ORAL SQUAMOUS CARCINOMA

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A treatise submitted in partial fulfilment
of the requirements for the degree of
Master of Dental Surgery

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1987
PREFACE

The purpose of this dissertation is an attempt by the author to critically evaluate the vast dental and medical literature on oral squamous cell carcinoma. I have endeavoured to provide a reasonable résumé of all relevant journal articles concerning certain aspects of oral squamous cell carcinoma over the last fifteen years, from 1970 to 1985 inclusive. During the last five years in particular, there has been an explosion in the number of articles written in an ever increasing number of scientific journals.

In addition to the increased volume of scientific literature on oral squamous cell carcinoma, many ambiguities, contradictions and misconceptions pervade most of the dental and medical literature. As a direct result of these I have attempted to devise concise definitions of terms commonly used – and often abused – by clinicians, researchers and academics alike. It is sincerely hoped that a clearer understanding of the topic by the reader will ensue from my efforts.

Briefly, a synopsis of this dissertation on oral squamous cell carcinoma involves a discussion on: (1) oral premalignancy; (2) general pathology of neoplasia; and (3) oral squamous cell carcinoma and variants, including aetiology, clinical features, pathology and diagnosis. I have chosen to restrict my study to oral squamous cell carcinoma and its variants, viz: verrucous carcinoma; adenoid squamous cell carcinoma; and spindle cell carcinoma. The reason being that this neoplasm is the commonest type of oral malignant neoplasia (oral cancer) and as such is the most fully researched and documented. From the outset it must be emphasized that most, if not all, reported information on oral cancer really refers to oral squamous cell carcinoma. Vague terms like "carcinoma" usually refer to this type of neoplasm, as well. I have not included epidemiology of oral squamous cell carcinoma because of the difficulties in comparing different studies and in interpreting their findings logically and meaningfully. Also, I have not included a review of current therapeutic régimes because of the difficulty a novice encounters in evaluating the efficacy of treatment modalities.

Oral premalignancy is a controversial subject with many unknowns and little agreement between authorities on definitions, clinical types, pathology, significance of histopathologic parameters and treatment
regimens. A brief discussion of the pertinent aspects of oral premalignancy is presented, which I consider important for a better understanding of the pathogenesis of oral squamous cell carcinoma.

Also presented is a review of certain relevant aspects of the general pathology of neoplasia and carcinogenesis. This is also an important prerequisite for a sound understanding of the intricacies of oral carcinogenesis and neoplasia.

The main subject of this treatise is oral squamous cell carcinoma and its variants. The aetiology, clinical features, pathology and diagnosis are reviewed. The chief aim of this review is to emphasize to the reader the importance of the early detection of oral squamous cell carcinoma, allowing for the formulation of a definitive diagnosis and the implementation of appropriate treatment at the earliest clinical stage to improve the prognosis.

I wish to indicate my indebtedness to the Head of the Department of Oral Medicine and Oral Surgery, Professor M. Jolly for access to departmental clinical and histopathological material and to Associate Professor N.H.H. Smith for his kind and sound advice, encouragement and assistance in making this treatise a physical reality, replete with illustrations: all histopathological photomicrographs presented are taken by the author; the clinical photographs are courtesy of the Department. Finally, to my wonderfully understanding family goes more appreciation than I can ever adequately express.

P.K.
Sydney, N.S.W.
Dedicated to my parents for the uncompromising principles that guide their lives, and for leading their children into intellectual pursuits.
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INTRODUCTION

Oral cancer research has burgeoned in a short period of time. Advances have taken place at many levels, ranging from basic research in oral carcinogenesis to clinical therapy.

This treatise endeavours to critically review the Western medical and dental literature on the clinicopathological features of oral squamous cell carcinoma: the most common type of oral malignant neoplasia. The aetiology, clinical features, pathology and diagnosis of oral squamous cell carcinoma are reviewed.

Oral premalignancy is a controversial subject with many unknowns and little agreement between authorities on definitions, clinical types, pathology and treatment modalities. A brief discussion of the pertinent aspects of oral premalignant lesions and conditions is presented in Chapter 1. An attempt is made to clarify the common misconceptions with a discussion on definitions and types of oral premalignant lesions and conditions. The histopathological examination of epithelial dysplasia in oral premalignancy is considered important as an histological sign of impending neoplasia. The methods of histopathological diagnosis and the difficulties encountered in standardizing and assessing the different degrees of epithelial dysplasia are presented.

A review of certain pertinent aspects of the general pathology of neoplasia and carcinogenesis is important for a sound understanding of the intricacies of oral carcinogenesis and neoplasia. Neoplasm invasiveness, metastasis and heterogeneity; attributes of neoplastic cells: altered growth properties, karyotypic changes, antigenic changes, metabolic and cell surface and membrane changes; carcinogens and carcinogenesis: chemical, radiation, oncogenic viruses, oncogenes; and cellular theories of carcinogenesis are reviewed in Chapter 2.

The general discussion on the pathology of neoplasia and carcinogenesis is logically followed by a detailed discussion of oral squamous cell carcinoma and its histological variants: verrucous carcinoma, spindle cell carcinoma and adenoid squamous cell carcinoma; which account for approximately ninety per cent of oral malignant neoplasia (see Chapters 3-7).
The aetiology of oral squamous cell carcinoma is reviewed in detail in Chapter 4. Although the precise aetiology, in biochemical terms, remains unknown there exists epidemiological and experimental evidence implicating several "risk factors", viz.: tobacco usage; excessive alcohol consumption; sideropenic dysphagia; chronic hyperplastic candidiasis and *Herpesvirus hominis*. Actinic irradiation in the aetiology of labial vermilion squamous cell carcinoma is discussed. Also reviewed are miscellaneous risk factors, *viz.*: socio-economic factors; occupation; diet and nutrition.

The clinical features of oral squamous cell carcinoma and its variants are presented in Chapter 5. Early asymptomatic lesions, presenting symptoms and oral squamous cell carcinoma at various anatomical sites are discussed. Also presented are the clinical features of multiple primary carcinoma and intra-osseous carcinoma.

The pathology and pathogenesis of oral squamous cell carcinoma is reviewed in Chapter 6. The main emphasis is on the histopathology: both light microscopic and ultrastructural; histochemistry and cell kinetics. Also discussed are the patterns and mechanisms of osseous invasion and metastasis of oral squamous cell carcinoma: lymphatic; trans-capsular; distant and perineural. A review of the immunology of oral squamous cell carcinoma is presented with emphasis on the stromal and lymph nodal response, immunological changes and neoplasm-associated antigens.

The logical conclusion of a review on the clinicopathological aspects of oral squamous cell carcinoma is a discussion on the diagnosis (see Chapter 7). The importance of the early detection of oral squamous cell carcinoma is emphasized, allowing the establishment of a definitive diagnosis and the implementation of appropriate treatment at the earliest stage to improve the prognosis. The diagnosis of early clinical stage oral carcinoma and the initial evaluation of suspected malignant lesion are discussed in terms of clinical and histopathological examinations. Toluidine blue vital staining and oral exfoliative cytology serve as useful adjunctive procedures to biopsy and histopathological examination. New diagnostic techniques currently under investigation, are also described, *e.g.*: ultrasonography; immunohistochemical techniques; monoclonal antibody techniques; radioimmunological techniques; and electron microscopy. The differential diagnosis of oral squamous cell carcinoma is considered with a discussion on inflammatory lesions, premalignant lesions, benign neoplasia, histological types of oral squamous cell carcinoma,
metastases to the oral tissues and malignant salivary neoplasms. Finally, a brief discussion is presented on the evaluation of histopathologically proven squamous carcinoma with comments on routine laboratory tests; radiographic examination: routine and new radiographic techniques; radionuclide scans; and panendoscopy.
CHAPTER 1
ORAL PREMALIGNANT LESIONS

1.1 Introduction

1.2 Types of precancerous lesions

1.3 Histopathology of oral premalignant lesions

1.3.1 Epithelial dysplasia

1.3.2 Oral carcinoma *in situ*
CHAPTER 1
ORAL PREMALIGNANT LESIONS

1.1 INTRODUCTION

The concept of premalignancy and of premalignant lesions and conditions is full of ambiguities, uncertainties and conjecture. The main underlying problem is the lack of consensus on nomenclature. The medical and dental "literature" is a miscellany of definitions, terms, descriptions and ideas which, contrary to their intended action, muddle and confuse issues rather than provide intellectual enlightenment. The following account is an attempt to clarify common misconceptions derived from the imprecise "communication" of information on the topic of premalignancy and neoplasia.

A "premalignant or precancerous lesion" is a lesion that may become cancerous or malignant, where malignant refers to a neoplasm that is non-encapsulated, tends to infiltrate, metastasize and in the absence of treatment terminate fatally; and a lesion means a circumscribed and well-defined abnormal change in the structure of an organ or tissue due to injury or disease. A precancerous lesion is defined by Findborg (1980) as a "... morphologically altered tissue in which cancer is more likely to occur than in its apparently normal counterpart ..." He had intended to refer primarily to an histological change. Grundmann (1983) defines premalignant lesions as "... certain histopathologic abnormalities of tissue in which cancer is more likely to occur than in the normal counterpart ...". Cawson (1975) uses the term "premalignant lesion" to imply an identifiable change preceding the development of cancer at the same site after a latent period. Marder and Deesen (1982) define premalignant or precancerous lesion as "... any lesion that displays the metabolic and histologic activity found in cancerous lesions and within whose boundaries it is possible, but not mandatory, for a carcinoma to develop ...". Johnson in MacKenzie et al. (1980b) has made the suggestion that it might be useful to confine the definition "premalignant" to lesions which, given adequate time, would inevitably become malignant; and to use the description "potentially malignant" for lesions which, on a statistical basis, have a high probability of becoming malignant neoplasms. Professor Kramer in MacKenzie et al. (1980c) suggests that the terms
"high risk lesion" and "low risk lesion" might form a more useful fundamental terminology and avoid the implications associated with terms such as "premalignant" and "precancerous". It should be also noted that in gynaecological pathology the term "pre-invasive" is used for lesions showing severe dysplastic cervical cells or carcinoma in situ, but again this term implies that malignant neoplasia is an inevitable sequela of all such lesions.

Currently, oral precancerous lesions consist of the following types: leukoplakia; erythroplakia; carcinoma in situ; and erosive oral lichen planus.

The term "precancerous condition" now deserves to be considered. Grundmann (1983) defines precancerous conditions as "... clinical states [disease entities] associated with a significantly increased risk of cancer ..." The conditions currently considered to fulfil this definition are: oral submucous fibrosis, tertiary syphilitic glossitis; sideropenic dysphagia (Paterson-Kelly or Plummer-Vinson syndrome); dyskeratosis congenita; xeroderma pigmentosum; chronic oral candidiasis; and chronic discoid lupus erythematosus.

A study of premalignant lesions is important in the overall management of oral malignant neoplasia. However, there still exists gaps in our knowledge on premalignant lesions of the oral cavity, particularly in respect of aetiology, statistical relations between premalignant lesions and squamous carcinoma, the identification of such lesions and assessment of the prognosis (Cawson, 1975). It must be pointed out that most of the information available on oral premalignant lesions relates to leukoplakias. It is an unfortunate fact that leukoplakia is over-emphasized as the most common premalignant lesion at the expense of other more potentially serious premalignant lesions: erythroplakia and carcinoma in situ, which prove to be in the majority of cases early, asymptomatic squamous carcinoma (Mashberg and Garfinkel, 1978; Mashberg, 1978). Consequently, 60 per cent of oral squamous carcinomata are well advanced at the time of discovery (Seidman et al., 1976, 1978). The main factor in this failure is, according to Mashberg and Garfinkel (1978), the propagation of ambiguous and inaccurate criteria for the diagnosis of cancerous and "potentially cancerous" oral mucosal lesions, and the emphasis on symptomatic carcinoma.
There is little information about the nature of the sequence of events of carcinogenesis and no evidence that detectable premalignant change is common (MacDonald, 1975). In fact Johnson (1977) found that as many as 80 per cent of cases of oral squamous cell carcinoma appear to be overtly invasive at presentation and are readily recognized histologically. It is likely, of course, that some histological change takes place in the tissues before frank carcinoma develops, but such a change is not necessarily visible clinically, nor likely to cause symptoms attributable to some premalignant lesion. Grundmann (1983) describes carcinogenesis as a continuum of events ranging from normal epithelium; cellular atypia; mild, moderate and severe dysplasia; carcinoma in situ; and frank squamous carcinoma. It is hypothesized that squamous cell carcinoma can develop from precancerous lesions: dysplasia or carcinoma in situ, or from normal epithelium de novo.

Premalignant change can only be diagnosed histologically. This depends on the recognition and evaluation of epithelial changes: cellular atypia and epithelial dysplasia. There are two inherent problems, viz.: (1) uncertainty as to whether the most informative area (area chiefly at risk) has been chosen for biopsy; and (2) the subjective nature of the evaluation of the cellular changes and the virtual impossibility, under routine conditions, of quantifying the abnormalities regarded as significant in order to reach a useful prognosis (Cawson, 1975). In considering the problems of evaluation, an analogy can be drawn between premalignant oral lesions and pre-invasive lesions of cervix uteri. In the latter, despite vast amounts of accumulated data, there is still no certainty as to the relation of carcinoma in situ to squamous carcinoma, and there is little evidence that surgical excision of these lesions has reduced the cervical cancer mortality.

1.2 TYPES OF PRECANCEROUS LESIONS

There are generally accepted to be four lesions which can be regarded as precancerous or premalignant lesions. These are: leukoplakia; erythroplakia; carcinoma in situ; and erosive oral lichen planus. Of these erythroplakia is the most important, exhibiting the greatest rate of malignant transformation. Shafer and Waldron (1975) found that 91 per cent of oral erythroplakia is either squamous cell carcinoma, carcinoma in situ or severe epithelial dysplasia. However, leukoplakias have had the
traditional reputation of being premalignant and consequently a vast body of data on all aspects of leukoplakia oris has been collected.

Nomenclature has traditionally posed a problem. Leukoplakia was first introduced by Schwimmer in 1877 as a clinical descriptive term: "... a white plaque on the oral mucosa ..." The term then underwent divergent definitions in the following century: one a clinical and the other an histological definition showing a significant degree of epithelial dysplasia. This variation in usage, has in the past, and still continues to lead to serious misunderstandings (Kramer, 1980). The definition recommended by the World Health Organisation Collaborating Centre for Oral Precancerous Lesions (1978) (W.H.O.) defines leukoplakia as "... a white patch or plaque that cannot be characterized clinically or pathologically as any other disease ..." This is a definition by exclusion that carries no histological connotations other than that histological examination does not suggest an alternative diagnosis. It may or may not show epithelial dysplasia (Kramer, 1980). However, it implicitly regards leukoplakia as a clinical entity, i.e., a disease which is diagnosed after all other differential diagnoses are disregarded. Unfortunately, the W.H.O. definition is currently in vogue. It would be better to restrict leukoplakia to the original clinical descriptive term of "... a white plaque on the oral mucosa ..." and to refer to premalignant white lesions as dysplastic leukoplakia or dyskeratotic leukoplakia as suggested by Cooke (1964).

There are many white lesions of the mouth, only some of which are premalignant. Jolly (1976a) has reviewed these white lesions of the oral cavity. They include: linea alba buccalis; leukoedema; white sponge naevus; traumatic keratosis; smokers' keratoses; candidiasis; syphilitic leukoplakia; chemical burns; oral lichen planus; lupus erythematosus; squamous cell carcinoma and idiopathic leukoplakia. The type of oral white lesions referred to as oral leukoplakia generally include only traumatic keratosis, smokers' keratoses and idiopathic leukoplakia, in accordance with the W.H.O. definition.

Leukoplakia is subdivided by Sugár and Bánóczy (1957, 1959) and Bánóczy (1983) into:

1. leukoplakia simplex: keratinized mucosa;
2. leukoplakia verrucosa: verrucous proliferations; and
3. leukoplakia erosiva: white lesions with erythematous areas and erosions.
Leukoplakia is subdivided by Pindborg et al. (1968) into:

1. Homogenous leukoplakia: plaque may be cracked or fissured; and
2. Speckled (nodular) leukoplakia: raised grey or white patches on an erythematous base.

The homogenous type of leukoplakia is equivalent to leukoplakia simplex and verrucosa, whilst speckled (nodular) leukoplakia is equivalent to leukoplakia erosion (see Plate 1).

An additional term encountered is preleukoplakia, a term defined by Pindborg et al. (1968) as "... a low grade or very mild reaction of the mucosa, appearing as grey or greyish white, but never completely white area with a slight lobular pattern and with indistinct borders blending into the adjacent normal mucosa ...". The validity of having such an additional definition is questionable since preleukoplakia implies that it is an antecedent lesion which becomes a definite disease entity: leukoplakia. This implication is in variance with the usage of leukoplakia as a purely descriptive term analogous to the term "rash".

Erythroplakia is defined analogously to leukoplakia by the W.H.O. Collaborating Reference Centre for Oral Precancerous Lesions (1978) and by Pindborg (1980) as "... a bright and velvety plaque which cannot be characterised clinically or pathologically as being due to any other condition ...". Again this is an exclusion definition because there are many erythematous lesions of the oral mucosa, e.g.: dermatoses; inflammatory conditions due to local infection: subacute or chronic atrophic candidiasis, tuberculosis. The term evolved from the anglicized version of the French erythroplasie, analogous with leukoplakia derived from leukoplakie. However, Queyrat (1911) initially described erythroplasie as a sharply defined, bright red, glistening, velvety lesion occurring on the glans penis and representing a precancerous lesion. Shear (1972) has reviewed oral erythroplakia and has classified it (a) clinically as:

1. Homogenous erythroplakia;
2. Erythroplakia interspersed with patches of leukoplakia; and
3. Granular or speckled erythroplakia: embracing the lesion described as speckled leukoplakia;

and (b) histologically as:

1. Neoplastic [and preneoplastic]: squamous cell carcinoma, carcinoma in situ; epithelial dysplasia; and
(2) inflammatory: candidiasis; tuberculosis; histoplasmosis; and miscellaneous specific and non-specific lesions. However, consistency is again lacking, with some researchers using erythroplakia as a clinical descriptive term and others as a "specific disease entity" (Shafer and Waldron, 1975) with the histological connotation of premalignancy.

