing tumour-related complications. The optimal curative radiation dose can be determined by the therapeutic ratio which is the probability of eradicating the tumour to the probability of causing severe late damage to normal tissue. The dose-response curves of normal tissues and tumours are similar and in close proximity and therefore, it is not possible to provide curative radiation without complications (Fleming et al., 1995).

For delivery of curative external beam radiotherapy, standard fractionation regimes comprise 5 equal fractions per week (Fleming et al., 1995). For some tumours, conventional fractionation may fail as a cure because of rapid proliferation or repopulation of the tumours during the radiotherapy. If repopulation of tumours is a potential problem, one approach is to increase the fractions per day and shorten the overall treatment time (Wilson and McNally, 1992).

Hyperfractionation comprises a larger number of smaller doses, 100 to 120 cGy twice per day, for a higher total dose and hopefully, improved tumour control without the risk of complications.

Accelerated fractionation comprises multiple daily fractions of 150 to 200 cGy per fraction, decreasing the total time of treatment and overall dose. Hypofractionation includes a smaller number of larger fractions with a reduced overall dose in attempts to avoid serious complications. Split-course radiation consists of larger daily fractions (>250 cGy daily) with a planned break in the middle so acute reactions can subside; the overall treatment time is also decreased (Fleming et al., 1995).

c. Complications of Radiation Therapy

(i). Acute Complications

There is a clinical lag phase from the start of radiotherapy when the epithelial stem cells are killed, until the supply of cells to replace the desquamated cells is cut off and the mucosa is thinned and ultimately denuded (mucositis). Capillary dilation and swelling of the endothelium account for

erythema and edema. Erythema is also related to the acute inflammatory infiltration of the lamina propria and submucosa. A confluent mucosal reaction ensues and may include ulceration and pseudomembrane formation consisting of cell debris, fibrin and leucocytes. Surviving stem cells attempt to repopulate the mucosa but with a radical dose of daily fractions of 55 Gy, mucositis typically starts by the end of the second week, is maximal by the middle of the third week, and within a month of a 6-7 week course of treatment, the epithelium has regenerated (Bomford 1993). An acute inflammatory reaction also occurs in the major salivary glands where serous cells undergo degeneration more rapidly than mucous cells, resulting in a thick, mucous secretion (Baker 1982; Squier 1990, Epstein 1994). Xerostomia becomes increasingly prominent as therapy progresses; after 40 - 60 cGy, 80% of patients experience permanent xerostomia and beyond 60 cGy, permanent xerostomia occurs in all individuals (Bomford 1993). Alteration in taste is also an early response to radiation and precedes xerostomia and mucositis. Bitter and sour sensation are more susceptible to change than salty and sweet tastes, and individuals with the greatest pre-radiation taste discrimination appear to experience the quickest loss of taste. The causes of taste alteration may be related to edema of the taste buds, changes in saliva flow and composition or secondary infections; taste alterations may be permanent depending upon radiation dose (Rubin and Doku, 1967; Baker 1992; Epstein 1994).

The advantage of interstitial implantation lies in its delivery of a high radiation dose to a small volume. However, following interstitial implantation, necrosis may occur in the soft tissues and in the adjacent bone. Necrosis is related to the treated volume of tissue and the proximity of the implant to bone. Necrosis of the soft tissues alone is more common following implantation than external beam irradiation, and usually heals with conservative management within a few weeks (Bomford 1993), and there are few late sequelae (van der Waal and Pindborg, 1986).

(ii). Chronic Complications

The mechanisms underlying chronic complications are less well understood than the acute

complications and overall, significant recovery from the cellular effects of radical therapeutic irradiation is limited because the doses are already very close to the tissue tolerance limits. When endothelium is damaged by ionizing radiation, endothelial cells are lost and underlying collagen is exposed which is thought to promote thrombus formation. In a process termed endarteritis obliterans, the thrombus may be incorporated into the vessel wall and the intimal lining may proliferate to narrow or obliterate the vessel lumen; the ensuing chronic vascular insufficiency leads to atrophy. Ionizing radiation may also lead to fibrosis of the tissues and the hypocellular, hypovascular and hypoxic tissues are unable to effectively remodel or repair. Consequently, irradiated tissues are at risk for secondary infection or necrosis from even minor trauma, and bone is susceptible to osteoradionecrosis. With conventional fractionation (usually 180-200 Gy/fraction; Bomford 1993), radionecrosis is rare when radiation dose is under 60 Gy; the incidence is 1-2% at radiation doses up to 70 Gy, and above 9% at radiation exposures above 70 Gy (Bomford 1993). Osteoradionecrosis is more likely to develop secondary to necrosis of the overlying soft tissues, and the mandible is especially susceptible because its vascular supply depends solely on unilateral inferior alveolar arteries as compared to the collateral circulation available in the maxilla. Prevention of osteoradionecrosis begins with prophylactic extraction of teeth with questionable prognoses, especially in patients with poor oral hygiene and questionable compliance with dental treatment, avoidance of mucosal trauma from dental prostheses or appliances, cessation of smoking and excessive alcohol consumption, and maintenance of adequate nutrition (reviewed by Rubin and Doku, 1976; Baker 1992; Epstein 1994).

Permanent xerostomia may result from bilateral radiation exposure of the major salivary glands and at three years postradiation, saliva volume may be reduced by 95% (Epstein 1994). Changes in saliva composition compromise its roles as a permeability barrier, oral lubricant and anti-desiccant (Section IV.B.2); salivary antimicrobial activity is also reduced and may result in oral fungal infections and altered oral flora which become more cariogenic. The increased acidity of saliva and its decreased buffering capacity further compromise the remineralization of teeth so that rampant

caries of the cervical thirds and incisal edges may result if oral hygiene is not meticulous, and if neutral fluoride is not applied consistently (reviewed by Rubin and Doku, 1976; Baker 1992; Epstein 1994).

Fibrosis and scarring of the head and neck musculature and temporomandibular joint may limit mandibular movement, soft palate function and tongue mobility. In addition, pain following irradiation is a universal complaint and may be related to tumour recurrence, the treatment, or be unrelated to the tumour. Nevertheless, pain has emotional components and social implications that should be addressed by healthcare providers (Epstein 1994), (see also Section VII.F).

d. Clinical Applications of Radiotherapy for Oral Cancer

In general, the larger the field of irradiation, the lower the possible dose and the less likely the tumour control. Therefore, small and early stage I and II lesions of the oral cavity, can be controlled by either surgery or radiotherapy. If radiation is selected as initial treatment, it may include external beam teletherapy and/or interstitial brachytherapy (Shaha and Strong, 1995; Shah and Lydiatt, 1995)

As radiation therapy can be given only once, it is generally utilized in more advanced conditions or for recurrent tumours, and for patients not suitable for surgical treatment. Management of advanced cancers of the head and neck such as stage III and IV typically employ combined therapy (i.e. surgery followed by radiation therapy) which can improve local control and increase 5-year survival rates as compared to rates demonstrated by surgery alone (Shaha and Strong, 1995). Indications for postoperative radiotherapy of the primary tumour include large tumours with significant risk of recurrence (T3 and T4), histologically positive surgical margins, and perineural or perivascular invasion. Indications for postoperative radiotherapy of the neck include gross residual tumour in the neck following neck dissection, multiple positive lymph nodes and extranodal extension of tumour (Shah and Lydiatt, 1995).

Lymph node spread is most likely to occur within two years following treatment, and 50% of block dissections are carried out within 3 months of completion of treatment of the primary (Bomford 1993). Elective treatment of N0 disease by radiotherapy includes necks that were not treated surgically but where the risk of micrometastasis is high, patients who are unlikely to be compliant with follow-up, and patients whose general condition contraindicates a radical neck dissection (Bomford (1993). In general, postoperative and elective radiotherapy covers the primary site and the entire neck, bilaterally, with recommended doses in excess of 6000 cGy and with a boost to high risk areas (Shah and Lydiatt, 1995).

In planning radiation teletherapy, the clinician defines the target volume which is the tumour and surrounding margins which are to be included in the treatment; the treatment volume refers to the area actually covered by the radiation beams (Bomford 1993). Attempts are made to spare uninvolved tissues or structures during radiation treatment-planning of target volumes, and by moving the uninvolved part out of the target volume by means such as mouth bites, or shielding or blocking out portions of the radiation field with lead. The target volumes are carefully controlled by immobilizing and reproducibly positioning the patient through the use of customized acrylic shells or head holders, etc. In teletherapy, two or more fields are typically used to converge and create the treatment volume (Bomford 1993). A parallel opposed pair comprises two directly opposing radiation fields; the radiation is distributed symmetrically about the axes of the two beams and irradiation is uniform to the tissues in the treatment volume; parallel opposed pairs are used when tumours are large or involve the midline. The box technique comprises two intersecting parallel pairs which can produce distributions of different shapes depending upon the angle between the axes of the two beams. Two beams may also be used with wedges to create a wedged pair. Wedges are a wedge-shaped piece of metal that is positioned within the radiation beam to attenuate the beam and thereby reduce the beam dose and tilt the isodose curves through an angle. This technique is used to deliver unilateral radiation fields (Bomford 1993).

e. Radiotherapy for Lingual Cancer

(i). Oral Tongue

Surgery and radiotherapy are equally effective for curing early (T1) well-differentiated SCCs. Interstitial implantation may be considered for T1 and small, noninfiltrating T2 tumours of the lateral border but larger T2 and T3 tumours may be too extensive for brachytherapy and should be treated by teletherapy, surgery or a combination (Bomford 1993). The target volume typically includes the primary tumour with at least a 2 cm margin, and the submandibular and upper deep cervical nodes. For lateral tongue tumours a lateral and anterior wedge pair are suitable, but if the tumours extends across the midline, a parallel opposed pair of fields is used (Bomford 1993). More advanced T3 or T4 tumours generally require surgical resection followed by radiotherapy, and inoperable tumours require radical irradiation to the primary and draining nodes (Bomford 1993).

(ii). Base of Tongue

Interstitial implantation of the BOT is rarely practical because of difficulty of access and the risk of serious complications which include necrosis and edema, and secondly, BOT lesions usually present at advanced stages that preclude implantation (Bomford 1993). Nevertheless, Zelefsky et al., (1992) and Harrison et al. (1992) advocated the use of external beam radiation followed by interstitial implantation for T1-T3 tumours of the BOT.

Surgery of the BOT may involve a total glossectomy and resultant major functional impairments and therefore, radiotherapy is the preferred treatment although it may also be used in conjunction with surgery (Zelefsky et al., 1992; Bomford 1993). Harrison et al. (1992) evaluated 36 patients treated with external beam and brachytherapy for BOT cancer. Soft tissue necrosis or osteonecrosis occurred in 25% of patients, but 93% of patients retained excellent speech articulation and 83% denied a restriction in diet, in place of eating or social companionship during eating; survival at 2 years was 87.5% (Harrison 1992). Harrison et al. (1994) compared the quality of life and functional outcome in patients with BOT SCC whose primary treatment was

either radiotherapy (external beam followed by interstitial implantation, 30 patients) or surgery followed by external beam radiotherapy (10 patients). Both early and advanced stages of disease were included in each treatment group but patients treated with radiation alone consistently reported better quality of life and had better performance in regards to eating, normalcy of diet and understandability of speech as compared to patients treated by surgery and radiotherapy ($p < 0.0001$). For patients treated primarily by surgery, functional status deteriorated significantly ($p = 0.0014$) when comparing T1-2 lesions with T3-4 lesions, an observation consistent the fact that larger tumours required more extensive excisions (Harrison et al., 1994).

As the incidence of regional lymphatic metastasis is very high for BOT tumours, the primary tumour and lymphatic levels 1-V are generally included in the target radiation volume. A parallel opposed pair of lateral wedged fields is used to cover the primary and upper neck; the spinal cord is shielded and the lower neck may receive a direct field (Bomford 1993).

3. Chemotherapy

a. Mechanisms of Action

The potential toxicities of chemotherapy include mucositis, bone marrow suppression, nausea and vomiting. Most chemotherapeutic agents exert the bulk of their toxicities and their antitumour effects by interfering with the synthesis or function of nucleic acids. Therefore, these agents are most effective against proliferating cells, characteristically those in S phase, and tumours with high growth fractions (Fleming et al., 1995). As well, a drug may be incompletely absorbed, rapidly metabolized and excreted, or incompletely converted to its active form. Drug resistance also develops due to selection pressure for drug-resistant cells which develop from spontaneous mutations, but chemotherapeutic agents may act as mutagens themselves by adding to the genetic instability of malignant cells and by increasing the rate of spontaneous mutations which promotes drug resistance (Fleming et al., 1995). Clones of resistant cells replace sensitive clones during treatment so that delays in treatment not only permit an increase in tumour burden, but can result in

a greatly increased probability of drug-resistant cells. Some malignancies appear resistant to chemotherapy because the tumour cells are anatomically inaccessible to the drugs such as tumours of the central nervous system (Fleming et al., 1995).

In many cases, a chemotherapeutic agent kills cancer cells in several ways, some of which are not well understood. Conventionally, these drugs are categorized as alkylating agents, antimetabolites, antitumour antibiotics, plant alkaloids, hormonal agents or as miscellaneous compounds. These categories are briefly reviewed below with a focus on agents that have been investigated in treatment of head and neck tumours.

Alkylating agents such as cyclophosphamide react with preformed nucleic acids, disrupting DNA replication and transcription; they tend to be active throughout all phases of the cell cycle making them attractive treatments for tumours with low growth fractions. However, alkylating agents also cause delayed, prolonged and even permanent marrow failure, and have a mutagenic effect on marrow stem cells so that the risk of developing acute myelogenous leukemia is directly proportional to the alkylating-drug dose (Fleming et al., 1995). Antimetabolite agents include 5-fluorouracil (5FU) and methotrexate; these agents interfere with nucleic acid synthesis and have maximum effects in S phase. Antimetabolites are not associated with delayed or prolonged myelosuppression and they present a minimal risk of carcinogenesis (Fleming et al., 1995).

The tumour antibiotics are all derived from species of *Streptomyces*. Bleomycin is a tumour antibiotic which acts by binding to DNA and causing breaks in the DNA stands. As a result, cells accumulate in G2 phase and there are no significant myelosuppressive effects (Salmon and Sartorelli, 1995). The plant alkaloids include the vinca alkaloids, vincristine and vinblastine, and the alkaloid ester, paclitaxel (Taxol). Paclitaxel enhances polymerization and stabilization of microtubules so that mitosis is disrupted; toxicities include neutropenia, thrombocytopenia and peripheral neuropathy (Salmon and Sartorelli, 1995). The miscellaneous compounds include

cisplatin and its analogue carboplatin. Cisplatin is an inorganic complex of the platinum atom, two amine groups and two chlorine atoms. Its mechanism of action and toxicity are thought to be analogous to that of alkylating agents. Cisplatin is most effective in G1 but kills cells in all stages of the cell cycle by binding to DNA and forming interstrand crosslinks. Cisplatin is nephrotoxic but has relatively little effect on the bone marrow; carboplatin has less renal toxicity but is myelosuppressive (Fleming et al., 1995; Salmon and Sartorelli, 1995).

b. Clinical Applications

A curative role for chemotherapy in tumours of the head and neck has not been established (Bomford et al., 1993), but chemotherapy may have a role in sensitizing tumours of the head and neck to radiation (Fleming et al., 1995). Chemotherapy may also be used as initial treatment of advanced tumours or recurrent disease when surgery or radiation is unlikely to result in cure of micrometastases, or in palliative therapy of symptomatic patients where other treatments have failed (Bomford 1993; reviewed by Epstein 1994).

Methotrexate, cisplatin and bleomycin are the most-tested single chemotherapy agents used in treatment of upper aerodigestive tract cancers and response frequencies vary depending on prior treatment and disease stage (Aisner et al., 1994). Where chemotherapy was used in the setting of recurrent or metastatic disease or where chemotherapy was only palliative, the response frequencies for complete clearance of the tumour varied from 20% to 60% for methotrexate, from 17% to 40% for cisplatin, from 6% to 30% for bleomycin, and 41% for paclitaxel (Aisner et al., 1994). Single-agent and combined chemotherapies used as initial or induction treatments have demonstrated enhanced rates of response (overall, about 50%) compared with similar treatments for recurrent disease; the combination of cisplatin/5FU in induction therapy had an overall response rate of greater than 80%, and carboplatin/5FU produced a 78% response rate (Aisner et al., 1994).

Chemotherapy agents such as bleomycin, 5FU and methotrexate have also been used in

combination with radiotherapy but myelosuppression and increased severity of mucositis often resulted in a delay or reduction of the chemotherapy dose, the radiotherapy dose, or both. Some trials demonstrated improved clearance rates and prolongation of time to tumour progression as compared to those treated with radiotherapy alone, but most trials failed to demonstrate a significant improvement in survival for the combined modality group (Aisner et al., 1994). However, cisplatin may have potential as a radiation-sensitizing agent as cisplatin and concurrent radiotherapy demonstrated 69% clearance rates and median survival of 24 months among poor-prognosis patients with advanced unresectable regional disease, although survival was not significantly altered. However, for patients with poor prognoses, chemoradiotherapy may offer a better quality of life including less anatomic deformity as compared to aggressive and often deforming aggressive surgical approaches, and less severe radiation complications as a result of smaller field sizes compared with large radiation-field, high dose radiotherapy, or both (Aisner et al., 1994).

4. Treatment of Tongue Cancer at the Memorial Sloan-Kettering Cancer Center

During the 50 years between 1927 and 1978, over 3100 patients with tongue cancer were treated at the Memorial Sloan-Kettering Cancer Center in New York, USA. The obvious advantage of such a large patient pool within one cancer center, is that it has permitted investigators to assess the impact of changing trends in the management of tongue cancer (Franceschi et al., 1993), and several publications from this Cancer Center have documented a significant improvement in the 5-year survival rates of tongue cancer over time (eg. Callery et al., 1984; Franceschi et al., 1993). Unfortunately, some earlier reports from this Cancer Center reported "cure rates" (eg. Frazell and Lucas, 1962) without indication of the time period involved, and some survival rates were calculated at 2 years (Harrison et al., 1992), some at 5 years (eg. Kraus et al., 1993) and some at 7 years (Zeleftsky et al., 1992). The criteria for calculating "cure rate" were not specified (eg. Frazell and Lucas, 1962, Spiro and Strong, 1972) and hence, it was not known if cure rate was an equivalent measure to disease-free survival (Section II.J). Moreover, some survival rates were

limited to oral tongue lesions (Franceschi et al., 1993) but were compared to survival rates for oral and BOT lesions (Callery et al., 1984). Consequently, survival and cure rates between studies cannot be readily compared (see also Sections II.D and II.J).

Prior to 1940, irradiation employed alone or in varying combinations with surgery, dominated as the treatment method and resulted in a cure rate of 25%. Between 1939 and 1953, the cure rate was 35.4% for tongue lesions of which 68% were T1,T2 and 32% were T3,T4 (Frazell and Lucas, 1962). The increase in cure rate from 25% to over 35% was attributed to the increased utilization of more aggressive surgery of the primary site and neck, although surgery was still used in combination with external beam radiotherapy and interstitial implantation (Frazell and Lucas, 1962).

Between 1957 and 1963, the 5-year cure rate for oral tongue cancers was 62.1% among a majority of stage I, II (93%) lesions treated primarily with partial glossectomy as radical neck dissections were reserved for management of subsequent clinical metastases (Spiro and Strong, 1972).

During this same time interval, an overall 5-year cure rate of 42% was reported for oral and BOT lesions treated primarily with surgery (Spiro and Strong, 1974). This cure rate included a rate of 47.7% for oral tongue lesions of which almost 37% were Stage III, IV, and a cure of 30% for BOT lesions of which 90% were stage III, IV. However, when patients with similarly-staged lesions were compared, the results of surgical treatment were the same regardless of whether the tumour arose in the oral portion or the BOT (Spiro and Strong, 1974).

The increase in the cure rate for tongue cancer from 25% to 42% from 1927 through 1963, was attributed to a decline in the proportion of patients with advanced disease and extensive lymph node involvement, as well as a shift to safer surgery and more effective radiotherapy (Callery et al., 1984). Between 1969 and 1978, treatment focus shifted again, this time to more conservative surgery than previously performed at this center. As well, adjuvant radiotherapy and elective neck

dissections were utilized more frequently, and the overall 5-year cure rate for tongue cancer rose by 2% to 44% (Callery et al., 1984). This was an insignificant increase from the earlier report (42%, Spiro and Strong, 1974) since the overall distribution of clinical stages did not change.

Nevertheless, the 5-year cure rate for stage I, II patients was 75%, and 37% for patients with stage III, IV disease (Callery et al., 1984), but the cure rates for oral tongue as compared to BOT lesions were not differentiated.

Between 1978 and 1987, management protocols for tongue cancer at Memorial Sloan-Kettering Cancer Center shifted to more conservative tongue and mandible-sparing surgeries along with increased use of elective neck dissections such as the supraomohyoid dissection, and increased use of postoperative radiotherapy, particularly in patients with high-stage tumours or involved surgical margins. As a result, treatment of tongue cancer has resulted in less morbidity and improved quality of survival (Franceschi et al., 1993). The overall 5-year survival from oral tongue cancer rose to 65% with survival for stage I, II tumours at 82%, and 49% for stage III, IV disease (Franceschi et al., 1993). However, it should be stressed that the 5-year rate of 65% reported by Franceschi et al. (1993) included only oral tongue lesions whereas the 5-year cure rate of 44% reported by Callery et al. (1984) included both oral and BOT lesions. Nevertheless, Franceschi et al. (1993) compared their rate of 65% to the 44% cure rate reported by Callery et al. (1984), and concluded that this improvement was statistically significant ($p=0.03$) because the distribution of patients by stage was the same (Franceschi et al., 1993). Moreover, the most significant improvement in survival between the two time periods, occurred in patients with advanced disease (37% versus 49% survival) and was attributable to more aggressive and effective treatment of the neck including elective dissections and postoperative radiotherapy (Franceschi et al., 1993).

The proportion of patients receiving adjunctive radiotherapy increased from 6% during 1957 to 1963 (Spiro and Strong, 1974), to 31% between 1969 and 1978 (Callery et al., 1984), and to 70% between 1978 and 1987 (Franceschi et al., 1993). Among stage III and IV patients in the 1993

study, the incidence of neck recurrence among patients who received adjunctive radiotherapy was 13% as compared to 29% for patients who did not receive radiotherapy. Although no survival advantage for radiotherapy in these advanced-stage patients could be demonstrated, the only long-term survivors (minimum follow-up was 5 years) among patient with stage IV disease were those who received postoperative radiotherapy (Franceschi et al., 1993).

The shift towards tongue preservation with combined external beam radiation and brachytherapy in lieu of surgery also affected treatment of BOT lesions at Memorial Sloan-Kettering Cancer Center. Zelefsky et al. (1992) reviewed 31 cases of BOT cancer treated between 1973 and 1986 with surgery followed by postoperative radiation, and reported a 7-year "control rate" of 81% but 21% incidence of neck failure. Kraus et al (1993) reviewed 100 patients with BOT SCC whose primary tumours were resected between 1979 and 1989. Overall, 62% of patients had a clinically-involved neck, and 80% of patients had stage III or IV disease that included 18 patients with a clinically N0 neck. Continuity of the mandible was maintained in 86% patients and the larynx was preserved in 80%. Postoperative radiation was administered to 63 patients due to positive resection margins, positive nodes or high tumour stage. Overall 5-year survival rates were 55%, and cause-specific 5-year survival was 65%. The 5-year cause-specific survival for stage I/II was 77%, stage III was 64% and stage IV was 59%. Kraus et al. (1993) concluded that surgery was a viable treatment option for patients with advanced BOT cancer but they admitted to failure of objective assessment of speech and swallowing function following treatment.

Harrison et al. (1989, 1992) reviewed BOT tumours treated with external beam radiation in combination with interstitial implantation and reported local "control rates" of 88% for T1-T4 lesions (Harrison et al., 1989; 1992) with no significant differences in local control by T stage (Harrison et al., 1992), and survival at 2 years was 87.5% (Harrison et al., 1992). As radiation therapy maintained a better performance status and provided similar local control and survival as compared to surgery for BOT cancer, radiation treatment (external beam and interstitial

implantation) was the preferred treatment strategy for BOT cancer at Memorial Sloan-Kettering Cancer Center (Zelevsky et al., 1992; Harrison et al., 1992; Harrison et al., 1994).

F. Follow-up Care and Prevention

Day et al. (1994a, b) followed a cohort of 1090 oral/pharyngeal cancer patients originally enrolled in a population-based case-control study of oral cancer in the United States (Blot et al., 1988), and evaluated patient characteristics and survival patterns of patients who developed second cancers. Second cancers were considered to be a second primary if the possibility of metastasis of the original oral cancer was excluded, if the second tumour was not found at the same time or within 2 months of the index oral cancer, if the second tumour was clearly malignant histologically, and if the second tumour was anatomically separated by normal-appearing mucosa. First primary tumours had been diagnosed over a 15-month period in the mid 1980's and patient follow-up ranged from 51 to 66 months (Day et al., 1994a). During that time, 64% of the 1090 patients had died, 2.5% were lost to follow-up, but 33.5% (365) were alive. A total of 107 patients (9.8% of the original 1090 patients) developed a second cancer; 12 patients (1.1% of the original 1090 patients) had their second tumour between 2 and 6 months after the first diagnosis, and 95 (8.7% of the original 1090 patients) were detected at 6 months or more after the index cancer. Most of the second cancers arose in the aerodigestive tract comprising the oral cavity, pharynx, oesophagus and lung, but the development and site of the second cancer was independent of the site of the index cancer as nearly identical percentages of second cancers occurred following index tumours of the tongue, pharynx or other oral site. The majority (92%) of index cancers were SCCs but histology of the second cancers typically differed from that of the index tumour as only 50% of second tumours were SCC, 30% were adenocarcinomas and 14% were some other type of carcinoma or non-epithelial tumour (Day et al., 1994a).

The overall rate of a second cancer among oral/pharyngeal cancer patients was 3.7% per year but the risk increased over time: for the first 2.5 years the rate was 3.4%, but the rate between 2.5 and

5 years was 4.4% (Day et al., 1994a). Patients aged 60-69 years had the highest annual rate of second primary tumours and the average age at initial diagnosis was an important determinant of whether a second cancer developed; the average age at index diagnosis for patients with a single tumour (60.9 years) was significantly ($p<0.001$) lower than the age of patients who subsequently developed second cancers (64 years). The mean time to occurrence of a second cancer was 27 months, the median time was 24 months. Survival was significantly ($p<0.001$) lower among patients with a second cancer. Conditional on survival for at least 3 years, survival at 5 years following the index diagnosis was 80.2% for patients without a second cancer and 62.2% for patients with a second cancer (Day et al., 1994a).