Carcinoma in situ is a misnomer, for the word "carcinoma" defines a malignant neoplasm of surface or lining epithelium, and this in itself implies infiltration of connective tissues and metastasis. Thus the term "carcinoma in situ" literally means a malignant neoplasm of surface or lining epithelium confined intra-epithelially. This is an obvious antilogy. In medical terminology carcinoma in situ is defined as foci of intra-epithelial anaplasia without evidence of penetration of the basement membrane. It represents a premalignant lesion in which severe epithelial dysplasia involving the whole or almost whole thickness of epithelium occurs (Grundmann, 1983; Lucas, 1984b). Shafer (1975) regards carcinoma in situ as an histopathological entity clinically manifested as leukoplakia, erythroplakia, ulcerations and Bowen's disease. However, Hayward and Regezi (1977) were unable to determine whether epithelial dysplasia and carcinoma in situ are separate clinico-pathological entities with similar end points, or whether they are part of a continuum in a spectrum of epithelial neoplasia. Kramer (1973) considers carcinoma in situ as a premalignant lesion: one which has undergone a malignant change, i.e. individual cells are neoplastic, but in which invasion has not yet occurred.

1.3 HISTOPATHOLOGY OF ORAL PREMALIGNANT LESIONS

The detection of "premalignant" or "precancerous" lesion can only be achieved by histopathological examination. The presence of epithelial dysplasia in leukoplakia oris, erythroplakia oris and erosive oral lichen planus is considered important as an histological sign of impending neoplasia as revealed by epidemiological and histological studies. It has been shown that the prevalence of epithelial dysplasia is highest in leukoplakia erosiva which also exhibits a higher tendency for malignant transformation (Mehta et al., 1969a; Bánóczy, 1977). In fact Gupta et al. (1980) found that dysplastic leukoplakia shows a fifteen-fold increased risk of malignant transformation than does non-dysplastic lesions.
Erythroplakia oris is a precancerous lesion which histologically proves to be epithelial dysplasia, carcinoma in situ or frank squamous cell carcinoma in all cases (Shafer and Waldron, 1975).

In a symposium on oral premalignancy, MacKenzie et al. (1980b) indicated a lack of consensus on basic definitions and clinical significance of oral premalignant lesions. There was concern expressed about the subjectivity of present methods of histopathological diagnosis and difficulties encountered in standardizing and assessing different degrees of epithelial dysplasia.

1.3.1 Epithelial dysplasia

The term "epithelial dysplasia" refers to an alteration, "deranged development" of adult epithelial cells characterized by variation in size, shape and organization. These irregular, atypical proliferative changes are in response to chronic irritation (including carcinogens) or inflammation (e.g. lichen planus). Cellular atypia refers to the individual cell changes whereas epithelial dysplasia is used to denote a lesion in which part of the thickness of the epithelium is replaced by atypical cells (Pindborg et al., 1977).

Signs of incipient or overt malignancy in stratified squamous epithelium reflect disturbances in several homeostatic mechanisms (see Table 1) (Johnson, 1977; Kocher et al., 1981; Incze et al., 1982). Although the criteria for the recognition of these individual dysplastic features have been described in detail, the final assessment is essentially subjective. Attempts have been made to record these features more precisely and to assess their relative importance. Two approaches in multifactorial analysis have been attempted: firstly by Smith and Pindborg (1960), and secondly, by Kramer et al. (1970, 1974).

Smith and Pindborg (1969) devised a grading system of thirteen epithelial features of epithelial dysplasia by reference to photographic standards, in an attempt to achieve a more reproducible assessment between different observers and between the same observer at different times. This is achieved by concentrating the observer's attention on one photographically standardized microscopic feature at a time and by enabling the observer to assess each feature individually and allocating a weighted score to each, the sum total of which gives a final score or "epithelial atypia index". The thirteen histological features of epithelial
dysplasia are shown in Table 2. Each character is graded as "absent", "slight" or "marked" and given a score. A grading of "absent" is scored as nought, whereas a grading of "slight" or "marked" is allocated a score of between one and ten. This allocation is entirely subjective. The total score may range from 0 to 75.

Recently, Katz et al. (1985b) conducted a critical evaluation of epithelial dysplasia in oral mucosal lesions using the Smith–Pindborg method of standardization. They found the use of the procedure as "... extremely valuable for the purposes of standardization ...". They regard the question of whether the weighting of the different characteristics proposed by Smith and Pindborg is sufficiently accurate, or whether it is too subjective, as being unresolved and they suggest further testing. They also suggest that further testing is required to establish if the histological criteria now used to assess the severity of epithelial dysplasia are, in fact, those which are of the greatest value in prognostic assessment of the potentiality of malignant change.

The second approach devised by Kramer et al. (1970a, b) is an attempt to introduce greater objectivity into the diagnostic histopathological analyses and to gain a greater insight into the present, largely subjective methods. The group chosen for study was "oral white lesions", which included clinical leukoplakia, "keratosis" and lichen planus. For each of these cases forty-one histological variables are identified and converted to a binary coding used in computer-aided analyses (Kramer et al., 1974). The two statistical analyses used were cluster analysis and discriminant analysis.

Cluster analysis is a statistical method using any number of parameters in "hyperspace" or multi-dimensional space (Kramer et al., 1970a). A computer is used to facilitate calculations; it allocates data into clusters or groups and it can express mathematically how much variation there is within each cluster, and the distance or difference between clusters. Analysis of the histological findings on an objective basis using a six cluster analysis programme provides a surprising degree of separation into the diagnostic groups: lichen planus, keratosis and leukoplakia, in view of the fact that each histological feature is assigned equal importance, there being no weighting of the histological changes thought to be most important in making a diagnosis. The computer using cluster analysis tends to select leukoplakias that are most likely to develop into carcinoma and to separate them from the bulk of cases in
TABLE 1: Disturbances in homeostatic mechanisms in incipient or overt squamous cell carcinoma.

| (1) Cell Division: | abnormal mitoses  
|                  | mitotic activity  
|                  | hyperchromatism  
|                  | nuclear-cytoplasmic ratio  
|                  | nuclear pleomorphism  
|                  | anisonucleosis  
|                  | hyperplasia/atrophy  
| (2) Cell maturation: | keratinization/parakeratinosis  
|                    | dyskeratinization  
|                    | irregular stratification  
|                    | nuclear alterations  
|                    | nuclear-cytoplasmic ratio  
|                    | cell pleomorphism  
|                    | hyperplasia/atrophy  
| (3) Cell aggregation: | intercellular space (spongiosis)  
|                     | acantholysis  
|                     | pseudopodia  
|                     | invasion  
| (4) Host reactions: | immune inflammatory response  
|                    | vascularity  
|                    | density of connective tissue fibres  
|                    | ground substance (proteoglycans) density  
| (5) Ultrastructural changes: | increased tonofilament bundles in basal cells  
|                          | abnormal cytoplasmic organelles  
|                          | abnormalities of basal lamina (wrinkling or folding, discontinuity, lamination)  
|                          | inflammatory cell invasion of epithelium with or without ulceration  
|                          | spongiosis with increased proteaceous débris  
|                          | desmosomal changes (ruptured desmosomes and decrease in number)  
|                          | decrease in number of gap junctions (nexi).  


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<td>Irregular epithelial stratifications</td>
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<td>Premature keratinization</td>
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<td>4</td>
<td>Basal cell hyperplasia</td>
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<td>5</td>
<td>Loss of intercellular adherence (acantholysis)</td>
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<td>6</td>
<td>Loss of polarity</td>
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<td>7</td>
<td>Hyperchromatic nuclei</td>
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<td>8</td>
<td>Increased nuclear-cytoplasmic ratio</td>
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<td>9</td>
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<td>12</td>
<td>Levels of mitoses in epithelium</td>
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<td>13</td>
<td>Bizarre mitoses</td>
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which malignant transformation has not occurred (Kramer et al., 1970a; Kramer, 1975).

Discriminant analysis (canonical variate analysis) is a statistical method for determining objectively and quantitatively the value of each of a series of variables for discriminating between two or more groups of objects (Kramer et al., 1970b). The computer programme produces objectively a weighting factor for each histological variable: the application of all these weighting factors produces the best possible separation or discrimination between the groups. The programme also yields a mathematical assessment of the degree of separation or discrimination between the groups. The programme also yields a mathematical assessment of the degree of separation thus achieved, and of the statistical significance of this separation. Discriminant analysis between leukoplakias, that become malignant and those that do not, reveals eight important histological characteristics, separating the future neoplastic groups from others (Kramer et al., 1970a, 1974; Kramer, 1975):

1. abnormal mitoses in stratum spinosum;
2. disturbance in polarity of basal cell layer;
3. abnormal mitoses in stratum basale;
4. nuclear hyperchromatism;
5. enlarged nucleoli in cells of the stratum spinosum;
6. epithelial cell pleomorphism;
7. intra-epithelial keratinization (dyskeratosis); and
8. Russell bodies in the lamina.

Of interest is the apparent prognostic importance of Russell bodies suggesting immunological implications of neoplasia. The intactness or otherwise of basement membrane is of little prognostic significance. Analysis of the total scores for the groups reveals a statistically significant discrimination between leukoplakia and lichen planus, keratosis and lichen planus; and leukoplakias with or without subsequent malignant transformation.

Polar vector graphs can be used to plot quantitatively the typical histopathological pattern of groups of cases placed together into a cluster, e.g., "severe" leukoplakia and carcinoma, "keratosis" and lichen planus (Kramer et al., 1974). Each histological variable is represented by

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1. Basement membrane refers to PAS-positive structure subjacent to the epithelium and not to the "basement membrane" in haematoxylin–eosin stained sections.
a radius and its quantitative importance in defining the characteristics of the cluster is indicated by the distance from the centre. This results in a computer-calculated "finger-print" of each diagnostic cluster or group. This allows any new case to be compared with characteristic patterns of known diagnoses.

The human observer bias element will be eliminated with the development in the future of a computer-linked, fully automated scanning device that will be able to examine an histological section; and identify the normal and abnormal features and their spatial relations. Methods do exist for more precise objective measurements of individual features but these are of no current practical value in diagnosis and prognosis. These include: immunological investigations, biochemical, histochemical, ultrastructural and stereological (cell kinetics) studies, which will be discussed later.

Epithelial dysplasia is divided into three grades in terms of increasing severity (Grundmann, 1983):

1. Grade I: a light or mild or slight dysplasia, shows hyperplasia of basal cells and disturbed polarity of these cells;
2. Grade II: moderate dysplasia characterized by basal cell hyperplasia, complete loss of polarity of basal cells, moderate cellular polymorphism, increased rate of mitoses, isolated dyskeratoses (i.e. individual keratinizing keratinocytes);
3. Grade III: severe dysplasia showing all features of Grade II plus more cellular polymorphism, high rates of mitosis, numerous dyskeratoses and severely disturbed epithelial architecture (see Plate 2 and Plate 3).

Severe epithelial dysplasia is in "fluid transition" with carcinoma in situ and often a clear distinction between the two lesions is difficult (W.H.O., 1978; Grundmann, 1983). Katz et al. (1985b) found 41 per cent of cases of epithelial dysplasia to be mild dysplasia, 32 per cent to be severe and 29 per cent to be moderate epithelial dysplasias. Also, bizarre mitoses occur less frequently in severe epithelial dysplasia.

The majority of homogeneous erythroplakia oris (66.7 per cent) and speckled erythroplakia oris (63 per cent) are found to be severe epithelial dysplasias, whereas the majority of dysplastic leukoplakias are mild epithelial dysplasias (Katz et al., 1985b). These findings are concordant with those of Shafer and Waldron (1975), Bánoczy and Csiba (1976), Burkhardt and Seifert (1977) and Mashberg (1980).
Longitudinal epidemiological studies have established the prevalence of malignant transformation of oral epithelial dysplasia: 6.6 per cent of oral epithelial dysplastic lesions (Pindborg _et al._, 1977); 11.1 per cent (Mincer _et al._, 1972) and 13.2 per cent (Banczy and Csiba, 1976) in dysplastic leukoplakia and 36 per cent in erythroleukoplakia (Silverman _et al._ 1984). Burkhardt _et al._ (1978) found 3 per cent of non-dysplastic leukoplakia, and 4 per cent with moderate dysplasia developed into squamous cell carcinoma, whereas 43 per cent of cases with severe epithelial dysplasia and 64 per cent of cases with carcinoma _in situ_ developed into squamous carcinoma. This emphasizes the fact that not all dysplastic cases or carcinomata _in situ_ will necessarily develop into squamous cell carcinoma.

The relationship between epithelial dysplasia, carcinoma _in situ_ and squamous cell carcinoma remains controversial. Cawson (1969) has stated that it should not be assumed that leukoplakia oris, epithelial dysplasia and carcinoma _in situ_ are inevitable, or even usual, preliminary stages of squamous cell carcinoma. MacDonald and Rennie (1975) have reported the occurrence of "epithelial" atypia in cases of chronic inflammatory hyperplasia induced by ill-fitting dental prostheses, lichen planus and squamous papillomata; lesions which are not considered premalignant. They suggest that a slight degree of cellular atypia may be present as a reactive change not reflecting a "premalignant potential".

On the other hand, Grundmann (1983) and Incze _et al._ (1982) regard the histomorphological abnormalities defined as cellular atypia, epithelial dysplasia or carcinoma _in situ_ as a part of the continuum of the transition from normal to malignant transformation. A "paradigm of carcinogenesis" as taught by Grundmann (1983) includes the progression from normal epithelium, through basal cell hyperplasia, mild, moderate and severe epithelial dysplasia, carcinoma _in situ_ to squamous cell carcinoma. The development of squamous cell carcinoma from apparently normal epithelial cells and from each of these precancerous lesions or steps has still to be considered a distinct possibility (see Figure 1). Incze _et al._ (1982) have shown that morphological abnormalities in normal appearing mucous membrane of the oral cavity occur in tobacco smokers with a proved susceptibility to carcinoma development in the upper aerodigestive tract. Of the twenty-eight ultrastructural abnormalities observed, those of alterations of maturation, pleomorphism of epithelial cells, defects in basal lamina and severe spongiosis are considered important. These
FIGURE 1: Development of squamous cell carcinoma from precancerous lesions. (Modified from Grundmann, 1983.)
morphological abnormalities are consistent with the concept that carcinogenesis is a multi-step process of sequential neoplastic development extending over a large period of time (Medline and Farber, 1981).

1.3.2. Oral carcinomata in situ

Carcinoma in situ is a lesion in which the epithelium is believed to have undergone a malignant change and in which invasion of subjacent tissues has not yet occurred. It is a precancerous lesion (Kramer, 1973; Grundmann, 1983). The term has been borrowed from gynaecological terminology, although in the oral mucosa there is little evidence for spontaneous regression occurring and progression to squamous cell carcinoma may be most rapid. Carcinoma in situ of the cervix uteri is described as foci of intramucosal anaplasia without evidence of penetration of the basement membrane. It is full thickness epithelial dysplasia. Sometimes the anaplastic cellular changes extend along the surface into underlying endocervical glands; however this should not be construed as invasion since the basement membranes of these glands are not breached (Robbins et al., 1984b).

Clinically, oral carcinoma in situ is manifested as leukoplakia, speckled erythroplakia or erythroplakia, presenting anywhere in the oral cavity, with the floor of the mouth, tongue and labial vermilion and labial mucosa being the most common sites (Shear, 1972; Kramer, 1973; Shafer, 1975). The "high risk" sites described by Mashberg (1978), viz.: floor of mouth, ventrolateral surface of tongue, soft palate complex, are areas where carcinoma in situ has a higher risk of progression into squamous cell carcinoma. Amagasa et al. (1985) found 50 per cent of oral carcinomata in situ to progress into squamous carcinomata over a decade.

Hayward and Regezi (1977) were unable to determine whether oral epithelial dysplasia and carcinoma in situ are separate clinico-pathological entities with similar end points or whether they are part of a continuum in a spectrum of epithelial neoplasia as believed by Grundmann (1983) and Incze et al. (1982).

The criteria used in the diagnosis of cervical lesions cannot be directly extended to oral mucous membrane because of the difference in the histological architecture of these different epithelia (Kramer, 1973). In particular cervical epithelium is not keratinized to the extent that is normal in some parts of the oral mucosa. The characteristic
histopathological features of carcinoma in situ of the oral mucosa includes (Shear, 1972; Shafer, 1975; Küffer and Flore-Donno, 1977; Incze et al., 1982):

1. architectural abnormalities:
   - irregular epithelial thickness (hyperplasia with areas of atrophy and normal thickness);
   - loss of normal polarity and irregular disposition (orientation);
   - variations in keratinization (para-, ortho- and non-keratinization);
   - spongiosis (widening of intercellular spaces);

2. abnormalities of maturation:
   - migration of basal cells into superficial epithelial strata;
   - mitotic activity in superficial strata;
   - delayed or arrested cell maturation resulting in loss of parakeratinization;

3. cellular abnormalities:
   - inversion of nuclear–cytoplasmic ratio;
   - poikilokarynosis (Darier's term for the formation of various types and arrangements of cells occurring in Bowen's disease);
   - nuclear abnormalities (irregular nucleochromatin distribution, several nucleoli, hypernucleochromatism, enlargement of nucleus);
   - cytoplasmic organelle abnormalities;
   - increase in tonofilament bundles in basal cells;
   - cellular mitosis through epithelial strata;
   - cellular dyskeratosis (regarded as the hallmark of the transformation of carcinoma in situ into squamous cell carcinoma);

4. inflammatory cell infiltrate of corium (mainly lymphocytic, normal host defensive response to lesion);

5. intact basal lamina: is by definition the most important criterion differentiating carcinoma in situ from squamous cell carcinoma.²

Shafer (1975) found that epithelial thickness: hyperplasia, normal thickness and atrophy is roughly in the same proportion (30:24:23) in oral carcinoma in situ. Prominent nuclear hyperchromatism and increased

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2. In squamous cell carcinoma there is wrinkling or folding, discontinuity and lamination of the basal lamina.
nucleocytoplasmic ratio are not a particularly common finding. The most conspicuous and almost uniformly consistent finding is the loss of orientation of cells: loss of orderly arrangement of cells, individually and with respect to neighbouring cells, and the loss of normal polarity. Occasionally, a well demarcated junction between carcinoma in situ and the contiguous normal epithelium can be observed. Also multiple foci of carcinoma in situ can be seen distributed throughout the epithelium analogous to multifocal cutaneous basal cell carcinoma (see Plate 4 and Plate 5).