Information on alcohol and tobacco consumption was obtained for 80 patients with second primary cancers (cases) and 189 sex-and-survival matched patients without a second primary (controls), (Day et al., 1994b). Nearly all patients with index tumours had been tobacco smokers and/or regular consumers of alcohol, and these longstanding habits were linked to their markedly elevated risk of second aerodigestive tract cancers although the effects of smoking were more pronounced than the effects of alcohol (Day et al., 1994b). The odds ratios for smoking (adjusted for alcohol) increased with intensity ($p=0.04$) and duration ($p=0.01$) of smoking and reached 4.7 (95% CI=1.3, 17) among smokers of 40 or more cigarettes per day for 20 or more years. Among current smokers as compared with ex-smokers and never-smokers, odds ratios reached 4.3 (95% CI=1.6, 12). The risk of a second cancer slowly declined with duration of smoking cessation, and the risk was lowest among persons who had quit at least 1 year before the index diagnosis. Among consumers of alcohol, only consumption of 15 or more drinks of beer demonstrated statistically significant ($p=0.02$) increased risks (OR 3.8, 95% CI=1.2, 12) and there was little or no excess risk for consumption of hard liquor or wine. However, among smokers who were also heavy drinkers (≥ 15 alcoholic drinks/week), the odds ratio was 4.5 (95% CI=1.2, 16), (Day et al., 1994b).

Macfarlane et al. (1995) investigated second cancers among 10,839 men with previous oral/pharyngeal cancers from Australia, Scotland and Slovenia. A total of 919 second primary tumours occurred, representing an incidence rate of 2.9 per 100 person years; 15% of the second cancers occurred in the oral cavity and 60% in the upper aerodigestive tract. In all three countries, the overall risk of a second cancer was highest in the first 5 years after diagnosis of the index cancer, and relative risks varied from 2 (95% CI=1.6, 2.4) in Scotland and Australia to 3.5 (3.0, 4.0) in Slovenia. After 5 years the risks diminished but not until 10 years after initial diagnosis, was the risk close to that of the general population (Macfarlane et al., 1995).

Since local/regional recurrence typically occurs in the first 2 to 3 years following initial treatment of head and neck cancer, and the risk of a second primary tumour rises at the rate of 3% (Day et al., 1994a) to 6% (Shah and Lydiatt, 1995) per year for the first 2 years, routine physical examinations of the head and neck, and chest radiographs are required on a periodic basis. For the first two years following treatment, follow-up examinations are typically scheduled every 2 months. For the following two years they are scheduled every 3-4 months and then no less than every 6 months for the balance of the patient's lifetime (Shah and Lydiatt, 1995).

Counselling and assistance with life-style changes to reduce exposure to carcinogens and reduce the risk of second primaries are also integral parts of follow-up care. Day et al. (1994b) observed that 36% of patients continued to smoke after the first diagnosis of oral/pharyngeal cancer but heavy smokers compared to moderate smokers were more likely to quit following the first diagnosis of oral/pharyngeal cancer. As well, most patients with oral/pharyngeal cancer continued to drink; 44% of heavy drinkers continued to drink at the same levels and only 33% of patients reduced their intake (Day et al., 1994b).

The length of survival following head and neck cancer may be an inadequate measure of the success of treatment as it ignores the quality of survival. Follow-up cancer treatment should also

monitor patients' well-being and coping mechanisms as survivors and patients with oral and pharyngeal cancer often suffer serious functional impairments. Moreover, head and neck cancer patients often have a history of alcohol abuse, heavy smoking, poor eating habits and lower socioeconomic status which may influence their quality of life and be associated with compromised coping skills (Routh and Hickman, 1991; Beeken and Calman, 1994; Bjordal et al., 1995b).

Beeken and Calman (1994) assessed 25 British patients between 18 months to 7 years after treatment with surgery and/or radiotherapy for oral/pharyngeal cancer. The major complaints included xerostomia, difficulty with eating, swallowing and speech, taste changes and sore mouth. Side-effects which specifically affected eating were reported by 80% of patients and the side effects perceived to be of greatest importance to quality of life all related to the ability to eat (Beeken and Calman, 1994).

At 12 months following treatment for oral and pharyngeal cancer, Languis and Lind (1995) classified 42 Swedish patients with regard to the extensiveness of their surgery. All patients had received surgery involving the tongue and 79% of patients also received radiotherapy. Overall, the patients perceived themselves as less well-informed about the psychological aspects of their disease and its treatment than the post-treatment-related conditions. The post-treatment conditions perceived as most severe by the patients included mouth dryness, difficulties in swallowing, eating, chewing, difficulties with speech, and inability to do things as before. Additional, less severe complaints included pain in relation to disease or treatment, disfigurement, altered taste and smell, and decreased social life. There were no significant correlations between perceived severity of complaints and extent of the surgery ($r=0.15$), age ($r=-0.3$) or gender ($r=0.24$). However, the less a patient's anxiety and the stronger a patient's sense of coping and perceived general health, the less serious was the patient's perception of conditions related to surgery or radiotherapy. Thus, personality rather than extensiveness of the treatment was the major determinant of a patient's perception of the seriousness of the situation. The authors concluded that pretreatment

psychological assessments would be useful in decision-making about treatment choices and supportive post-treatment measures (Languis and Lind, 1995).

Bjordal et al. (1995a) assessed 50 Norwegian patients at 1 to 6 years following treatment for head and neck cancer. The patients reported reduced quality of life and post-treatment side effects that included xerostomia, oral candidiasis, fibrosis, edema of the neck, personality disorders, pain, anxiety and depression, trismus, cosmetic problems and problems with speech, eating, swallowing as well as problems with teeth. Overall, the patients reported lower quality of life and increased frequency of symptoms and problems as compared with assessments provided by clinicians. The authors concluded that clinicians were not sensitive enough to patients' problems but clinicians' abilities to accurately assess problems could be improved by using standardized examination programmes comprising patient self-report questionnaires, clinician-rated questionnaires, clinical interviews and clinical examinations. In addition, patient self-reports may be a useful tool for improving communication between clinicians and patients (Bjordal et al., 1995a).

Bjordal et al. (1995b) contend that knowledge about a patient's quality of life before cancer is not available, and therefore the distress of cancer patients cannot easily be separated from the effects of the disease and its treatment. These investigators assessed 204 survivors from a prospective randomized clinical trial of two different but radiobiologically-equivalent radiotherapy fractionation regimes, and the patients were matched to 766 community controls that were participants in a large population health survey in Norway. Compared with controls, the patients reported significantly lower satisfaction with life and physical health but these findings could not be explained by clinical treatment variables or socio-demographic variables. Although head and neck cancer patients surviving 5 years are considered to be cured, physical limitation and changes of appearance after treatment are constant reminders of disease and a source of chronic stress. Moreover, head and neck cancer patients may have an increased risk of developing psychosocial problems due to the association of their disease with alcohol abuse and low socioeconomic class. As well, the

increased ages of the typical oral/pharyngeal cancer patients may compromise their functional abilities, quality of life and increase the likelihood of intercurrent illnesses. Overall, head and neck cancer patients have a high risk of long-term psychosocial morbidity, and psychological interventions may result in significant improvements in quality of life for these patients (Bjordal et al., 1995b).

G. Summary

This Chapter provided criteria for evaluation of the literature (Section II) and reviewed the normal structure and functions of the tongue (Section III) and oral epithelium (Section IV). Possible mechanisms of carcinogenesis, risks factors (Section V) and oral premalignancy (Section VI) were reviewed as well as the epidemiology and treatment of oral/pharyngeal cancer (Section VII).

Overall, general conclusions across studies and between countries over time for trend data cannot easily be drawn, but the tongue remains a common site for oral/pharyngeal cancers which represent about 3% of all body cancers. The balance of this thesis reviews 328 cases of SCC of the tongue that were admitted to the Vancouver Branch of the BCCA over the 16-year period between 1979 and 1994.

Tumour

TX	minimum requirements to assess primary tumour cannot be met
T0	No evidence of primary tumour
Tis	carcinoma in situ
T1	tumour ≤ 2 cm in greatest dimension
T2	tumour > 2 cm but ≤ 4 cm
T3	tumour > 4 cm
T4	tumour invades adjacent structures, eg through cortical bone, into deep (extrinsic) muscle of tongue, maxillary sinus, skin (invasion of intrinsic tongue muscles is not classified as T4)

Nodes

NX	minimum requirements to assess regional lymph nodes cannot be met
N0	no evidence of regional lymph node involvement
N1	single ipsilateral lymph node, ≤ 3 cm in greatest dimension
N2a	single ipsilateral lymph node, > 3 cm but ≤ 6 cm (midline nodes are considered ipsilateral nodes)
N2b	multiple ipsilateral lymph nodes, ≤ 6 cm
N2c	bilateral or contralateral lymph nodes, ≤ 6 cm
N3a	ipsilateral node > 6 cm
N3b	bilateral nodes > 6 cm

Metastasis

MX	minimum requirements to assess distant metastases cannot be met
M0	no evidence of distant metastases
M1	distant metastases present

Staging

Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T3	N0	M0
	T1,T2,T3	N1	M0
Stage IV	T4	any N	M0
	any T	N2,N3	M0
	any T	any N	M1

Table 1.12. TNM Classification of the lips, oral cavity and oropharynx. T refers to the extent of the primary tumour, N refers to the presence or absence of regional lymph node metastasis and M refers to the presence or absence of distant metastases. For assessment of the T category, both clinical examination and radiography is required (but not for the lips); for the N category, only clinical examination is required; and for the M category, both clinical examination and radiography are required (van der Waal and Pindborg, 1986). If there is doubt concerning the correct T, N or M category to which a case should be allotted, the lower category is selected. For lip, oral and oropharyngeal tumours, the clinical (TNM) and postsurgical histopathological (pTpNpM) classifications coincide, but the clinical classification is used for purposes of reporting and evaluation.

Adapted from van der Waal and Pindborg, 1986; UICC TNM Atlas, Third Edition, 1990; and American Joint Committee on Cancer, Manual for Staging for Cancer (3rd edition), Philadelphia: J. B. Lippincott; 1988.

<u>1991</u>		<u>Number</u>	<u>Percent</u>
Total New Cancers Diagnosed		109442	
Oral Cavity and Pharynx		3017	2.8
Tongue		516	.47
<u>1993</u>			
Total Cancer Deaths		56144	
Oral Cavity and Pharynx		995	1.7
Tongue		224	.43
<u>1996 Estimates</u>			<u>Death:Cases</u>
Oral Cavity and Pharynx Cancers			
Estimated New Cases		3090	0.35
Male		2200	0.35
Female		890	0.34
Estimated Deaths		1070	
Male		770	
Female		300	
Age-standardized Incidence Rate		<u>Rate per 10⁵ population</u>	
Male		15	
Female		5	
Age-standardized Mortality Rate			
Male		5	
Female		2	
<u>Lifetime Probabilities</u>		<u>Percent</u>	<u>One in:</u>
Developing Cancer			
Male		41.6	2.4
Female		37.1	2.7
Developing Oral/Pharyngeal Cancer			
Male		1.8	57.1
Female		data not available	
Dying from Cancer			
Male		26.7	3.7
Female		22.4	4.5
Dying from Oral/Pharyngeal Cancer			
Male		0.6	169.5
Female		data not available	

Table 1.13. Summary of Cancer Statistics for Canada. Data summarized from Canadian Cancer Statistics 1996; National Cancer Institute of Canada 1996.

Age-standardized Rates**Rate per 10⁵ BC population**

<u>Incidence</u>	<u>Male</u>					<u>Female</u>				
	1990	1991	1992	1993	1994	1990	1991	1992	1993	1994
All Cancers (excluding skin)	316	326	330	342	327	283	285	288	291	282
Tongue Cancer	2.4	2.1	2.1	2.0	2.0	1.3	0.8	1.1	1.1	0.7
Mouth Cancer	3.6	3.7	3.5	2.8	3.0	1.9	2.2	2.7	2.5	2.3
Pharynx Cancer	3.6	3.9	4.3	4.2	3.8	1.7	1.6	1.7	2.1	1.6
<u>Mortality</u>										
All Cancers (excluding skin)	149	149	147	144	143	126	124	121	122	125
Tongue	0.8	0.9	0.9	1.0	0.9	0.6	0.5	0.1	0.5	0.5
Mouth	0.8	1.2	0.8	0.6	0.7	0.5	0.4	0.5	0.2	0.7
Pharynx	2.1	2.0	1.7	1.5	1.9	0.8	0.8	0.9	0.5	0.6

1994

	<u>Number</u>	<u>Percent</u>
Total New Cancers Diagnosed	14971	
Mouth	125	0.84
Tongue	60	0.4
Pharynx	124	0.83
Total Cancer Deaths	6945	
Mouth	37	0.53
Tongue	36	0.52
Pharynx	63	0.91

1996 Estimates

Estimated New Cases	16858	
Mouth	153	0.91
Tongue	81	0.48
Pharynx	136	0.81
Estimated Cancer Deaths	7444	
Mouth	35	0.47
Tongue	32	0.43
Pharynx	66	0.89

Table 1.14. Summary of Cancer Statistics for British Columbia. Data summarized from 1995-1996 Annual Report, BC Cancer Agency, BC Cancer Research Centre, BC Cancer Foundation

CHAPTER 2

METHODS

In 1994 and 1995, all the files of patients with tongue cancer admitted to the Vancouver Branch of the British Columbia Cancer Agency (BCCA), Vancouver, British Columbia, Canada from 1979 to 1994 were reviewed by Dr. J. Hay, Dr. J. Epstein and Dr. Eric van der Meij. The review included a total of 332 cases of tongue cancer; 4 cases were adenocarcinomas which were subsequently omitted to leave 328 cases of lingual SCC. For discussion purposes in this thesis, the series of 328 cases of lingual SCC is referred to as the BCCA-1994 case series.

The data fields comprising patient and tumour characteristics, treatment modalities and outcomes were designated according to criteria established by Dr. J. Epstein and Dr. Van der Meij. The data were entered into a computer and analyzed by Dr. N. Le. Multiple independent chi-squared tests were performed in a search for associations that could generate hypotheses (Chapter 1, Section II.F.3.b) and evidence of associations between various characteristics was assessed using the likelihood ratio (LR) test (Parmar and Machin, 1996). Some comparisons included cells with very small numbers of cases (i.e. less than 5 cases) and consequently, those p values were considered to be unreliable. The p values were included in the results only if $p < 0.05$, and if they were considered to be reliable (i.e. there are no cells with less than 5 cases per cell).

Survival analyses relied upon the criteria assessed at patient follow-up, and the criteria used to determine the clinical status at last follow-up are shown in Table 2.1. Survival time was measured from the start of treatment, to a subsequent event. Survival was calculated for the following events: survival to local recurrence of cancer, survival to regional recurrence of cancer, survival to distant metastases, survival to death from all causes, survival to death related to the tongue cancer or its treatment. The first evidence of local disease recurrence was treated as an event. That is, it was assumed that the patient did not have disease recurrence at the last time of follow-up and a similar approach was used for regional recurrence and distant metastases. Patients for whom the

event of interest was not observed, were censored (eg. Sheps 1995; Parmar and Machin, 1996).

The survival curves were obtained based on this approach.

Alive

- a. Without disease
- b. With local disease
- c. With regional disease
- d. With both local and regional disease
- e. With local or regional and distant disease
- f. With distant disease, only
- g. Status unknown

Dead

- h. NED
- i. With local disease
- j. With regional disease only
- k. With both local and regional disease
- l. With distant mets only
- m. With local or regional and distant mets
- n. With another primary
- o. With complications from surgery
- p. Unrelated cause with disease
- q. Status unknown
- r. Lost to follow-up

Alive

- s. NED but with another cancer

Table 2.1. Criteria for Clinical Status at Last Follow-up used in Survival Analyses.

For calculation of overall (all-cause) survival, status codes (h) to (q) were considered as an event, and the remaining status codes were censored.

For calculation of DS (disease-specific or cause -secific) survival, status codes (i) to (m), and (q) were considered as an event, and the remaining status codes were censored.

NED refers to "No Evidence of Disease."

CHAPTER 3

RESULTS

This chapter is divided into 2 sections which correspond to (I) Descriptive/Association Statistics and (II) Survival Analyses of the data. The tables and figures that pertain to each section are presented in order, at the end of either Section I or II.

I. Descriptive/Association Statistics

A. Cases by Year and Sample Demographics

1. Cases by Year

In the 16-year period between 1979 and 1994, 328 patients with SCC of the tongue were admitted to the Vancouver Branch of the British Columbia Cancer Agency (BCCA). Approximately 81% (n=265 cases) of the cases were admitted between 1980 and 1988 (Table 3.1A, Figure 3.1).

2. Gender, Age and Stage of Disease

A greater proportion of tongue cancers occurred in males (~60%) than females (~40%), (Table 3.1B, Figure 3.2), and the male:female ratio for cancer of the tongue was 1.50:1.0. The mean age of all patients was 61.1 years and ranged from 18 years to 96 years.

The majority of patients had an early stage of disease (~55% had carcinoma in situ, stage I or stage II disease), (Table 3.1C, Figure 3.3). In similar fashion, the majority of all male patients (~55%) as well as the majority of all female patients (~54%) had an early stage of disease (carcinoma in situ, stage I or stage II disease), (Table 3.1D, Figure 3.4).

In both males and females, the majority of the tongue cancers occurred in individuals between the ages of 50 and 79 years (n=248 cases, ~76%), (Tables 3.1C, D; Figures 3.3, 3.4). In this age group, the majority of the tumours were an early stage of disease (~54%), (Table 3.1C, Figure 3.3) and the majority involved the oral tongue (76%, Table 3.1E).

Ten percent of the tongue cancers (33 cases) occurred in individuals less than 40 years of age and in this age group, males (~79%) represented the majority with a male:female ratio of 3.7:1.0 (Tables 3.1B,C,D; Figure 3.2). Among individuals less than 40 years of age, the majority of tumours (24 cases) were an early stage of disease (~73%, Table 3.1C, Figure 3.3) and the majority involved the oral tongue (85%, Table 3.1E).

B. Risk Factors

Patient associations with risk factors are presented in Table 3.2 and Table 3.3.

1. Alcohol

The majority of patients (~43%) admitted consuming 4 or more drinks of alcohol per day (Table 3.2A). The majority of heavy alcohol consumers were between the ages of 50 and 79 years (118 cases, ~84%), (Table 3.3).

Among heavy consumers of alcohol, the majority had stage IV (~32%) or stage II (~31%) followed by stage I, stage III disease, and carcinoma in situ (1 case), (Table 3.3). However, among heavy consumers of alcohol, ~35% were also heavy smokers of over 20 cigarettes per day (see B.2.below).

Among moderate drinkers, stages I through III of disease were more evenly distributed, followed by stage IV disease (Table 3.3). Among non or light drinkers, the majority had stage II disease followed by stage I, stage III, stage IV disease and carcinoma in situ (5 cases, ~4%), (Table 3.3).

Among heavy drinkers, and among non drinkers or light drinkers the majority of tumours occurred on the dorsal lateral borders of the oral tongue, followed by the BOT and the inferior ventral surface (Table 3.3). Among moderate drinkers, the majority of tumours involved the dorsal/lateral borders followed by the inferior ventral surface and the BOT (Table 3.3).

2. Tobacco

If the categories of “unknown” history of alcohol (17 cases) and tobacco (6 cases) use are ignored (23 cases), then among all of the remaining patients (305), only 65 patients (~21%) admitted to consuming no or less than 1 alcoholic drink per week, and having never smoked or having stopped over 10 years ago (Tables 3.2B, C). Thus, all of the remaining patients (240 cases, ~79%) admitted to a positive history of alcohol and tobacco use within the past 10 years.

Over half the patients (~56%) smoked over a 20 cigarettes per day (Table 3.2B), and the majority of heavy smokers (~83%) were between 50 and 79 years old (Table 3.3). Among patients who were nonsmokers or had stopped smoking over 10 years ago, the majority had stage I disease followed by stages II, III and IV disease, and carcinoma in situ (5 cases, ~6%), (Table 3.3). Among patients who had stopped smoking between 1 and 10 years ago, the majority had stage III disease; in contrast, among light or moderate smokers; the majority had stage II disease (Table 3.3). Among heavy smokers, the majority had stage IV or stage II disease followed by stages I or III disease, and carcinoma in situ (1 case, ~1%), (Table 3.3).

Among all categories of known tobacco use, the majority of tumours were located on the dorsal/lateral borders of the oral tongue. Among light, moderate or heavy smokers, a greater proportion of tumours were located on the BOT than on the inferior ventral surface (Table 3.3).

C. Symptoms

The patient symptoms are presented in Table 3.4, Figure 3.5, and Figure 3.6. The majority of patients denied symptoms in association with the tumour but among symptoms, soreness of the tongue was most common symptom and was experienced by ~66% of the patients (Table 3.4A-H, Figure 3.5). Most patients (~36%) experienced a sore tongue between 1 and 6 months, ~17% had a sore tongue over 6 months, and ~13% had a sore tongue for less than a month (Table 3.4A, Figure 3.5). Among individuals who experienced a sore tongue, ~53% were heavy smokers and

44% were heavy consumers of alcohol. The association between sore tongue and tumour location was significant ($p < 0.001$), and most of the sore tongue lesions were located on the dorsal/lateral borders (~70%) followed by the inferior ventral surface (~20%) and the BOT (~10%), (Table 3.4H, Figure 3.6).

Additional symptoms in decreasing frequency included a lump on the tongue, referred pain to the ear, dysphagia, a mass in the neck, voice change, and bleeding, (Table 3.4 A-H, Figure 3.5, Figure 3.6). For each symptom, the majority of patients were aware of symptoms between 1 and 6 months (Table 3.4 A-G, Figure 3.5).

The predominant location of the tumour varied with the symptom (Table 3.4H, Figure 3.6). For symptoms of sore tongue, lump on the tongue, ear pain and bleeding, the majority of tumours were located on the dorsal/lateral borders of the oral tongue. For symptoms of a neck mass, voice changes and dysphagia, the majority of tumours were located on the BOT (Table 3.4H, Figure 3.6).

Among those patients who were aware of their weight (324 cases), ~35% claimed weight loss as a symptom and most patients (~20%) lost up to 10 pounds (Table 3.4J), but the time frames over which the losses occurred were not known. Among individuals who experienced weight loss (114 cases), the majority were heavy smokers (64%) and heavy consumers of alcohol (49%).

D. Tumour Characteristics

Tumour characteristics are presented in Table 3.5.

1. Tumour Location

The 328 SCC tumours were distributed as follows: 71 were located on the base of the tongue, posterior to the vallate papillae; 257 were located on the oral tongue, and included 61 lesions on the

inferior ventral surface and 196 lesions on the dorsal surface and lateral borders anterior to the vallate papillae (Table 3.5A).

The association between tumour location and T status ($p=0.00001$) as well as N status ($p=0.0001$) were significant. Most of the lesions of the dorsal/lateral borders of the oral tongue were T2 (46%), NX/N0 (82%) and stage II (~39%), (Table 3.5F). Lesions of the inferior ventral surface were mainly Tis, T1 status (~39%) or T2 (~36%), NX/N0 (77%), and stage I (~34%) or stage II (~31%), (Table 3.5F). Overall, the majority (~67%) of all oral tongue SCCs were an early stage of disease (carcinoma in situ, stage I or stage II), (Table 3.5F).

In contrast, the majority (~92%) of SCCs of the BOT were an advanced (stage III or IV) stage of disease. Lesions involving the BOT were predominantly T3/T4 (~58%), N2 (~35%) and stage IV (~63%), (Table 3.5F).

For lesions involving the oral tongue, the majority occurred in heavy smokers (~53%), and in heavy consumers of alcohol (~43%). However, about 28% of oral tongue lesions occurred in individuals who never smoked or stopped more than 10 years ago, and ~38% occurred in nondrinkers or consumers of less than 1 alcoholic drink per week (see also Risk Factors above and Table 3.3).

The majority of BOT lesions occurred in heavy smokers (~66%) and heavy drinkers (~42%), (see also Risk Factors above and Table 3.3).

2. Cell Type

Histologic assessment of tumour cell type revealed that about 3% of the tumours were carcinoma in situ, ~14% were nonkeratinizing SCCs, and the vast majority (~83%) were keratinizing SCCs (Table 3.5B). The majority of the keratinizing SCCs occurred in heavy smokers (~58%) and

heavy drinkers of alcohol (~44%). The majority of the keratinizing SCCs were located on the dorsal/lateral borders of the oral tongue (~63%), and the keratinizing tumours were predominantly T2 (~43%), NX/N0 (68%), and stage II (33%).

3. Cell Differentiation

Histologic assessment of cell differentiation revealed that ~43% of the tumours were well-differentiated, 39% were moderately differentiated, and 18% were poorly differentiated (Table 3.5C). Most of the tumours were either well-differentiated keratinizing SCCs (~41%), or moderately-differentiated keratinizing SCCs (~37%). Within each grade of differentiation, the majority of cases occurred in heavy smokers and heavy consumers of alcohol.

The association between cell differentiation and tumour location was significant ($p=0.003$), (Table 3.5G). The oral tongue demonstrated the vast majority of well- or moderately-differentiated tumours (86%) whereas the BOT demonstrated a more similar distribution of differentiation grade (41% moderate, 32% poor, 27% well), (Table 3.5G).

Most of the well-differentiated tumours were T2 (~44%), NX/N0 (~76%) and stage II (~34%).

Most of the moderately-differentiated tumours were also T2 (~41%), NX/N0 (~66%) and stage II (~31%). In contrast, most of the poorly-differentiated tumours were T3/T4 (~47%), NX/N0 (50%) and stage IV (~43%).

4. Tumour Status, Node Status and Stage of Disease

The T status and N status are presented in Tables 3.5D, E and Figure 3.7. The association between N and T status was significant ($p=0.0001$). The majority of patients presented with T2, NX/N0 tumours (~31%), followed by Tis/T1, NX/N0 tumours (~24%), and T3/T4, NX/N0 tumours (~12%). Most patients were stage II (~31%), followed by stage IV (~25%), stage I (~23%) and stage III (~21%), (Table 3.1F).