Shear (1972) showed that the loss of epithelial differentiation, i.e., maturation, is associated with the complete loss of histochemically demonstrable glycogen. This represents a metabolic defect in atypical cells and is a useful diagnostic aid in distinguishing a true atypia from "pseudo-atypia" arising from inflammation. The anaplastic carcinoma in situ has an extremely limited capacity for keratinization: this plus epithelial atrophy results in a clinically erythematous rather than leukoplakic lesion. In homogenous erythroplakias and in speckled erythroplakia the erythematous areas are non-keratinized. The white foci seen in speckled erythroplakia may represent either localized keratinization, foci of necrosis with bacterial and/or fungal colonies or areas of superficial epithelial oedema.
CHAPTER 2
PATHOLOGY OF NEOPLASIA

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CHAPTER 2
PATHOLOGY OF NEOPLASIA

At this point it would be feasible to review certain aspects of the general pathology of neoplasia and carcinogenesis. Only a brief discussion will be attempted as this topic covers a vast area of experimental and clinical research. The discussion will be limited to the following topics: neoplasm invasiveness; metastasis; neoplasm heterogeneity; attributes of neoplastic cells; carcinogenic agents and carcinogenesis; and cellular theories of carcinogenesis.

2.1 NEOPLASM INVASIVENESS

Next to the development of metastases, invasiveness is the most reliable feature that differentiates malignant from benign neoplasms. Invasiveness is the characteristic infiltrative, erosive growth of the malignant neoplasm into the adjacent tissue: connective tissue stroma; lymphatics; blood vessels; perineural spaces; cartilage and bone.

The importance of the extracellular tissue matrix and the basal lamina in neoplasm invasions has been recently reviewed by Liotta (1984). The extracellular tissue matrix is a complex, three-dimensional lattice-work of collagen and elastin, embedded in a visco-elastic ground substance composed of proteoglycans and glycoproteins. The matrix acts as a scaffold which isolates tissue compartments, mediates cell attachment and determines tissue architecture. It also acts as a selective macromolecular filter and influences growth morphology and cytodifferentiation. The matrix is hypothesized to exert mechanical and chemical influences on the shape and biochemistry of the cell via the plasma membrane. The basal lamina is a dense reticulum of Type IV collagen, glycoproteins such as laminin and entactin, and proteoglycans. Type V collagen may exist at the interface between the basal lamina and the stroma. General and widespread changes occur in the organization, distribution, and quantity of the epithelial basal lamina during the transition from benign to invasive neoplasia. In invasive neoplasia there are defects in the basal lamina, e.g. discontinuities, fragmentation or focal reduplication.
Mechanisms of neoplastic invasiveness are still poorly understood and remain controversial (Marcel, 1980). They include: (1) physical pressure; (2) reduced adhesiveness and cohesiveness of neoplastic cells; (3) increased motility of neoplastic cells; (4) loss of "contact inhibition"; and (5) the release of enzymes e.g. collagenases, lysosomal hydrolysates and plasminogen activators. More recently, a three-step hypothesis of neoplasm invasion has been proposed by Liotta (1984) consisting of: (1) neoplastic cell attachment to the extra-cellular matrix, which may be mediated by specific attachment factors such as laminin¹ (in the case of basal lamina) and fibronectin;² (2) local degradation of the matrix by neoplastic cell-associated proteases, which degrade both the attachment proteins as well as the structural collagenous proteins. Proteolysis may be localized at the neoplastic cell surface where the amount of the active enzyme outbalances the natural protease inhibitors present in the matrix; (3) neoplastic cell locomotion into the region of the matrix modified by proteolysis. The direction of locomotion may be influenced by chemotaxins. Continued invasion of the extracellular matrix may take place by the cyclic repetition of these steps.

Neoplasm invasion is facilitated by a cascade of proteases including: thiol proteases, heparanases, serine proteases such as plasminogen activator; and Type IV and V collagenases (Liotta, 1984). A Type IV collagenolytic metalloproteinase degrades Type IV collagen of the basal lamina. Its action results in one major cleavage localized to a position 25 per cent of the distance from the N-terminus of the Type IV collagen molecule, representing a cleavage at the opposite end when compared to the cleavage of the "classic" collagenase which cleaves Type I, II and III collagens. A separate metalloproteinase degrades Type V collagen. Dense, fibrous, collagenous connective tissue such as membranes, tendons, joint capsules can resist degradation and neoplastic invasion for long periods of time. Arteries are more resistant than veins due to their thicker walls, elastin fibres and elaboration of protease inhibitors (Rifkin and Crowe, 1975). Cartilage is the most resistant tissue to neoplastic invasion due to: (1) the physicochemical characteristics of the matrix; (2) the biological stability and slow turnover of cartilage; and (3) the

1. Laminin is a glycoprotein of the basal lamina which normally regulates cell attachment, growth, morphology and cell migration.  
2. Fibronectin is a cell-surface glycoprotein involved in fibroblast adhesion to the extracellular matrix (Meyer et al., 1984).
elaboration of inhibiting substances, e.g. anti-angiogenesis factor, or inhibitors of the enzymes involved in the growth and invasiveness of neoplastic cells (Kuettner et al., 1978; Brem and Folkmann, 1975).

2.2 METASTASIS

Metastasis is the ability of malignant neoplasms to disseminate throughout the body establishing metastases: neoplastic implant discontinuous with the primary neoplasm. All cancers by definition metastasize except for most gliomata and basal cell carcinomata which rarely metastasize.

Pathways of dissemination or spread involve (Robbins et al. 1984a):
(1) direct "seeding" of body cavities or surfaces (trans-coelomic spread);
(2) transplantation; (3) lymphatic spread; and (4) haematogenous spread. Transplantation is the mechanical transport of neoplasm fragments by instruments, aspiration biopsy or gloved hands; it is very rare in humans due mainly to surgical techniques or the inability of neoplastic cells to survive when artificially displaced. Lymphatic spread is the most common pathway for the initial dissemination of carcinomata. Invasive neoplastic cells gain access into the lymphatic lumina by "reverse diapedesis": the active migration through inter-endothelial cell junctions; and then embolize to the regional lymph nodes draining the area. However, local lymph nodes may be by-passed ("skip metastasis") because of venous anastomoses, or when there has been inflammatory or radiation-induced obliteration of the lymphatics.

Neoplastic cells may be destroyed in the lymph nodes by either neoplasm-specific immune responses initiated by "tumour-specific" antigens, or by sensitized "natural killer" cells (NK). Lymphadenopathy can be the result of either metastasis or follicular hyperplasia, proliferation of paracortical T cells, and sinus histiocytosis (proliferation of sinus endothelial cells and histiocytes) initiated by "tumour antigens" and/or neoplastic, cellular débris.

The essential steps in the formation of a metastatic lesion involves a complex sequence of interdependent events best conceptualized as a "staircase" (Hart and Fidler, 1980, 1981; Poste and Fidler, 1980). These

3. "Tumour angiogenesis factor (TAF)" promotes vascularization of the stroma and permits the growth of solid neoplasms (Folkman and Cotran, 1976).
steps are: (1) progressive growth of primary neoplasm; (2) vascularization; (3) invasion of lymphatic and vascular channels; (4) detachment of neoplastic cells; (5) embolization; (6) survival in circulation by evasion of non-specific, humoral and cell-mediated immune responses; (7) arrest in capillary beds of distant viscera by either adherence to endothelial cells or attachment to vascular basement membrane exposed by endothelial cell retraction; (8) extravasation; (9) evasion of host defence system; (10) progressive growth; and (11) metastasis. Recently, it has been suggested that laminin, a glycoprotein of the basal lamina plays a significant role in haematogenous metastasis (Liotta, 1984). Laminin receptors of neoplastic cells stimulate haematogenous metastases by two mechanisms, viz.: (1) if the receptor is unoccupied, it can be used by the cell to bind directly to host laminin; and (2) if the receptor is occupied with laminin, the cell can utilize the cell surface laminin as an attachment bridge through the globular end regions of the molecule which bind to Type IV collagen. The fragment of laminin which binds to the receptor, but lacks the globular end regions, inhibits both of these mechanisms.

2.3 NEOPLASM HETEROGENEITY

All human cancers are made up of diverse subpopulations of cells having different biological characteristics, e.g.: antigenic properties, karyotypes, growth rate, hormone receptors, the ability to invade and metastatic capabilities. This neoplasm heterogeneity may be a reflection of the polyclonal origin of a single neoplasm, but it is most likely the result of acquired mutations in the genetically unstable replicating cells of monoclonal origin (Fidler and Kripke, 1977). It is closely linked to "tumour progression", which is the progressive acquisition by a neoplasm of such individual and separable characteristics as invasiveness, metastatic potential, humoral responsiveness and so on. "Progressive" implies that over the course of its evolution a cancer, or particular subpopulations or clones, acquire, for example, an increased growth rate or the capacity to invade or to metastasize.

Metastatic potential is an attribute that may or may not be acquired by one or more cell sublines within a malignant neoplasm during its evolution. An important finding is that neoplastic cells within vascular channels in a microscopic slide of neoplasm, although ominous, cannot be equated with metastasis because of the many interdependent events
involved. Experimental models (Fisher and Fisher, 1959) have shown that only 0.1 to 1.0 per cent of intravenously injected neoplastic cells survive for more than twenty-four hours. Cell death occurs by macrophages and natural killer (NK) cells before an immune response is mounted. Furthermore, the location of metastases, in experiments, has been shown to be non-random. Certain events occur that affect the location of neoplastic cell arrest and survival (Karpatin and Pearlsstein, 1981; Nicolson et al., 1981). These include: homotypic adhesion of the neoplastic cells to form multicell emboli, and adhesion to platelets, lymphocytes and non-circulating host cells. Surface properties of circulating neoplastic cells and host normal cells, possibly receptors, have been shown to lead to specific localization of metastases.

Comparison between benign and malignant neoplasms reveals the following characteristics (Robbins et al. 1984a): (1) benign neoplasms are characterized by: a well-differentiated structure usually typical of tissue of origin; usually progressive and slow rate of growth which may come to a standstill or regress; usually encapsulated but rarely a capsule may be lacking and are generally cohesive and expansile; and absence of metastasis; (2) malignant neoplasms sometimes lack differentiation, exhibiting anaplasia; exhibit erratic rate of growth which may be slow to rapid, usually with numerous and abnormal mitotic figures; invasion without encapsulation, usually infiltrative but may be seemingly cohesive and expansile; metastases are frequently present, the larger and less differentiated the primary the more likely are the metastases.

2.4 Attributes of Neoplastic Cells

Exposure of normal cells in vitro to carcinogens, e.g. chemical carcinogens, oncogenic viruses, radiant energy, results in the acquisition of many altered characteristics involving growth behaviour and morphology. These cells are said to have undergone "transformation". Transformation followed by malignant potential is a dynamic process involving more than one generation of cells, each exhibiting greater deviation from the norm. Cell proliferation is required to permanently establish the "transformed state".

The precise intracellular molecular event(s) that bring about the neoplastic transformation remains unknown. The phenotype of the neoplastic cell, however, exhibits the following salient features (type
et al., 1980): (1) altered growth properties; (2) morphological changes which may be subtle and deceptively absent; (3) karyotypic changes; (4) antigenic changes; (5) metabolic changes; and (6) cell surface and membrane changes.

2.4.1 Altered growth properties

Altered growth properties include: (1) unregulated proliferation with continued replication in the absence of mitogenic stimuli (Hull, 1981), and loss of response to regulatory controls (Vaheri and Mosher, 1978); (2) failure to mature thereby living longer because of the absence of terminal differentiation; (3) transplantability of individual neoplastic cells; (4) "immortality" of neoplastic cells allowing indefinite subculturing in vitro or transplantation into an appropriate host; (5) loss of "contact inhibition" resulting in the piling up of neoplastic cells, creating multilayered disorderly masses; (6) neoplastic cells require lower serum concentrations for optimal growth, do not require "anchorage" and so will grow in semisolid or fluid media (Hull, 1981); and (7) neoplastic cells achieve higher densities in culture than normal host cells, and do not arrest at $G_0$ stage in the cell cycle at high density or in low serum media. Consequently, neoplastic cells are less subject to "density-dependent inhibition of growth" (Allen and Iype, 1979).

2.4.2 Karyotypic changes

Antigenic changes are expressed by transformed cells. A range of such antigens is expressed (Damjanov and Knowles, 1983), e.g.: species-specific antigens; tissue or organ-specific antigens; ABO blood group antigens; and "tumour-associated" antigens. The first three antigen types are also found in normal cells. "Tumour-associated" antigens provide the basis for "markers" for the immunocytochemical identification of neoplastic cells. These "neo-antigens" of experimentally transformed cells are membrane-associated, immunologically distinct from histocompatibility antigens, and will induce transplantation immunity. They are referred to as "tumour-specific transplantation antigens" (TSTAs) or "tumour-associated rejection antigens" (TARAs).
Chemical carcinogens evoke TSTAs that are unique to the particular neoplasm and are immunologically distinct from those found in other neoplasms produced by the same agent, whether in the same host or an unrelated host (Embelton and Baldwin, 1980). The TSTAs of chemically-induced cancers arise as the result of a specific interaction between "target cells" and a carcinogen.

All neoplasms evoked by a specific oncogenic virus have the same "tumour-specific antigens", in contrast to chemically-induced and radiation-induced neoplasms in which the antigens evoked are "private" or unique to a particular neoplasm (Law et al., 1980; Old, 1981). Additionally, cells transformed by certain DNA oncoviruses, e.g. SV40 and polyoma viruses, also express a nuclear T antigen or several T antigens which are immunologically distinct from TSTAs but constitute separable fractions of the same polypeptide chain. TSTAs are probably new products not ordinarily expressed by the genetic code of the normal cell, and are not necessary for malignant transformation since many chemically-induced and most spontaneous neoplasms lack them. Thus, they are best viewed as the consequence of malignant transformation rather than having an intrinsic role in the fundamental change leading to the malignant phenotype. The nuclear T antigens of DNA viruses, on the other hand, are intrinsic to the transformation process.

Most human malignant neoplasms totally lack "tumour-specific antigens", or at most, are very weakly immunogenic. They do, however, have "tumour-associated antigens", some of which are foetal antigens (oncofoetal antigens) normally found in embryonic cells, e.g. alpha-foetoprotein associated with hepatocarcinomata, yolk-sac neoplasms and non-neoplastic conditions (Abelev, 1971); carcino-embryonic antigen associated with carcinomata of the colon, respiratory tract, pancreas, mamma, etc. (Gold and Freedman, 1965). Oncofoetal antigens are not "tumour-specific".

"Ca antigen" has recently been described in a wide variety of malignant neoplasms, but is not uniquely a marker for human neoplastic cells. The validity of this "tumour" antigen for human cancer remains to be established (Ashall et al., 1982; McGee et al., 1982).

Indirect evidence of an apparent immune response to human malignant neoplasia is provided by histological evidence suggesting a cell-mediated response to "tumour-associated antigens" in carcinomata of the lung, testis, mamma, melanocarcinoma and Hodgkin's disease; and by the
increased frequency of malignant neoplasms in immuno-compromised individuals (Ioachim, 1976).

2.4.4 Metabolic changes

Some generalizations can be made about metabolic changes observed in neoplasia (Robbins et al., 1984c): (1) the better the differentiation and the slower the rate of division of the neoplastic cell, the closer its metabolism approximates that of its normal predecessor and vice versa; (2) all highly anaplastic cells tend towards a common simplified metabolic and enzymic pattern, sometimes referred to as "the biochemical convergence of tumours"; (3) none of the metabolic deviations constitutes a hallmark of the malignant phenotype; and (4) none of the metabolic deviations is thought to be the fundamental event responsible for the emergence of the neoplastic phenotype, rather appear to be secondary to neoplastic transformation. Biochemical and metabolic analyses of neoplastic cells have yielded no profound insights into the nature of the neoplastic transformation.

2.4.5 Cell surface and membrane changes

Cell surface and cytoplasmic membrane changes seem to be critical to the aggressive behaviour of the malignant phenotype. Well-established changes include (Nicolson, 1976; Trump et al., 1980): (1) a loss, diminution, or in some cases acquisition of such surface specializations as microvilli, pseudopodia and filopodia; (2) alteration in cell junctions; (3) inconstant cyto-skeletal alterations, viz. disorganization of microtubules and microfilaments; (4) changes in surface-associated glycoproteins and other proteins, particularly enzymes affecting membrane transport and possibly invasiveness; (5) changes in glycolipids and lipids affecting permeability, surface receptors and surface antigens; (6) enhanced lectin agglutinability; (7) changes in responsiveness to inhibitory and stimulatory putative growth factors; and (8) alterations of surface-associated and intracellular ions.

There is enhancement of transmembrane transport influx of nutrients and efflux of metabolites, potentiating increased metabolic activity and replication. There is also increased synthesis, incorporation, and, in particular, exploitation of surface and membrane components (Black, 1980).
The cell surface glycoprotein, formerly known as LETS (large external transformation-sensitive) protein, shows an imperfect correlation between the level of carcinogenicity and the extent of loss by shedding fibronectin from the cell surfaces (Chen et al., 1979). This loss may contribute to the reduced adhesiveness and cohesiveness of neoplastic cells. Exfoliated fibronectin is found in plasma as cold-insoluble globulin which may contribute to the coagulopathy occasionally seen. Other procoagulant factors are also released by certain neoplastic cells (Dvorak et al., 1979).

Surface glycolipids, some of which are receptors, are also reduced in neoplasia. These receptors play roles in localization of metastases, the action of hormones and possibly of growth-stimulatory and growth-inhibitory substances, e.g. "chaiones". Their detection may be important in understanding loss of growth regulation (Robbins et al., 1984d). Neoplastic cells exhibit increased permeability of solutes from the environment which may play a role in the diminished density-dependent inhibition of growth by conferring an advantage in the competition for nutrients.

Recent studies (Jesudason and Iseri, 1980) reveal that a normal complement of desmosomes may be present in the primary neoplastic lesion and within hepatic metastases suggesting that detachment and dissemination of neoplastic emboli to distant sites occurs despite retention of desmosomes. However, gap (nexus) or communicating junctions are frequently diminished or absent, accounting for the diminished contact inhibition characteristics of neoplastic cells.

Lectins comprise of plant and invertebrate divalent molecules that cross-link polysaccharides on adjacent cells. An increase in lectin agglutinability correlates well with a loss of growth regulation (Robbins et al., 1984a).

Changes in membrane enzymes include (Robbins et al., 1984d): the production and release of serine protease, plasminogen activator, protease galactosyl-transferase and protein phosphorylating kinase (a major enzyme in the transforming activity of certain oncogenic viruses). Another surface-related enzyme that mediates the synthesis of cyclic-adenosine monophosphate (c-AMP), an intracellular messenger, from adenosine triphosphate (ATP) is adenylylcyclase. Reduced levels of c-AMP are found in neoplastic cells suggesting a lack of regulatory signals involved in control of cell mitosis and growth. Underlying the lower levels of c-AMP
are reductions in membrane adenylcyclase and increases in cyclic nucleotide phosphodiesterase involved in its katabolism.