For all stages of disease, heavy smokers represented the majority of patients; in stage I, 50% of patients with stage I disease were heavy smokers, 53% in stage II, ~55% in stage III and ~68% in stage IV. Heavy consumers of alcohol also represented the majority of patients in stage II (43%), stage III (~37%) and stage IV disease (~56%), (see also Table 3.3).

E. Treatment

1. Treatment Modalities

Treatments are presented in Table 3.6 and Table 3.7.

Seven patients received no treatment and 31 patients received only treatment with a palliative intent. Among the patients who received either no treatment or received treatment with a palliative intent, the vast majority (92%) had advanced disease (stage III or IV). Palliative-intent treatments included radiotherapy, chemotherapy and/or surgery (Table 3.6).

Treatment with curative intent of the remaining 290 patients included 16 patients (~5%) who received surgery alone, 144 (~50%) who received radiation therapy alone, 129 (~45%) who received a combination of radiation and surgery, and 1 patient who received a combination of radiation and chemotherapy (Table 3.6). Among the patients who received treatment with a curative intent, 55% had an early stage of disease (carcinoma in situ, stage I or II) and 45% had an advanced stage of disease.

Surgical treatments of the primary site included hemiglossectomies (~42%), local excision (~32%) and composite resection or total glossectomy (~26%), (Table 3.7). The surgical margins were negative in 80% of the surgical cases, and positive or close (<5mm) in the balance of the surgical cases. Surgical treatments of the neck included ipsilateral dissections (~85%), bilateral dissections (~13%) and contralateral dissections (~2%). Following neck dissection, the surgical pathology was negative in 23% of cases whereas unilateral nodes were positive in ~70% of cases, and

bilateral in 7% of cases. Positive extra-nodal disease was evident in ~48% of cases (Table 3.7).

Radiation treatment included external beam treatment alone (~47%), brachytherapy alone (~43%), and combined brachytherapy/external beam therapy (~10%), (Table 3.7).

2. Complications of Treatment

There were no recorded complications of surgical treatment.

Complications of radiotherapy included necrosis of the soft tissues and necrosis of the bone (post radiation osteonecrosis, PRON). In the BCCA-1994 case series, necrosis of the soft tissues was defined as a nonhealing mucosal deficit that persisted for at least 3 months, without exposure of the underlying bone and without radiographic evidence of osseous changes (J. Epstein, personal communication, 1997). Typically, soft tissue necrosis involved the tongue, FOM or the buccal mucosa. PRON was defined as exposure of bone persisting for at least 3 months (Epstein et al., 1987; 1997b).

Almost 10% of patients receiving radiotherapy experienced necrosis of the soft tissues (Table 3.7). The gender of the patient had no effect on necrosis of the soft tissues but age had a statistically significant ($p=0.024$) negative effect on soft tissue necrosis. After adjusting for age, brachytherapy including the total dose ($p=0.001$), total time ($p=0.0004$), and number of fractions ($p=0.0001$), was shown to have statistically significant effects on soft tissue necrosis.

Almost 6% of patients receiving radiotherapy experienced PRON (Table 3.7). Gender of the patient had no effect on PRON but age had a statistically significant ($p=0.011$) negative effect. After adjusting for gender and age, only the number of brachytherapy fractions ($p=0.035$) was shown to have a statistically significant effect on PRON.

A. Cases by Year

	<u>Number</u>	<u>Percent</u>
1979	1	0.3
1980	24	7.3
1981	27	8.2
1982	30	9.1
1983	34	10.4
1984	43	13.1
1985	32	9.8
1986	33	10.1
1987	22	6.7
1988	20	6.1
1989	11	3.4
1990	12	3.7
1991	21	6.4
1992	9	2.7
1993	8	2.4
1994	<u>1</u>	<u>0.3</u>
	<u>328</u>	<u>100</u>

B. Gender and Age

	<u>Male</u>	<u>Female</u>	<u>Total</u>	<u>Percent</u>
< 40 years	26	7	33	10.1
40-49 years	12	11	23	7.0
50-59 years	58	28	86	26.2
60-69 years	52	35	87	26.5
70-79 years	42	33	75	22.9
> 80 years	<u>7</u>	<u>17</u>	<u>24</u>	<u>7.3</u>
	<u>197</u>	<u>131</u>	<u>328</u>	<u>100</u>

C. Age and Stage of Disease

	<u>Age in Years</u>						<u>Total Number</u>
	<u><40</u>	<u>40-49</u>	<u>50-59</u>	<u>60-69</u>	<u>70-79</u>	<u>>80</u>	
Carcinoma in situ	1		1	1	2	1	6
Stage I	7	5	20	22	15	5	74
Stage II	16	6	25	19	28	6	100
Stage III	5	7	14	21	13	7	67
Stage IV	<u>4</u>	<u>5</u>	<u>26</u>	<u>24</u>	<u>17</u>	<u>5</u>	<u>81</u>
	<u>33</u>	<u>23</u>	<u>86</u>	<u>87</u>	<u>75</u>	<u>24</u>	<u>328</u>

D. Gender and Stage of Disease

	<u>Male</u>	<u>Female</u>	<u>Total</u>	<u>Percent</u>
Carcinoma in situ	3	3	6	1.8
Stage I	50	24	74	22.6
Stage II	56	44	100	30.5
Stage III	35	32	67	20.4
Stage IV	<u>53</u>	<u>28</u>	<u>81</u>	<u>24.7</u>
	<u>197</u>	<u>131</u>	<u>328</u>	<u>100</u>

E. Age and Tumour Location

	Age in Years					
	<40	40-49	50-59	60-69	70-79	>80
Oral Tongue						
D/L	20	15	48	50	43	21
I/V	8	3	14	16	17	3
BOT	<u>5</u>	<u>5</u>	<u>24</u>	<u>21</u>	<u>15</u>	<u>1</u>
	<u>33</u>	<u>23</u>	<u>86</u>	<u>87</u>	<u>75</u>	<u>24</u>

Table 3.1. Cases of Lingual SCC by Year and Patient Demographics.

- A. Number of cases of SCC of the tongue by year of admission to the BCCA
 B. Gender and age of the cases
 C. Age and stage of disease
 D. Gender and stage of disease
 E. Age and location of the tumour. D/L refers to the dorsal/lateral borders of the oral tongue;
 I/V refers to the inferior ventral surface of the oral tongue; BOT refers to the base of tongue.

Figure 3.1. Number of Cases Admitted to Vancouver Branch, BCCA by Year, 1979 - 1994

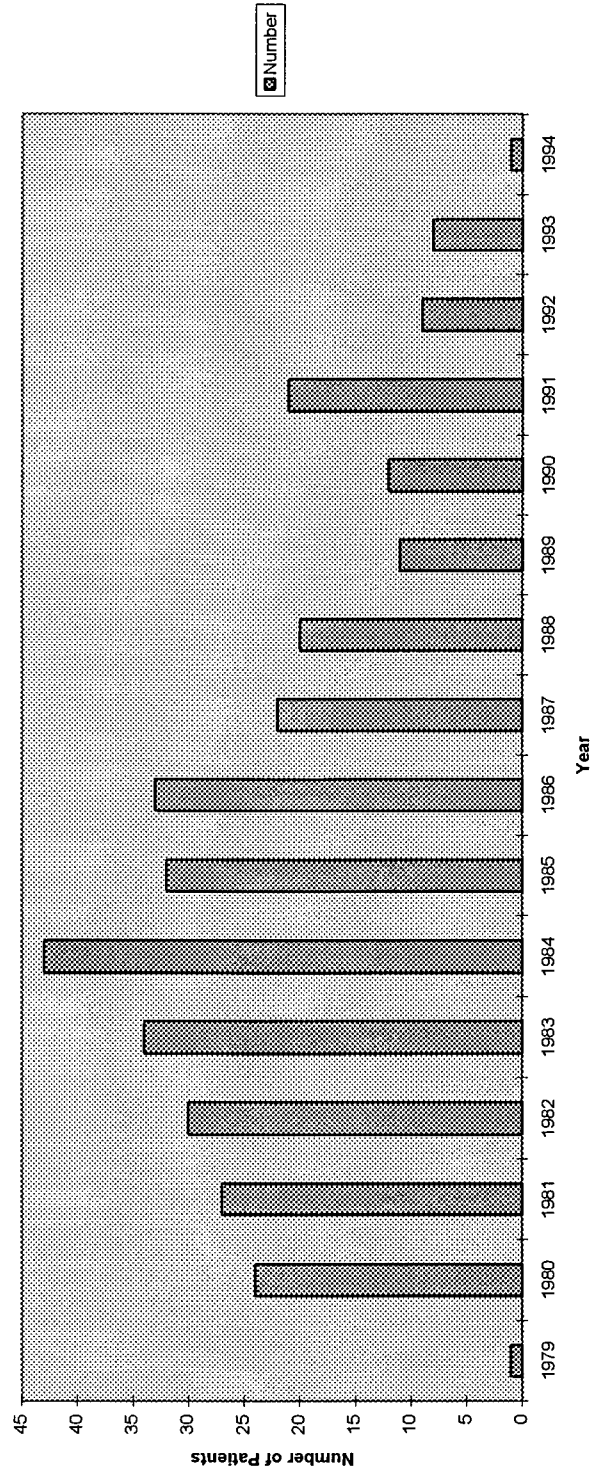


Figure 3.2. Gender and Age Distribution of Patients

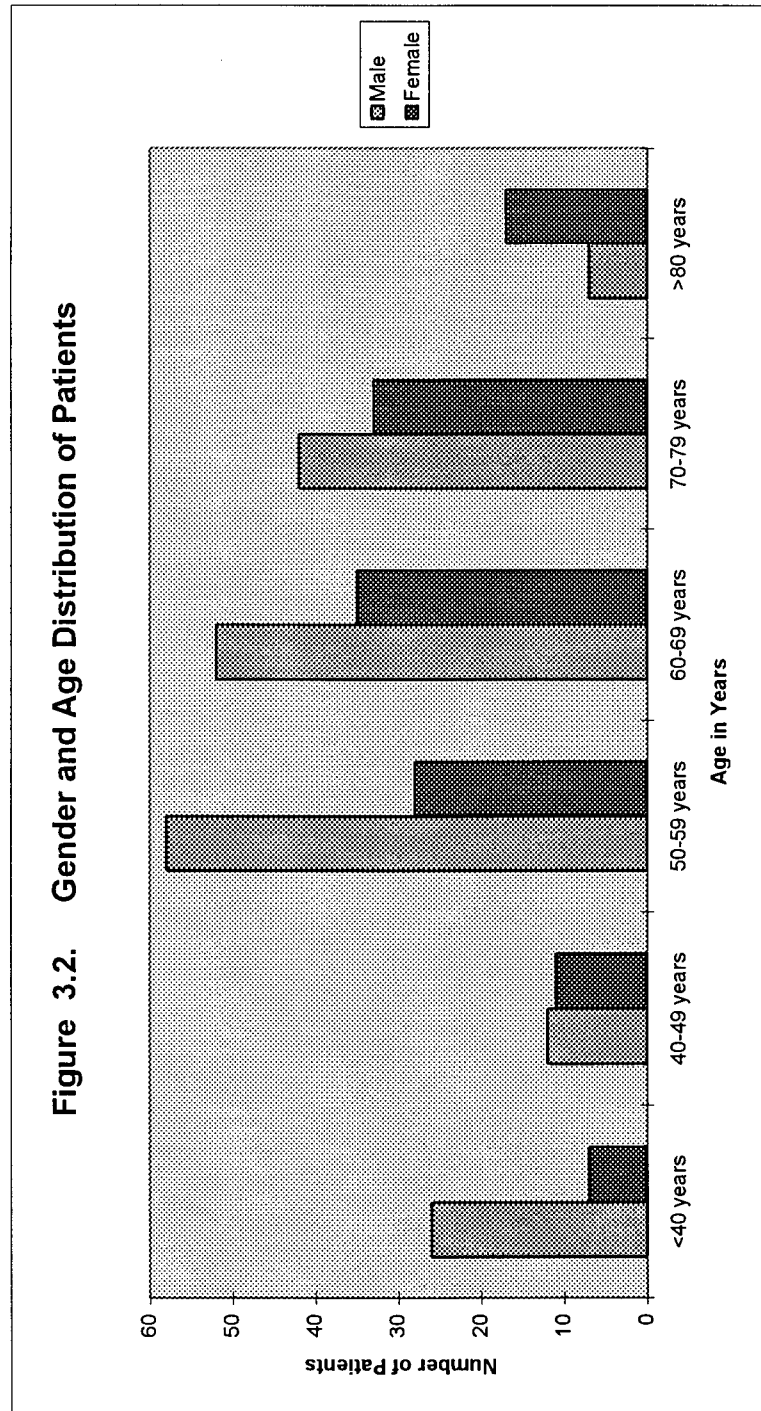


Figure 3.3. Distribution of Stage of Disease by Age Groups

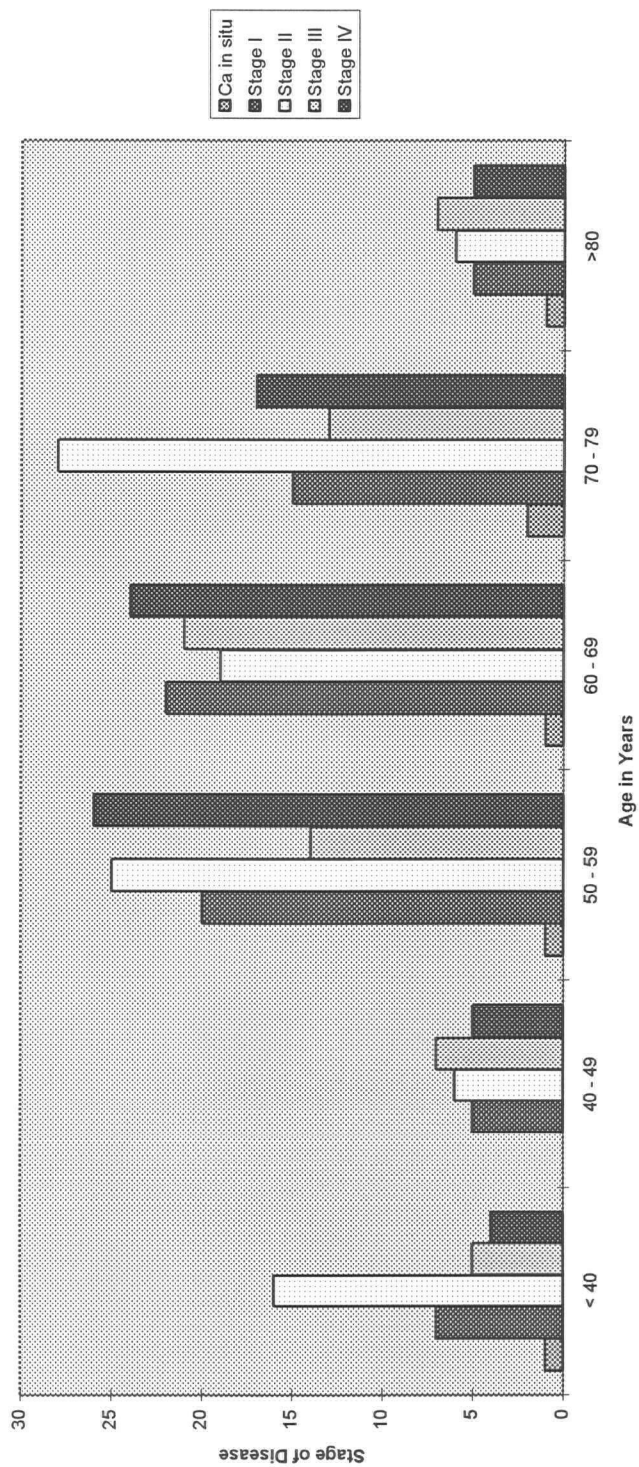
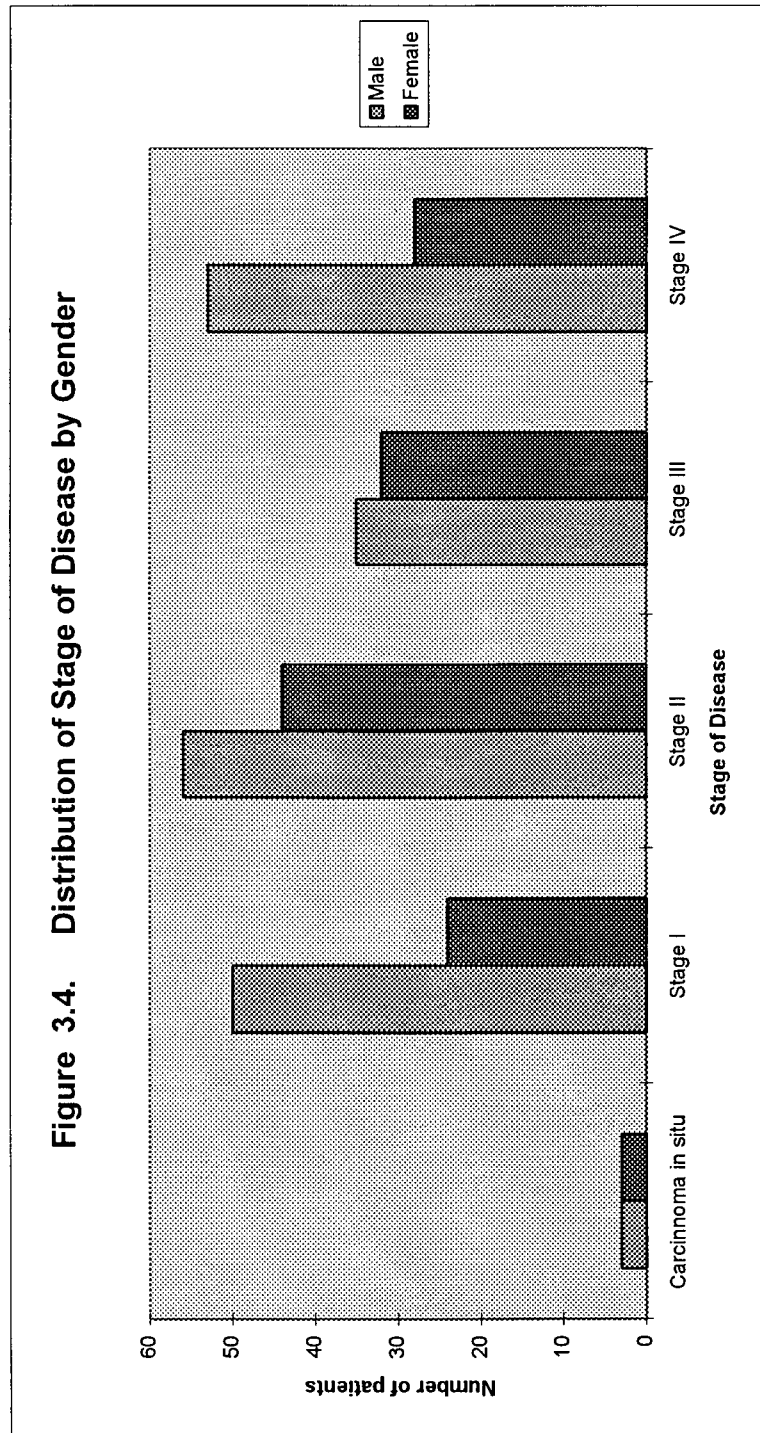


Figure 3.4. Distribution of Stage of Disease by Gender



A. Alcohol Consumption

	<u>Number</u>	<u>Percent</u>
Unknown	17	5.2
None or < 1 drink/wk	120	36.6
Moderate	51	15.5
Heavy (≥ 4 drinks/day)	<u>140</u>	<u>42.7</u>
	<u>328</u>	<u>100</u>

B. Tobacco Consumption

	<u>Number</u>	<u>Percent</u>
Unknown	6	1.8
Never or stopped >10 yrs	82	25
Stopped >1 yr \leq 10 yrs ago	23	7.0
Light (1-10 cig/day)	10	3.0
Moderate (11-20 cig/day)	18	5.5
Heavy (>20 cig/day)	183	55.8
Pipe smoker	<u>6</u>	<u>1.8</u>
	<u>328</u>	<u>100</u>

Table 3.2. Risk Factors Associated with Patients.

A. Patient History of Alcohol Consumption

B. Patient History of Tobacco Consumption

Risk Factor	Ages (~%) 50-79 yrs	Stage of Disease (~%)				Tumour Location (~%)		
		I	II	III	IV	BOT	D/L	Oral V/I
Alcohol								
0 or <1 drink/wk (120 cases)	64	27	34	19	16	18	69	13
moderate (51 cases)	82	25	28	28	19	26	47	27
heavy (≥4 drinks/day) (140 cases)	84	19	31	18	32	22	59	19
Tobacco								
never, stopped >10 yrs (82 cases)	52	34	31	16	13	11	73	16
stopped >1yr≤10 yrs ago (23 cases)	88	13	26	40	21	17	65	18
light (1-10 cig/day) (10 cases)	60	20	40	10	30	30	60	10
moderate (11-20 cig/day) (18 cases)	89	11	45	22	22	28	61	11
heavy (>20 cig/day) (183 cases)	83	20	29	20	30	26	54	20

Table 3.3. Alcohol and Tobacco Consumption and Percentage Distribution by Age, Stage of Disease and Tumour Location. Within each row, the percentages represent the proportion of cases per risk category that are within the age group (50-70 years), each stage of disease and tumour location. The number of cases in each risk category is given in parentheses beneath each category. For tumour location, D/L refers to the dorsal/lateral borders of the oral tongue, V/I refers to the ventral/inferior surface of the oral tongue; BOT refers to the base of tongue. For stage of disease, cases staged as carcinoma in situ were not included and they represent 4% of 0 or <1 drink/wk and <1% of heavy drinkers; 6% of never or stopped > 10 yrs ago, and ~1% of heavy smokers.

A. Sore Tongue

	<u>Number</u>	<u>Percent</u>
None	110	33.5
0-<1 month	42	12.8
1-6 months	119	36.3
over 6 months	<u>57</u>	<u>17.4</u>
	<u>328</u>	<u>100</u>

B. Lump on Tongue

	<u>Number</u>	<u>Percent</u>
None	234	71.3
0-<1 month	16	4.9
1-6 months	56	17.1
over 6 months	<u>22</u>	<u>6.7</u>
	<u>328</u>	<u>100</u>

C. Referred Pain to Ear

	<u>Number</u>	<u>Percent</u>
None	262	79.9
0-<1 month	17	5.2
1-6 months	42	12.8
over 6 months	<u>7</u>	<u>2.1</u>
	<u>328</u>	<u>100</u>

D. Bleeding

	<u>Number</u>	<u>Percent</u>
None	323	98.5
0-<1 month	2	0.6
1-6 months	1	0.3
over 6 months	<u>2</u>	<u>0.6</u>
	<u>328</u>	<u>100</u>

E. Mass in Neck

	<u>Number</u>	<u>Percent</u>
None	280	85.4
0-<1 month	11	3.4
1-6 months	36	11.0
over 6 months	<u>1</u>	<u>0.3</u>
	<u>328</u>	<u>100</u>

F. Voice Change

	<u>Number</u>	<u>Percent</u>
None	317	96.6
0-<1 month	1	0.3
1-6 months	8	2.4
over 6 months	<u>2</u>	<u>0.6</u>
	<u>328</u>	<u>100</u>

G. Dysphagia

	<u>Number</u>	<u>Percent</u>
None	271	82.6
0-<1 month	10	3.0
1-6 months	43	13.1
over 6 months	<u>4</u>	<u>1.2</u>
	<u>328</u>	<u>100</u>

H. Symptom and Tumour Location

<u>Symptom</u>	<u>Number Symptomatic cases</u>	<u>Dorsal/lateral Oral Tongue</u>		<u>Inferior ventral Oral Tongue</u>		<u>Base of Tongue</u>	
		<u>No.</u>	<u>%</u>	<u>No.</u>	<u>%</u>	<u>No.</u>	<u>%</u>
sore tongue	218	153	70.2	44	20.2	21	9.6
lump on tongue	94	53	56.4	25	26.6	6	6.38
ear pain	66	36	54.6	6	9.1	24	36.4
bleeding	5	3	60	1	20	1	20
mass in neck	48	11	22.9	2	4.2	35	72.9
voice changes	11	4	36.4	2	18.2	5	45.5
dysphagia	<u>57</u>	<u>18</u>	31.6	<u>5</u>	8.8	<u>34</u>	59.6
	499	278		85		126	

J. Weight Loss

	<u>Number</u>	<u>Percent</u>
Unknown	4	1.2
None	210	64
0-10 pounds	64	19.5
11-20 pounds	22	6.7
>20 pounds	<u>28</u>	<u>8.5</u>
	<u>328</u>	<u>100</u>

Table 3.4. History of Patient Symptoms. Patient history of A. sore tongue, B. lump on tongue, C. referred pain to ear, D. bleeding from site of the cancer, E. mass in the neck, F. voice change, G. dysphagia. H. symptoms and location of tumour. H. history of weight loss.

Figure 3.5. Patients Symptoms and Duration of Symptoms

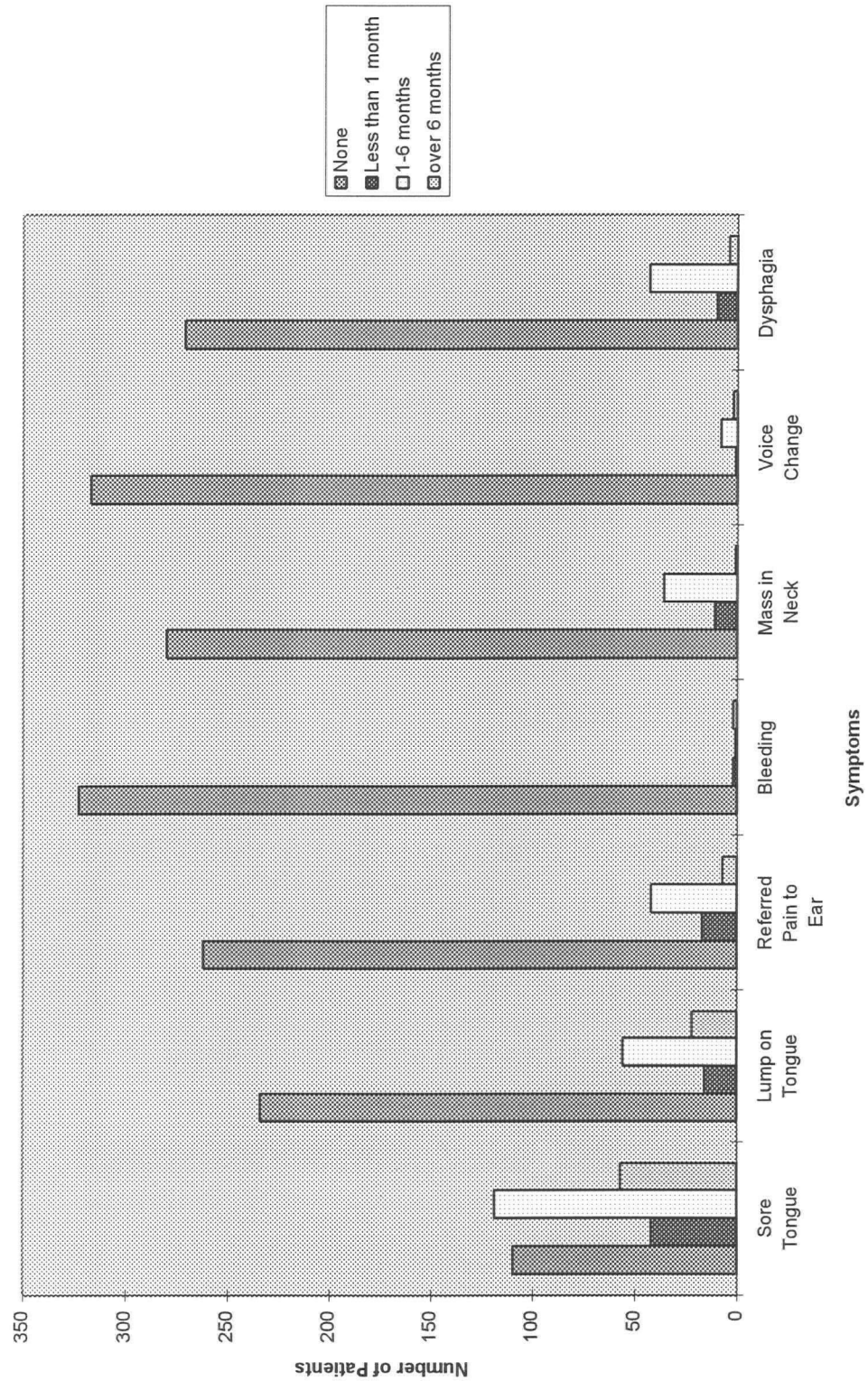
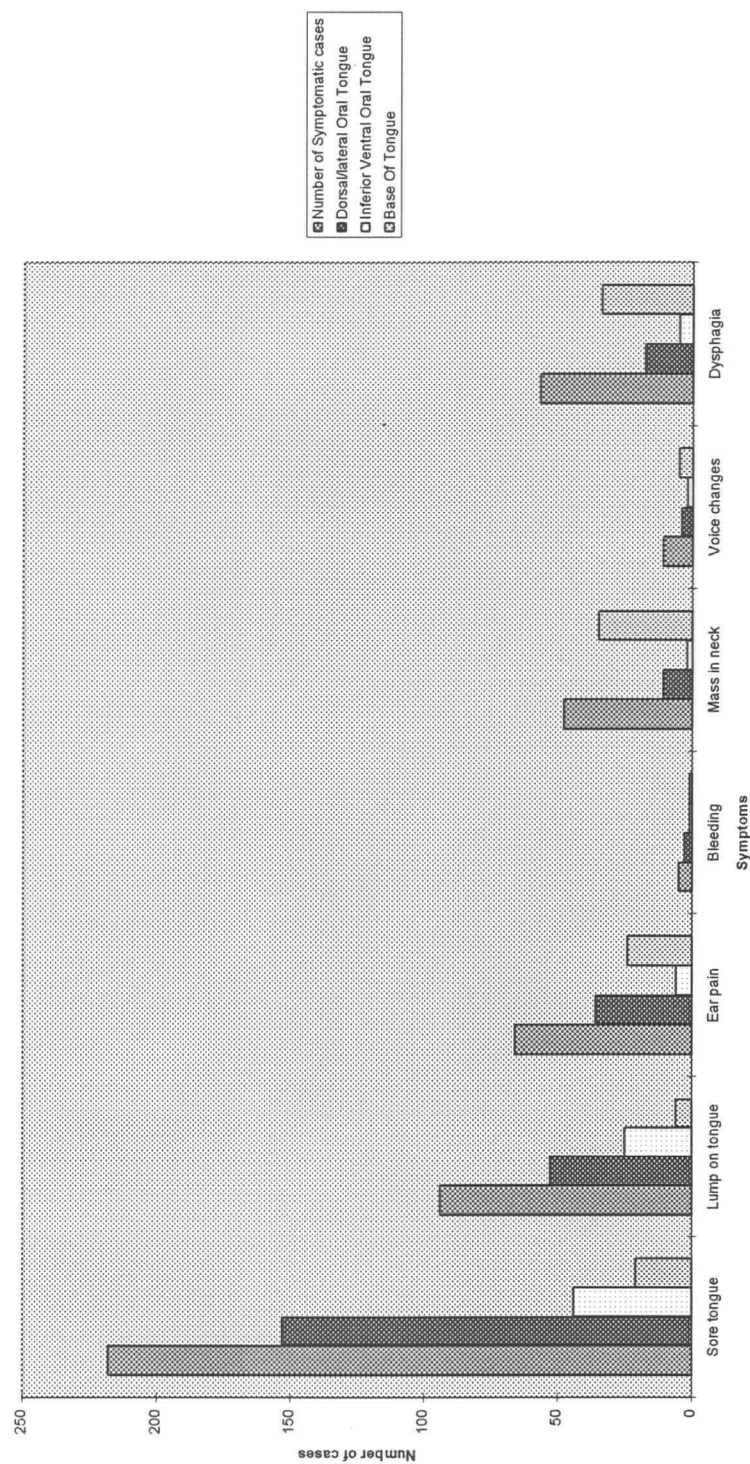


Figure 3.6. Distribution of Patient Symptoms by Tumour Location



A. Tumour Location

	<u>Number</u>	<u>Percent</u>
Oral} inferior/ventral	61	18.6
dorsal/lateral	196	59.8
Base of Tongue	<u>71</u>	<u>21.6</u>
	<u>328</u>	<u>100</u>

B. Cell Type

	<u>Number</u>	<u>Percent</u>
Carcinoma in situ	9	2.7
nonkeratinizing SSC	48	14.6
keratinizing SCC	<u>271</u>	<u>82.6</u>
	<u>328</u>	<u>100</u>

C. Cell Differentiation

	<u>Number</u>	<u>Percent</u>
well	140	42.7
moderate	130	39.6
poor	<u>58</u>	<u>17.7</u>
	<u>328</u>	<u>100</u>

D. Tumour Status

	<u>Number</u>	<u>Percent</u>
Tis, T1	87	26.5
T2	133	40.5
T3, T4	<u>108</u>	<u>32.9</u>
	<u>328</u>	<u>100</u>

E. Node Status

	<u>Number</u>	<u>Percent</u>
NX, N0	222	67.7
N1	40	12.2
N2a, 2b, 2c	39	11.9
N3a, b	<u>27</u>	<u>8.2</u>
	<u>328</u>	<u>100</u>

F. Tumour Location and Stage of Disease

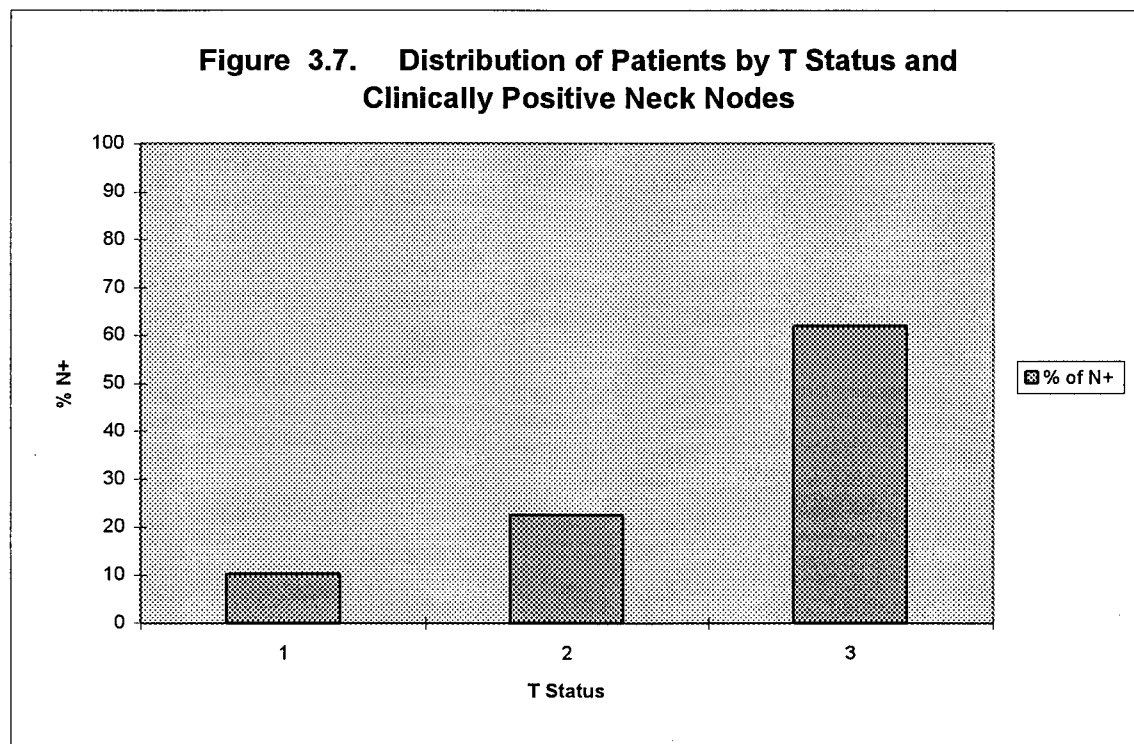
	Oral D/L	Tongue V/I	BOT	Total Number
Tis (in situ)	4	2		6
Stage I	52	21	1	74
Stage II	76	19	5	100
Stage III	38	9	20	67
Stage IV	<u>26</u>	<u>10</u>	<u>45</u>	<u>81</u>
	196	61	71	328

G. Tumour Location and Cell Differentiation

	Oral D/L	Tongue V/I	BOT	Total Number
Well	91	30	19	140
Moderate	79	22	29	130
Poor	<u>26</u>	<u>9</u>	<u>23</u>	<u>58</u>
	196	61	71	328

Table 3.5. Tumour Characteristics. The numbers and percentages of tumours according to A. location, B. cell type, C. cell differentiation, D. tumour status, E. node status; F. the number of cases by tumour location and stage of disease, G. The number of cases by tumour location and cell differentiation. For tumour location, D/L refers to the dorsal/lateral borders of the oral tongue, V/I refers to the ventral/inferior surface of the oral tongue, and BOT refers to the base of tongue.

Figure 3.7. Distribution of Patients by T Status and Clinically Positive Neck Nodes



	<u>Number</u>	<u>%</u>	<u>Number (~%)</u>	
			<u>Ca in situ, Stage I, II</u>	<u>Stage III, IV</u>
No treatment	7		1	6
Palliative Intent (all types)	<u>31</u>		<u>2</u>	29
	38			
Curative Intent:				
Surgery alone	16	5.5	16	0
Radiation alone	144	49.7	82	62
Planned surgery; radn in 3 mos	1	0.3	0	1
surgery followed by radn	11	3.8	8	3
radn; surgery within 3 mos	12	4.2	4	8
radn; followed by surgery >3 mos	104	35.9	67	37
split course radn + surgery	1	0.3	0	1
radn + chemotherapy	<u>1</u>	<u>0.3</u>	<u>0</u>	<u>1</u>
	290	100	180	148
			(~55%)	(~45%)

Table 3.6. Summary of Treatments for SCC of the Tongue by Stage of Disease.
Radn refers to radiation treatment and includes both external beam therapy and brachytherapy.

A. Surgical Treatment (145 patients total)		Surgical Pathology	
Local Treatment			%
hemiglossectomy	42	Negative Margins	80
local excision	32	Positive or	
total glossectomy	26	<5 mm Margins	20
	<u>100</u>		<u>100</u>
Neck Treatment			
ipsilateral	85	Negative Nodes	23
bilateral	13	Positive-unilateral	70
contralateral	2	-bilateral	7
	<u>100</u>		<u>100</u>
B. Radiation Treatment (274 patients total)		Complications	
external beam alone	47	None	84
brachytherapy	43	Soft tissue necrosis	10
combined	10	<u>PRON</u>	6
	<u>100</u>		<u>100</u>

Table 3.7. Surgical Treatment, Radiation Therapy, Surgical Pathology and Complications.

A. Surgical treatments of primary site and neck, and surgical pathology. There were no recorded complications following surgical treatment.

B. Radiation treatments and complications. PRON refers to post-radiation osteonecrosis.

III. Survival Analyses

Survival analyses were based on follow-up of patients ranging from 0 months to 154 months. Except for the calculation of survival proportions by stage of disease, survival analyses were based on a sample of 328 patients and comprised 38 patients who received no treatment or received treatment with a palliative intent, as well as 290 patients who received treatment with a curative intent. The sample size for calculating survival proportions by stage of disease (TNM stage I - IV) was 322 patients because 6 patients were categorized as carcinoma in situ and this category was not included in survival analyses by stage of disease. It was not known if patients had a concomitant or prior history of cancer of the oral/pharyngeal region, or had received prior treatment for these cancers, at a centre other than the Vancouver branch of the BCCA. That is, it was not known if the sample was limited to patients receiving first treatment of a first primary cancer involving the oral/pharyngeal region.

Survival analyses are summarized in Table 3.8 and Table 3.9.

A. Overall All-Cause Survival

Based on the follow-up of patients between 0 months and 154 months, the overall all-cause survival of patients with SCC of the tongue was ~41% (40.74%).

Based on the follow-up of patients between 0 months and 154 months, overall all-cause survival was significantly associated with stage of disease, tumour location, age, and cell differentiation (Table 3.8). Overall all-cause survival was not associated with stage groups (T3, N0 or any T, N1), gender, smoking or alcohol use, or cell type.

At 5 years (60 months), the greatest all-cause survival proportions were associated with well-differentiated, stage I tumours located on the inferior/ventral borders of the oral tongue in patients aged <40 years (Table 3.9). The poorest survival proportions at 5 years were associated with

poorly-differentiated, stage IV tumours located on the BOT in patients aged >80 years (Table 3.9).

B. Disease Specific Survival

Based on the follow-up of patients between 0 months and 154 months, disease specific (DS) survival was significantly associated with stage, tumour location, age, cell differentiation, and cell type (Table 3.8). DS survival was not associated with stage groups, gender, smoking or alcohol use.

At 5 years (60 months), the greatest DS survival proportions were associated with well-differentiated, carcinoma in situ or stage I tumours located on the oral tongue in patients aged <40 years (Table 3.9). The poorest survival proportions at 5 years were associated with poorly-differentiated, nonkeratinizing stage IV tumours located on the BOT in patients aged >80 years (Table 3.9).

C. Local Recurrence

Based on the follow-up of patients between 0 months and 154 months, survival to local recurrence of disease was significantly associated with stage of disease, tumour location, and cell differentiation (Table 3.8). Local recurrence of disease was not associated with age, gender, smoking or alcohol use, cell type or stage groups .

At 5 years (60 months), the greatest survival proportions were associated with moderate or well-differentiated stage I tumours or the oral tongue (Table 3.9).

D. Regional Recurrence

Based on the follow-up of patients between 0 months and 154 months, survival to regional recurrence of disease was significantly associated with stage of disease, tumour location, cell differentiation, and cell type (Table 3.8). Survival to regional recurrence of disease was not

associated with age, gender, smoking or alcohol use, or stage groups.

At 5 years (60 months), the greatest survival proportions were associated with carcinoma in situ or well-differentiated stage I tumours of the oral tongue (Table 3.9).

E. Distant Metastasis

Based on the follow-up of patients between 0 months and 154 months, survival to distant metastasis was significantly associated only with stage of disease (Table 3.8).

At 5 years (60 months), the greatest survival proportions were associated with stage II or stage I disease (Table 3.9).

III. Summary of Results

The results are summarized in Table 3.10.

Survival to	Prognostic Factors	p Value
Local Recurrence	stage of disease	<0.001
	tumour location	0.016
	cell differentiation	0.099
Regional Recurrence	stage of disease	<0.001
	tumour location	0.049
	cell differentiation	0.002
	cell type	0.011
Distant Metastases	stage of disease	0.017
Overall All-Cause Survival		
	stage of disease	<0.001
	tumour location	0.001
	age	<0.001
	cell differentiation	0.002
Disease-Specific Survival		
	stage of disease	<0.001
	tumour location	<0.001
	age	<0.001
	cell differentiation	<0.001
	cell type	0.082

Table 3.8. Prognostic Factors for Survival and p Values.

Prognostic factors with a statistically significant ($p < 0.05$) association with survival are shown. P values refer to the survival analyses ranging 0 months to 154 months. The sample size for calculation of survival proportions and stage of disease was 322 patients; the sample size for the balance of the calculations was 328 patients.

Prognostic Factors	Survival Proportions (~%) at 5 years					
	All-Cause Survival	DS Survival	Local Recurrence	Regional Recurrence	Distant Metastasis	
Stage of Disease	I	61	82	77	63	95
	II	48	65	63	45	98
	III	36	54	46	44	89
	IV	<u>13</u>	<u>22</u>	<u>28</u>	<u>31</u>	<u>77</u>
	Means	40	56	54	46	90
Tumour Location	Dorsal/Lateral	38	62	63	50	}51
	Oral Tongue{ Inferior/Ventral	56	65	57	52	
	Base of Tongue	<u>27</u>	<u>34</u>	<u>43</u>	<u>35</u>	
	Means	40	54	54	46	
	Age	<40 years	79	79		
40-49 years		42	66			
50-59 years		44	63			
60-69 years		42	57			
70-79 years		26	39			
>80 years		<u>8</u>	<u>33</u>			
Means	40	56				

Prognostic Factors	Survival Proportions (~%) at 5 years				
	All-Cause Survival	DS Survival	Local Recurrence	Regional Recurrence	Distant Metastasis
Cell Differentiation					
Well	47	65	56	56	
Moderate	37	54	60	44	
Poor	<u>29</u>	<u>42</u>	<u>45</u>	<u>31</u>	
Means	38	54	54	44	
Cell Type					
Carcinoma in situ		89		90	
nonkeratinizing SCC		46		31	
keratinizing SCC		<u>57</u>		<u>46</u>	
Means		64		56	

Table 3.9. Prognostic Factors and Survival Proportions at 5 years

Except for stage of disease, survival analyses were based on a sample of 328 patients in which 38 patients received no treatment or treatment with a palliative intent, and 290 patients who received treatment with a curative-intent. The sample size for calculating survival proportions by stage of disease was 322 patients. Only prognostic factors with a statistically significant ($p < 0.05$) association with survival are shown (refer to Table 3.8); survival proportions are shown in ~%. DS refers to disease-specific survival.

<u>Patients:</u>	328 patients: 71 with SCC of the BOT 257 with SCC of the oral tongue	<u>Age:</u> mean age 61 years range 18 - 96 years <u>male:female ratio:</u> 1.5:1.0
<u>Main Symptom:</u>	sore tongue, present 1-6 months	
<u>Risk Factors</u>	-79% of the patients had positive history of alcohol and tobacco use -56% of all patients smoked over 20 cigarettes per day -43% of all patients consumed 4 or more drinks of alcohol per day	
<u>TNM stage of disease:</u>	55% carcinoma in situ, stage I or stage II 45% stage III or stage IV	
<u>Stage and Tumour Location</u>	-oral tongue stage of disease: 67% carcinoma in situ, stage I or stage II -BOT stage of disease: 92% stage III or stage IV	
<u>Tumour Type:</u>	41% well-differentiated keratinizing SCC 39% moderately-differentiated keratinizing SCC	
<u>Tumour Type and Tumour Location</u>	-oral tongue tumour type: 86% well or moderately differentiated tumours -BOT tumour type: 68% well or moderately differentiated tumours	
<u>Treatments:</u>	38 patients received no treatment or received treatment with palliative intent; 92% were stage III or IV 290 patients received treatment with a curative intent; 55% were stage I or II 5% surgery alone 50% radiation therapy alone 45% combined surgery and radiation therapy-	
	Complications of radiation therapy:	10% soft tissue necrosis 6% PRON

Survival Analyses: based on 328 patients including both curative-intent, palliative-intent, and no treatment groups, except for survival analyses by stage of disease (TNM stage I-IV) which was based on 322 patients because 6 patients were categorized as carcinoma in situ. It was not known if the sample included patients with a concomitant or prior history of oral/pharyngeal cancer, or included patients who had received prior treatment at another cancer center.

Patient Follow-up: ranging 0 to 154 months overall all-cause survival was ~41%

Stage of disease significantly related to

- survival to local recurrence
- survival to regional recurrence
- survival to distant metastases
- overall all-cause survival
- DS survival

Tumour Location significantly related to

- survival to local recurrence
- survival to regional recurrence
- overall all-cause survival
- DS survival

Cell Differentiation significantly related to

- survival to local recurrence
- survival to regional recurrence
- overall all-cause survival
- DS survival

Cell Type significantly related to

- survival to regional recurrence
- DS survival

Age significantly related to

- overall all-cause survival
- DS survival

Overall All-Cause Survival

at 5 years, average 40% survival proportion by stage of disease
at 5 years, best survival proportions associated with

- stage I
- oral tongue
- age <40 years
- well differentiated

Disease Specific Survival

at 5 years, average 56% overall by stage of disease
at 5 years, best survival proportions associated with

- stage I
- oral tongue
- age <40 years
- well differentiated
- carcinoma in situ

Table 3.10. Summary of Patient and Tumour Characteristics, Treatment and Survival of Patients with SCC of the Tongue Admitted to Vancouver Branch, BCCA 1979 - 1994.

CHAPTER 4

DISCUSSION

During the 16-year period from 1979 to 1994, 328 patients with lingual SCC were admitted to the Vancouver Branch of the BCCA. For discussion purposes in the thesis, this series of 328 cases is referred to as the BCCA-1994 case series (i.e. compiled by Dr. J. Hay, Dr. J. Epstein, Dr. E. van der Meij and Dr. N. Le).

The tables for Chapter 4, Table 4.1 and Table 4.2, are located at the end of the chapter.

I. Descriptive Associations

A. Demographics, Stages of Disease, Symptoms and Risk Factors

In the BCCA-1994 case series, the preponderance of male cases as compared to female cases (1.50:1.0 ratio), and the mean age of cases (mean age 61 years) were consistent with the demographics reported for oral/pharyngeal cancer in general (see Chapter 1, Section VII.D; eg. Garfinkel 1995a) and SCC of the tongue in particular (eg. Callery et al., 1984; Franceschi et al., 1993).

In the BCCA-1994 case series, the percentage of cases aged less than 40 years (10%) was greater than the reported incidence of 1%-3% of oral/pharyngeal SCC in patients 40 years of age and younger (Burzynski et al. 1992). Moreover, this percentage was also greater than the 3.6% incidence reported by McGregor et al. (1983) for tongue and lower oral cavity SCCs admitted to the BCCA between 1972 and 1981. However, McGregor et al. (1983) also reported that an increasing number of young people with oral cancers were being seen at the BCCA, with a high of three patients in 1979, and that a total of approximately 70 new patients per year with these oral cancers were being seen (McGregor et al., 1983). This increased proportion of oral/pharyngeal and tongue cancers in younger patients seen at the BCCA may be part of the international trend

reported by others (see Chapter 1, Section VII.D.1; eg. MacFarlane et al., 1992; Blot et al., 1994; Llewelyn and Mitchel, 1994; Devesa et al., 1995). It is probable that cases included in the McGregor et al. (1983) series were also included in the BCCA-1994 series; the former study reported a female preponderance (female to male ratio of 3:2) whereas there was a male majority (male to female ratio of 3.7:1.0) in the BCCA-1994 case series which is consistent with trends reported by others (eg. Burzynski et al., 1992; Jovanovic et al., 1993b; Sarkaria and Harari, 1994; Ostman et al., 1995). In the BCCA-1994 case series, the majority (73%) of patients aged less than 40 years presented with early stage disease (carcinoma in situ, stage I or II disease) which is also consistent with other reports (eg. McGregor et al., 1983; Sarkaria and Harari, 1994).

In the BCCA-1994 case series, the majority of patients with tongue cancer had an early stage of disease (~55% were carcinoma in situ, stage I or stage II). The majority of patients with cancers of the oral tongue presented with an early stage of disease (~68%); in contrast, the majority of patients with BOT lesions were stage III or IV of disease (~92%). Similar overall stages of disease, and differences in stage of disease in relationship to tumour site have been reported (Chapter 1, Section VII.B; eg. Callery et al., 1984; Franceschi et al., 1993; Kraus et al., 1993; see also Table 4.1).

In the BCCA-1994 case series, the majority of patients denied symptoms, but among admitted symptoms, soreness of the tongue was the most common complaint, similar to reports by Gehanno et al., (1992), Franceschi et al., (1993) and Krupala and Gianoli (1993), (see also Chapter 1, Section VII. B). In the BCCA-1994 case series, the majority of patients (~79%) had a positive history of alcohol and tobacco use, a proportion that was within the range of reported percentages of patients admitting these habits (eg. 70%, Franceschi et al., 1993; 89%, Blot et al., 1988; 92%, Day et al., 1994b).

B. Tumour Characteristics

In the BCCA-1994 case series, most of the lingual SCCs were either well- or moderately-

differentiated keratinizing SCCs, similar to most oral SCCs (eg. Regezzi and Sciubba, 1989; Paterson et al., 1996). The differentiation of oral/pharyngeal SCCs may differ between different sites. The mouth demonstrates higher proportions of well- and moderately- differentiated SCCs as compared to the nasopharynx which demonstrates a majority of poorly-differentiated SCCs; the hypopharynx and oropharynx both demonstrate a more equal distribution of differentiation grades (reviewed by Biorklund and Wenersberg, 1994). In the BCCA-1994 case series, differentiation and tumour location were significantly associated ($p=0.003$); the oral tongue demonstrated the vast majority of well- or moderately-differentiated tumours whereas the BOT (located in the oropharynx) demonstrated a more similar distribution of differentiation grades.