2.5 CARCINOGENIC AGENTS

There are three major categories of carcinogenic agents, viz.: (1) chemical carcinogens; (2) radiant energy; and (3) oncogenic viruses (oncoviruses).

2.5.1 Chemical carcinogens

Chemical carcinogens consist of two categories (Miller, 1978; Harris, 1979; Reiner et al., 1977; Rosner, 1976; Bergsagel et al., 1979):

(1) Direct-acting (activation-independent) compounds:
   (a) alkylating agents:
       beta-propiolactone (2-propanolactone)
       methylisotrosoarea
       dimethylsulphate
       methylmethanesulphonate
       diepoxybutane
       antineoplastic mustards (e.g. cyclophosphamide, chlorambucil, busulfan, melphalan, etc.)
   (b) acylating agents:
       1-acetylimidazole
       dimethylcarbamyl chloride

(2) Procarcinogens that require metabolic conversion in vivo, usually in the liver, or enzymatic conversion in vitro to produce metabolites capable of transforming cells called "ultimate carcinogens":
   (a) polycyclic and heterocyclic aromatic hydrocarbons (Sims, 1980; Miller and Miller, 1981; Taussig, 1984a):
       1-benzanthracene
       3:4-benzpyrene
       1:2, 5:6-dibenzanthracene
       1:2, 3:4-dibenzanthracene (weak carcinogen)
       3-methylcholanthrene
       7, 12-dimethylbenzanthrene
   (b) aromatic amines, amides, azo-dyes (Kleinfeld et al., 1966):
       2-naphthylamine
benzidine
2-acetylaminofluorene
N, N-dimethylaminobenzene ("butter yellow")
"scarlet red" dye
(c) natural plant and microbial products (Miller and Miller, 1976; Peers et al., 1976; Sumithran and MacSween, 1979):
aflatoxin B₁ (produced by strains of Aspergillus flavus)
mptomycin C
griseofulvin
cycasin
safrole
betel nuts
(d) others:
nitrosamines and nitrosamides
carbon tetrachloride (tetrachloromethane)
etionine.

The polycyclic, aromatic hydrocarbons represent some of the most potent carcinogens known. The ultimate carcinogens for many of these are dihydrodiol epoxides which are strong electrophilic reactants that combine covalently with nucleophilic sites in target cells, including ribonucleic acids and proteins. Polycyclic hydrocarbons are produced in the combustion of tobacco, particularly with cigarette smoking, or possibly in the process of cooking especially broiling or smoking of meats and fats.

2.5.2 Chemical carcinogenesis

Chemical carcinogenesis is a dynamic process involving sequential generations of cells over a variable span of time, depending mainly on the cell type, species, reactivity of the carcinogen or its metabolites, and especially on dosage. Measurements of the latent period in relation to dosage have demonstrated two related concepts of chemical carcinogenesis (Taussig, 1984b); viz.: (1) that their effects are irreversible; and (2) that there is no measurable threshold dose below which exposure has no effect.

The steps involved in chemical carcinogenesis can be explained by the "two-stage theory of neoplasia" (Farber, 1979; van Duuren et al., 1978):
(1) Initiation which is irreversible, resulting from the exposure of cells to an appropriate dose of carcinogen causing a permanently altered cell with neoplastic potential; and

(2) Promotion, in which promoters applied after the initiating agent in amounts above a specific threshold level increase the neoplastic response. It is reversible, however, since initiation is irreversible, even withholding the promoting agent for months or years still has been shown to result in the production of cutaneous neoplasms. Examples of promoters include: phenols (found in tobacco tars), phenobarbitone, saccharin, cyclamates, various hormones, notably oestrogens (Pitot et al., 1981; Cohen et al., 1979). A further stage in chemical carcinogenesis has been postulated called "progression" from benign to malignant neoplasia. In vivo, hormones may function as co-carcinogens, able to moderate both promotion and progression (Taussig, 1984a).

The salient features of chemical carcinogenesis:

(1) most chemical carcinogens are mutagenic in at least one test system (Ames, 1979; Straus, 1981); however, not all carcinogens are mutagens and vice versa (Rinkus and Lagator, 1979);

(2) cell replication is required for "fixation" of the transformed state. The process of mitosis leads to ever greater errors in the genetic code introduced by the initiating "lesion". Regenerative activity, including hyperplasia, following cell death favours the development of neoplasia (Columbano et al., 1981). "Pre-neoplastic" enzyme-altered foci occurring during the latent period have been described in chemical model systems and cell proliferation is thought to be obligatory to their induction (Farber and Cameron, 1980);

(3) No "safe" threshold level exists for carcinogens since neoplastic transformation can be the result of the additive effects of multiple, small exposures to initiators;

(4) diverse oncogenic influences may act in concert to induce cell transformation, i.e. co-carcinogenesis, e.g. tobacco smoke residue contains polycyclic aromatic hydrocarbons, nitrosamines and other chemicals which act as co-carcinogens, and phenolic compounds which act as promoters; and

(5) neoplastic transformation by chemical agents in a multi-stage and possibly "multi-hit" process involving successive generations of cells, each approaching more closely the malignant phenotype. There is initiation, promotion and neoplasm progression with the evolution of
successive clones of cells one or more, which ultimately escapes regulatory controls.

2.5.3 Radiation carcinogenesis

There are two types of radiant energy (Störer, 1982; Upton, 1982): (1) ultraviolet actinic (solar) electromagnetic radiation (wave-length 3 x 10^{-7} to 3 x 10^{-10} metre); and (2) ionizing radiation, which may be electromagnetic (x and gamma-rays, wavelength smaller than 3 x 10^{-10} m) or particulate (alpha, beta particles, nucleons), which can result in neoplasia.

Ultraviolet electromagnetic irradiation induces cutaneous squamous cell and basal cell carcinomata, as well as melanocarcinomata (Urbach, 1982; Viola and Houghton, 1982). The degree of risk depends on the intensity of exposure and the quality of high-absorbing melanin pigmentation. Radiobiological effects include: (1) inhibition of mitosis; (2) inactivation of enzymes; (3) induction of mutations; (4) cell death; and (5) carcinogenesis. The carcinogenicity is attributed to the formation of pyrimidine dimers in the deoxyribonucleic acid (DNA), which if not repaired, may lead to large transcriptional errors and sometimes neoplasia. Other carcinogenic mechanisms may be DNA chain breakage, formation of abnormal cross-links and nucleotide base destruction. Murine studies suggest that ultraviolet irradiation simultaneously activates T-suppressor cells and so inhibits cell-mediated immunity permitting the emergence of highly antigenic cutaneous neoplasms (Kripke, 1981; Fisher and Kripke, 1982).

Supportive evidence for the mutagenic effects of ultraviolet irradiation comes from a small group of autosomal recessive hereditary diseases, all characterized by some anomaly in DNA metabolism, particularly repair mechanisms. These include: xeroderma pigmentosum; De Sanctis–Cacchione syndrome; ataxia telangiectasia; Fanconi's anaemia; and Bloom's syndrome. Xeroderma pigmentosum is characterized by extreme photophobia and high incidence of ultraviolet light-induced cutaneous carcinomata, but no increased incidence of the common lethal cancers such as carcinomata of the lungs, mamma or colon. It is caused by a deficiency of the endonuclease required to commence excision repair of DNA dimers (Cleaver and Bootsma, 1975; Cairns, 1981). In de Sanctis–Cacchione syndrome, a variant of xeroderma pigmentosum, the enzyme
missing is probably involved in post-replication repair rather than excision repair of DNA dimers (Taussig, 1984b). Ataxia telangiectasia is characterized by cerebellar ataxia (muscular inco-ordination) and oculocutaneous telangiectases, a predisposition to leukaemias and other lymphoreticular neoplasms. The host cells are more sensitive to gamma-radiation than to ultraviolet radiation, and are apparently unable to excise and repair the gamma-ray modified DNA bases. There is also a marked immunodeficiency, which may be more important than the defect in excision, in the development of neoplasia. In Fanconi's anaemia there is also the tendency to develop leukaemias; it is associated with an inability to excise thymine dimers after ultraviolet irradiation, although the molecular defect is not well-defined (Remsen and Cerutti, 1976). In Bloom's syndrome there is either a defect in DNA repair or replication or both. It is marked by an increased incidence of all forms of malignant neoplasia (German et al., 1977).

Ionizing radiation, electromagnetic and particulate, includes irradiation from several sources, e.g. therapeutic, occupational (radiologists, watch-dial painters, miners of radioactive ores) and thermonuclear reactions (Court-Brown and Doll, 1965; Hirohata, 1976; Boice, 1981).

Several theories on the mechanism of radiation carcinogenesis have been proposed (Robbins et al., 1984e). They include: (1) the direct ionization of critical cellular macromolecules inducing a somatic mutation; (2) "indirect" theory whereby radiation first interacts with water or molecular oxygen to produce free radicals that mediate damage to DNA inducing a somatic mutation; (3) activation of latent oncogenic viruses (in certain forms of murine neoplasia); and (4) radiation-induced neoplastic transformation being the result of cell-killing followed by regenerative replication. The carcinogenicity of ionizing radiation best correlates with its mutagenicity which depends on the quality of incident radiation, dosage, dose rate, DNA repair and host factors including age, e.g. foetus, infants and children are more susceptible; immune competence; hormonal influences; and cell type. Normal cells with high radiosensitivity include: lymphoid, haemopoietic, germ cells, intestinal epithelial and ovarian follicular cells. Those with fairly high radiosensitivity include: epidermal cells, epidermal adnexae (hair follicle, sebaceous gland), oropharyngeal stratified epithelial cells, urinary bladder, oesophageal, gastric glandular and ureter epithelial cells. Medium radiosensitivity is experienced by
connective tissue, neuroglia, endothelia, growing cartilage and bone. Fairly low radiosensitive cells are mature chondrocytes or osteocytes, mucous or serous glandular epithelial pulmonary, renal, hepatic, pancreatic, pituitary, thyroid, adrenal and nasopharyngeal cells. Muscle cells and ganglion cells have the lowest radiosensitivity.

2.5.4 Oncogenic viruses

Oncogenic viruses have been shown to induce malignant neoplasia in a wide range of species from amphibians to primates. However, there currently exists no conclusive evidence of the viral aetiology of any type of human malignant neoplasia, even though several viruses have been implicated, *e.g.* Herpesvirus hominis* type 1 and 2, Epstein–Barr virus, hepatitis B virus and molluscum contagiosum virus.

There are two categories of oncogenic viruses (Wyke, 1981), *viz.:* (1) RNA oncogenic viruses; and (1) DNA oncogenic viruses.

The RNA oncogenic viruses (oncogenic–RNA viruses or oncornaviruses or retroviruses) all code for a reverse transcriptase enzyme which is critical to their transforming function. They consist of two genera: (1) Type B oncovirus, *e.g.* mouse mammary tumour virus (MMTV) which has an eccentric viral nucleoid; and (2) Type C oncovirus with a centrally placed viral nucleoid, which can induce lymphomata, leukaemias, sarcomata, and mammary carcinomata in chickens, rodents, cats, cattle and non-human primates. All RNA oncogenic viruses have the following common features (Wyke, 1981):

1. single-stranded RNA genome enclosed within a virus-specific protein envelope;
2. some are competent viruses, possessing all the genes necessary for viral replication; others are defective viruses that can be "rescued" by co-cultivation with helper viruses;
3. most have centrally placed viral nucleoid, *i.e.* Type C viruses;
4. all have reverse transcriptase coded by a viral genome which mediates the synthesis of a complementary copy of DNA, using the viral RNA as a template. The DNA may then be integrated into the genome of the host cell as a "provirus"; and
5. they either carry a specific transforming gene (oncogene) or become associated with an indigenous cellular "proto-oncogene" critical to their transforming capacity.
Methods of transmission of retroviruses in their normal hosts vary with the particular agent, e.g. congenitally via ovum or transplacentally; or horizontally via saliva or milk. Recently a unique type of C-retrovirus designated HTLV or LAV has been associated with an uncommon form of human T-cell lymphoma and/or leukaemia (Gallo and Wong-Staal, 1982; Kadin and Kamoun, 1982; Catovsky et al., 1982). It is a possible cause of various forms of neoplasia seen in patients with acquired immune deficiency syndrome (AIDS).

The DNA oncogenic viruses consist of five families capable of causing neoplasia in animals. They are: (1) papovaviruses; (2) adenoviruses; (3) herpesviruses; (4) poxviruses; and (5) hepatitis B virus. The papovavirus family contains three members, viz. papilloma virus, polyoma virus and vacuolating virus (the original name for SV40 virus). "Papova" is the acronym derived from these three member viruses. The papovaviruses cause verrucae, condylomata acuminata and laryngeal papillomata in humans (Editorial, Lancet, 1983). Occasionally, condyloma acuminatum and epidermodysplasia verruciformis may progress to squamous cell carcinoma. Papovavirus has also been implicated in squamous carcinoma of the cervix uteri (Zur Hansen, 1982).

Three human strains of adenoviruses (type 12, 18 and 31) are strongly oncogenic in neonate hamsters and rodent cells in culture, but have no effect in humans. In humans, only three types of herpesviruses are implicated as being oncogenic, e.g. Epstein-Barr virus, Herpesvirus hominis type 1 and 2. Epstein-Barr virus is strongly associated in the aetiology of African Burkitt's lymphoma and anaplastic nasopharyngeal carcinoma (Epstein and Achong, 1979; Klein, 1979; Editorial, Lancet, 1982). Additional factors involved in its oncogenic potential, e.g. concomitant malaria and other disorders impairing immunocompetence, enable the Epstein-Barr virus (EBV) to replicate freely within B lymphocytes and so activate clonal lymphoblastosis. This lymphoblastosis favours the appearance of mutations, such as translocations from chromosome numbers 8 to 14, and less often to chromosome numbers 2 and 22, favouring enhanced expression of the "oncogene" (Lenoir et al., 1982). In immunocompromised male homosexuals with acquired immune deficiency syndrome there is a high rate of malignant neoplasia, either Kaposi's sarcoma or Burkitt's lymphoma (Ziegler et al., 1982; Koziner et al., 1982; Drew 1982). Sero-epidemiological data and the detection of virus in
neoplastic cells link *Herpesvirus hominis* type 2 with squamous cell carcinoma of the cervix uteri (MacDougall *et al.*, 1980).

Of the poxviruses: Shope fibroma virus, Yaba virus and molluscum contagiosum virus, none have been associated with human neoplasms. Hepatitis B virus has been implicated as a possible aetiological factor in human hepatocellular carcinoma.

The mechanisms of action of oncogenic viruses have been reviewed by Wyke (1981) and Gallo and Wong–Staal (1982). RNA oncogenic viruses achieve a stable association with the host cell by integration of the provirus into the genome of infected cells. Cell replication leads to viral replication when the DNA provirus contains a linear sequence of the three genes necessary for viral replication, \(^4\) *viz:* (1) "gag" gene which codes for a polyprotein that can be cleaved into virion core proteins; (2) "pol" gene which codes for reverse transcriptase; and (3) "env" gene which codes for envelope glycoproteins. At both the 3' and 5' ends of this proviral gene sequence there are segments, referred to as long terminal repeat (LTR) units (see Figure 2). The LTR unit appears to be a control–promoter sequence that probably has importance in regulating replication of the contiguous proviral genome. The loss of one of the three genes yields a defective, incompetent virus incapable of replication, which can however, be rescued by concomitant infection with non–defective "helper viruses".

RNA oncogenic viruses can be subdivided into two further categories: (1) viruses that also contain the genetic information for transforming cells, *i.e.* have "oncogenes"; and (2) viruses without such transforming "oncogenes" (Bishop, 1981; Wong–Staal *et al.*, 1981). Retroviruses having oncogenes are likely to have acquired them as captives during infections of their evolutionary antecedents, and normal cells in both animals and humans possess apparent "proto–oncogenes": the "enemy within". What activates these oncogenes is not clearly understood; it may depend on genetic or epigenetic control mechanisms or on some mutation in the proto–oncogene itself. It has been suggested that the way retroviruses not possessing their own oncogenes act may depend on the chance insertion of a provirus with its promoter LTR next to a cellular oncogene resulting in carcinogenesis. In fact the complete

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4. Acronyms derived from internal proteins (group antigens) to give "gag"; reverse transcriptase (*polymerase*) to give "pol"; and envelope proteins to give "env".
RNA VIRAL GENOME

R

5' U5, GAG, POL, ENV, U3 3'

DNA PROVIRUS

R = repeat sequence nucleotides
U = unique sequence of nucleotides
LTR = long terminal repeat sequence

FIGURE 2: RNA viral genome.
viral sequence is not needed, the long terminal repeat sequence (LTR) alone may suffice suggesting that the neoplastic transformation hinges on the regulation or mutation of cellular-potential oncogenes (Weinberg, 1983). The synthesis of oncogene-coded transforming protein may be involved in the cell transformation pathway (Hamlyn and Sikora, 1983).

DNA oncogenic viruses cannot be isolated from transformed cells since transformation is incompatible with replication of the virus. Neoplastic cells transformed by all DNA oncogenic viruses are synthesizing virus-coded proteins called "T antigens" (Huebner et al., 1983). Like retroviruses, only small pieces of oncogenic DNA viral genome are required for the transformation of cells in vitro or in vivo (Israel et al., 1979). Carcinogenesis is the consequence of the integration of non-infectious, incomplete genomes or fragments into non-permissive cells that, in some way, perturbs the homeostasis of cells and leads to the formation of transforming proteins.

2.5.5 Oncogenes

Investigations regarding molecular biology of neoplasia have shown that aspects of the malignant character of human neoplasms are governed by the "activation" and/or the inappropriate expression of certain cellular genes resembling retroviral transforming genes: oncogenes. Some twenty such cellular oncogenes (c-onc genes) have been described (Buick and Pollak, 1984).

Cellular and viral oncogenes are similar but not identical (Busch, 1984). Retroviral oncogenes have developed point mutations and have unique coding elements. They are derived from cellular oncogenes. Cellular oncogenes (c-onc genes) constitute a structurally and functionally heterogeneous group of genes, members of which may co-operate with one another in order to achieve transformation of cells (Land et al., 1983).

The relationship of cellular oncogenes to carcinogenesis is attributed to point-mutations in the c-onc gene sequence creating an abnormal gene product, or to a loss of regulation of transcription of c-onc genes arising through processes such as gene amplification, or translocation to a transcriptionally active area of a chromosome. Protein products of oncogenes are heterogeneous with respect to function and intracellular localization: many are membrane-bound protein kinases, others are nuclear in location (Buick and Pollak, 1984). There are two functionally distinct
"complementation" groups of oncogenes, viz.: Group A: oncogene products are homologous nuclear proteins concerned with "immortalization"; and Group B: oncogene products are heterogeneous cytoplasmic proteins concerned with other aspects of the malignant phenotype.