C. Treatment

1. Treatment Modalities

In the BCCA-1994 case series, 290 patients received treatment with a curative intent. The majority received radiation therapy alone (~50%), followed by a combination of radiation and surgery (~45%), and surgery alone (~5%). Radiation treatment included external beam radiation alone (~47%), brachytherapy alone (~43%), and combined external beam irradiation and brachytherapy (~10%). In the BCCA-1994 case series, surgical treatment alone was limited to patients with an early stage of disease, and the majority of patients with either an early or advanced stage of disease received radiation alone, followed by radiation and surgery more than 3 months later.

The distribution of treatment modalities in the BCCA-1994 case series differs from other reports and probably reflects the treatment biases at the different cancer centers (eg. see Chapter 1, Section VII.E.4) as well as differences in the distributions of the stage of disease in the different studies (see Table 4.1). For example, Table 4.1 illustrates that among patients with SCC of the oral tongue, the proportion of patients with early stage disease (stage I, II) ranged from 52% (Nyman et al., 1993) to 100% (Shibuya et al., 1993). The treatment focus for SCC of the oral tongue varied and included primarily surgical treatment (eg. Spiro and Strong, 1971, 1974), primarily

radiotherapy (eg. Hareyama et al., 1992), or primarily a combined radiotherapy/surgical approach (eg. Nyman et al., 1993).

In similar fashion, among patients with SCC of the BOT, the proportion of patients with early stage disease ranged from 3% (Zelevsky et al., 1992) to 33% (eg. Foote et al., 1992). Treatment of SCC of the BOT also varied and included primarily surgical treatment (Foote et al., 1992; Spiro and Strong, 1974), or primarily combined radiation/surgical therapy (Callery et al., 1984; Harrison et al., 1989) as compared to radiotherapy only (Table 4.1).

2. Complications of Treatment

In general, complications of treatment are related to the type of treatment rendered and to proportion of patients receiving a particular type of treatment (i.e. surgery, radiation or a combination). In the BCCA-1994 case series, ~50% of patients received a surgical procedure but there were no reported complications following surgical treatment. In contrast, at Memorial Sloan Kettering Cancer Center, the focus of treatment of SCC of both the oral tongue and BOT has been primarily surgical (Table 4.2; see also Chapter 1, Section VII. E.4), and complications from surgery included 17% among patients treated definitively for SCC of the oral tongue, (Franceschi et al., 1993), 23% among patients treated definitively for SCC of either the oral tongue or BOT (Callery et al., 1984), and 40% (Kraus et al., 1993) among patients treated definitively for SCC of the BOT (Table 4.2). At the Mayo Clinic where all SCCs of the BOT were treated surgically, Foote et al. (1992) reported surgical complications in 49% of patients. (Table 4.2).

In the BCCA-1994 case series, complications of treatment were limited to complications of radiation therapy; ~10% of patients receiving radiotherapy experienced necrosis of the soft tissues and ~6% experienced PRON. A comparison of radiotherapy complications among different studies is thwarted by differing definitions of soft tissue necrosis and PRON (eg. Shibuya et al., 1993; Pernot et al., 1994; Epstein et al., 1987, 1997b) as well as by the failure of some authors to

define their criteria for necrosis altogether (eg. Harrison et al., 1989; Hareyama et al., 1992; Franceschi et al., 1993; Kraus et al., 1993; Nyman et al., 1993). Nevertheless, a summary of complications following radiotherapies for lingual SCCs is provided in Table 4.2 which illustrates that the proportion of soft tissue necrosis ranged from less than 2% (Nyman et al., 1993) to 29% (Harrison et al., 1989), and the proportion of PRON ranged from less than 2% (Kraus et al., 1993) to 13% (Hareyama et al., 1992; Shibuya et al., 1993).

The incidence of PRON of the mandible following radiotherapy of head and neck tumours ranges from 2.6% to 22% (reviewed by Epstein et al., 1997b) but the most commonly-reported range is 5% to 15% (reviewed by Epstein et al., 1987; Epstein et al., 1997b). Epstein et al. (1987), reviewed 26 BCCA cases of PRON of the mandible following radiotherapy of the head and neck between 1977 and 1984, and Epstein et al. (1997b) reviewed the long-term follow-up of 26 BCCA cases treated between 1975 and 1989; it is possible that cases from the present BCCA-1994 case series (1979-1994) of tongue SCC were also included in these two reports. Both Epstein et al. (1987) and Marx and Johnson (1987) differentiated between spontaneous or idiopathic cases of PRON and cases related to trauma such as from tooth extraction, other surgery or irritation from prostheses. These categories were not distinguished in the BCCA-1994 case series of lingual SCC, but spontaneous cases of PRON accounted for ~46% (Epstein et al., 1987) and 49% (Marx and Johnson, 1987) of cases treated for head and neck tumours by radiation.

In the BCCA-1994 case series, age had a significant negative effect on post-radiation soft tissue necrosis and osteonecrosis, and Epstein et al. (1987) noted that the majority of patients who developed PRON were aged 50 years or older. The negative effects of age upon post-radiation necrosis may reflect the overall age-related atrophy, decreased vascularization and decreased healing capacity of the oral tissues (Adams 1975). After adjusting for age in the BCCA-1994 case series, brachytherapy was significantly associated with both post-radiation soft tissue necrosis and PRON. The brachytherapy dose, total time and number of brachytherapy fractions were

significantly related to soft tissue necrosis but only the number of brachytherapy fractions was significantly related to PRON.

Denham (1992) suggested that necrosis associated with brachytherapy (implantation) was independently related to the dose rate of radiation, the size of the implant and spacing of the radiation sources. Mazeron et al. (1991) investigated the factors associated between necrosis and brachytherapy (interstitial implantation of iridium-192) of 279 T1 or T2 SCCs of the oral tongue and FOM. These authors defined necrosis as "either soft tissue ulceration occurring or persisting longer than 3 months after implantation or osteonecrosis", and graded necrosis according to patient symptoms and treatment approaches required for management of the necrosis. Necrosis was not related to dose ($p=0.08$) but was significantly correlated to the dose rate independently of the total dose, although only at dose rates below rates of 0.5 Gy/hour ($p=0.058$). They concluded that doses of 65-70 Gy delivered at a dose rate of 0.3-0.5 Gy/hour and with intersource spacings less than 15 mm could maximize local control of disease and minimize necrosis (Mazeron et al., 1991, 1992). Tumour diameter (T1 vs. T2, $p=0.04$) and tumour location (oral tongue vs FOM, $p<0.01$) also influenced the risk of necrosis. At 5 years, 33% of T1 sites experienced necrosis as compared to 44% of T2 sites, and 28% of oral tongue sites as compared to 58% of FOM sites experienced necrosis.

In contrast, Simon et al. (1993) considered oral tongue and FOM as separate sites in their analyses of iridium-192 implants. Among oral tongue cancers treated by iridium-192 implantation, necrosis was significantly related to the activity of the implant (dose rate, $p=0.066$) and tumour diameter ($p=0.065$), but for patients treated in similar fashion for FOM cancer, neither of these factors was related to necrosis.

II. Survival

The reporting of survival of SCC of the tongue has been done in many ways. Unfortunately, there has been little conformity to a common standard among different studies and consequently, comparison of survival rates between studies is difficult (see Table 4.1).

Survival data may be displayed in tables or plotted as survival curves. The estimated survival curves are plotted as a step function where the estimated curves remain at a plateau between successive patient deaths, when the curve drops instantly. The curves start from 100% (all of the patients are alive), decline towards 0, but reach 0 only if the patient with the longest follow-up has died (Parmar and Machin, 1996). Survival curves provide a useful summary of the data and some reports of lingual SCC have included survival curves (eg. Hareyama et al., 1992; Franceschi et al., 1993; Shibuya et al., 1993) but in each instance, the overall scale (size) of the graphs differs and the gradations of time on the abscissa differ. Consequently, it is very difficult to compare survival proportions among different studies based solely on the shapes of the survival curves. An alternative method of describing survival data is to report the survival proportions at fixed time points, such as at 5 years (Table 4.1), (Parmar and Machin, 1996).

A common approach for survival analyses has been to aggregate all patients together and determine an average result for 5-year survival, without any regard for stage of disease, location of the tumour, the intent of treatment or the mode of treatment (reviewed by Weber et al., 1993). For example, some reports categorized the patients by tumour status but failed to identify the node status or TNM stage of disease (eg. Weber et al., 1993). Some reports identified the tumour location as the tongue but failed to identify the tongue as either the oral or the BOT (eg. Dearnaley et al., 1991), and some reports combined oral tongue and FOM tumours in their survival analyses (eg. Mazon et al., 1991; Bachaud et al., 1994). Some reports failed to identify the intent of treatment (eg. Zelefsky et al., 1992; Nyman et al., 1993; Pernot et al., 1994; Table 4.1), some reports clearly included patients treated with either a palliative or curative intent (eg. BCCA-1994

case series), and some reports limited their samples to patients treated with a curative intent (eg. Foote et al., 1992; Hareyama et al., 1992; Franceschi et al., 1993; Kraus et al., 1993; Shibuya et al., Table 4.1). Some reports may also have included patients with prior or concomitant oral/pharyngeal tumours and/or patients who received prior treatment for these tumours (eg. Spiro and Strong, 1971; Mazon et al., 1991; Pernot et al., 1994). In contrast, many reports clearly restricted their samples to patients who received initial treatment of first primary tongue and oral/pharyngeal cancers (eg. Hareyama et al., 1992; Kraus et al., 1993; etc., Table 4.1).

It is important that all survival results be examined for what they represent. Some studies calculated survival as survival from death due to all causes of death (all-cause survival) as opposed to survival from death specifically in relation to the tongue cancer (disease or cause-specific survival), (Table 4.1; see also Chapter 1, Section II.J; Sheps 1995; Parmar and Machin 1996). For cancers such as oral/pharyngeal cancers that occur predominantly in patients in the fifth and sixth decades of life (eg. Shaha and Strong, 1995), all-cause survival rates are typically lower than disease-specific survival rates because patients in this age group are at increased risk of dying from intercurrent illnesses unrelated to the cancer. This trend in differing survivals is also supported by the differences observed between all-cause and DS survival rates at 5 years in the BCCA-1994 case series data (Chapter 3, Table 3.8). It is noteworthy that in the BCCA-1994 case series data, no differences were observed between all-cause and DS survivals for patients aged less than 40 years; this observation may reflect the better general health status generally expected in these younger patients, or the predominance of early stage disease of the oral tongue in this age group.

Both all-cause and DS survival analyses include patients that have survived death, however; the surviving patients may have local or regional failures or a second primary. Some studies (Table 4.1) reported survival as disease-free survival (eg. Franceschi et al., 1993; Mazon et al., 1991) or as cure-rates (eg. Spiro and Strong, 1971, 1974;), and these survival rates may be lower than either all-cause or DS survival rates calculated for the same sample of patients (Sheps 1995).

From the foregoing discussion and Table 4.1, it should be apparent that unless there is conformity among investigators for a common approach to survival analyses, general conclusions about survival from SCC of the tongue across studies are difficult to draw. Yet despite differences among studies between proportions of disease stage (Table 4.1) of either oral or base of tongue cancers (Table 4.1), in the focus of treatments (Table 4.1), and in different approaches for calculating survival (Table 4.1), there is nonetheless general agreement among studies that patients with early stages of disease have a more favourable survival prognosis than those with advanced disease (stage III or IV). In similar fashion, tumours of the oral tongue have a more favourable prognosis than BOT lesions although this observation may be related to the higher proportion of advanced-stage tumours involving the BOT (eg. Weber et al., 1993; see Chapter 1, Section VII.B).

In the BCCA-1994 case series, the overall all-cause survival proportion was ~41% for follow-up ranging 0-154 months. At 5 years (60 months), the overall all-cause survival proportion (by stage of oral and BOT cancer combined) was an average of 40%, and the DS survival proportion was an average of 56% (Chapter 3, Table 3.9). These values are within the general range of survival reported for oral and BOT cancer (Table 4.1). Nevertheless, it is worth noting that survival proportions may have been higher if the BCCA-1994 case series had been limited to patients treated with a curative intent as well as to patients being treated for their first primary tumour of the oral/pharyngeal region. That is, 92% of patients treated with a palliative intent had advanced disease (stage III or IV) which has a poorer survival than early stage disease, and patients treated with recurrent or second primaries of the oral/pharyngeal region have a lower survival than patients with a first primary (eg. Day et al., 1994; see Chapter 1, Section VII.F).

In the BCCA-1994 case series, stage of disease was significantly related to survival of local and regional recurrence, survival of distant metastasis, all-cause survival and DS survival. Similar associations were observed between survival and tumour location, age, cell type and differentiation

(Chapter 3, Tables 3.8-3.10), although tumour differentiation in general has not been significantly associated with local control (Pernot et al., 1994) or survival (Weber et al., 1993). These associations may reflect the higher proportions of early stage disease among tumours located on the oral tongue, among well or moderately-differentiated tumours, and among patients under 40 years of age.

In conclusion, the BCCA-1994 case series is similar to other reports of SCC of the oral tongue and BOT with respect to patient demographics, patient risk factors, tumour characteristics and survival. Squamous cell carcinomas of the tongue have a good prognosis if they are detected early; consequently, screening and case finding strategies are essential and should concentrate efforts on individuals at highest risk due to alcohol and tobacco use.

Study	# pts	Tongue Site	I	II	III	IV	Treatment Focus (%) Radn Surg Comb only only	Survival (%)
1	185	oral	51	42	7		100	5 yr cure rate 62.1%
2 }	314	oral	16	47	35	2	2 93	{ 5 yr cure rate 42.1% overall
	126	BOT	<1	12	63	25	7 75	
3 }	252	oral	37	28	24	11	10 76	{ 5 yr disease-free survival 44% overall
	160	BOT	10	9	32	49	33 8	
4	297	oral	37	34	21	8	6 74	5 yr disease-free survival 65% overall stage I, II Ds-free survival 82% stage III, IV Ds-free survival 49%
5	17	BOT		12	35	53	29	2 yr disease-free survival 87%
6	31	BOT		3	26	71	not clearly described	7 yr disease-free survival 64% overall
7	100	BOT	6	14	44	36	37	5 yr disease specific survival 65% overall stage I, II Ds-specific survival 77% stage III Ds-specific survival 64% stage IV Ds-specific survival 59%

Study	# pts	Tongue Site	Stage (%)				Treatment Focus (%)		Survival (%)
			I	II	III	IV	Radn only	Surg Comb only	
8	55	BOT	15	18	38	29	89	11	5 yr disease-specific survival 65%
9	228	oral	24	28	26	22	29	25	46 5 yr disease-specific survival 54% 5 yr overall all-cause survival 37%
10	448	oral	26 (1% TXNX)	34	33	6	60	40	5 yr disease-specific survival 45% 5 yr overall all-cause survival 44%
11	177	oral	46* (*oral tongue and FOM cases)	45*	7*	2*	radiation; perhaps surgery (details not provided)		5 yr survival of local disease 82% (rate for oral tongue alone)
12	130	oral	15	42	31	12	96	24	5 year disease specific survival 65% overall stage I Ds-specific survival 85% stage II Ds-specific survival 69% stage III Ds-specific survival 65% stage IV Ds-specific survival 40%
13	370	oral	24	76			55	45	5 year disease-specific survival 78% overall stage I Ds-specific survival 84% stage II Ds-specific survival 75%

Table 4.1. Survival of SCC of the Oral Tongue and BOT by Study, Site, Stage of Disease and Treatment Focus.

The data included in this table may not be accurate as the required information was not clearly presented in many of the studies and therefore, the required data was calculated or inferred from the data that was available. Treatment focus refers to the overall treatment of patients including treatment of the primary tumour, treatment of the neck as well as salvage treatment, as best as could be determined from the results reported. Radiation treatment includes external beam irradiation and brachytherapy. Combination treatment typically refers to combination of radiotherapy and surgery, but also includes some cases treated with chemotherapy in addition to radiotherapy and surgery. (eg. Study # 9).

In the legend below, the asterisk denotes studies in which it was clearly stated that only patients with first primary tumours that had not received prior treatment were included. The symbol "+" indicates studies that stated that the sample was limited to patients treated with a curative-intent. The type of survival analyses were typically not clearly described and therefore, may not be correctly described in the table above.

<u>Study #</u>	<u>Authors</u>	<u>Years of Study</u>	<u>Site of Study</u>
1	Spiro and Strong, 1971	1957-1963	Memorial Sloan Kettering Cancer Center
2 *	Spiro and Strong, 1974	1957-1963	Memorial Sloan Kettering Cancer Center
3 * †	Callery et al., 1984	1969-1978	Memorial Sloan Kettering Cancer Center
4 * †	Franceschi et al., 1993	1978-1987	Memorial Sloan Kettering Cancer Center
5 * †	Harrison et al., 1989	1977-1986	Memorial Sloan Kettering Cancer Center
6 *	Zelevsky et al., 1992	1973-1986	Memorial Sloan Kettering Cancer Center
7 * †	Kraus et al., 1993	1979-1989	Memorial Sloan Kettering Cancer Center
8 * †	Foote et al., 1992	1971-1986	Memorial Sloan Kettering Cancer Center
9 *	Nyman et al., 1993	1970-1988	Mayo Clinic Sweden
10	Pernot et al., 1994	1972-1986	France
11 †	Mazeron et al., 1991	1970-1988	France
12 * †	Hareyama et al., 1992	1974-1984	Japan
13 * †	Shibuya et al., 1993	1966-1988	Japan

Study	Tongue Site	Radiation Treatment	Radiotherapy Complications
Hareyama et al., 1992	oral	brachytherapy alone or brachytherapy/external beam; (surgery with initial treatment failure)	26/130 pts (20%) with tongue ulceration 17/132 pts (13%) with bone complications 8/17 with PRON 9/17 with osteomyelitis + PRON
Franceschi et al., 1993	oral	external beam either alone or in combination with surgery	3/90 pts (3.3%) with PRON
Nyman et al., 1993	oral	external beam alone or with brachytherapy	3/171 pts (1.8%) with soft tissue necrosis 19/171 pts (11%) with PRON
Shibuya et al., 1993	oral	brachytherapy alone or brachytherapy/external beam; (surgery with initial treatment failure)	81/370 pts (22%) with soft tissue compl. 49/370 pts (13%) with bone compl.
Pernot et al., 1994	oral	brachytherapy and neck surgery (181 pts) brachytherapy and external beam (267 pts)	81/448 pts (18%) with soft tissue compl. 42/448 pts (9%) with bone compl.
Mazeron et al., 1991	oral	brachytherapy alone or with external beam	28% necrosis (soft tissue, bone combined) due to treatment of oral tongue SCC alone
Callery et al., 1984	oral BOT	external beam only (25 pts); adjunctive external beam (35pts) external beam only (53 pts); adjunctive external beam (93 pts)	5/60 pts (8%) with radionecrosis 8/146 pts (5%) with radionecrosis
Kraus et al., 1993	BOT	external beam as adjunct to surgery in 63 pts	1/63 pts (1.6%) with PRON

Harrison et al., 1989	BOT	brachytherapy and external beam	5/17 pts (29%) with soft tissue ulceration 1/17 pts (5.8%) with PRON
-----------------------	-----	---------------------------------	---

Table 4.2. Complications of Radiotherapy by Study, Site and Treatment Focus.

The details regarding each study such as stage of disease, survival and sample characteristics are described in Table 4.1.

CHAPTER 5

FUTURE DIRECTIONS

The 5-year survival rates for oral/pharyngeal cancer “average 52%” and over the last 15 years, the rates have demonstrated “little significant change” (Garfinkel 1995a). Although efforts are made to improve survival rates through different treatment modalities (Chapter 1, Section VII.E), perhaps the best way to control cancer is to find means of preventing it or, in other words, to find ways of reducing the risk of cancer (Garfinkel 1995b). Despite all the knowledge that has accumulated about the lifestyle causes of oral/pharyngeal cancer (i.e. tobacco and alcohol consumption), not all cancers can be prevented from occurring. Discoveries about genes associated with various cancers have implications for identifying persons at increased risk so that these individuals may be monitored closely in order that cancers that do develop may be detected at an early stage (Chapter 1, Section V).

The detection of premalignant and malignant lesions in the oral/pharyngeal region may be hampered by the inaccessibility of some sites to visual inspection by patients and clinicians alike. Nevertheless, programs to educate patients about the warning signs of head and neck cancer are important as many patients, for a variety of reasons, may not be involved in professional surveillance of their head and neck regions. Health-care providers (i.e. dentists, hygienists and physicians) must recognize the biological significance of precursor lesions in carcinogenesis (Chapter 1, Section VI) and should be actively involved in case-finding and screening programs so that patients with suspicious signs and/or symptoms may be referred to specialists or centers experienced in the diagnosis and treatment of oral/pharyngeal precancer and cancer. Centers and protocols exist for the treatment and follow-up of lesions diagnosed as carcinoma in situ or invasive cancer. However, similar centers for the treatment and follow-up of precancer do not exist in most communities, perhaps because the prevailing perception among many clinicians is that nothing can be done about precancer (Sporn 1993). That is, other than the elimination of obvious

risk factors such as alcohol and tobacco use, the clinician is typically unaware of interceptive or preventive treatments for precancer, and aware that the lesion could even disappear spontaneously. Moreover, the clinician may not wish to alarm the patient unnecessarily with a diagnosis of potential “cancer” (Sporn 1993). Consequently, precancerous lesions are often simply observed until changes in signs or symptoms prompt further investigations that may result in a histological diagnosis of cancer, at which time the patient is referred to a cancer center for management .

The key objective of early detection of precancer and cancer is to reduce cancer mortality and morbidity through early intervention. That is, intervention must be initiated either before carcinogenesis or before carcinogenesis progresses to invasive disease, at a stage when the process may still be stopped, slowed or reversed (Greenwald et al., 1995; Chapter 1, Sections II.J and VIII.D.1). As reviewed in Chapter 1, Section V, carcinogenesis is a long, multi-step process initiated by changes at the genetic level, and followed by a number of potentially-reversible promotion steps and progression steps that ultimately lead to malignancy. Because most cancers have very long latency periods, opportunities exist for intervention at early as well as later stages of carcinogenesis (Sporn 1993). Based on a history of precursor lesions, genetic factors, environmental and lifestyle exposures, or a combination thereof, it may be feasible for clinicians to develop risk profiles that could provide a rationale for defining specific interventions to modulate the risks (Greenwald et al., 1995). In addition to elimination of lifestyle risk factors (Chapter 1, Section V.F), another approach to cancer prevention is chemoprevention.

The term “chemoprevention” was coined by Sporn (1976) to describe the inhibition or reversal of carcinogenesis by the “use of nontoxic nutrients or pharmacologic agents to enhance intrinsic physiological mechanisms that protect against the development and progression of mutant clones of malignant cells” (Sporn 1993). For epithelial cancers, chemopreventive intervention measures may be taken during the development of the neoplastic process and up to the stage of in situ carcinoma (Band et al., 1989).

Inhibitors of carcinogenesis may be classified according to the stage in the process of carcinogenesis in which they are effective. Chemopreventive agents may be effective in the initiation phase if they prevent the absorption or formation of carcinogens, or prevent carcinogens from reaching or reacting with DNA targets (Sporn 1993; Greenwald et al., 1995). Such therapeutic interventions during the initiation and early reversible promotion phases are described as "physiologic" by Band et al. (1989). Agents may also be effective during promotion by suppressing the expression of neoplasia in cells that have already been exposed to durations and doses of carcinogens that would otherwise cause cancer (Sporn 1993; Greenwald et al., 1995); such interventions are referred to as "pharmacologic" chemoprevention (Band et al., 1989).

Chemopreventive agents include micronutrients such as vitamins (eg. Vitamins A, C, E), minerals (eg. selenium and calcium), natural products (carotenoids such beta-carotene) and synthetics (eg. Vitamin A or D derivatives), (Band et al., 1989; Shklar and Schwartz, 1993; Greenwald et al., 1995). Retinoids including both natural and synthetic derivatives of vitamin A (retinol; Chapter 1, Section III.C.4) have been widely investigated and their clinical usefulness has been demonstrated by the ability of systemic synthetic retinoids to arrest or reverse the progression of leukoplakia (Hong et al., 1986; Chiesa et al., 1992; reviewed by Kaugars et al., 1996), and to reduce the occurrence of second primary carcinomas of the head and neck but not prevent recurrence of the original tumours (Hong et al., 1990). In vitro, retinoids can suppress malignant transformation caused by chemical carcinogens and ionizing radiation, restore anchorage-dependent growth and contact inhibition to transformed cells (reviewed by Band et al., 1989; see also Chapter 1, Section IV.C.4). Retinoids may affect the initiation step of carcinogenesis but their main anticarcinogenic action stems from their antipromotion effect and to their role in regulating epithelial proliferation and differentiation. Overall, the experimental and epidemiological evidence support the role of physiological levels of vitamin A and its analogs in inhibiting the initiation and/or reversible promotion processes (Band et al., 1989). However, as precancerous lesions develop, it becomes apparent that this protective physiologic effect has been overwhelmed (Band et al., 1989).

The use of retinoids in chemoprevention depends upon the timing and dosage in relation to the stage of cancer development. In later and advanced stages of carcinogenesis such as signified by the presence of premalignant lesions, physiological levels of retinoids are likely to be ineffective, and pharmacologic levels are required. Consequently, Band et al. (1989) recommended that vitamin A and retinoids be considered as drugs and investigated accordingly in the appropriate clinical trials.

Combinations of biomarkers (Chapter 1, Section V.C) are being investigated as surrogate or intermediate measures of cancer to help determine the etiology and stage in the carcinogenesis process. Biomarkers may also monitor the efficacy of chemopreventive agents and provide information about disease maintenance or disease progression, and recurrence (Lippman et al., 1990; Pillai et al., 1992; Sporn 1993). However, the use of biomarkers as intermediate markers of cancer must still be validated using appropriate epidemiological methods of investigation (eg. Lippman et al., 1990; Chapter 1 Section II). Unfortunately, clinical investigations into oral premalignant and malignant lesions have been plagued by inconsistencies and inappropriate methodologies that have rendered comparisons and conclusions between studies difficult and probably invalid (Chapter 1, Section II). The following recommendations are made in efforts to raise the calibre of investigation and to provide a more uniform approach to investigation that may facilitate comparison and analysis of data (i.e. meta analysis) from several sources.