The overall function of cellular oncogenes, under normal circumstances, is the regulation of cellular proliferation (Buick and Pollak, 1984). Normal stem cell proliferation is regulated by a balance of endogenous proliferation and differentiation-inducing factors. Genes that code for these factors, their receptors, and the intracellular proteins involved in the mediation of these effects might have the "transforming" properties of activated oncogenes if they were altered by mutation and/or inappropriately expressed.

Five separate mechanisms of proto-oncogene activation have been found to date (Buick and Pollak, 1984; Busch, 1984).

The precise mechanism responsible for the creation of activated oncogenes in spontaneously arising neoplasms remains obscure. It is widely assumed that these oncogenes are formed by somatic mutation. A single oncogene, acting alone, has limited powers of carcinogenesis: the co-operative interaction between different oncogenes is required to explain the multi-step process of spontaneous or chemical carcinogenesis; each step reflecting a requirement for the activation of a distinct cellular gene such as an oncogene. The possibility exists that the number of separate cellular genes involved in the entire process of carcinogenesis may be limited to as few as three. Activation of each of these genes may define an essential step in the carcinogenic process.

The procedures of gene transfer and molecular cloning have made it possible to detect some of the centrally important determinants of carcinogenesis (Land et al., 1983). These determinants, the oncogenes, act pleiotropically,\(^5\) since their gene products affect complex regulatory cascades within the cell. The majority of these oncogenes are tissue-specific. However, despite the optimism that hyperactivation or mutation of proto-oncogenes may result in neoplasia, no well-substantiated evidence corroborates this hypothesis for human neoplasia. Moreover, there is no convincing evidence that such genes relate to the initiation or promotion events for virus-negative neoplasia (Busch, 1984). There is no statistically satisfactory evidence for the qualitative changes such as

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5. Pleiotropically: i.e. producing many effects in the phenotype.
deletions, point mutations, recombinations, transposons or other transposable elements, or other translocated elements that alter the functions of c-onc genes in "real" human malignant neoplasia. Even when suggestive findings have been made, further evidence for increased activity or altered function, has not been forthcoming.

2.6 CELLULAR MECHANISMS OF NEOPLASIA

Several cellular mechanisms of neoplasia have been proposed (Taussig, 1984a). They include: (1) the aberrant differentiation hypothesis; (2) mutation hypothesis; (3) acquisition of viral information; (4) cellular oncogene hypothesis; (5) stem cell model; and (6) gene transcription hypothesis.

2.6.1 Aberrant differentiation hypothesis

The neoplastic cell is postulated to arise as a result of an altered pattern of expression of normal genes which does not require a mutational change in DNA. The difference between a normal and neoplastic cell involves only the pattern of gene expression and de-repression. The hypothesis argues that since, for example, hepatocytes and renal cells follow their distinct, stable pathways of differentiation without requiring gene loss or mutation, it is unnecessary to invoke mutation in order to explain inheritable change in neoplasia. The term "epigenetic" is used to describe inheritable changes such as those which occur in normal differentiation and do not involve alteration of the genetic material. The repression of genes normally expressed and the inappropriate de-repression of other genes lends support to the concept of neoplasia as an abnormal differentiation pathway; moreover, the types of inappropriate products expressed by neoplastic cells (ectopic hormones, foetal proteins, etc.) are often characteristic of a particular type of neoplasm (Taussig, 1984c).

2.6.2 Mutation hypothesis

A more widely accepted view is that alterations in DNA are involved in neoplasia (Taussig, 1984a). Supportive evidence include the frequent demonstration of chromosomal abnormalities in neoplastic cells and the
fact that the vast majority of chemical and physical carcinogens react with DNA and are mutagenic. Mutations affecting either regulatory or structural genes are envisaged, the former leading to inappropriate gene depression, and the latter to the loss of function of specific proteins. The transforming genes are often enzymes, potentially with many different substrates, having complex and far-reaching cellular consequences. Mutation per se only initiates neoplasia, promotion being necessary for the expression of the mutant gene. Some of the cellular abnormalities associated with neoplasia may be secondary changes which occur on promotion or progression.

All chemical carcinogens are electrophiles which react with nucleophilic centres of DNA, e.g. guanine being the preferential base of attack usually at N7 or C8 position. Bivalent reagents such as the sulphur mustards can cross-link guanines on adjacent DNA strands to produce guanine dimers. Other reagents, such as the acridines intercalate in the DNA, i.e. slot between two bases before binding with them. Ultraviolet light causes the formation of dimers of adjacent thymines on the same DNA chain, while ionizing radiation leads to physical breaks and other chromosomal changes. The results of these reactions is to damage DNA producing point mutations, which can be either base-pair substitutions or frame-shift mutations by the intercalation of new additional base pairs in the nucleotide sequence. More complex chromosomal changes can also occur. All these mutations are "fixed" permanently in the genome, being passed on to daughter cells and becoming permanently inheritable.

2.6.3 Acquisition of viral information

The acquisition of viral information constitutes a third mechanism whereby the introduction into the cell genome of new viral information transforms the cell. Infection by an oncogenic virus is essentially an effective means of presenting to the cell an activated form of one of its own growth-controlling genes (Taussig, 1984a).

2.6.4 Cellular oncogenic hypothesis

The cellular oncogene hypothesis is a unifying theory of neoplasia (Taussig, 1984a). The basic tenet is that neoplasia is the result of the
activation of, or mutation in, a cellular gene responsible for normal growth control. When activated or altered the cellular gene becomes an oncogene or transforming gene and directs the neoplastic change. Under normal circumstances in differentiated cells, the expression of growth-controlling genes is regulated to ensure that they are only activated in response to tissue requirements, growth factors, etc. Their permanent activation or alteration leads to uncontrolled cell division; thus growth-controlling genes of normal cells are potential oncogenes.

A number of different routes may lead to the activation of cellular transforming genes, viz.: (1) mutation at critical regulatory sites by chemical or physical carcinogens; (2) the integration of non-oncogene viral DNA close to the cellular gene, e.g. leukaemia viruses; or (3) the acquisition of the activated retroviral form of the gene, e.g. sarcoma viruses. Alternatively, mutation in a cellular gene may alter the function of its product sufficiently to cause uncontrollable growth, e.g. bladder cancer gene. Thus, in this schema cellular oncogenes are primarily genes essential to normal cell function, but lead to oncogenesis when expressed abnormally. Where their products are enzymes with several possible substrates, the diversity of the cellular abnormalities associated with neoplasia can be readily accounted for.

The existence of cellular oncogenes has been established by homology with retroviral transforming genes (viral oncogenes) and by the technique of "transfection", in which fragments of purified DNA from neoplastic or normal cells are taken up by normal recipient cells in culture, integrated into their genomes and induce neoplastic transformation in them. Among human malignant neoplasms in which transforming genes have been demonstrated by transfection include: urinary bladder, lung, mammary and colonic carcinomata; myeloma, and T and B cell lymphomata. In all these neoplastic cells an oncogene is active which behaves in a dominant fashion and can transform a normal recipient murine cell.

2.6.5 Stem cell model

In the stem cell model of carcinogenesis, neoplasms are regarded as stem cell systems in which a minority of cells have the proliferative capacity to maintain the neoplasm, whereas the majority of cells demonstrate differentiation features and have limited proliferation
potential (Buick and Pollak, 1984). In this model there is no need to propose the process of "de-differentiation" as a mechanism of neoplastic growth. The hypothesis proposes that the changes associated with carcinogenic insult cause a loss of control and dissociation of normal stem cell function, cell renewal and cell differentiation. The ability of the subsequent neoplasm to organize tissue-specific differentiation (to varying degrees in different neoplasms) would be a function of the extent to which normal stem cell function had been deranged by the carcinogenic event. One end point of the damage might be a loss of control of cell renewal and commitment to differentiation resulting in neoplasia rather than maintaining a steady-state equilibrium.

Supportive evidence of stem cell origin and growth of human neoplasms, especially of the haemopoietic and epithelial tissues, includes: the documentation of tissue-specific differentiation in neoplastic cell populations; the implication of small target size for neoplasm control with radiotherapy; and the evidence for neoplasm "clonality" generated by the analysis of glucose-6-phosphate dehydrogenase heterozygotes.

A common way by which to represent the properties of a cell renewal system is an hierarchy of cells with a spectrum of proliferative potential, viz. stem cell, transitional cells and end cells (Buick and Pollak, 1984). Steady-state control can be based on the premise that proliferative potential and differentiation are opposing processes; i.e.: as cells differentiate they lose the proliferative potential such that terminally differentiated cells are proliferatively inert. Stem cells have the unique combination of properties of being able to initiate and maintain such clonal hierarchies, the distinguishing feature being their capacity for self-renewal. Stem cells are normally regarded as being a minority subpopulation of cells in any particular tissue.

Normal stem cell proliferation is regulated by a balance of endogenous proliferation and differentiation-reducing factors. Genes that code for these factors, their receptors, and the intracellular proteins involved in the mediation of their effects might have the "transforming" properties of activated oncogenes if they were altered by mutation and/or inappropriately expressed. Abnormalities leading to the constitutive expression of genes coding for growth factors by proliferation-competent cells (stem cells), that are normally the targets of growth factor action, could lead to an "auto-stimulatory" mechanism of neoplasia.
The "immortalization" and relative homogeneity of cells of established clonogenic lines, as compared to primary cells, is explained in terms of the stem cell model as a consequence of a loss of regulation of genes normally involved in the control of stem cell self-renewal. The normal function of cellular homologues of the "immortalizing" complementation group of oncogenes might be related to stem cell self-renewal modulation; transcription of these genes would normally be closely regulated by growth factors that influence stem cell proliferation. In neoplastic stem cells, although the stem cell self-renewal is not as high as in "immortalized" cells, it is no longer under appropriate physiological control, and together with the loss of regulation of cell cycle entry leads to both the uncontrolled and heterogeneity of differentiation. The self-renewal gene, as well as the cell cycle controlling genes, are inappropriately expressed and modulated by growth stimulating or inhibiting factors.

2.6.6 Gene transcription hypothesis

In a review of the origin of human cancers Cairns (1981) found that there exists some evidence to suggest that most human neoplasia is more the result of large scale genetic and chromosomal re-arrangements rather than localized somatic mutations produced by environmental chemical mutagens. These genetic transpositions, as manifested by abnormal karyotypes, are detected by chromosomal banding techniques.

Genetic transposition is the shuffling of certain modules of genetic information ("transposons") so that new combinations of genes can be tested for survival advantage. The process depends on certain short sequences of DNA that are substrates for various recombinational enzymes (transposases). It represents a more drastic form of genetic variation than the localized changes in base sequence because it can alter the expression of whole regions of the genome. Transpositions can be site specific or non-specific; are usually solitary, but can occur in clusters of variable frequency; can be programmed to occur at any predetermined rate; can be concerned with any number of alternative states; and can be made virtually irreversible.
2.6.7 DNA repair mechanisms

Mutagenic and other damaging effects of chemical and physical carcinogens can be prevented by DNA repair mechanisms. There are basically two types of DNA repair mechanisms: (1) excision repair in which the segment of altered DNA is enzymatically removed and resynthesised correctly before DNA replication; and (2) post-replication repair in which the DNA is first replicated despite the damage, but gaps opposite the lesions in the template are left which are later filled in. Some of the enzymes involved in these processes show genetic variation and certain individuals having an inherited deficiency in the repair enzyme show special sensitivity to neoplasia. Thus, DNA repair is believed to play an important role in preventing mutations which could lead to cancer (Taussig, 1984a).

Excision repair mechanism involves the recognition of the distortion of the DNA helix by a bulky chemical substituent or thymine dimer by the enzyme endonuclease which "nicks" or makes an incision near the lesion. This allows the enzyme exonuclease to excise the lesion and degrade the DNA while polymerase resynthesizes new DNA using the opposite strand as a template. The enzyme ligase completes the repair by uniting the newly synthesized segment to the free end. Since repair takes place outside the S-phase of the cell cycle it is sometimes called "unscheduled" DNA synthesis. The efficiency of excision repair is not absolute. This results in residual carcinogenic moieties which must be removed by a "back-up" system. Also, excision repair may not function where only small alkyl groups are bound to DNA, as after methylation, because the distortion is insufficient to be recognized by the endonuclease.

Post-replication repair or recombination repair involves a complex mechanism. DNA replication proceeds up to the thymine dimers not removed by excision repair where it stops and then re-initiates at a point beyond the damage. The gaps produced in the replicated strand are filled in with the correct nucleotide sequences by a mechanism involving recombination. These events are blocked by caffeine, greatly increasing the lethality of various alkylating agents in cultured cells and the frequency of chromosomal aberrations induced by such agents, or by ultraviolet light, indicating the importance of post-replication repair in cell survival after exposure to these carcinogens.
Defective DNA repair mechanisms which predispose to neoplasia are seen in xeroderma pigmentosum, De Sanctis–Cacchione syndrome, ataxia telangiectasia and Fanconi's anaemia. However, for the majority of human malignant neoplasms there is yet no reason to believe that repair of DNA is abnormal.

2.6.8 Conclusion

Despite all the advances in the elucidation of cellular and molecular mechanisms of carcinogenesis, human neoplasia remains a complex problem. Results of experimental studies cannot be extrapolated directly to humans. Human malignant neoplasms are primary, complex clonal aggregates with both morphological and biochemical heterogeneity that is manifested as they grow, and frequently more so as they metastasize (Busch, 1984). Neoplasms of a given tissue origin differ widely in their neoplastic and associated cell populations. "Supportive" cells include: muscular endothelial cells, connective tissue cells, lymphocytes, etc. The actual population of neoplastic cells is small, with non-growing (G0) and growing cells varying from 5 to 95 per cent of the total cell population. Additionally, neoplastic cells vary in the percentage of the population in G0, G1, S, G2 and M phases of the cell cycle.

Heterogeneity with respect to growth rate and phenotypic differentiation within the various cells of a neoplasm is related to the heterogeneity with respect to metastasis and other biological properties. Populations of cells vary with respect to karyotype, antigenicity, growth, immunogenicity, susceptibility to chemotherapeutic agents and biochemical properties (Busch, 1984).

Carcinogenesis is a developmental process which appears to begin as a rapid and irreversible alteration in isolated cells or cell populations. However, the subsequent changes in the initiated cells which culminate in neoplasia are not inevitable, but may depend on other carcinogenic or co-carcinogenic environmental factors. Observations based primarily on in vivo models and on clinico-pathological evidence in a variety of human tissues provide a conceptual basis for carcinogenesis of the oral tissues (Solt, 1980). These include: (1) a multistage developmental process of carcinogenesis; (2) following the rapid initiation event the subsequent development of neoplasia is invariably of long duration; (3) random distribution and clonal nature of the initiated cell populations detected in
tissues previously exposed to potent carcinogens; their subsequent evolution to neoplasia is not inevitable, but may depend on carcinogenic or co-carcinogenic environmental factors such as dietary, humoral, chemical, physical, biological, *inter alia* which act in part by causing the selective proliferation of variant clones of initiative cells; and (4) although successive stages of carcinogenesis may be partially reversible, the initial event is essentially irreversible and persistent throughout the normal lifespan of the individual.

In terms of the present concepts of cellular mechanisms of carcinogenesis little is known about the precise aetiology of human oral neoplasia. Despite this fact most authorities refer to risk factors such as tobacco usage, alcohol, liver cirrhosis, industrial occupational hazards as "aetiological factors" of oral squamous carcinoma. This imprecision in nomenclature only further confounds the reader about the already enigmatic process of carcinogenesis. More on the aetiology of squamous cell carcinoma will be discussed subsequently.
CHAPTER 3
TYPES OF ORAL SQUAMOUS CELL CARCINOMATA

3.1 Squamous cell carcinoma

3.2 Verrucous carcinoma

3.3 Spindle cell carcinoma

3.4 Adenoid squamous cell carcinoma
CHAPTER 3
TYPES OF ORAL SQUAMOUS CARCINOMATA

Oral squamous cell carcinoma and its histological variants, viz.: verrucous carcinoma, spindle cell carcinoma and adenoid squamous carcinoma, account for 90 per cent of all oral malignant neoplasms, with other forms of carcinoma and sarcoma accounting for the remaining percentage (Lucas, 1984). The malignant neoplasms of the oral cavity and oropharynx include the following: squamous cell carcinoma; adenoid cystic carcinoma; transitional cell carcinoma; pleomorphic adenocarcinoma; mucoepidermoid carcinoma; nasopharyngeal carcinoma; anaplastic carcinoma; histiocytic lymphoma; lymphocytic lymphoma and other types of lymphoma; Kaposi's sarcoma; rhabdomyosarcoma and leukaemic infiltration (Voss, 1985). Excluded from this dissertation because of their low prevalence in oral tissues are: basal cell carcinoma; primary intra-osseous squamous carcinoma derived from odontogenic cysts and epithelial rests; carcinoma of the sinus maxillaris; metastatic carcinomata to the oral tissues; and salivary gland neoplasms.

3.1 SQUAMOUS CELL CARCINOMA

Squamous cell carcinoma of the oral soft tissues is usually well-differentiated and readily recognizable histologically. Anaplastic lesions, however, can present histopathological diagnostic problems, viz.: differentiation between lymphoma, Ewing's tumour, neuroblastoma and metastases from a primary lesion elsewhere in the body. Squamous cell carcinoma also has to be distinguished from kerato-akanthoma, pseudo-epitheliomatosus hyperplasia, verrucous carcinoma and spindle cell carcinoma. Squamous cell carcinoma can affect the labial vermilion, labial mucosa, buccal mucosa, gingivae, floor of the mouth, soft palate, hard palate and tongue (Pindborg, 1980; Lucas, 1984c).

3.2 VERRUCOUS CARCINOMA

Verrucous carcinoma is a well-known variant of squamous cell carcinoma described in detail first by Ackerman (1948). It is a locally invasive, late or non-metastasizing squamous carcinoma with characteristic
clinical, histological and cytokinetic features (Medina et al., 1984). Clinically, it manifests as a verrucous, fungating, ulcerated tumour often associated with leukoplakia (McCoy and Waldron, 1981; Slootweg and Müller, 1983). Verrucous carcinoma commonly affects the oral cavity, but has also been described occurring in the larynx, oesophagus, nasal fossae, skin, genitalia and leg (Burns et al., 1976; Schwade et al., 1976; Prieleau et al., 1980; Shear and Pindborg, 1980; McCoy and Waldron, 1981; Bohmfalk and Zallen, 1982; Slootweg and Müller, 1983; Mizuno et al., 1983; Medina et al., 1983). Verrucous carcinoma has also been described arising in an odontogenic cyst (Enriquez et al., 1980).