1. The disease of interest must be clearly defined such as through the
 - a. use of uniform definitions for premalignant lesions as advocated by Axell et al., 1996 (Chapter 1, Section VI)
 - b. use of uniform histological criteria or grading systems for grading of dysplasia or malignancy (Chapter 1 Sections VI and VII)
 - c. use of uniform codes for describing the location of lesions such as the ICD-9 and the clearly stated inclusion/exclusion of specific cancers or sites (Chapter 1, Section II.)

2. The statistical evaluation and reporting of results should be uniform such as the
 - a. use of clearly defined numerators, denominators and time periods for calculation of rates (Chapter 1, Section II)
 - b. use of the same reference populations for calculation of standardized rates (Chapter 1, Section II)
 - c. use of standardized categories for stratification of data and calculation of risks. That is, uniform measures of alcohol consumption that account for differences between wine, beer and liquor, and uniform measures of tobacco consumption that account for differences between the type of tobacco (blond, dark), the type (filter/nonfilter) and number of cigarettes smoked, the method of smoking, etc., as well as the duration of the habits (eg. number of cigarettes or packs, or ml of alcohol per year over x years)
 - d. use of clearly-specified criteria for the calculation of survival rates (ie. all-cause, cause-specific or disease-free survival), (Chapter 1, Section II) and samples limited to patients with first primary lesions treated initially with a curative-intent
 - e. avoidance of data dredging (Chapter 1, Section II)
4. Routine reporting of kappa scores for both the clinical and histological evaluations in a study, similar the to routine reporting of standard deviations as an assessment of the dispersion of data (Chapter 1, Section II.)
5. Standardized protocols between centers for the use of chemopreventive agents and patient follow-up.

The impetus for standardizing investigative and reporting protocols among investigators may lie with journal editorial boards and/or with specialty societies. For example, the American Academy of Periodontology (1977) which represents periodontists internationally and publishes its own research journal, has recently published guidelines for design of clinical trials and for analyses in

periodontal research; a similar approach may be considered by journals such as Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics which is the official publication for several of these respective societies in the United States.

The dental profession is in a unique and enviable position for detecting precancerous and cancerous lesions as well as monitoring the clinical effects that chemopreventive measures may exert upon the precancerous lesions. In contrast to precancerous and cancerous lesions in most other body sites (excluding skin lesions), most sites within the oral cavity are visible to the clinician. Moreover, these sites are generally accessible for palpation and for obtaining tissue samples (biopsy), as well as mucosal brushings (micronuclei, Chapter 1, Section V.C.8) which can be obtained noninvasively. Given the recent advances and interest in the use of biomarkers and the chemoprevention of epithelial neoplasms and premalignancy, there is an opportunity for clinicians and researchers in the lower mainland region of British Columbia to establish a clinic to which patients with histologically-proven oral dysplastic lesions could be referred for follow-up and management.

NOMENCLATURE

List of Abbreviations Used in the Thesis

AR	attributable risk
BCCA	British Columbia Cancer Agency
BOT	base of tongue
CDKs	cyclin-dependent kinases
cGy	centrigray
CI	confidence interval
cig	cigarettes
CRABPs	cellular retinoic acid-binding proteins
CT	computed tomography
D/L	dorsal lateral
DNA	deoxyribonucleic acid
DS	disease specific
EBV	Epstein Barr virus
ECM	extracellular matrix
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
5FU	5-fluorouracil
FOM	floor of the mouth
g	gram
G phase	gap phase
Gy	gray
HPV	human papillomavirus
HSV	herpes simplex virus
ICAM	intercellular adhesion molecule
ICD	International Classification of Diseases

IHC	immunohistochemistry
K	keratin
LR	likelihood ratio
M phase	mitosis phase
MHC	major histocompatibility
ml	milliliter
mRNA	messenger ribonucleic acid
MR	magnetic resonance
NED	no evidence of disease
NK	natural killer
nm	nanometer
NPV	negative predictive value
NS	not significant
OR	odds ratio
PCR	polymerase chain reaction
PRON	post-radiation osteonecrosis
PTL+	post test likelihood of a positive test
PTL-	post test likelihood of a negative test
RA	retinoic acid
radn	radiation
RAR	retinoic acid receptor
RAREs	retinoic acid responsive elements
Rb	retinoblastoma
RNA	ribonucleic acid
ROC	receiver operating characteristic
RR	relative risk
RXR	receptor for retinoic acid metabolites

S phase	synthesis phase
SCC	squamous cell carcinoma
SCM	sternocleidomastoid
SEER	Surveillance, Epidemiology and End Results
ST	smokeless tobacco
TGF α	transforming growth factor alpha
TGF β	transforming growth factor beta
TIMP	tissue inhibitor of metalloproteinases
V/I	ventral inferior
yrs	years
Z	atomic number

BIBLIOGRAPHY

- Abbey, L. M., Kaugars, G. E., Gunsolley, J. C., Burns, J. C., Page, D. P., Svirsky, J. A., Eisenberg, E., Krutchkoff, D. J. and Cushing, M. (1995). Intraexaminer and interexaminer reliability in the diagnosis of oral epithelial dysplasia. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 80:188-191.
- Adams, D. (1975). Age changes in the oral structures. In C. L. B. Lavelle (editor): "Applied Physiology of the Mouth." Bristol: John Wright and Sons Limited, pp 310-333.
- Aisner, J., Jacobs, M., Sinibaldi, V. Gray, W. and Eisenberger, M. (1994). Chemoradiotherapy for the treatment of a regionally advanced head and neck cancers. *Seminars in Oncology* 21:35-44.
- Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K. and Watson, J. D. (1989). "Molecular Biology of the Cell". New York: Garland Publishing, Inc., pp. 661-674; 737; 746-760; 791-799; 966-971, 1187-1218.
- Ali, S., Tiwari, R. M., and Snow G. B. (1985). False-positive and false-negative neck nodes. *Head Neck Surgery* 8:78-82.
- American Academy of Periodontology (1977). *Annals of Periodontology*. Volume 2, Number 1. Proceedings of the 1996 Joint Symposium on Clinical Trial Design and Analysis in Periodontics. Editors: R J. Genco, R. C. Page. Chicago, Illinois.
- American Joint Committee on Cancer. Manual for Staging of Cancer (3rd edition). Philadelphia: J. B. Lippincott; 1988. Cited by Epstein, J. B. (1994). Oral Cancer. In M. A. Lynch, Brightman, V. J. and Greenberg, M S. (Editors): "Burket's Oral Medicine", Ninth Edition. Philadelphia: J. B. Lippincott Company, p. 205.
- Anneroth, G., Batsakis, J. and Luna, M. (1987). Review of the literature and a recommended system of malignancy grading in oral squamous cell carcinomas. *Scan J Dent Res* 95:229-249.
- Ansari, B. and Hall, P. A. (1992). The Kinetic Organisation of Tissues. In P. A. Hall, D. A. Levison and N. A. Wright (editors): "Assessment of Cell Proliferation in Clinical Practice". London: Springer-Verlag, pp. 45-62.
- Atkinson, M. E. and White, F. H. (1992). "Principles of Anatomy and Oral Anatomy for Dental Students". New York: Churchill Livingstone, pp. 26-29, 35-49, 327-333, 337-340, 365, 382, 485-493.
- Atula, S., Kurvinen, K., Grenman, R. and Syrjanen, S. (1996). SSCP pattern indicative of p53 mutation is related to advanced stage and high-grade of tongue cancer. *Eur J Cancer* 32B:222-229.
- Axell, T., Pindborg, J. J., Smith, C. J., van der Waal, I. and an International Collaborative Group on Oral White Lesions. (1996). Oral white lesions with special reference to precancerous and tobacco-related lesions: conclusions of an international symposium held in Uppsala, Sweden, May 18-21, 1994. *J Oral Pathol Med* 25:49-54.

- Bacallao, R., Bomsel, M., Stelzer, E. H. K. and De Mey, J. (1990). Guiding Principles of Specimen Preservation for Confocal Fluorescence Microscopy. In J. B. Pawley (editor): "Handbook of Biological Confocal Microscopy." New York: Plenum Press, pp. 197-205.
- Bachaud, J. M., Delannes, M., Allouache, N., Cenchalal, N., Alzieu, C., David, J. M., Serrano, E., and Daly-Schveitzer, N. J. (1994). Radiotherapy of Stage I and II carcinomas of the mobile tongue and/or floor of the mouth. *Radiotherapy and Oncology* 31:199-206.
- Baker, D. G. (1982). The radiobiological basis for tissue reactions in the oral cavity following therapeutic X-irradiation. *Arch Otolaryngol* 108:21-24.
- Band, P. R., Deschamps, M., Israel, L. (1989). Retinoid chemoprevention timing and dose intensity. *Cancer Investigation* 7:205-210.
- Banoczy, J. (1977). Follow-up studies in oral leukoplakia. *J Maxillofac Surg* 5:69-75.
- Banoczy, J. and Csiba, A. (1976). Occurrence of epithelial dysplasia in oral leukoplakia; analysis and follow-up of 120 cases. *Oral Surg Oral Med Oral Pathol* 42:766-774.
- Banoczy, J., Rigo, O. and Albrecht, M. (1993). Prevalence study of tongue lesions in a Hungarian population. *Community Dent Oral Epidemiol* 21:224-226.
- Barasch, A., Morse, D. E., Krutchkoff, D. J. and Eisenberg, E. (1994). Smoking, gender, and age as risk factors for site-specific intraoral squamous cell carcinoma. *Cancer* 73:509-513.
- Baretton, G., Li, X., Stoll, C., Fischer-Brandies, E., Schmidt, M., and Lohrs, U. (1995). Prognostic significance of DNA ploidy in oral squamous cell carcinomas. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 79:68-76.
- Barnett, M. L. and Mathisen, A. (1997). Tyranny of the p-value: the conflict between statistical significance and common sense. *J Dent Res* 1:534-536.
- Beeken, L. and Calman, F. (1994). A return to "normal eating" after curative treatment for oral cancer. What are the long-term prospects? *Oral Oncol, Eur J Oncol* 30B:387-392.
- Biorklund, A. and Wennersberg, J. (1994). Epidemiology of cancer of the oral cavity and oropharynx. In H. D. Pape, U. Ganzer, G. Sachmitt (editors): "Carcinoma of the Oral Cavity and Oropharynx". Berlin: Springer-Verlag, pp. 1-8.
- Bjordal, K., Freng, A., Thorvik, J. and Kaasa, S. (1995a). Patient self-reported and clinician-rated quality of life in head and neck cancer patients: a cross-sectional study. *Oral Oncol, Eur J Cancer* 31B:235-241.
- Bjordal, K., Mastekaasa, A. and Kaasa, S. (1995b). Self-reported satisfaction with life and physical health in long-term cancer survivors and a matched control group. *Oral Oncol, Eur J Cancer* 31B:340-345.
- Blot, W. J., Devesa, S. S., McLaughlin, J. K. and Fraumeni, J. F. (1994). Oral and pharyngeal cancers. *Cancer Surveys; Trends in Cancer Incidence and Mortality* 19/20:23-42.
- Blot, W. J., McLaughlin, J. K., Winn, D. M., Austin, D. F., Greenberg, R. S., Preston-Martin, S., Bernstein, L., Schoenberg, J. B., Stemhagen, A. and Fraumeni, J. F. (1988). Smoking and drinking in relation to oral and pharyngeal cancer. *Cancer Res* 48:3282-3287.

- Boffetta, P., Mashberg, A., Winkelmann, R. and Garfinkel, L. (1992). Carcinogenic effect of tobacco smoking and alcohol drinking on anatomic sites of the oral cavity and oropharynx. *Int J Cancer* 52:530-533.
- Boffetta, P., Merletti, F., Magnani, C. and Terracini, B. (1994). A population-based study of prognostic factors in oral and oropharyngeal cancer. *Oral Oncol Eur J Cancer* 30B:369-373.
- Bomford, C. K., Kunkler, I. H., and Sherriff, S. B. (1993). "Walter and Miller's Textbook of Radiotherapy". Edinburgh: Churchill Livingstone, pp. 3-269, 311-326.
- Boring, C. C., Siquiers, T. S. and Tong, T. (1991). Cancer statistics. *CA: Cancer J Clinicians* 41:19-36.
- Boukamp, P., Breitkreutz, D., Stark, H. J. and Fusenig, N. E. (1990). Mesenchyme-mediated and endogenous regulation of growth and differentiation of human skin keratinocytes derived from different body sites. *Differentiation* 4:150-161.
- Boyle, J. P., Hakin, J., Koch, W., van der Riet, P., Hruban, R. H., Roa, R. A., Correo, R., Eby, Y. J., Rupert, J. M. and Sidransky, D. (1993). The incidence of p53 mutations increases with progression of head and neck cancer. *Cancer Research* 53:4477-4480.
- Brickley, M. R., Cowpe, J. G. and Shepherd, J. P. (1996). Performance of a computer simulated neural network trained to categorise normal, premalignant and malignant oral smears. *J Oral Pathol Med* 25:424-428.
- British Columbia Cancer Agency, British Columbia Cancer Research Centre, British Columbia Cancer Foundation Annual Report 1995-1996.
- Browman, G. P., Wong, G., Hodson, I., Sathya, J., Russell, R., McAlpine, L., Skingley, P. and Levien, M. N. (1993). Influence of cigarette smoking on the efficacy of radiation therapy in head and neck cancer. *N Eng J Med* 328:159-163.
- Brown, R. S., Bottomley, W. K., Puente, E. and Lavigne G. L. (1993). A retrospective evaluation of 193 patients with oral lichen planus. *J Oral Pathol Med* 22:69-72.
- Brunette, D. M. (1996). Critical Thinking. Understanding and Evaluating Dental Research". Chicago: Quintessence Publishing Co., Inc., pp. 39-51, 63-203.
- Brugere, J., Guenel, P., Leclerc, A. and Rodriguez, J. (1986). Differential effects of tobacco and alcohol in cancer of the larynx, pharynx and mouth. *Cancer* 57:391-395.
- Bryne, M., Nielsen, K., Koppang, H. S. and Dabelsteen, E. (1991a). Reproducibility of two malignancy grading systems with reportedly prognostic value for oral cancer patients. *Oral Pathol Med* 21:369-372.
- Bryne, M., Nielsen, K., Koppang, H. S. and Dabelsteen, E. (1991b). Grading of nuclear pleomorphism in oral carcinomas. Higher prognostic value than stereological assessment of nuclear volume. *APMIS* 99:333-339.
- Bryne, M., Koppang, H. S., Lilleng, R. and Kjaerheim, A. (1992). Malignancy grading of the deep invasive margins of oral squamous cell carcinomas has high prognostic value. *J Pathol* 166:375-381.

- Bundgaard, T., Bentzen, S. M. and Wildt, J. (1994). The prognostic effect of tobacco and alcohol consumption in intra-oral squamous cell carcinoma. *Oral Oncol, Eur J Cancer*, 30B:323-328.
- Burkhardt, A. (1996). Oncogenes and Growth Factor Receptors as Diagnostic and Prognostic Markers in Precancers and Cancers of the Oral Mucosa. In G. Seifert (editor): "Oral Pathology, Actual Diagnostic and Prognostic Aspects". Berlin: Springer-Verlag, pp. 223-239.
- Burns, J. E., McFarlane, R., Clark, L. J., Mitchell, R., Robertson, G., Soutar, D. and Parkinson, E. K. (1994). Maintenance of identical p53 mutations throughout progression of squamous cell carcinomas of the tongue. *Eur J Oncol* 30B:335-337.
- Burzynski, N. J., Flynn, M. B., Faller, N. M. and Ragsdale, T.L. (1992). Squamous cell carcinoma of the upper aerodigestive tract in patients 40 years of age and younger. *Oral Surg Oral Med Oral Pathol* 74:404-408.
- Callery, C. D., Spiro, R. H. and Strong, E. W. (1984). Changing trends in the management of squamous carcinoma of the tongue. *Am J Surgery* 148:449-454.
- Cannell, H. (1971). Dyskeratosis congenita. *Br J Oral Surg* 9:8-20.
- Cawson, R. A. (1975). Premalignant lesions in the mouth. *Br Med Bull* 31:164-168.
- Cawson, R. A. and Binnie, W. H. (1980). Candida Leukoplakia and Carcinoma: A Possible Relationship. In I. C. Mackenzie, E. Dabelsteen, C. A. Squier (editors): "Oral Premalignancy". Iowa City: University of Iowa Press, pp. 59-66.
- Chiesa, F., Tradati, N., Marazza, M., Rossi, N., Boracchi, P., Mariani, L., Clerici, M., Formelli, F., Barzan, L., Carrassi, A., Pastorini, A., Camerini, R., Giardini, R., Zurrida, S., Minn, F. L., Costa, A., De Palo, G., and Veronesi, U. (1992). Prevention of local relapses and new localizations of oral leukoplakias with the synthetic retinoid fenretinide (4-HPR). *Eur J Cancer* 28B:97-102.
- Clarke, A. R., Purdie, C. A., Harrion, D. J., Morris, R. G., Bird, C. C., Hooper, M. L., and Wyllie, A. H. (1993). Thymocyte apoptosis induced by p53-dependent and independent pathways. *Nature* 362:849-851.
- Cooper, D., Schermer, A. and Sun, T. T. (1985). Classification of human epithelia and their neoplasms using monoclonal antibodies to keratins: strategies, applications and limitations. *Lab Investigation* 52:243-254.
- Cox, B., Taylor, K. and Treasore, E. (1995). Trends in oral cancer by subsite in New Zealand. *Oral Oncol, Eur J Cancer* 31B:113-117.
- Crecco, M., Vidiri, A., Palma, O., Floris, R., Squillaci, E., Mattioli, M., Marzatti, F. and Squillaci, S. (1994). T stages of tumours of the tongue and floor of the mouth: correlation between MR with gadopentetate dimeglumine and pathologic data. *AJNR Am J Neuroradiol* 15:1695-1702.
- Crowe, D. L., Hu, L., Gudas, L. J. and Rheinwald, J. G. (1991). Variable expression of retinoic acid receptor beta mRNA in human oral and epidermal keratinocytes; relation to keratin 19 expression and keratinization potential. *Differentiation* 48:199-208.

- Dabelsteen, E. (1980). Cytological and Immunological Approaches to Prognosis of Oral Premalignant Lesions. In I. C. Mackenzie, E. Dabelsteen, C. A. Squier (editors): "Oral Premalignancy". Iowa City: University of Iowa Press, pp. 335-350.
- Dabelsteen, E. and Mackenzie, I. C. (1987). The Scientific Basis for Oral Diagnosis. In I. C. Mackenzie, C. A. Squier, E. Dabelstein (editors): "Oral Mucosal Diseases: Biology, Etiology and Therapy". Copenhagen: Laegeforeningens forlag, pp. 99-102.
- Daniels, T. E. (1984). Human mucosal Langerhans Cells: postmortem identification of regional variation in oral mucosa. *J Invest Dermatol* 82:21-24.
- Daniels, T. E. (1987). Immune Functions of Epithelial Dendritic Cells. In: I. C. Mackenzie, C. A. Squier, E. Dabelstein (editors): "Oral Mucosal Diseases: Biology, Etiology and Therapy". Copenhagen: Laegeforeningens forlag, pp. 41-44.
- Darmon, M. (1991). Retinoic acid in skin and epithelia. *Sem Dev Biol* 2:219-228.
- Darnell, J., Lodish, H. and Baltimore, D. (1986). "Molecular Cell Biology". New York: Scientific American Books, pp. 894-899.
- Day, G. L., Blot, W. J., Austin, D. F., Berstein, L., Greenberg, R. S. and Preston-Martin, S. (1993). Racial differences in risk of oral and pharyngeal cancer: alcohol, tobacco, and other determinants. *J Natl Cancer Inst* 85:465-473.
- Day, G. L., Blot, W. J., Shore, R. E., Schoenberg, J. B., Kohler, B. A., Greenberg, R. S., Liff, J. M., Preston-Martin, S., Austin, D. F., McLaughlin, J. K. and Fraumeni, J. F. (1994a). Second cancers following oral and pharyngeal cancer: patients' characteristics and survival patterns. *Oral Oncol, Eur J Oncol* 30B:381-386.
- Day, G. L., Blot, W. J., Shore, R. E., Schoenberg, J. B., Kohler, B. A., Greenberg, R. S., Liff, J. M., Preston-Martin, S., Austin, D. F., McLaughlin, J. K. and Fraumeni, J. F. (1994b). Second cancers following oral and pharyngeal cancer: the role of tobacco and alcohol. *J Natl Cancer Inst* 86:131-137.
- De Araujo, V. C., Louola, A. M., Santos Pinto Jr., D. D., Borra, R. C., and De Araujo, N. S. (1997). P53 biopsies of oral squamous cell carcinoma. A comparative study with a malignancy grading system. *Oral Oncol* 33:5-9.
- Dearnaley, D. P., Dardoufas, C., A'Hearn, R. P., and Henk, J. M. (1991). Interstitial irradiation for carcinoma of the tongue and floor of the mouth: Royal Marsden Hospital Experience 1970-1986. *Radiotherapy and Oncology* 21:183-192.
- Denham, J. W. (1992). Implantation of the tongue and floor of mouth - what factors really do contribute to necrosis? *Radiotherapy and Oncology* 24:64-66.
- Denk, H. (1987). Immunohistochemical Methods for the Demonstration of Tumor Markers. In: G. Seifert (ed): "Morphological Tumor Markers." Berlin: Springer-Verlag, pp. 47-69.
- Denk, H., Lackinger, E., Zatloukal, K. and Franke, W. W. (1987). Turnover of cytokeratin polypeptides in mouse hepatocytes. *Exp Cell Res* 173:137-143.
- Devesa, S. S., Blot, W. J., Stone, B. J., Miller, B. A., Tarone, R. E. and Fraumeni, J. F. (1995). Recent cancer trends in the United States. *J Nat Cancer Institute* 87:175-182.

- Dowell, S. P. and Ogden, G. R. (1996). The use of antigen retrieval for immunohistochemical detection of p53 overexpression in malignant and benign oral mucosa: a cautionary note. *J Oral Pathol Med* 25:60-64.
- Duke, D. R., Ojcius, D. M. and Young, J. D. (1996). Cell suicide in health and disease. *Scientific American* December 1996:80-87.
- Einhorn, J. and Wersall, J. (1967). Incidence of oral carcinoma in patients with leukoplakia of the oral mucosa. *Cancer* 20:2189-2193.
- Eisenberg, E. (1992). Lichen planus and oral cancer: is there a connection between the two? *JADA* 123:104-108.
- Eisenberg, E. and Krutchkoff, D. J. (1992). Lichenoid lesions of oral mucosa. Diagnostic criteria and their importance in the alleged relationship to oral cancer. *Oral Surg Oral Med Oral Pathol* 73:699-704.
- Ephros, H. (1997). Editorial: Leukoplakia and malignant transformation. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 83:187.
- Epstein, J. B. (1992). Oral complications of cancer chemotherapy: etiology, recognition and management. *Can J Oncology* 2:83-95.
- Epstein, J. B. (1994). Oral Cancer. In M. A. Lynch, Brightman, V. J. and Greenberg, M. S. (Editors): "Burket's Oral Medicine", Ninth Edition. Philadelphia: J. B. Lippincott Company, pp. 203-239.
- Epstein, J. B., Wong, F. L. W., Stevenson-Moore, P. (1987). Osteoradionecrosis: clinical experience and a proposal for classification. *J Oral Maxillofacial Surg* 45:104-110.
- Epstein, J. B., Oakley, C., Millner, A., Emerton, S., van der Meij, E. and Nhu, L. (1997a). The utility of toluidine blue application as a diagnostic aid in patients previously treated for upper aerodigestive tract cancers. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 83:537-547.
- Epstein, J. B., van der Meij, E., McKenzie, M., Wong, F., Lepawsky, M., and Stevenson-Moore, P. (1997b). Postradiation osteonecrosis of the mandible. *Oral Surg Oral Med Oral Pathol* 83:657-662.
- Eversole, L. R. (1992). Editorial. *Oral Surg Oral Med Oral Pathol* 73:707.
- Eversole, L. R. (1993). Oral Mucosal Diseases; Review of the Literature. In H. D. Millard and D. K. Mason (editors): "2nd World Workshop on Oral Medicine". Michigan: BMC Media Production, University of Michigan, pp 105-186.
- Eversole, L. R. (1997). Editorial: Papillomaviruses and oral cancer. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 83:187-188.
- Fakih, A. R., Rao, R. S., Borges, A. M. and Patel, A. R. (1989). Elective versus therapeutic neck dissection in early carcinoma of the oral tongue. *Am J Surgery* 158:309-313.
- Fiore, M. C. (1992). Trends in cigarette smoking in the United States. *Medical Clinics North America* 76:289-304.