Histologically, verrucous carcinoma is characterized by: a verrucous, densely keratinized surface; a sharply circumscribed, deep margin; bulbous, well-oriented rete ridges, often with central desquamation and composed of well-differentiated squamous epithelium; a "pushing" rather than an infiltrating type of advancing margin; and an associated chronic inflammatory infiltrate (Prieleau et al., 1980; Medina et al., 1984). It needs to be differentiated from diffuse verrucous lesions of the oral mucosa, viz.: exophytic papillary squamous cell carcinoma; verrucous hyperplasia; and chronic hyperplastic candidiasis (candidal leukoplakia) (Shear and Pindborg, 1980; Eversole and Papanicolaou, 1983; Slootweg and Müller, 1983).

3.3 SPINDLE CELL CARCINOMA

Spindle cell carcinoma is an uncommon variant of squamous cell carcinoma with a large spindle cell component. Synonyms commonly used indiscriminantly include: carcinosarcoma; pleomorphic carcinoma; and pseudosarcoma, depending upon which pathogenesis is believed. It can affect the oral cavity, oropharynx, larynx, oesophagus, skin and mamma. It mainly affects elderly males. The oral neoplasms occur most frequently on the lower lip, tongue and alveolar ridge as either polypoid, exophytic, or sessile, endophytic lesions (Someren et al., 1976; Ellis and Corio, 1980).

The histogenesis of the spindle cell element is subject to controversy, with the current consensus favouring an epithelial origin (Harris, 1982; Someren et al., 1976; Battifora, 1976; Ellis and Corio, 1980; Takagi and Ishikawa, 1982). Histopathologically, the lesion consists of fasciculated, myxomatous and streaming patterns of spindle cells, with variable mitotic activity, pleomorphism, benign and atypical multinucleated
giant cells, a mononuclear inflammatory cell infiltrate, and infiltration of subjacent structures, e.g. skeletal muscle, bone, salivary glands and nerves.

3.4 ADENOID SQUAMOUS CELL CARCINOMA

Adenoid squamous cell carcinoma is a variant of squamous carcinoma, generally occurring in the skin of the head and neck, labial vermillion, tongue, gingivae and floor of mouth (Jacoway et al., 1971; Tomich and Hutton, 1972; Weitzner, 1974; Takagi et al., 1977; McLatchie et al., 1984). It is a slow growing neoplasm that seldom metastasizes (Takagi et al. 1977). Cutaneous lesions show a male predominance occurring mainly in the over fifty years of age group, amongst those with fair complexion and outdoor occupation. It frequently occurs in pre-existing actinic keratosis. The clinical appearance of labial adenoid squamous cell carcinoma resembles that of usual squamous carcinoma. The lesion may be ulcerated, hyperkeratotic, "rough" or granular, or slightly elevated or nodular. However, unlike normal squamous carcinoma, adenoid squamous cell carcinoma occurs with some frequency on the maxillary labial vermillion (Tomich and Hutton, 1972).

Histopathologically, adenoid squamous cell carcinoma is characterized by dysplastic squamous epithelium with lateral or deep extensions of the neoplastic epithelium as solid and tubular ductal structures. The ductal structures are lined by an intact layer of cuboidal cells. Acantholytic and dyskeratotic epithelial cells are commonly found within these structures (Tomich and Hutton, 1972; Takagi et al., 1977; McLatchie et al., 1984). It may resemble kerato–acanthoma leading to mis–diagnosis.

The pathogenesis of adenoid squamous cell carcinoma is uncertain. It may arise as a new distinct carcinoma from the usual squamous cell carcinoma or a single metamorphic differentiation (Takagi et al., 1977). Another possibility is that adenoid squamous cell carcinoma of the labial vermillion arises in areas of actinic keratosis (Tomich and Hutton, 1972; Weitzner, 1974).
CHAPTER 4
AETIOLOGY OF ORAL SQUAMOUS CELL CARCINOMA

4.1 Tobacco Usage

4.2 Alcohol Usage

4.3 Syphilis

4.4 Dental Factors

4.5 Iron Deficiency Anaemia

4.6 Chronic Hyperplastic Candidiasis

4.7 Herpesvirus hominis

4.8 Ultraviolet Light and Labial Carcinoma

4.9 Miscellaneous Risk Factors
   4.9.1 Socio-economic factors
   4.9.2 Occupation
   4.9.3 Diet and nutrition

4.10 Summary
CHAPTER 4
AETIOLOGY OF ORAL SQUAMOUS CARCINOMA

The precise biochemical event(s) which result in neoplasia remain a mystery. The aetiology of oral neoplasia is likewise unknown. Epidemiological studies, both prospective and retrospective, have provided certain "risk factors" which have come to be commonly accepted. By the examination of geographic variables; demographic variables: sex, age distribution, ethnicity, religion, occupation, education; tobacco usage: cigarettes, cigars, pipes, chewing tobacco and "snuff dipping"; and alcohol usage, circumstantial evidence implicating certain factors as possible aetiological agents has emerged. These "risk factors", since they should not be considered at present as definitive aetiological factors include: tobacco in its various forms, alcoholic beverages, syphilis, oral sepsis and chronic dental trauma, iron deficiency, chronic candidiasis, Herpesvirus hominis type 1, ultraviolet irradiation, ionizing irradiation and certain occupational hazards. The role of diet and nutrition may also be relevant in neoplasia (Wynder and Stellman, 1977; Sellers, 1979; Pindborg, 1980; Decker and Goldstein, 1982; Binnie et al., 1983).

Oral neoplasia is a multifactorial disease produced by both extrinsic and intrinsic factors acting together, some synergistically. Many of these factors may act as primary carcinogens or as co-carcinogens in the promotion of carcinogenesis following initiation. Premalignant lesions and conditions have been identified. The premalignant lesions include: leukoplakia oris; erythroleukaemia oris; carcinoma in situ; and chronic erosive oral lichen planus. Premalignant conditions include: oral submucous fibrosis; sideropenic dysphagia (Paterson-Kelly, Plummer-Vinson syndrome); dyskeratosis congenita; xeroderma pigmentosum; ataxia telangiectasia; chronic candidiasis; and chronic discoid lupus erythematosus.

4.1 TOBACCO USAGE

Tobacco usage, viz.: cigarette smoking, cigar and pipe smoking, chewing tobacco with betel nut, "snuff dipping" etc., has been implicated as the primary risk factor of oral neoplasia (Moore, 1980; Binnie et al., 1983). There is ample epidemiological evidence to support the premise
that tobacco consumption is a dose/time related entity in the aetiology of intra-oral squamous cell carcinoma (Binnie et al., 1983). Rothman (1978) has stated categorically that tobacco is an established risk factor for oral and pharyngeal squamous cell carcinoma. Mashberg et al. (1981) have reported the relative risk for smoking tobacco, adjusted for alcohol consumption, rose from 3.2 to 4.5 to 5.0 for smokers of 10–19, 20–30, 40 or more cigarettes a day, respectively. It has further been found that the chance of developing a second primary squamous carcinoma is dependent principally on the intensity, i.e. quantity and duration, of the smoking and drinking habit prior to the onset of the first neoplasm. Furthermore, it appears that the continuation of smoking habits after diagnosis of the "index carcinoma" increase the risk of the development of a second primary lesion (Wynder et al., 1977). Cigar and pipe smokers have a risk similar to cigarette smokers for oral squamous cell carcinoma, but low risk for bronchial and laryngeal carcinomata. The risk of developing tobacco-related squamous cell carcinoma decreases with the extent of ex-smoking, in comparison with persons who continue to smoke, approaching the level of non-smokers after about fifteen years; and with the smoking of "low-tar" filter cigarettes. Unfortunately, since greater smoking habits and lesser cessation rates are noted amongst the lower socio-economic groups, these groups will bear an ever increasing proportion of the burden of tobacco-related malignant neoplasia (Wynder and Stellman, 1977).

Cultural differences in the use of tobacco products have led to variations in geographic and anatomical incidences of oral and pharyngeal cancers in accordance with the dose–response principle (Decker and Goldstein, 1982). For example, there are four main ways of smoking tobacco in Mainpuri, India (Wahi, 1968, 1976; Sanghvi, 1982): "bidi", "chilum", "hookah" and cigarettes. Cigars are also smoked. "Bidi" is the local form of cigarette, about 5–7.5 cm long, made by rolling in the fingers a quarter to a half a gramme of tobacco flakes in a rectangular piece of dried temburni leaf (Diospyros melanoxylon). The "chilum" is a conical clay pipe usually 10 cm long; a pebble is inserted above to prevent the tobacco from dropping down when the pipe is filled and lit. The "hookah" is a kind of water-pipe in which the tobacco smoke from the upper bowl passes through a wooden tube and through the water of the receptacle and then enters the mouth of the smoker through a long pipe. In the state of Andhra Pradesh, India "chutta" smoking is most

1. ex-smoking: meaning "having stopped smoking", i.e. are ex-smokers (American jargon).
prevalent, especially among women. The "chutta" is a special variety of cheroot which is smoked with the burning end inside the mouth. The local effects of the heat and smoke are focused on the palatal mucosa resulting in chronic stomatitis, leukoplakia and squamous cell carcinoma. The palatal leukoplakia is different from leukokeratosis nicotina palati (stomatitis palatina) of the Western pipe smokers (Sellers, 1979; Pindborg et al., 1971; Reddy et al., 1975; Wahi, 1976). The relative risk of developing squamous cell carcinoma is forty-seven times that of the non-smoking female in those who have the reverse-smoking habit (Reddy et al., 1975).

The indigenous tobacco smoking and chewing habits of India are seen to be primarily responsible for the high incidence of neoplasia of the upper alimentary and respiratory tracts: oral cavity, pharynx, oesophagus and lung, which account for more than half of the malignant neoplasms in males and a quarter in females (Sanghvi, 1981). Chewing and smoking habits act synergistically and persons with mixed habits form a substantial fraction of the high risk population. The primary site of action of "bidi" smoke is the oropharynx. The habit of cigarette smoking is a comparatively recent phenomenon in India and is confined to the affluent sections of the Indian society.

Chemical analysis of "bidi" smoke reveals that a single "bidi" delivers about one and a half times the amount of carcinogenic hydrocarbons delivered by a single cigarette when it is smoked with two puffs per minute, and almost three times as much when the cigarette is smoked under standard United States of America smoking conditions (Sanghvi, 1981). A Bombay "bidi" smoker takes on average five puffs per minute, thus receiving much larger amounts of toxic chemicals, e.g.: carbon monoxide, hydrogen cyanide, phenol, benzanthracene and benzpyrene. "Hookah" smoke is generally smoked for a long duration each time but less frequently during the day. The water filter is efficient, reducing the "tar" and nicotine levels to values comparable to the mildest cigarettes.

An aetiological relationship between pipe smoking and labial vermilion squamous cell carcinoma has traditionally been assumed. However, data correlating pipe smoking to labial squamous cell carcinoma are inconclusive (Anderson, 1971; Keller, 1970; Spitzer et al., 1975; La Riviere and Pickett, 1979). It has been suggested by Keller (1970) that the association of pipe smoking with labial vermilion squamous cell carcinoma is the result of case-control disparities in age, residence,
occupation, or a combination of these and related factors, and that the statistical correlation of residence, nativity, "outdoor" occupations and age are stronger overall than that for tobacco, smoked or unsmoked in the occurrence of labial squamous carcinomata. Spitzer et al. (1975) in an evaluation of the risk factors of labial squamous carcinoma of Newfoundland fishermen found that the use of tobacco in general, i.e. cigarette, pipe, chewing tobacco, is not an important risk factor. However, the risk ratio for pipe smoking alone is 1.50 (p < 0.05). It is now recognized that all forms of tobacco use increase the risk of labial squamous cell carcinoma. An additional risk may accrue from the chronic thermo-mechanical irritation caused by the pipe stem, especially occurring in Irish peasant women, Negresses and Swedish women who are pipe smokers (Decker and Goldstein, 1982).

Epidemiological Studies

Many of the current studies examine the relative risk attributable to tobacco usage in concert with other possible carcinogens (Fortier, 1975; Bross and Coombs, 1976; Wynder et al., 1977; Kelier, 1977; Graham et al., 1977; Wynder and Stellman, 1977; Smith, 1979; McCoy et al., 1980; Moore, 1980; Mashberg et al., 1981; Wynder et al., 1981; Stich and Rosin, 1983; Scrimgeour and Jolley, 1983; Wynder, 1983; Freni, 1984).

Fortier (1975) found that oral squamous cell carcinoma occurs predominantly in French males residing in Montréal and Québec City in the province of Québec, Canada. Ninety per cent of heavy alcohol drinkers also smoke heavily, especially cigarettes. The age of intra-oral carcinoma patients using both cigarettes and alcohol excessively is significantly younger than that of other cancer patients. Eighty per cent of intra-oral carcinomata occur in the susceptible "U-shaped" area of the mouth consisting of ventrolateral tongue, floor of mouth, retromolar areas and palato-glossal arches. An epidemiological study on tobacco usage in France by Wynder et al. (1981) revealed that 82 per cent of males and 56 per cent of females surveyed smoked cigarettes. Pipes were smoked by older men (4.5 per cent at ages 55–89 years) and chewing tobacco was used by only 1.9 per cent. The observed differences between smoking practices of the French and those in the United Kingdom and United States of America, i.e. decrease in inhalation, "drooping" or carrying the lighted cigarette in the mouth without inhaling, use of non-filtered and
relatively higher alcohol consumption amongst older Frenchmen, may be factors associated with the observed low rates of lung cancer and high rates of oesophageal, laryngeal and oral carcinomata. It is possible that the lesser inhalation practices in older Frenchmen are the result of the high pH of French black tobacco (Gauloises, Gitanes) which yield a larger amount of free nicotine and thus contribute to the decreased need for deep inhalation. "Drooping" also makes deep inhalation less likely, although it possibly increases the passive inhalation of sidestream smoke, which is known to contain more toxic components than mainstream tobacco smoke. The alcohol facilitates the reactivity, i.e. enhances the tobacco-associated initiators of neoplasia.

Bross and Coombs (1976) have also found an earlier onset of oral squamous carcinoma in females of the State of New York, detected some fifteen years or earlier than in females who do not use either alcohol or tobacco. Exposure to smoking only produces a smaller age shift, but exposure to alcohol only does not produce any clear shift in the age of onset. It was suggested that in data where co-carcinogens are involved, the age of onset of disease may be the most useful detection device available for potential environmental hazards.

In a detailed, large, retrospective, comparative epidemiological study of tobacco-related cancers, Wynder and Stellman (1977) found the following results: (1) 13 per cent of male oral squamous cell carcinoma cases smoke only cigars and pipes, with relative risk of 8.0 and 11.0 for 11 + cigars or pipe bowls per day, respectively; (2) most cigarette smokers inhale ("at least beyond the throat", 93 per cent of cases), whereas most cigar and pipe smokers inhale little, if not all, suggesting an explanation for the lower risk for lung cancer observed in cigar and pipe smokers; (3) the effect of the cessation of smoking is to reduce the relative risk of neoplasia, approaching the risk of a non-smoker after fifteen years of continuous cessation. This decline in the risk is greatest for bronchial, oral and laryngeal carcinomata; (4) dose-response analysis reveals for all anatomical sites (except the urinary bladder) an approximately linear increase in the relative risk with dosage (i.e. quality), "tar" levels and duration of smoking; (5) combined relative risk of 3.4 for smokers of forty or more cigarettes per day who also consume 200 ml or more of alcohol per day. Within each class of tobacco usage, daily consumption of alcohol generally increases the risk of neoplasia. Among non-smokers no significant increase in risk with alcohol
consumption occurs. Also the sex ratio showing a preponderance of males is explicable by the long-term smoking habits of either sex. However, notable exceptions are squamous cell carcinomata of the tongue, buccal mucosa, supraglottis and oesophagus, which are more common in non-smoking women than non-smoking males, mainly because of subclinical Plummer-Vinson syndrome. Alcohol is regarded as a promoter of tobacco carcinogenesis. A strong correlation between socio-economic status and tobacco-related concerns exists: low socio-economic status males with heavy smoking and alcohol consumption, and associated nutritional deficiencies will experience the highest rates of malignant neoplasia of the oral cavity, larynx and oesophagus.

Graham et al. (1977) reported a synergistic relationship between heavy alcohol consumption, heavy tobacco consumption and poor dentition, indicating an increase risk of oral squamous cell carcinoma of 7.7 times in males possessing all three traits. Each trait considered separately, and controlled for the other factors, carried an increased risk. Poor dentition enhances the risk more so than the other traits. No dietary factors, including meats, fats, vitamin A and C, result in any important relationship. The study, however, did not support the concept of synergy of alcohol and tobacco with the exclusion of dental factors. It is hypothesized that a mechanism for inadequate dentition in which trauma to the oral tissue provides an easier "portal of entry for viral or chemical carcinogens". Keller (1977) has also reported a positive association between excessive consumption of whisky, tobacco plus alcohol, and mixed alcoholic beverages, and oral and hepatic carcinomata. Oral, pharyngeal and digestive tract lesions are significantly excessive among hepatic cirrhotic patients. In a review of the epidemiology of oral and pharyngeal cancers in the United States of America, Smith (1977) found that tobacco and alcohol are the "major independent aetiological agents", their effects being associated with age, sex, and religion-ethnicity. Other factors, viz.: geographic location, race, socio-economic status, nutrition, dental factors and concurrent disease are less consistently implicated as risk factors. The association found between hepatic cirrhosis and intra-oral squamous cell carcinoma, particularly of the floor of the mouth, is attributable "... most likely to heavy use of alcohol and is not causal in nature ...". Social and behavioural components may alter risk, stage of disease at diagnosis, treatment and survival of oral squamous cell carcinoma.
Recent studies on trends in tobacco consumption and incidence rates of upper aerodigestive tract neoplasia serve to emphasize the importance of tobacco in oral carcinogenesis. In Papua–New Guinea there is an increase in the average annual age-standardized incidence rate of oral squamous cell carcinoma in females, related to an increase in consumption of tobacco, especially flue-cured tobacco, or alcohol, or both, in a population in which habitual betel nut chewing was prevalent. Papua–New Guinean tobacco has a "tar" concentration in the middle range, and although lower than air-cured tobacco, the flue-cured tobacco burns at higher temperatures and increased concentrations of carcinogens are produced. Also more smoke is inhaled by the indigenous population (Scrimgeour and Jolley, 1983).