- Fleming, I. D., Brady, L. W., Mieszkalski, G. B., Cooper, M. R. and Cooper, M. R. (1995). Basis for Major Current Therapies for Cancer. In G. P. Murphy, W. Lawrence, Jr., R. E. Lenhard, Jr. (Editors): "American Cancer Society Textbook of Clinical Oncology". Washington: American Cancer Society, pp. 96-134.
- Folkman, J. and Moscona, A. (1978). Role of cell shape in growth control. *Nature* 273:345-349.
- Foote, R. L., Olsen, K. D., Davis, D. L., Buskirk, S. J., Stanley, R. J., Kunselman, S. J., Schaid, D. J. and DeSanto, L. W. (1992). Base of tongue carcinoma: patterns of failure and predictors of recurrence after surgery alone. *Head and Neck* 15:300-307.
- Franceschi, S., Barra, S., La Vecchia, C., Bidoli, E., Negri, E. and Talamini, R. (1992). Risk factors for cancer of the tongue and the mouth. *Cancer* 70:2227-2233.
- Franceschi, D., Gupta, R., Spiro, R. H., and Shah, J. P. (1993). Improved survival in the treatment of squamous carcinoma of the oral tongue. *Am J Surgery* 166:360-365.
- Frazell, E. L. and Lucas, J. C. (1962). Cancer of the tongue. Report of the management of 1554 patients. *Cancer* 15:1085-1099.
- Fukano, J., Matsuura, H., Hasegawa, Y., and Nakamaura, S. (1997). Depth of invasion as a predictive factor for cervical lymph node metastasis in tongue carcinoma. *Head Neck* 19:205-210.
- Gandour-Edwards, R. F., Donald, P. J. Yu, T. L-C., Howard, R. R. and Teplitz, R. L. (1994). DNA content of head and neck squamous carcinoma by flow and image cytometry. *Arch Otolaryngol Head Neck Surg* 120:294-297.
- Garfinkel, L. (1995a). Cancer Statistics and Trends. In G. P. Murphy, W. Lawrence, Jr., R. E. Lenhard, Jr. (Editors): "American Cancer Society Textbook of Clinical Oncology". Washington: American Cancer Society, pp. 1-9.
- Garfinkel, L. (1995b). Perspectives on cancer prevention. *CA - a cancer journal for clinicians* 45:5-8.
- Gehanno, P., Guedon, C., Barry, B., Depondt, J. and Kebaili, C. (1992). Advanced carcinoma of the tongue: total glossectomy without total laryngectomy. Review of 80 cases. *Laryngoscope* 102:1369-1371.
- Gopalakrishnan, R., Weghorst, C. M., Lehman, T. A., Calvert, R. J., Bijur, G., Sabourin, C. L. K., Mallery, S. R., Schuller, D. E., and Stoner, G. D. (1997). Mutated and wild-type p53 expression and HPV integration in proliferative verrucous leukoplakia and oral squamous cell carcinoma. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 83:471-477.
- Gorsky, M., Littner, M. M., Sukman, Y. and Begleiter, A. (1994). The prevalence of oral cancer in relation to the ethnic origin of Israeli Jews. *Oral Surg Oral Med Oral Pathol* 78:408-411.
- Greenblatt, M. S., Bennett, W. P., Hollstein, M. and Harris, C. C. (1994). Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. *Cancer Research* 54:4855-4878.

- Greenstein, G. and Lamster, I. (1995). Understanding diagnostic testing for periodontal diseases. *J Periodontol* 66:659-666.
- Greenwald, P., Kelloff, G., Burch-Whitman, C. and Kramer, B. S. (1995). Chemoprevention. *CA - Cancer J for Clinicians* 45:31-49.
- Gregory, W. M. (1992). Cell Proliferation and the Principles of Cancer Therapy. In P. A. Hall, D. A. Levison and N. A. Wright (editors): "Assessment of Cell Proliferation in Clinical Practice". London: Springer-Verlag, pp. 193-205.
- Greider, C. W. and Blackburn, E. H. (1996). Telomeres, telomerase and cancer. *Scientific American* Feb 1996:
- Gridley, G., McLaughlin, J. K., Block, G., Blot, W. J., Gluck, M. and Fraumeni, J. F. (1992). Vitamin supplement use and reduced risk of oral and pharyngeal cancer. *Am J Epidemiol* 135:1083-1092.
- Gudas, L. J., Sporn, M. B. and Roberts, A. B. (1994). Cellular Biology and Biochemistry of the Retinoids. In M. B. Sporn, A. B. Roberts and D. S. Goodman (editors). "The Retinoids: Biology, Chemistry, and Medicine". New York: Raven Press, Ltd., pp 443-520.
- Gupta, P. C., Mehta, F. S., Pindborg, J. J., Aghi, M. B., Bhonsle, R. B., Daftary, D. K., Murti, P. R. and Shah, H. T. (1986). Intervention study for primary prevention of oral cancer among 36,000 Indian tobacco users. *Lancet* 1:1235-1238.
- Gupta, P. C., Bhonsle, R. B., Murti, P. R., Kaftary, D. K., Mehta, F. A. and Pindborg, J. J. (1989). An epidemiologic assessment of cancer risk in oral precancerous lesions in India with special reference to nodular leukoplakia. *Cancer* 63:2247-2252.
- Harrison, L. B., Sessions, R. B., Strong, E. W., Fass, E. D., Nori, D., and Fuks, A. (1989). Brachytherapy as part of the definitive management of squamous cancer of the base of tongue. *Int J Radiat Oncol Biol Phys* 17:1390-1312.
- Harrison, L. B., Zelefsky, M. J., Sessions, R. B., Fass, D. E., Armstrong, J. G., Pfister, D. G. and Strong, E.W. (1992). Base-of-tongue cancer treated with external beam irradiation plus brachytherapy: oncologic and functional outcome. *Radiology* 184:267-270.
- Harrison, L. B., Zelefsky, M. J., Armstrong, J. G., Carper, E., Gaynor, J. J. and Sessions, R. B. (1994). Performance status after treatment for squamous cell cancer of the base of tongue - a comparison of primary radiation therapy versus primary surgery. *Int J Radiation Oncology Biol Phys* 30:953-957.
- Hecht, S. S., Peterson, L. A. and Spratt, T. E. (1994). Tobacco-specific Nitrosamines. In K. Hemminki, A. Dipple, D. E. G. Shuker, F. F. Kadlubar, D. Segerback and H. Bartsch (editors): "DNA Adducts: Identification and Biological Significance". Lyon: International Agency for Research on Cancer, pp. 91-106.
- Hennekens, C. H. and Buring, J. E. (1987). *Epidemiology in Medicine*. Boston: Little, Brown and Company, pp. 3-177, 243-323.
- Heyden, A., Huitfeldt, H. S., Koppang, H. S., Thrane, P. S., Bryne, M. and Brandtzaeg, P. (1992). Cytokeratins as epithelial differentiation markers in premalignant and malignant oral lesions. *J Oral Pathol Med* 21:7-11.

- Hill, M. W. and Mackenzie, I. C. (1989). The influence of subepithelial connective tissues on epithelial proliferation in adult mouse. *Cell Tissue Res* 255:179-182.
- Hirshberg, A. and Buchner, A. (1995). Metastatic tumours to the oral region. An overview. *Eur J Cancer* 31B:355-360.
- Hlatky, M. A., Pryor, D. B., Harrell, R. E. Jr., Califf, R. M. and Rosati, R. A. (1984). Factors affecting sensitivity and specificity of exercise electrocardiography. *Am J Med* 77:64-71.
- Hlatky, M. A., Mark, D. B., Harrell, F. E. Jr., Lee, K. L., Califf, R. M. and Pryor D. B. (1987). Rethinking sensitivity and specificity. *Am J Cardiol* 59:1195-1198.
- Hollywood, D. P. and Lemoine, N. R. (1992). Growth Factors, Oncogenes and Tumour Suppressor Genes. In P. A. Hall, Levison, D. A. and N A. Wright (editors): "Assessment of Cell Proliferation in Clinical Practice". London: Springer-Verlag, pp. 27-43.
- Holmstrup, P. (1992). The controversy of a premalignant potential of oral lichen planus is over. *Oral Surg Oral Med Oral Pathol* 73:704-706.
- Holmstrup, P., Thorn, J. J., Rindum, J. and Pindborg, J. J. (1988). Malignant development of lichen planus-affected oral mucosa. *J Oral Pathol* 17:219-225.
- Hong, W. K., Endicott, J., Itri, L. M., Doos, W., Batsakis, J. G., Bell, R., Fofonoff, S., Byers, R., Atkinson, E. N., Vaughan, D., Toth, B. B., Kramer, A., Dimery, I. W., Skipper, P., and Stong, S. (1986). 13-cis-retinoic acid in the treatment of oral leukoplakia. *New Engl J Med* 315:1501-1505.
- Hong, W. K., Lippman, S. M., Itri, L. M., Karp, D. D., Lee, J. S., Byers, R. M., Schantz, S. P., Kramer, A. M., Lotan, R., Peters, L. J., Dimery, I. W., Brown, B. W., and Goepfert, H. (1990). Prevention of second primary tumours with isotretinoin in squamous cell carcinoma of the head and neck. *New Engl J Med* 323:795-801.
- Horwitz, A. F. (1997). Integrins and health. *Scientific American* May 1997:68-75.
- Horwitz, A. F. and Thiery, J. P. (1994). Cell-to-cell contact and extracellular matrix. *Curr Opin Cell Biol* 6:645-647.
- Hu, L., Crowe, D. L., Rheinwald, J. G., Chambon, P. and Gudas, L. J. (1991). Abnormal expression of retinoic acid receptors and keratin 19 by human oral and epidermal squamous cell carcinoma cell lines. *Cancer Res* 51:3972-3981.
- International Agency for Research in Cancer Monographs. (1995). *Studies of Cancer in Humans*. 64:189-192.
- International Classification of Diseases (ICD), 9th Revision, Clinical Modification, 4th Edition (1995). California: Practice Management Information Corporation, pp. 62-64.
- Ismail, A.I, Burt, B. A., Hendershot, G. E., Jack, S. and Corbin, S.B. (1987). Findings from the dental care supplement of the National Health Interview Survey, 1983. *JADA* 114:617-621.

- Jakobsson, P. A., Eneroth, C. M., Killander, D., Moberger, G. and Martensson, B. (1973). Histologic classification and grading of malignancy in carcinoma of the larynx (a pilot study). *Acta Radiol Ther Phys Biol* 12:1-8. Cited and reviewed in Anneroth, G., Batsakis, J. and Luna, M. (1987). Review of the literature and a recommended system of malignancy grading in oral squamous cell carcinomas. *Scan J Dent Res* 95:229-249.
- Jetten, A. M., Fujimoto, W., Zhang, L., Marvin, K., Austin, S., Mills, K. J., Bernacki, S. H. and Saunders, N. A. (1994). Regulation of Gene Expression During Squamous Differentiation by Multiple Retinoic Acid Signalling Pathways. In M. A. Livrea and G. Vidali (editors): "Retinoids: From Basic Science to Clinical Application". Basel: Birkhauser Verlag, pp 243-252.
- Johnson, N. W., Evans, A. W., Morgan, P. R. and Butcher, R. G. (1980). Biochemical and Histochemical Changes in Oral Premalignancy. In I. C. Mackenzie, E. Dabelsteen, C. A. Squier (editors): "Oral Premalignancy". Iowa City: University of Iowa Press, pp. 312-334.
- Johnston, W. D. and Ballantyne, A. J. (1977). Prognostic effect of tobacco and alcohol use in patients with oral tongue cancer. *Am J Surg* 134:444-447.
- Jovanovic, A., Schulten, E. A. J. M., Kostense, P. J., Snow, G. B. and van der Waal, I. (1993a). Tobacco and alcohol related to the anatomical site of oral squamous cell carcinoma. *Oral Pathol Med* 22:459-462.
- Jovanovic, A., Schulten, E. A. J. M., Kostense, P. J., Snow, G. B. and van der Waal, I. (1993b). Squamous cell carcinoma of the lip and oral cavity in the Netherlands; an epidemiological study of 740 patients. *J Cranio-Max-Fac Surg* 21:149-152.
- Kannan, R., Bijur, G. N., Mallery, S. R., Beck, F. M., Sabourin, C. L. K., Jewell, S. D., Schuller, D. E. and Stoner, G. D. (1996). Transforming growth factor- α overexpression in proliferative verrucous leukoplakia and oral squamous cell carcinoma. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 82:69-74.
- Karabulut, A., Reibel, J., Therkildsen, M. H., Praetorius, F., Hielsen, H. W. and Dabelsteen, E. (1995). Observer variability in the histologic assessment of oral premalignant lesions. *J Oral Pathol Med* 24:198-200.
- Karring, T., Comming, B. R., Oliver, R. C. and Loe, H. (1972). The origin of granulation tissue and its impact on post-operative results of mucogingival surgery. *J Periodontol* 46:577.
- Karring, T., Lang, N. P. and Loe, H. (1975). The role of connective tissue in determining epithelial differentiation. *J Periodont Res* 10:1.
- Kaugars, G. E. (1995). Discussion. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 80:191.
- Kaugars, G. E., Silverman, S., Lovas, J. G. L., Thompson, J. S., Brandt, R. D., and Singh V. N. (1996). Use of antioxidant supplements in the treatment of human oral leukoplakia. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 81:5-14.
- Kautsky, M. B., Fleckman, P. and Dale, B. A. (1995). Retinoic acid regulates oral epithelial differentiation by two mechanisms. *J Invest Dermatol* 104:546-554.

- King, W. W. K., Lam, P. K. and Arthur, D. C. (1995). DNA ploidy as a predictor of cervical metastasis in advanced squamous carcinoma of the tongue. *Acta Otolaryngol* 115:455-458.
- Kinsella, A. R., Bowie, G. L., Field, J. K. and Jones, A. S. (1994). Expression of the cell-cell adhesion molecule E-cadherin in tongue carcinoma cell lines. *J Laryngology and Otology* 108:957-961.
- Kleinman, D. V., Swango, P. A., Pindborg, J. J. and Gupta, P. (1993). Toward assessing trends in oral mucosal lesions: lessons learned from oral cancer. *Adv Dent Res* 7:32-41.
- Kowalski, L. P., Franco, E. L., Torloni, H., Fava, A. S., Sobrinho, J., Ramos, G., Oliveira, B. V. and Curado, M. P. (1994). Lateness of diagnosis of oral and oropharyngeal carcinoma: factors related to the tumour, the patient and health professionals. *Oral Oncol, Eur J Cancer* 30B:167-173.
- Kramer, I. R. H. (1980a). Basic Histopathological Features of Oral Premalignant Lesions. In I. C. Mackenzie, E. Dabelsteen, C. A. Squier (editors): "Oral Premalignancy". Iowa City: University of Iowa Press, pp. 23-34.
- Kramer, I. R. H. (1980b). Prognosis from Features Observable by Conventional Histopathological Examination. In I. C. Mackenzie, E. Dabelsteen, C. A. Squier (editors): "Oral Premalignancy". Iowa City: University of Iowa Press, pp. 304-311.
- Kraus, D. H., Vastola, P., Huvos, A. G. and Spiro, R. H. (1993). Surgical management of squamous cell carcinoma of the base of the tongue. *Am J Surgery* 166:384-388.
- Krogh, P., Hald, B. and Holmstrup, P. (1987). Possible mycological etiology of oral mucosal cancer: catalytic potential of infecting *Candida albicans* and other yeasts in production of N-nitrosobenzylmethylamine. *Carcinogenesis* 8:1543-1548.
- Krolls, S. O. and Hoffman, S. (1976). Squamous cell carcinoma of the oral soft tissues: a statistical analysis of 14,253 cases by age, sex, and race of patients. *JADA* 92:571-574.
- Krupala, J. L. and Gianoli, G. J. (1993). Carcinoma of the oral tongue. *J Louisiana State Medical Society* 145:421-426.
- Kulasegaram, R., Downer, M. C., Jullien, J. A., Zakrzewska, J. M. and Speight, P. M. (1995). Case-control study of oral dysplasia and risk habits among patients of a dental hospital. *Oral Oncol, Eur J Cancer* 31B:227-231.
- Lane, D. P. (1993). A death in the life of p53. *Nature* 362:786-787.
- Languis, A. and Lind, M. G. (1995). Well-being and coping in oral and pharyngeal cancer patients. *Oral Oncol, Eur J Cancer* 31B:242-249.
- Laskaris, G. (1988). "Color Atlas of Oral Disease". New York: Thieme Medical Publishers, Inc., pp. 14, 219-229.
- La Vecchia, C., Negri, E., D'Avanzo, B., Boyle, P. and Franceschi, S. (1991). Dietary indicators of oral and pharyngeal cancer. *Int J Epidemiol* 21:39-44.
- Lavelle, C. L. B. (1975a). Deglutition. In C. L. B. Lavelle (editor): "Applied Physiology of the Mouth." Bristol: John Wright and Sons Limited, pp 243-248.

- Lavelle, C. L. B. (1975b). The Metabolism of the Oral Mucosa. In C. L. B. Lavelle (editor): "Applied Physiology of the Mouth." Bristol: John Wright and Sons Limited, pp 33-44.
- Lavelle, C. L. B. (1975c). The Healing of Wounds. In C. L. B. Lavelle (editor): "Applied Physiology of the Mouth." Bristol: John Wright and Sons Limited, pp 334-344.
- Lawley, P. D. (1994). From Fluorescence Spectra to Mutational Spectra, a Historical Overview of DNA-reactive Compounds. In K. Hemminki, A. Dipple, D. E. G. Shuker, F. F. Kadlubar, D. Segerback and H. Bartsch (editors): "DNA Adducts: Identification and Biological Significance". Lyon: International Agency for Research on Cancer, pp. 3-22.
- Lazarus, P., Farewal, H. S., Scuibba, J., Zwiebel, N., Calcagnotto, A., Air, A., Schaefer, S. and Richer, J. P. Jr. (1995). A low incidence of p53 mutations in premalignant lesions of the oral cavity from non-tobacco users. *Int J Cancer* 60:458-463.
- Leedy, D. A., Trune, D. R., Kronz, J. D., Weidner, N. and Cohen, J. I. (1994). Tumor angiogenesis, the p53 antigen, and cervical metastasis in squamous cell carcinoma of the tongue. *Otolaryngol Head Neck Surg* 111:417-422.
- Leeson, T. S. and Leeson, C. R. (1970). "Histology". Philadelphia: W. B. Saunders Company, pp. 256-258.
- Leong, I. T., Main, J. H. P., and Birt, B.D. (1995). Cancer of the tongue. *CDAJ* 61:792-796.
- Levine, M. J., Jones, P. D., Loomis, R. E., Reddy, M. S., Al-Hashimi, I., Bergey, E. J., Bradway, S. D., Cohen, R. E. and Tabak, L. A. Functions of Human Saliva and Salivary Mucins: An Overview. In: I. C. Mackenzie, C. A. Squier, E. Dabelstein (editors): "Oral Mucosal Diseases: Biology, Etiology and Therapy". Copenhagen: Laegeforeningens forlag, pp. 7-9.
- Lindberg, K. and Rheinwald, J. G. (1989). Suprabasal 40kd keratin (K19) expression as an immunohistologic marker of premalignancy in oral epithelium. *Am J Oral Pathol* 134:89-98.
- Lippman, S. M., Lee, J. S., Lotan, R., Hittleman, W., Wargovich, M. J., and Hong, W. K. (1990). Biomarkers as intermediate end points in chemoprevention trials. *National Cancer Inst Journal* 82:555-560.
- Llewellyn, J. and Mitchell, R. (1994). Smoking, alcohol and oral cancer in South East Scotland: a 10-year experience. *Br J Oral Maxillofac Surg* 32:146-152.
- Lotan, R. (1994). Relationships among Nuclear Retinoic Acid Receptors, Squamous Differentiation and Head and Neck Carcinogenesis and Prevention. In M. A. Livrea and G. Vidali (editors): "Retinoids: From Basic Science to Clinical Application". Basel: Birkhauser Verlag, pp 253-266.
- Lovas, J. G. L., Harsanyi, B. B. and El Geneidy, A. K. (1989). Oral lichenoid dysplasia: a clinicopathologic analysis. *Oral Surg Oral Med Oral Pathol* 68:57-63.
- Love, J. M. and Gudas, L. J. (1994). Vitamin A, differentiation and cancer. *Current Opinion in Cell Biology* 6:825-831.
- Lowe, S.W., Schmitt, E. M., Smith, S. W., Osborne, B. A., and Jacks, T. (1993). p53 is required for radiation-induced apoptosis in mouse thymocytes. *Nature* 362:847-849.

- Lumerman, J. H., Freedman, P. and Kerpel, S. (1995). Oral epithelial dysplasia and the development of invasive squamous cell carcinoma. *Oral Surg, Oral Med, Oral Pathol* 79:321-329.
- Ma, R., Epstein, J. B., Emerton, S. and Jay, J. H. (1995). A preliminary investigation of an association between dental restorations and carcinoma of the tongue. *Oral Oncol, Eur J Cancer* 31B:232-234.
- MacFarlane, T. W. (1975). Defense Mechanisms of the Mouth. In C. L. B. Lavelle (editor): "Applied Physiology of the Mouth." Bristol: John Wright and Sons Limited, pp 180-190.
- Macfarlane, G. J., Boyle, P. and Scully, C. (1992). Oral cancer in Scotland: changing incidence and mortality. *Br Med J* 305:1121-1123.
- Macfarlane, G. J., McCredie, M., Pompe-Kirn, V., Sharpe, L. and Coates, M. (1995). Second cancers occurring after cancers of the mouth and pharynx; data from three population-based registries in Australia, Scotland and Slovenia. *Oral Oncol, Eur J Oncol* 31B:315-318.
- Mackenzie, I. C. and Hill, M.W. (1984). Connective tissue influences on patterns of epithelial architecture and keratinization in skin and oral mucosa of the adult mouse.. *Cell Tissue Res.* 235:551-559.
- Maden, C., Beckman, A. M., Thomas, D. B., McKnought, B., Sherman, K. J., Ashley, R. L., Corey, L. and Daling, J. R. (1992). Human papillomavirus, herpes simplex viruses, and the risk of oral cancer in men. *Am J Epid* 135:1093.
- Maier, H., Zoller, J., Herrmann, A., Kreiss, M. and Heller, W. D. (1993). Dental status and oral hygiene in patients with head and neck cancer. *Otolaryngol head neck surg* 108:655-661.
- Maroudas, N. G. (1973a). Chemical and mechanical requirements for fibroblast adhesion. *Nature* 244:353-354.
- Maroudas, N. B. (1973b). Growth of fibroblasts on linear and planar anchorages of limiting dimensions. *Exp Cell Res* 81:104-110.
- Marx, R. E. and Johnson, R. P. (1987). Studies in the radiobiology of osteoradionecrosis and their clinical significance. *Oral Surg Oral Med Oral Path Oral Radio* 64:379-390.
- Mashberg, A. (1980). Clinical Features of Oral Malignancy in Relation to Prognosis. In I. C. Mackenzie, E. Dabelsteen, C. A. Squier (editors): "Oral Premalignancy". Iowa City: University of Iowa Press, pp.292-334.
- Mashberg, A. and Samit, A. (1995). Early diagnosis of asymptomatic oral and oropharyngeal squamous cancers. *CA Cancer J Clin* 45:328-351.
- Mashberg, A., Garfinkel, L. and Harris, S. (1981). Alcohol as a primary risk factor in oral squamous carcinoma. *CA Cancer J Clin* 31:146-155.
- Mashberg, A., Morrissey, J. B. and Garfinkel, L. (1973). A study of the appearance of early asymptomatic oral squamous cell carcinoma. *Cancer* 32:1436-1445.
- Matthews, B. (1975). Mastication. In C. L. B. Lavelle (editor): "Applied Physiology of the Mouth." Bristol: John Wright and Sons Limited, pp. 199-242.

- Mazeron, J. J., Simon, J. M., Le Pechoux, C., Crook, J. M., Grimard, L., Piedbois, P., Le Bourgeois, J. P., and Pierquin, B. (1991). Effect of dose rate on local control and complications in definitive irradiation of T1-2 squamous cell carcinomas of mobile tongue and floor or mouth with interstitial iridium-192. *Radiotherapy and Oncology* 21:39-47.
- Mazeron, J. J., Simon, J. M., Le Pechoux, C., Crook, J. M., Grimard, L., Piedbois, P., Le Bourgeois, J. P., and Pierquin, B. (1992). Reply to the Editors. *Radiotherapy and Oncology* 24:65-66.
- McGregor, G. I., Davis, N. and Robins, R. E. (1983). Squamous cell carcinoma of the tongue and lower oral cavity in patients under 40 years of age. *Am J Surgery* 146:88-92.
- McLaughlin, J. K., Gridley, G., Block, G., Winn, D. M., Preston-Martin, S., Schonberg, J. B., Greenberg, R. S., Stemhagen, A., Autin, D. F., Ershow, A. G., Blot, W. J. and Fraumeni, J. F. (1988). Dietary factors in oral and pharyngeal cancer. *J Nat Cancer Inst* 80:1237-1243.
- McLean, W. H. and Lane, E. B. (1995). Intermediate filaments in disease. *Curr Opin Cell Biol* 7:118-125.
- Miller, D. S. and White, D. K. (1996). Human papillomavirus expression in oral mucosa, premalignant conditions, and squamous cell carcinoma. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 82:57-68.
- Mills, J. L. (1993). Data torturing. *N Eng J Med* 329:1196-1199.
- Mincer, H. H., Coleman, S. A. and Hopkins, K. P. (1972). Observation on the clinical characteristics of oral lesions showing histologic epithelial dysplasia. *Oral Surg Oral Med Oral Pathol* 33:389-399.
- Moll, R. (1987). Epithelial Tumor Markers: Cytokeratins and Tissue Polypeptide Antigen. In: G. Seifert (ed): *Morphological Tumor Markers*. Berlin: Springer-Verlag, pp. 71-101.
- Moll, R., Franke, W. W. and Schiller, D. L. (1982). The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. *Cell* 31:11-24.
- Monasebian, D. M. and Ruskin, J. D. (1994). Flow cytometric analysis of squamous cell carcinoma of the tongue. *J Oral Maxillofac Surg* 52:574-578.
- Moore, C. and Catlin, D. (1967). Anatomic origins and locations of oral cancer. *Am J Surg* 114:510-513.
- Morgan, P. R. and Su, L. (1994). Intermediate filaments in oral neoplasia. I. Oral cancer and epithelial dysplasia. *Oral Oncol, Eur J. Cancer* 30B, No. 3, pp 160-166.
- Morton, R. P., Ferguson, C. M., Lambie, N. K. and Whitlock, R. M. L. (1994). Tumor thickness in early tongue cancer. *Arch Otolaryngol Head Neck Surg* 120:717-720.
- Mukherji, S. K., Weeks, S. M., Castillo, M., Ynnkaskas, B. C., Krishnan, L. A. G. and Schiro, S. (1996). Squamous cell carcinomas that arise in the oral cavity and tongue base: can CT help predict perineural or vascular invasion? *Radiology* 198:157-162.

- Mulshine, J. L., Jett, M., Cuttitta, F., Treston, A. M., Qinn, K., Scott, F., Iwai, N., Avis, I., Linnoila, I., and Shaw, G. L. (1993). Scientific basis for cancer prevention. *Cancer* 72:978-983.
- Murnane, J. P. and Schwartz, J. L. (1993). Cell checkpoint and radiosensitivity. *Nature* 365:22.
- Murti, P. R., Bhonsale, R. B., Gupta, P. C., Daftary, D. K., Pindborg, J. J. and Mehta, F. S. (1995). Etiology of oral submucous fibrosis with special reference to the role of areca nut chewing. *J Oral Pathol Med* 24:145-152.
- Murti, P. R., Daftary, D. K., Bhonsle, R. B., Gupta, P. C., Mehta, F. S. and Pindborg, J. J. (1986). Malignant potential of oral lichen planus: observations in 722 patients from India. *J Oral Pathol* 15:71-77.
- Nathan, C. and Sporn, M. (1991). Cytokines in context. *J Cell Biol* 113:981-986.
- National Cancer Institute of Canada. Candian Cancer Statistics 1995:18.
- National Cancer Institute of Canada. Canadian Cancer Statistics 1996.
- Newcomb, P. A. and Carbone, P. P. (1992). The health consequences of smoking. *Medical Clinics North America* 76:305-332.
- Nielsen, H., Norrild, B., Vedtofte, P., Praetorius, F., Reibel, J. and Holmstrup, P. (1996). Human papillomavirus in oral premalignant lesions. *Oral Oncol, Eur J Cancer* 32B:264-270.
- Nielsen, J. (1942). Om cancer i de ovre luft-og spiseveje. *Tandlaegebladet* 46:1-23. Cited in: van der Waal, I. and Pindborg, J. J. (1986). "Diseases of the Tongue". Chicago: Quintessence Publishing Co, Inc., p. 163.
- Norman, G. R. and Streiner, D. L.. (1986). PDQ Statistics. Toronto: B. C. Decker Inc.
- Nyman, J., Mercke, C. and Linstrom, J. (1993). Prognostic factors for local control and survival of cancer of the oral tongue. *Acta Oncologica* 32:667-673.
- Odell, E. W., Jani, P., Sherriff, M., Ahluwalia, S. M., Hibbert, J., Levison, D. A. and Morgan, P. R. (1994). The prognostic value of individual histologic grading paramenters in small lingual squamous cell carcinomas. *Cancer* 74:789-794
- Odland, G. and Ross, R. (1968). Human wound repair. I. Epidermal regeneration. *J Cell Biol* 39:135-151.
- Ogden, G. R. (1997). The future role for oral exfoliative cytology - bleak or bright? *Oral Oncol* 33:2-4.
- Ogden, G. R., Connor, E. and Chisholm, D. M (1988). Dyskeratosis congenita: report of a case and review of the literature. *Oral Surg Oral Med Oral Pathol* 65:586-591.
- Ogden, G. R., Cowpe, J. G. and Green, M. W. (1991). Detection of field change in oral cancer using oral exfoliative cytologic study. *Cancer* 68:1611-1615.

- Ogden, G. R., Cowpe, J. G., Chisholm, D. M. and Lane, E. B. (1994). DNA and keratin analysis of oral exfoliative cytology in the detection of oral cancer. *Eur J Cancer* 30B:405-408.
- Oreggia, F., De Stefani, E., Correa, P. and Fierro, L. (1991). Risk factors for cancer of the tongue in Uruguay. *Cancer* 67:180-183.
- Ostman, J., Anneroth, G., Gustaffsson, H. and Tavelin, B. (1995). Malignant oral tumours in Sweden 1960-1989 - an epidemiological study. *Oral Oncol, Eur J Cancer* 31B:106-112.
- Ostwald, C., Muller, P., Barten, M., Rutsatz, K., Sonnenburg, M., Milde-Langosch, K. and Loning, T. (1994). Human papillomavirus DNA in oral squamous cell carcinomas and normal mucosa. *J Oral Pathol Med* 23:220-225
- Ouhayoun, J. P., Sawaf, M. H., Fofflaux, J. C., Etienne, D., and Forest, N. (1988). Re-epithelialization of a palatal connective tissue graft transplanted in a non-keratinized alveolar mucosa: a histological and biochemical study in humans. *J. Perio. Res.* 23:127-133.
- Palefsky, J. M., Silverman, S., Abdel-Salaam, M., Daniels, T. E., and Greenspan, J. S. (1995). Association between proliferative verrucous leukoplakia and infection with human papillomavirus type 16. *J Oral Pathol Med* 24:193-197.
- Parmar, M. K. B. and Machin D. (1996). "Survival Analysis. A Practical Approach". New York: John Wiley and Sons, pp 1-50, 140-151.
- Parker, S. L., Tong, T., Bolden, S. and Wingo, P. A. (1996). Cancer statistics, 1996. *CA Cancer J Clin* 46:5-27.
- Parker, S. L., Tong, T., Bolden, S. and Wingo, P.A. (1997). Cancer statistics, 1997. *CA Cancer J Clin* 47:5-27.
- Paterson, I. C., Eveson, J. W. and Prime, S. S. (1996). Molecular changes in oral cancer may reflect aetiology and ethnic origin. *Oral Oncol, Eur J Cancer* 32B:150-153.
- Peck, G. L. and DiGiovanna, J. J. (1994). Synthetic Retinoids in Dermatology. In M. B. Sporn, A. B. Roberts and D. S. Goodman (editors). "The Retinoids: Biology, Chemistry, and Medicine". New York: Raven Press, Ltd., pp 631-158.
- Perera, F. (1993). Biomarkers and molecular epidemiology of occupationally related cancer. *J Toxicol Environmental Health* 40:203-215.
- Pernot, M., Lamissard, L., Hoffstetter, S., Luporsi, E., Peiffert, D., Aletti, P., Kozminski, P. and Bey, P. (1994). The study of tumoral, radiobiological, and general health factors that influence results and complications in a series of 448 oral tongue carcinomas treated exclusively by irradiation. *Int J Radiation Oncology Biol Phys* 29:673-679.
- Pfahl, M. (1994). Retinoid Response Pathways. In M. A. Livrea and G. Vidali (editors): "Retinoids: From Basic Science to Clinical Applications". Basel: Birkhauser Verlag, pp. 115-136.
- Pillai, R., Garewal, H. S., Wood, S., and Watson, R. R. (1992). Biological monitoring of cancer chemoprevention. *J Surgical Oncology* 51:195-202.

- Pindborg, J. J. (1980). Lesions of the Oral Mucosa to be Considered Premalignant and Their Epidemiology. In I. C. Mackenzie, E. Dabelsteen, C. A. Squier (editors): "Oral Premalignancy". Iowa City: University of Iowa Press, pp. 2-14.
- Pindborg, J. J. (1994). Clinical Relevance of Precancerous Lesions of Oral Mucosa. In H. D. Pape, U. Ganzer, Schmitt, G. (Editors): "Carcinoma of the Oral Cavity and Oropharynx". Springer-Verlag, pp. 9-16.
- Pindborg, J. J., Reibel, J. and Holmstrup, P. (1985). Subjectivity in evaluating oral epithelial dysplasia, carcinoma in situ and initial carcinoma. *J Oral Pathol* 14:698-708.
- Porter, S. R., Kirby, A., Olsen, I. and Barrett, W. (1997). Immunological aspects of dermal and oral lichen planus. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 83:358-366.
- Pukkala, E., Soderholm, A. L. and Lindqvist, C. (1994). Cancers of the lip and oropharynx in different social and occupational groups in Finland. *Oral Oncol, Eur J Cancer* 30B:209-215.
- Potter, R. H. (1994). Significance level and confidence interval. *J Dental Res* 73:494-496.
- Price, C. (1988). Oral Radiology. OMSS 426, 1988-1989 Course Manual. Faculty of Dentistry, University of British Columbia, British Columbia, Canada.
- Raybaud-Diogene, H., Tetu, B., Morency, R., Fortin, A. and Monteil, R. A. (1996). p53 overexpression in head and neck squamous cell carcinoma: review of the literature. *Oral Oncol, Eur J Cancer* 32B:143-149.
- Regezi, J. A. and Sciubba, J. J. (1989). "Oral Pathology - Clinical-Pathological Correlations". Philadelphia: W. B. Saunders Company, pp. 70-73, 105-118, 139-141, 337-348.
- Regezi, J. A., Zarbo, R. J., Regev, E., Pisanty, S., Silverman, S., and Gazit, D. (1995). p53 protein expression in sequential biopsies of oral dysplasias and in situ carcinomas. *J Oral Pathol Med* 24:18-22.
- Reichert, P. A. (1995). Oral cancer and precancer related to betel and miang chewing in Thailand: a review. *J Oral Pathol Med* 24:241-243.
- Riber, E. and Kaaber, S. (1978). Barrier properties of inflamed denture-loaded palatal mucosa to water. *Scand J Dent Res* 86:386-391.
- Riva, C., Lavieille, J.P., Reyt, E., Brambilla, E., Lunardi, J., and Brambilla, C. (1995). Differential c-myc, c-jun, c-raf and p53 expression in squamous cell carcinoma of the head and neck: implication in drug and radioresistance. *Oral Oncol, Eur J Cancer* 31B:384-391.
- Ross, M. H. and Reith, E. J. (1985). "Histology - A Text and Atlas". New York: Harper & Row, Publishers, J. B. Lippincott Company, pp. 291, 306, 338-339; 370-411.
- Romanes, G. J. (1986). "Cunningham's Manual of Practical Anatomy". New York: Oxford University Press, pp. 130-137, 166-171, 301-307.
- Rosenberg, D. and Cretin, S. (1989). Use of meta-analysis to evaluate tolonium chloride in oral cancer screening. *J Oral Surg* 67:621-627.

- Routh, A. and Hickman, B. (1991). The influence of socio-economic status on prognosis of patients with cancer of the tongue after radiotherapy. *Radiation Medicine* 9:26-28.
- Rubin, R. L. and Doku, H. C. (1976). Therapeutic radiology - the modalities and their effects on oral tissues. *JADA* 92:731-739.
- Rubright, W. C., Hoffman, H. T., Lynch, C. F., Kohout, F. J., Robinson, R. A., Graham, S.G., Funk, G. and McCulloch, T. (1996). Risk factors for advanced-stage oral cavity cancer. *Arch Otolaryngol Head Neck Surg* 122:621-626.
- Ruoslahti, E. (1996). How cancer spreads. *Scientific American* September 1996:72-77.
- Rugg, T., Saunders, M. I. and Dische, S. (1990). Smoking and mucosal reactions to radiotherapy. *Br J Radiology* 63:554-556.
- Sackett, D. L. (1979). Bias in analytic research. *J Chron Dis* 32:51-63.
- Sackett, D. L., Haynes, R. B., Guyatt, G. H. and Tugwell, P. (1991). "Clinical Epidemiology. A Basic Science for Clinical Medicine". Boston: Little, Brown and Company, pp. 3-18; 69-189, 199-220, 285-295.
- Saito, T., Sato, J., Satoh, A., Notani, K., Fukuda, H., Mizuno, S., Shindoh, M. and Amamiya, A. (1994). Flow cytometric analysis of nuclear DNA content in tongue squamous cell carcinoma: relation to cervical lymph node metastasis. *Int J Oral Maxillofac Surg* 23:28-31.
- Sakata, K. (1996). Alterations of tumor suppressor genes and the H-ras oncogene in oral squamous cell carcinoma. *J Oral Pathol Med* 25:302-307.
- Salmon, S. E. and Sartorelli, A. C. (1995). Cancer Chemotherapy. In B. G. Katzung (editor): *Basic and Clinical Pharmacology*. A Lange Medical Book, Sixth edition. Connecticut: Appleton and Lange pp. 823-857.
- Sarkaria J. N. and Harari, P. M. (1994). Oral tongue cancer in young adults less than 40 years of age: rationale for aggressive therapy. *Head and Neck* 16:107-111.
- Sauter, E. R., Ridge, J. A., Gordon, J. and Eisenberg, B. L. (1992). p53 overexpression correlates with increased survival in patients with squamous carcinoma of the tongue base. *Am J Surg* 164:651-653.
- Schechter, M. T. (1995). Personal Communication. HCEP 504 Clinical Epidemiology. Department of Health Care and Epidemiology, Faculty of Medicine, University of British Columbia. HCEP 504 Course Manual.
- Schechter, M. T. and Sheps S. S. (1985). Diagnostic testing revisited: pathways through uncertainty. *Can Med Assoc J* 132:755-760.
- Scholes, A. G. M. and Field, J. K. (1996). Genomic Instability in Head and Neck Cancer. In G. Seifert (editor): "Oral Pathology, Actual Diagnostic and Prognostic Aspects". Berlin: Springer-Verlag, pp. 223-239.
- Schwartz, W. B., Wolfe, H. J. and Pauker, S. G. (1981). Pathology and Probabilities. A new approach to interpreting and reporting biopsies. *N Eng J Med* 305:917-923.

- Scott, J. H. and Symons, N. B. B. (1974). "Introduction to Dental Anatomy". London: Churchill Livingstone, pp. 294-303.
- Scott, J. and Dixon, A. D. (1972). "Anatomy for Students of Dentistry". Edinburgh: Churchill Livingstone, pp. 329-332.
- Scully, C. (1996). New Aspects of Oral Viral Diseases. In G. Seifert (editor): "Oral Pathology, Actual Diagnostic and Prognostic Aspects". Berlin: Springer-Verlag, pp. 29-96.
- Scully, C. and Field J. K. (1997). Genetic aberrations in squamous cell carcinoma of the head and neck (SCCHN), with reference to oral carcinoma (review). *Int J Oncol* 10:5-21.
- Scully, C., El-Kabir, M. and Samaranayake, L. (1980). Infectious Diseases: Candidosis. In H. D. Millard and D. K. Mason (editors): "2nd World Workshop on Oral Medicine". Michigan: BMC Media Production, University of Michigan, pp 27-51.
- Shafer, W. G. (1980). Basic Clinical Features of Oral Premalignant Lesions. In: I. C. Mackenzie, E. Dabelsteen, C. A. Squier (editors). Iowa City: University of Iowa Press; pp. 15-22.
- Shah, J. P. and Lydiatt, W. (1995). Treatment of cancer of the head and neck. *CA Cancer J Clin* 45:352-368.
- Shaha, A. R. and Strong, E. W. (1995). Cancer of the Head and Neck. In G. P. Murphy, W. Lawrence, Jr., R. E. Lenhard, Jr. (Editors): "American Cancer Society Textbook of Clinical Oncology". Washington: American Cancer Society, pp. 355-377
- Shapiro, S., Castellana, J. V., and Sprafka, J. M. (1996). Alcohol-containing mouthwashes and oropharyngeal cancer: a spurious association due to underascertainment of confounders? *Am J Epid* 144:1091-1095.
- Sheps, S. (1995). Personal Communication. HCEP 502: Epidemiology: Measuring the Health of Human Populations, Department of Health Care and Epidemiology, Faculty of Medicine, University of British Columbia. HCEP 502 Course Manual.
- Shibuya, H., Hoshina, M., Takeda, M., Mastsumoto, S., Suzuki, S. and Okada, N. (1993). Brachytherapy for stage I and II oral tongue cancer: an analysis of past cases focusing on control and complications. *Int J Radiation Oncology Biol Phys* 26:51-58.
- Shklar, G. and Schwartz, J. (1993). Oral cancer inhibition by micronutrients. The experimental basis for clinical trials. *Oral Oncol, Eur J Cancer* 29B:9-16.
- Sigurgeirsson, B. and Lindelof, B. (1991). Lichen planus and malignancy. *Arch Dermatol* 127:1684-1687.
- Silverman, S. (1991). Lichen planus. *Current Opinion in Dentistry* 1:769-772.
- Silverman, S. and Rozen, R. D. (1968). Observations on the clinical characteristics and natural history of oral leukoplakia. *JADA* 76:772-777.
- Silverman, S., Gorsky, M. and Lozada, F. (1984). Oral leukoplakia and malignant transformation. A follow-up study of 257 patients. *Cancer* 53:563-568.

- Silverman, S., Gorksy, M. and Lozada-Nur, F. (1985). A prospective follow-up study of 750 patients with oral lichen planus: persistence, remission, and malignant association. *Oral Surg Oral Med Oral Pathol* 60:30-34.
- Silverman, M. A., Gorsky, M. and Kaugars, G. E. (1996). Leukoplakia, dysplasia, and malignant transformation. *Oral Surg Oral Med Oral Pathol* 82:117.
- Simon, J. M., Mazon, J. J., Pohar, S., Le Pechoux, C., Crook, J. M., Grimard, L., Peidbois, P., Le Bourgeois, J. P., and Pierquin, B. (1993). Effect of intersource spacing on local control and complications in brachytherapy of mobile tongue and floor of mouth. *Radiotherapy and Oncology* 26:19-25.
- Slaughter, D. P., Southwick, H. W. and Smejkal, W. (1953). "Field cancerization" in oral stratified squamous epithelium. *Cancer* 6:963-968.
- Slootweg, P. J. (1996). Suppressor Protein p53 and its Occurrence in Oral Tumors. In G. Seifert (editor): "Oral Pathology, Actual Diagnostic and Prognostic Aspects". Berlin: Springer-Verlag, pp. 179-200.
- Smith, C. J. (1980). Connective Tissue Influences on Epithelial Malignancy or Premalignancy. In I. C. Mackenzie, E. Dabelsteen, C. A. Squier (editors): "Oral Premalignancy". Iowa City: University of Iowa Press, pp. 148-163.
- Smith, C. G. and Pindborg J. J. (1969). Histological Grading of Oral Epithelial Atypia by the Use of Photographic Standards. Copenhagen. Cited in Pindborg, J. J., Reibel, J. and Holmstrup, P. (1985). Subjectivity in evaluating oral epithelial dysplasia, carcinoma in situ and initial carcinoma. *J Oral Pathol* 14:698-708.
- Sperber, G. H. (1973). "Craniofacial Embryology". Bristol: John Wright and Sons Ltd., pp. 9-46, 102-108.
- Spiro, R. H. and Strong, E. W. (1971). Epidermoid carcinoma of the mobile tongue, treatment by partial glossectomy alone. *Am J Surg* 122:707-710.
- Spiro, R. H. and Strong, E. W. (1974). Surgical treatment of cancer of the tongue. *Surgical Clinics of North America* 54:759-765.
- Spiro, R. H., Huvos, A. G., Wong, G. Y., Spiro, J. D., Gnecco, C. A. and Strong, E. W. (1986). Predictive value of tumour thickness in squamous carcinoma confined to the tongue and floor of the mouth. *Am J Surgery* 152:345-350.
- Spitz, M. R. (1994). Epidemiology and risk factors for head and neck cancer. *Sem Oncol* 21:281-288.
- Sporn, M. B. (1976). Approaches to prevention of epithelial cancer during the preneoplastic period. *Cancer Res* 36:2699-2702.
- Sporn, M. B. (1993). Chemoprevention of cancer. *Lancet* 342:1211-1213.
- Squier, C. A. (1980). Structure of Normal Oral Mucosa. In I. C. Mackenzie, E. Dabelsteen, C. A. Squier (editors): "Oral Premalignancy". Iowa City: University of Iowa Press, pp. 119-147.

- Squier, C. A. (1987). Barrier Functions of Oral Epithelia. In I. C. Mackenzie, C. A. Squier, E. Dabelstein (editors): "Oral Mucosal Diseases: Biology, Etiology and Therapy". Copenhagen: Laegeforeningens forlag, pp. 7-9.
- Squier, C. A. (1990). Mucosal Alterations. NCI monographs 9:169-172.
- Squier, C. A. and Hall, B. K. (1985). The permeability of skin and oral mucosa to water and horseradish peroxidase as related to the thickness of the permeability barrier. *J Invest Dermatol* 84:176-179.
- Squier, C. A. and Johnson, N. W. (1975). Permeability of oral mucosa. *Br Med Bull* 31:169-175.
- Stilloto, A. M. (1975). Taste and olfaction. In C. L. B. Lavelle (editor): "Applied Physiology of the Mouth." Bristol: John Wright and Sons Limited, pp. 345-358.
- Su, L., Morgan, P. R., Thomas, J. A. and Lane, E. B. (1993). Expression of keratin 14 and 19 mRNA and protein in normal oral epithelia, hairy leukoplakia, tongue biting and white sponge nevus. *J Oral Pathol Med* 22:183-189.
- Su, L., Morgan, P. R., and Lane, E. B. (1996). Keratin 14 and 19 expression in normal, dysplastic and malignant oral epithelia. A study using in situ hybridization and immunohistochemistry. *J Oral Pathol Med* 25:293-301.
- Syrjanen, S. M., Syrjanen, K. J., and Happonen, R. P. (1988). Human papillomavirus (HPV) DNA sequences in oral precancerous lesions and squamous cell carcinoma demonstrated by in situ hybridization. *J Oral Pathol* 17:273-278.
- Taubes, G. (1995). Epidemiology faces its limits. *Science* 269:164-169.
- Teichgraeber, J. F. and Clairmont, A. A. (1984). The incidence of occult metastases for cancer of the oral tongue and floor of the mouth: treatment rationale. *Head Neck Surgery* 7:15-21.
- Templeton, D. J. and Weinberg, R. A. (1995). Principles of Cancer Biology. In G. P. Murphy, W. Lawrence and R. E. Lenhard (editors): "American Cancer Society Textbook of Clinical Oncology". Atlanta: The American Cancer Society, Inc., pp. 164-177.
- Terenghi, G. and Fallon, R. A. (1990). Techniques and Applications of In Situ Hybridization. In J. C. E. Underwood (editor): "Pathology of the Nucleus". New York: Springer Verlag, pp. 289-337.
- Thoma, K. H. (1941). Oral Pathology: A Histological, Roentgenological and Clinical Study of the Diseases of the Teeth, Jaws, and Mouth. St. Louis: C. V. Mosby Co., p. 1222. Cited in Shafer, W. G. (1980). Basic Clinical Features of Oral Premalignant Lesions. In: I. C. Mackenzie, E. Dabelsteen, C. A. Squier (editors). Iowa City: University of Iowa Press; p16.
- Triegeer, N., Ship, I. I., Taylor, G. W. and Weisberger, D. (1958). Chirrhosis and other predisposing factors in carcinoma of the tongue. *Cancer* 11:357-362.
- "UICC/International Union Against Cancer TNM Atlas. Illustrated Guide to the TNM/pTNM Classification of Malignant Tumours", Third Edition. (1990). Speissl, B., Beahrs, O. H., Hermanek, P., Hutter, R. V. P., Scheibe, O., Sobin, L. H. and Wagner, G. (editors). Berlin: Springer-Verlag, pp. 5-25.

- van der Waal, I. and Pindborg, J. J. (1986). "Diseases of the Tongue". Chicago: Quintessence Publishing Co, Inc., pp. 11-20, 151-176.
- van der Waal, I., Banoczy, J., Axell, T., Hansen, L., Holmstrup, P., Mehta, F. S., Shear, M. and Silverman, S. (1986). Diagnostic and therapeutic problems of oral precancerous lesions. *Int J Oral Maxillofac Surg* 15:790-798.
- van Poppel, G. (1993). Carotenoids and cancer: an update with emphasis on human intervention studies. *Eur J Cancer* 29A:1335-1344.
- Vogelstein, B. and Kinzler, K. W. (1993). The multistep nature of cancer. *Trends in Genetics* 9:138-141.
- Warnakulasuriya, K. A. A. S. and Johnson, N. W. (1994). Association of overexpression of p53 oncoprotein with the state of cell proliferation in oral carcinoma. *J Oral Pathol Med* 23:246-250.
- Warnakulasuriya, K. A. A. S. and Johnson, N. W. (1996). Importance of Proliferation Markers in Oral Pathology. In G. Seifert (editor): "Oral Pathology, Actual Diagnostic and Prognostic Aspects". Berlin: Springer-Verlag, pp. 147-177.
- Weber, P. C., Myers, E. N., and Johnson, J. T. (1993). Squamous cell carcinoma of the base of tongue. *Eur Arch Otorhinolaryngol* 250:63-68.
- Weinberg, R. A. (1996). How cancer arises. *Scientific American* Sept 1996:62-70.
- Weisburger, J. H. and Williams, G. M. (1995). Causes of Cancer. In G. P. Murphy, W. Lawrence, Jr., R. E. Lenhard, Jr. (Editors): "American Cancer Society Textbook of Clinical Oncology". Washington: American Cancer Society, pp. 10-39.
- Williams, D. M. (1996). Mucocutaneous Conditions Affecting the Mouth. In G. Seifert (editor): "Oral Pathology, Actual Diagnostic and Prognostic Aspects". Berlin: Springer-Verlag, pp. 1-28.
- Wilson, G. D. and McNally, N. J. (1992). Measurement of Cell Proliferation Using Bromodeoxyuridine. In P. A. Hall, Levison, D. A. and N A. Wright (editors): "Assessment of Cell Proliferation in Clinical Practice". London: Springer-Verlag, pp. 113-139.
- Wilson, G. D., Richman, P. I., Dische, S., Saunders, M. I., Robinson, B., Daley, F. M., and Ross, D. A. (1995). p53 status of head and neck cancer: relation to biological characteristics and outcome of radiotherapy. *Br J Cancer* 71:1248-1252.
- Wingo, P. A., Tong, T. and Bolden, S. (1995). Cancer Statistics, 1995. *CA Cancer J Clin* 45:8-30.
- Winn, D. M. (1995). Diet and nutrition in the etiology of oral cancer. *Am J Clin Nutr* 61(suppl):437S-445S.
- Winn, D. M., Blot, W. J., McLaughlin, J. K., Austin, D. F., Greenberg, R. SA., Preston-Martin, S., Shoenberg, J. B. and Fraumeni, J. F. (1991). Mouthwash use and oral conditions in the risk of oral and pharyngeal cancer. *Cancer Research* 51:3044-3047.

- World Health Organization (WHO) Collaborating Center for Oral Precancerous Lesions. (1978). An aid to studies on oral precancer. *Oral Surg, Oral Med, Oral Pathol* 46:518-539.
- Wulff, H. R. (1976). "Rational Diagnosis and Treatment". Oxford: Blackwell Scientific Publications.
- Xu, X. C., Ro, J. Y., Lee, J. S., Shin, D. M., Hong, W. K. and Lotan, R. (1994). Differential expression of nuclear retinoid receptors in normal, premalignant, and malignant head and neck tissues. *Cancer Research* 54:3580-3587.
- Yamamoto, E., Miyakawa, A. and Kohama, G. (1973). Mode of invasion and lymph node metastasis in squamous cell carcinoma of the oral cavity. *Head Neck Surgery* 6:938-947.
- Yan, J. J., Tzeng, C. C. and Jin, Y. T. (1996) Overexpression of p53 protein in squamous cell carcinoma of buccal mucosa and tongue in Taiwan: an immunohistochemical and clinopathological study. *J Oral Pathol Med* 25:55-59.
- Zelevsky, M. J., Harrison, L. B. and Armstrong, J. G. (1992). Long-term treatment results of postoperative radiation therapy for advanced stage oropharyngeal carcinoma. *Cancer* 70:2388-2395.