In the Netherlands Antilles, abuse of alcohol and tobacco, mineral deficiencies, malnutrition, the use of sorghum in the daily diet, and reverse smoking by females are factors believed to be responsible for the high incidence of oral, pharyngeal and oesophageal squamous cell carcinomata in the past (Freni, 1984). With the exception of tobacco and alcohol abuse, these factors have changed considerably in a favourable direction resulting in a decrease in incidence rate, once amongst the highest in the world, of approximately 3 per cent per year during the period from 1958 to 1981.

Stich and Rosin (1983) have described a method for quantitating the synergistic effect of smoking and alcohol consumption with the micronucleus test on human buccal mucosal cells, which shall be discussed later. An elevated frequency of micronucleated buccal mucosal cells was found only among smokers and alcoholic beverage drinkers. The frequency of micronucleated buccal cells and the average number of micronuclei per cell appears to depend on the number of cigarettes smoked. An approximately eight-fold increase in micronucleated mucosal cells is seen among alcohol drinkers who smoke three or more packages of cigarettes per day, and an approximately 4.2 fold elevation when one to two packages are consumed. Neither smoking alone of up to over sixty cigarettes per day nor alcohol drinking of up to 1.2 litres per day lead to a detectable elevation of micronucleated buccal mucosal cells. Open to question remains whether the strong synergistic effect between smoking and alcohol consumption, as seen by the frequency of micronuclei in buccal mucosal cells, is related to their synergistic effect in the induction of oral squamous cell carcinoma.
Tobacco Chewing Habits

Tobacco chewing habits such as "pan" and "khaini" are commonly practised on the Indian subcontinent. Betel nut chewing, with or without tobacco, is also commonly practised in India, southeast Asia and Papua–New Guinea (Sellars, 1979; Ramanthan and Lakshimi, 1976). It has become established that the topical use of tobacco is a definite risk factor in oral squamous cell carcinoma (Hirayama, 1966; Wahi, 1968, 1976; Mahoubi, 1977; Jayant et al., 1977; Malaowalla et al., 1976; Gupta et al., 1980; Sanghvi, 1981; W.H.O., 1984). The relative risks of oral cancer from various tobacco habits has been summarized by W.H.O. (1984) as the following: betel quid only, 1–4; smoking only, 3–6; betel quid incorporating tobacco, 8–15; betel quid and smoking, 4–25; and betel quid incorporating tobacco and smoking 20. Approximately 90 per cent of oral cancers in south and southeast Asia can be attributed to tobacco chewing and smoking habits. This accounts for one-third of total malignant neoplasms in these areas. The highest risks occur in those who use tobacco in a betel quid and also smoke tobacco, with a ten to twenty times greater risk than people who neither chew nor smoke, giving them a lifetime risk of developing oral cancer of about 10 per cent (or 1:10). This risk is also increased by increasing the use of tobacco, and is higher for people who begin chewing tobacco at an early age, who chew tobacco for a long time, who chew betel quids frequently and continually, or who keep a quid in the mouth overnight.

Tobacco chewing and "snuff dipping" is reported as prevalent in southeastern United States of America among rural female workers resulting in "snuff dipper's cancer" (Winn et al., 1981; Schottenfeld, 1981; McGuirt, 1983). "Snuff dipping" is also practised in Sweden (Sundström et al., 1982; Hirsch and Johansson, 1983). In southern Saudi Arabia a native form of oral snuff called "Shammah" is associated with oral preneoplasia and neoplasia (Salem et al., 1984). In the Union of Soviet Socialist Republics the chewing of "nass", which consists of tobacco powder, wood ash and lime, has been linked to the relative high incidence of oral squamous cell carcinoma (Paches and Milievskaya, 1980).

The form tobacco chewing takes in India involves the chewing of a betel nut quid called "pan". This quid is composed of three separate ingredients: areca nut (Areca catechu, commonly referred as "betel nut"), slaked lime and catechu (Acacia catechu) wrapped in two betel leaves
(Piper betle). To this is normally added flakes of tobacco and/or other spices (Mehta et al., 1969b; Burton-Bradley, 1979; Mehta et al., 1981; W.H.O., 1984). The slaked lime is procured from burnt sea shells, corals, or mountain lime (calcium hydroxide).

Some confusion remains as to which ingredient(s) of the betel nut quid are carcinogenic. The matter is complicated by the combination of various smoking habits with tobacco and "pan" chewing. Indeed, Mehta et al. (1981) have shown that whereas the effect of combining "bidi" smoking (a cheap native cigarette) with "pan" chewing appears to be synergistic for the incidence of leukoplakia, the combination does not produce a synergistic effect for the malignant transformation of leukoplakia.

Jussawalla and Deshpande (1971) have estimated the crude relative risk of oral, pharyngeal and oesophageal carcinoma- attributable to separate, as well as, to the combined habits of smoking and chewing. Compared to non-chewers and non-smokers the risks are 10.14 for oral, 81.72 for oropharyngeal, 16.86 for hypopharyngeal and 20.09 for laryngeal carcinoma. Jayant et al. (1977) estimated the respective proportions of carcinoma that would occur or could be prevented in the absence of tobacco habits as 70 per cent for oral, 84 per cent for oropharyngeal, 74 per cent for hypopharyngeal and 50 per cent for oesophageal carcinoma.

However, Burton-Bradley (1979) suggests that the clinical literature on the putative association between betel chewing and human oral carcinoma is not impressive, and presumably unconvincing. He points out that betel chewing (without tobacco) is practised daily by more than 200 million people, the vast majority of whom do not have oral carcinomata. Be this as it may, experimental animal studies (Ranadive et al., 1979) have confirmed (1) the specific carcinogenicity of certain betel quid ingredients like betel nut, particularly its tannin-containing polyphenolic fraction and its combinations with tobacco and lime; and (2) the direct relationship of the frequency of chewing with the induction of oral carcinogenesis in the hamster buccal pouch animal model. The high incidence of gastric lesions (39–48 per cent) induced suggests a direct association between chewing habits and increasing oesophageal and gastric lesions in the Kashmiri and other Indian populations.

The hazards of intra-oral snuff usage, so-called "snuff dipping", have recently emerged from epidemiological studies in the United States of America (Christen, 1980; Goldsmith and Winn, 1980; Winn et al., 1981; Schottenfeld, 1981; McGuirt, 1983). In the U.S.A. and Sweden snuff is
not sniffed as in Europe, instead a pinch of snuff composed of finely ground or powdered tobacco, is placed (or "dipped") in the oral cavity, usually in the lower buccal or labial sulci. It is very common among men and women of both races, Caucasian and Negroid, in the southeastern United States of America. "Smokeless tobacco" can produce many harmful changes in the soft and hard oral tissues, e.g. oral leukoplakia, gingival recession, tooth abrasion and periodontal destruction (Christen et al., 1979). "Snuff dipping" has also been implicated as a factor in the aetiology of gingival and buccal squamous cell carcinoma.

In a case-control study in North Carolina involving women, Winn et al. (1981) found that the mortality rate of oral squamous cell carcinoma exceeded that of women in the North by 30 per cent in urban and 90 per cent in rural areas. This finding in Caucasian women is primarily related to the chronic use of oral snuff. The relative risk associated with "snuff dipping" among Caucasian non-smokers is 4.2 and among chronic users the risk approached 50-fold for carcinomata of the gingivae and buccal mucosa: tissue directly in contact with tobacco powder. In the absence of snuff dipping, oral and pharyngeal squamous cell carcinoma resulted mainly from the combined effects of cigarette smoking and alcohol consumption.

Oral lesions found to be induced by "snuff-dipping" in Swedish males include verrucous carcinoma, verrucoid squamous cell carcinoma, ulcerative squamous cell carcinoma, as well as dysplastic leukoplakia oris (Sundström et al., 1982). In an American population McGuirt (1983) found that "snuff dipper's carcinoma" occurs predominantly in Caucasian women older than sixty years of age with a history of "snuff dipping" longer than forty years duration. The carcinomata appear preponderantly in the buccal (47.5 per cent) and alveolar regions (31.6 per cent), often appearing clinically as a verrucous lesion which proves to be histologically well-differentiated squamous cell carcinoma. Fifty-eight per cent of patients experience recurrence or a contiguous second primary neoplasm. Almost half (47.3 per cent) have concurrent leukoplakia oris and 14 per cent have had leukoplakia previously excised. All reacted to the progressive pan-mucosal insult.

The effect of long-term application of snuff on the oral mucosa has been studied experimentally using a suitable rat model (Hirsch and Johansson, 1983). In general, the rat model, after exposure to standard snuff (pH 8.3) and highly alkaline snuff (pH 9.3) for 9-22 months, exhibits
a markedly higher frequency of hyperorthokeratinized, atrophic and ulcerated, mildly dysplastic and fibrotic lesions compared to controls. In human "snuff dippers" fewer atrophic and ulcerated lesions occur, as is the occurrence of vacuolated cells. These differences may reflect species-related differences or snuff exposure differences. In the U.S. tobacco products such as fermented snuff, high levels (29.1 p.p.m.) of N'-nitrosonornicotine (NNN), the first organic carcinogen isolated in unburnt tobacco, and other tobacco specific N-nitrosamines e.g. 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N'-nitrosoanatabine (NAT) have been extracted from snuff during "dipping" by analysis of saliva (Hoffman and Adams, 1981). Concentrations of these chemicals vary with the specific brand of snuff tested. These chemicals, especially N'-nitrosonornicotine are believed to be the primary carcinogens in snuff tobacco (Goldsmith and Winn, 1980; Winn et al., 1981; Hirsch and Johannson, 1983). The N'-nitrosonornicotine is partly derived from bacterial or enzyme action of nicotine during curing and high levels are generated from unburnt tobacco incubated in saliva, presumably through the action of salivary nitrites. A high pH may also contribute significantly to more rapid absorption of NNN and nicotine (Hecht et al., 1975, 1978).

The effect of oral snuff and Herpesvirus hominis type 1 on rat oral mucosa and its possible associations with the development of squamous cell carcinoma has been studied by Hirsch et al. (1984). The results suggest that Herpesvirus hominis type 1 in combination with snuff exposure may be associated with oral carcinogenesis. The snuff may act as a co-carcinogen due to its restrictive effects on cytolytic herpesvirus infections, a prerequisite for virus-induced neoplasia. It is postulated that the nitrosamines in tobacco may act in two ways: as direct inducers of neoplasia or as indirect inhibitors of herpes simplex infection, thereby facilitating transformation of cells by the Herpesvirus hominis.

"Smokeless tobacco" and oral cancer is rapidly becoming a problem for concern in the United States of America because of the increased promotion and use of both snuff and chewing tobacco aimed specifically at children and adolescents (Squier, 1984). Although epidemiological information is lacking in the U.S.A., the vast amount of information from Indian studies suggest that oral squamous cell carcinoma and preneoplastic lesions occur almost solely among those with tobacco habits. The rate of malignant transformation of premalignant lesions is not greater than in
Western countries, and the relative risk of developing oral neoplasia is similar in India and the U.S.A., increasing with duration of use. Consequently, it seems likely that increased usage of "smokeless tobacco" in the U.S.A. will eventually lead to an increased incidence of preneoplastic and neoplastic oral lesions in North Americans.

"Khaini" tobacco, a powdered tobacco with the addition of lime, is commonly used by the residents of Bihar, India. The mixture is placed on the inner side of the lower lip within the gingivo-labial groove. Carcinomata, called "Khaini cancers", develop at the site where the tobacco is in close contact with the mucosa. An elevated frequency in micronuclei of cells of the oral mucosa of all "Khaini" tobacco chewers was found by Stich et al. (1982). Micronuclei indicate the occurrence of chromosome aberrations, "genotoxic effect" in the dividing cell population of the stratum basale. The induction of micronucleated mucosal cells seems to be due to the genotoxic agents released from the tobacco/lime mixture. In vitro tests exclude lime (calcium hydroxide) as the genotoxic agent. The question of the relevance of the prolonged formation of micronucleated cells in the oral mucosa of "Khaini" tobacco users, raw betel nut or pan chewers, in the development of oral carcinomata remains unanswered. The non-random formation of particular chromosome alterations as well as survival within a tissue must be met before a "potential" neoplastic cell may give rise to a neoplasm. Whatever the ultimate significance of chromosome aberrations in neoplasia may prove to be, they seem to represent an easily detectable marker indicating a tissue at high risk of developing neoplasia. The micronucleus test on exfoliated cells seems to provide evidence of exposure to carcinogens, and a measure of the degree of exposure in the tissue from which carcinomata develop.

Experimental Evidence

Several experimental studies support the idea that tobacco consumption represents an important aetiological factor in the development of oral squamous cell carcinoma (McCoy et al., 1980; Trell et al., 1981; Trushin et al., 1985; Pisker and Philipson, 1984; Korsgaard et al., 1984; Kobayashi and Hayatsu, 1984; Mohtashamipur et al., 1984; Trell et al., 1984).
The cyclic nitrosamines such as $N$-nitrosopyrrolidine and $N'$-nitrosornornicotine are important environmental carcinogens. $N$-nitrosopyrrolidine is found in mainstream and sidestream tobacco smoke while $N'$-nitrosornornicotine is the major tobacco specific carcinogen occurring in tobacco and tobacco smoke. Microsomal alpha-hydroxylation of both these chemicals result in the formation of ultimate carcinogens. The hydroxylation of either alpha-carbon gives rise to alpha-hydroxy-$N$-nitrosopyrrolidine, an unstable intermediate, which undergoes ring opening, loss of $\text{N}_2$ and hydroxide to give 4’-oxobutylcarbonium ion, which may be the ultimate carcinogen. This ion reacts with water to give 4’-hydroxybutanal which exists predominantly as the cyclic hemiacetal 2’-hydroxytetrahydrofuran (McCoy et al., 1980; Trushin et al., 1985). The alpha-hydroxylation of $N'$-nitrosonornicotine results in two products: a keto-alcohol from 2’-hydroxylation and a lactol from 5’-hydroxylation. Chronic ethanol consumption increases the rates of alpha-hydroxylation of $N$-nitrosopyrrolidine and 5’-hydroxylation of $N'$-nitrosonornicotine in liver microsomes of hamsters (McCoy et al., 1980) and in nasal mucosa of rats, suggesting that chronic ethanol consumption could influence the organospecificity of $N'$-nitrosonornicotine, altering its carcinogenic potency (Trushin et al., 1985) (see Figure 3).

Verrucous hyperplasia and verrucous carcinoma have been induced experimentally in rat oral mucosa by Fisker and Philipsen (1984) using the carcinogen 4-nitroquinoline-1-oxide, further supporting the aetiological significance of tobacco consumption.

The activation of polycyclic aromatic hydrocarbons to electrophils or ultimate carcinogens in cells is mediated by a component enzyme system of microsomal mixed-function oxidase complex, commonly referred to as aryl hydrocarbon hydroxylase (AHH), which hydroxylates polycyclic aromatic hydrocarbons to epoxides (Trell et al., 1981). These microsomal metabolites form covalent bonds with DNA, RNA and proteins, thereby inducing mutations. However, it must be remembered that most human neoplasms are not caused by conventional mutagens, but are more likely the result of genetic transpositions, i.e. large scale changes in the genome caused by rearrangements and deletions (Cairns, 1981). The association of AHH activity and polycyclic aromatic hydrocarbon-induced neoplasia has been widely debated and most laboratory studies have been inconclusive or contradictory. Trell et al. (1981) found high AHH inducibility more common ($p < 0.001$) in cigarette smokers with oral
squamous cell carcinoma, suggesting that smoking is a prominent exogenous factor associated with the occurrence of oral squamous cell carcinoma. A further study adding support to the concept that AHH is a major activator of the polycyclic aromatic hydrocarbon carcinogens was provided by Korsgaard et al. (1984).

Smoking tobacco has been shown to elicit mutagenic effects. A short-term study of the urinary mutagenicity caused by cigarette smoking reveals an increase in mutagenicity rapidly after the start of smoking, decreasing after smoking is stopped to the non-smoking level after six to thirteen hours (Kobayashi and Hayatsu, 1984). There is no great difference among individuals regarding this rapid time-dependent response. Using the same bacterial microtitre fluctuation test Mohtashamipur et al. (1984) exposed rats separately to main- and side-stream smoke of a commercial brand of cigarette. Exposure to both side-stream and main-stream smoke of at least two cigarettes results in significant excretions of frameshift mutagens in urine within twenty-four hours. Doubling the exposure to side-stream smoke results in reduced water intake by the animals and thus toxic effects of urine concentrates on the test bacteria. Treil et al. (1984) studied the effects of smoking tobacco on mutagen sensitivity and enzyme induction in healthy middle-aged male humans. Unscheduled DNA synthesis, i.e. excision repair of N-acetoxy-2-acetylaminofluorene (NA-AAF) damage to DNA of human lymphocytes and the levels of tritium-labelled NA-AAF bound to DNA (carcinogen binding) of the lymphocytes after eighteen hours of culturing, reveals an association with smoking (p < 0.01) with increasing levels of both. Under normal circumstances smoking may modulate the risk of neoplasia and cardiovascular disease by influencing individual mutagen sensitivity.

4.2 ALCOHOL USAGE

The use of the term "alcohol" is preferred to "ethanol" because the latter has not been shown experimentally to be carcinogenic or co-carcinogenic. Furthermore, it is generally believed that alcoholic beverages as a whole are important risk factors in oral carcinogenesis. However, the role of alcohol consumption in the aetiology of oral neoplasia is confused (Binnie, 1976; Mashberg et al., 1981; Binnie et al., 1983). The most commonly cited reason for the lack of definitive evidence on the carcinogenic capacity of alcohol is the difficulty in
isolating heavy alcohol consumption from tobacco smoking. Wynder et al. (1967) showed an association between oral cancer and frequent, heavy drinking, especially American whisky. In France, Schwartz et al. (1962) in an analysis of the association between alcohol consumption and squamous carcinoma of the tongue, hypopharynx, larynx, oesophagus, buccal cavity, and oropharynx in 3937 Frenchmen showed a definite relationship after excluding the possible influence of smoking.

Before recent evidence showing an increased rate of oral carcinoma in alcohol consumers can be assumed to be absolute, one must reconcile the decreasing or static incidence of the disease in the U.S.A. and United Kingdom with the vast increase in alcohol consumption per caput (sic) over the same time span (Binnie et al., 1983). Intra-oral squamous cell carcinoma in England and Wales has been decreasing in incidence over the last three or four decades while alcohol consumption has been rapidly increasing (Binnie, 1976).

Epidemiological Studies

Several epidemiological studies have investigated the effects of alcohol and cigarette consumption on oral squamous cell carcinoma, both separately and combined (Fortier, 1975; Bross and Coombs, 1976; Wynder et al., 1977; Wynder and Stellman, 1977; Graham et al., 1977; Keller, 1977; Tuyns, 1979; Smith, 1979; Mashberg et al., 1981).

Mashberg et al. (1981) regards alcohol as a primary risk factor in oral squamous cell carcinogenesis. The relative risk for drinkers, adjusted for smoking, is 3.3, 15.2 and 10.6 for those who drink less than six, six to nine, and ten or more "whisky equivalents" (i.e. approximately 30 ml of 86-proof whisky) per day, respectively. An interesting finding was that beer and wine drinkers have a much higher relative risk than whisky drinkers. The adjusted relative risk for whisky drinkers consuming ten or more "whisky equivalents" a day is 7.3, whereas for beer/wine drinkers it is 20.4. The study confirms the synergist effect of alcohol and tobacco, but in addition shows that alcohol drinkers, especially beer and wine consumers, have a greater risk than heavy smokers of forty or more cigarettes a day. The age of intra-oral squamous cell carcinoma patients using both cigarettes and alcohol heavily is significantly younger than would occur in non-smokers and non-drinkers, fifteen years or more earlier among women (Fortier, 1975; Bross
and Coombs, 1976). Wynder and Stellman (1977) found that the risk of developing tobacco-related cancers increases with the quantity of liquor consumed. The highest proportion of heavy alcohol usage in each tobacco use category occurs for oral, laryngeal and oesophageal carcinoma. Among smokers, heavy alcohol consumption specifically enhances the risk of carcinomata of the oral cavity, larynx and oesophagus. Graham et al. (1977) also showed a higher risk of developing oral squamous cell carcinoma to be associated with heavy smoking, heavy drinking and poor dentition. Moreover, heavy smokers and drinkers with poor dentition and males with all three traits have a substantially higher risk (7.7 times) than would be expected if the traits are considered additively.

Keller (1977) has reported an association between liver cirrhosis and carcinomata of the oral cavity and pharynx. Excessive use of whisky, tobacco plus alcohol, and mixed alcoholic beverages are positively associated with oral and hepatic carcinomata.

Both prospective and retrospective epidemiological studies have shown an increased risk of developing carcinomata of the mouth, pharynx, larynx and oesophagus, as well as liver and lung. For carcinomata of the buccal cavity, pharynx, larynx and oesophagus the effect of drinking has been shown to be associated with the effect of smoking (Tuyns, 1979). However, the mechanisms by which this increase in risk occurs remains as yet unknown. There is no evidence indicating that ethanol per se should be or is carcinogenic (Graham et al., 1977; Tuyns, 1979; Wynder et al., 1977). There appears to be a synergistic effect between tobacco and alcohol consumption, with alcohol acting as a promoter or enhancer (Wynder et al., 1977; Wynder and Stellman, 1977; Wynder, 1984), at least in regards to effects on the oral cavity, larynx (glottis and supraglottis) and oesophagus. Nutritional deficiencies arising from either malnutrition and/or as a direct consequence of alcohol intake, i.e. impaired absorption or enhanced elimination, could play a role in the aetiology of head and neck carcinoma (Wynder and Stellman, 1977; Wynder et al., 1977; McCoy et al., 1980).

Experimental Evidence

Experimental evidence of the effect of alcohol in enhancing the carcinogenesis of the locally applied chemical carcinogen, dimethylbenzanthracene (DMBA), to hamster buccal pouch has been reported
by Freedman and Shklar (1978). The DMBA animals given 10 per cent solution of "alcohol" develop dysplastic leukoplakia and squamous cell carcinomata two weeks ahead of water-drinking control animals. The neoplasms grow to a larger size, are more invasive and more anaplastic histologically. The action of ethanol is considered that of a co-
carcinogen. Other experimental studies reveal that ethanol consumption increases the rate of liver microsomal alpha-hydroxylations of N-
nitrosopyrrolidine and N'-nitrosonornicotine (McCoy et al., 1980).
Furthermore, ethanol consumption can influence the organospecificity of
N'-nitrosonornicotine and can alter its carcinogenic potency (Trushin et 
el., 1984).

In a quantitative study of the synergistic effect of smoking and alcohol consumption using the micronucleus test on human buccal mucosal
cells, Stich and Rosin (1983) found an eight-fold increase in the
frequency of micronucleated cells among alcohol drinkers who smoke
three or more packages of cigarettes per day, and an approximately 4.2
fold increase when one to two packages are consumed. Neither smoking
alone nor alcohol drinking alone lead to a detectable elevation of
micronuclei.

Studies of the prevalence and consumption of illegally produced spirits, indigenous to certain parts of the world, suggest the possible
significance of carcinogenic contaminants or congeners in the ethanol
beverage. Massé (1972) demonstrated that the highest incidence of
eosophageal carcinoma in France is found in the apple-growing areas of
Brittany and Normandy whose populations traditionally consume crude,
pot-still "calvados". Similarly, contrasting oral squamous cell carcinoma
in Puerto Rico with the mainland U.S.A., Pischman and Martinez (1977)
suggested the prevalence of home-made rum might, in part, account for
the higher incidence reported in Puerto Rico. Mashberg et al. (1981) have
reported a greater risk for beer and wine drinkers than for whisky
drinkers. Graham et al. (1977) hypothesizes that the various ingredients
in whisky, beer, wine, gin etc. work in conjunction with ethanol to
produce neoplasia. McCoy (1978) admits that ethanol facilitates the entry
of carcinogens into exposed cells, but suggests that the effect of ethanol
may be more readily explained by alterations in metabolism in the oral
and oesophageal epithelia. He further proposes that oxidation of ethanol
by the epithelial cells of the target tissue can alter intracellular
metabolism, creating a more favourable environment for metabolic
activation of procarcinogens. There also exists the possibility that alcohol-compromised liver can have a decreased ability to detoxify potential carcinogens and that the nutritional deficiencies in alcoholics probably further potentiate the systemic effects of alcohol in the aetiology of oral carcinoma.

4.3 SYPHILIS

Luetic or chronic interstitial glossitis of tertiary syphilis has long been associated with an increased risk of developing oral squamous cell carcinoma. The pathological co-existence of chronic interstitial syphilitic glossitis, carcinoma in situ and squamous cell carcinoma has been well documented in the last century. The relative risk of lingual squamous cell carcinoma in patients with positive serology for Treponema pallidum is approximately 3.1 (Sellars, 1979; Binnie et al., 1983). Wynder et al. (1957) found a similar figure of 2.6 after eliminating the other confounding factors such as ethanol and smoking. However, Wynder et al. (1957) questioned whether the lingual neoplasms may be related to arsenical and heavy metals therapy commonly used prior to the advent of antibiotics. Meyer and Abey (1970) also cast doubt about the aetiological role of syphilis by finding only 6 per cent of 243 cases of primary lingual squamous cell carcinoma to have a positive history of syphilis.

More recent studies have revealed a reduced correlation between syphilis and oral squamous cell carcinoma (Decker and Goldstein, 1982). Improved chemotherapy for syphilis and the discontinuation of arsenic therapy have been proposed to account for this decline. A commensurate decline in the incidence of lingual squamous cell carcinoma, which would be expected if syphilis were a major risk factor, has not occurred, and evidence incriminating arsenic as a cause of lingual neoplasia has not emerged, even though arsenic is carcinogenic for epidermis.

Chronic interstitial syphilitic glossitis is manifested clinically as macroglossia, lobulated and irregular in shape, with areas of atrophy and hypertrophy seen as leukoplakia. The diffuse gummatous infiltration of tertiary syphilis produces at first a diffuse vasculitis leading eventually to endarteritis obliterans. This relative ischaemia leads to marked lingual epithelial atrophy. Depapillation followed leading to a "bald tongue". Lobulation and irregular deep fissuring may also result (Plumara, 1976). Chronic syphilitic interstitial glossitis is a preneoplastic lesion which can
undergo neoplastic transformation into squamous cell carcinoma. The site of these lingual preneoplastic and neoplastic lesions is the anterior two-thirds of the dorsum linguæ, an unusual site for oral squamous cell carcinoma today.

It is unknown whether this chronic specific infection is primarily involved in malignant changes in the oral cavity or whether those exposed to syphilis are also more susceptible to oral carcinoma through socio-economic and behavioural factors (Sellars, 1979).

Despite the recent increase in primary syphilis, the mortality rate and incidence rate of late syphilis has been steadily declining. Modern methods of treatment will ensure that late stage syphilis will never be prevalent again, thereby resulting in a low incidence of chronic interstitial glossitis and lingual squamous cell carcinoma associated with tertiary syphilis (Binnie et al., 1983).

4.4 DENTAL FACTORS

Dental conditions such as poor oral hygiene, jagged restorations, sharp edges of teeth, trauma and ill-fitting dental prostheses have been implicated as possible aetiological factors of oral squamous cell carcinoma. Wynder et al. (1957) used "edentia" as an "objective" criterion for poor oral hygiene and found that 44 per cent of oral cancer patients were edentulous compared to 28 per cent of controls. Graham et al. (1977) demonstrated a three-fold increase in risk associated with inadequate dentition after controlling for smoking and drinking of alcohol. Browne et al. (1977) reported a higher incidence in poor oral hygiene and ill-fitting dentures associated with an increase in the incidence of oral squamous cell carcinoma in Stoke-on-Trent, England.

Thumfart et al. (1978) observed a topographic coincidence of oral neoplasia, dysplasia and hyperkeratosis and chronic mechanical trauma from poorly-fitting dental prostheses, sharp remaining teeth and efficient dental restorations. In the presence of alcohol and tobacco abuse chronic trauma may act synergistically to give rise to preneoplastic and finally overtly neoplastic lesions. Oral lesions were induced experimentally in 63 per cent of hamster buccal pouches by chronic trauma and chemical carcinogen exposure (Ranadive et al., 1979). Continuous contact with betel nut pieces in the hamster buccal pouch closely simulates the situation in the mouth of the habitual betel quid chewer.
It has been hypothesized by Graham et al. (1977) that inadequate
dentition allowing trauma to the periodontium, tongue etc., provides an
easier "... portal of entry for a viral or chemical carcinogen ...". The
synergistic risk of dental inadequacy and heavy use of tobacco and
alcohol can also be explained in the same fashion. However, these dental
factors may be spurious associations with socio-economic strata that
differentiate dental health status (Smith, 1979; Graham et al., 1977). It is
possible that most persons with oral squamous cell carcinoma are members
of lower socio-economic strata, who are more likely to have poor oral
health and less likely to make use of dental services. They also indulge
more heavily in alcohol and tobacco usage.

A commonly held belief among cancer therapists in the United
Kingdom is that the observed decrease in oral cancer incidence is due to
improved dental health. However, this assumption is not based on any
definitive evidence. Neither is there any evidence to the contrary
disproving any association (Binnie et al., 1983).

4.5 IRON DEFICIENCY ANAEMIA

Sideropenic dysphagia (Patterson-Kelly or Plummer-Vinson
syndrome) consists of a complex of iron deficiency anaemia, atrophic
glossitis, angular cheilitis and dysphagia. It is associated with oral,
pharyngeal and oesophageal mucosal atrophy and a submucous fibrosis,
which is characteristically localized in the post-cricoid region of the
pharynx (Sellers, 1979).

Patterson-Kelly syndrome is associated with post-cricoid,
hypopharyngeal and oral carcinoma in non-smoking Swedish women
(Larsson et al., 1975). Clinically, the syndrome consists of kolonychia,
atrophic glossitis, angular cheilitis, microstomia and a thin labial
vermilion, smooth facial skin, achlorhydria and atrophic gastritis, and
post-cricoid dysphagia. The post-cricoid dysphagia is due to post-cricoid
oesophageal strictures or "webs" consisting of a fold of mucosa or
structure with chronic inflammation of the muscle layers of the
oesophagus. The atrophic glossitis and angular cheilitis are probably due
to tissue depletion of iron, and may appear before the development of
anaemia. Angular cheilitis is a less specific abnormality, development of
which is favoured by edentia and ill-fitting dental prostheses. Xerostomia
may also be present. The lingual mucosa is smooth, erythematous and
painful. These mucosal changes predispose to leukoplakia, oral and pharyngeal carcinomata (Rose and Kaye, 1983b).

The post-cricoid oesophageal "webs" and strictures are characterized microscopically by fibrosis, epithelial atrophy and hyperplasia, sometimes combined with chronic inflammation. Preneoplastic epithelial changes may also be present. The characteristic roentgenological findings consist of one or more "webs" in the lower part of the hypopharynx or upper part of the oesophagus. Sometimes a more or less pronounced stricture is evident. Small "webs" are best visualized by cinematographic technique and may be found in patients without subjective dysphagia (Larsson et al., 1975).

The true pathogenesis of "sideropenic" epithelial lesions and Patterson–Kelly syndrome is still obscure. Iron deficiency is certainly important but there are probably other factors involved. Iron deficiency in tropical regions does not seem to give rise to the peculiar epithelial signs observed in Scandinavia, United Kingdom and United States of America (Larsson et al., 1975). Other nutritional deficiencies, viz.: riboflavin, thiamin, pyridoxine, proteins; and hereditary factors have been suggested as important. It is well documented that in rural areas of northern Sweden, where sideropenic epithelial syndrome is common, the diet was nutritionally inadequate. This was especially true during the long winter season when fresh vegetables, meat and fish were not available, leading to deficiencies in iron and vitamins: ascorbate, riboflavin and thiamin.

Recently, in the U.K., Rennie et al. (1982) have shown that in human oral epithelium quantitative histological changes are demonstrable in iron–deficiency anaemia. There is a significant reduction in the total epithelial thickness of the buccal epithelium due to a reduction in the thickness of the natural compartment and the pattern of epithelial change is the same for both sexes. From this study it was not possible to differentiate between the effects of iron deficiency and anaemia. However, the majority of available evidence supports deficiency of iron as the prime aetiological factor in the development of epithelial lesions, and that anaemia is merely a late manifestation of iron deficiency. Cell kinetic studies have shown an increase in cell production indicating that despite atrophy, the epithelium is "turning over" more rapidly. Consequently, Binnie et al. (1983) suggest that there may be an increased susceptibility to chemical carcinogens because there is an increased,
susceptible population of dividing cells, and also the epithelium may be more permeable when hyperplastic (Squier and Hall, 1985).

There is no doubt that iron is essential for the overall integrity and health of epithelia of the upper gastro-intestinal tract, and its importance may lie in its contribution to normal enzyme systems (Binnie et al., 1983). Joynson et al. (1972) demonstrated an impairment of lymphocyte transformation and migration inhibition factor production in iron deficient patients when challenged immunologically by Candida antigen and purified protein derivative (PPD). The intradermal injection of these immunogens produces a delayed-hypersensitivity skin reaction in only a minority of iron-deficient subjects. This result, it was suggested, carries important implications with regard to the pathogenesis of malignant neoplasia and chronic infections such as candidiasis.

At present, an epidemiological relationship between chronic sideropenia (Patterson-Kelly syndrome) and oral and post-cricoid carcinoma in Swedish women exists (Larsson et al., 1975). However, no cause-and-effect relationship has been demonstrated to date (Binnie et al., 1983).

4.6 CHRONIC HYPERPLASTIC CANDIDIASIS

Chronic hyperplastic candidiasis (candidal leukoplakia) is generally accepted as being a premalignant condition of the oral mucosa. Evidence for a relationship between chronic hyperplastic candidiasis and oral squamous cell carcinoma is provided by Cawson and Binnie (1980). The candidal leukoplakia is frequently speckled in character and proves to be epithelial dysplasia or carcinoma. The development of malignancy in candidal leukoplakia is more frequent than in other leukoplakias and unlike other types of leukoplakia a possible mechanism can be demonstrated: chick embryo ectoderm undergoes squamous metaplasia and proliferative activity which produces hyperplastic plaques when infected with Candida albicans (Jones and Russell, 1973; Russell and Jones, 1973). Furthermore, ultrastructural studies show that Candida albicans, an intracellular parasite of epithelial cells, causes changes in organelle structure which might affect the behaviour of epithelia (Montes and Wilborn, 1968; Cawson and Rajasingham, 1972).
Iron deficiency has also been shown to have profound effects on the oral mucosa, and to be associated with both oral and pharyngeal cancer and with chronic candidiasis (Higgs and Wells, 1972). An impairment of cell-mediated immunity in iron-deficient patients has also been shown (Joynson et al., 1972). In fact oral candidiasis is a common feature of acquired immune deficiency syndrome: Candida tropicalis accounts for up to 40 per cent of candidal infections (Greenspan et al., 1984). An unusual hyperkeratosis of the dorsum linguae is present (called "hairy leukoplakia" on account of its long hair-like processes). It is suggested that this type of leukoplakia may presage acquired immune deficiency syndrome, as well as, being associated with both papillomavirus and herpesvirus. It may also offer clues as to the pathogenesis of other forms of oral epithelial hyperplasia and dysplasia.

Oral mucosa has been shown to have permeability barriers which are at the level of the stratum granulosum in keratinized mucosa and at the surface of non-keratinized mucosa, corresponding to the levels at which membrane-coating granules are found ultrastructurally. Ethanol facilitates permeation, most probably by simple diffusion, and Candida hyphae penetrate only to the permeability barrier level. These facts may have importance in the alcohol drinking, tobacco smoking and chewing individual (Reade and MacKenzie, 1984).

4.7 HERPESVIRUS HOMINIS

The possibility that Herpesvirus hominis type 1 might be associated with oral squamous cell carcinoma was given impetus following gynaecological studies implicating Herpesvirus hominis type 2 with cervical squamous cell carcinoma. The similarities between carcinomata of the cervix uteri and oral mucosa extend to the fact that both may be preceded by premalignant lesions, both are usually squamous cell carcinomata and, in both sites, herpes simplex is common. Although Herpesvirus hominis type 2 is strongly associated with genital herpes simplex and type 1 virus with oral herpetic infection, either virus type can and does infect either site, with an increasing proportion of oral lesions being caused by Herpesvirus hominis type 2 (Scully and Ward-Booth, 1984).
The evidence for viral oncogenesis in oral carcinoma has been recently reviewed by Scully and Ward-Booth (1984) and that of *Herpesvirus hominis* specifically by Shillitoe and Silverman (1979). Herpesviruses are enveloped DNA viruses enclosed within an icosahedral capsid of about 100 to 150 nm in diameter. Of the four herpesviruses known to infect man, there is evidence implicating three in the development of some neoplasms, *viz.*: Epstein-Barr virus (EBV); Cytomegalovirus (CMV) and *Herpesvirus hominis* (HVH; known erroneously as herpes simplex virus: herpes simplex is the disease caused by HVH). Little evidence for the oncogenic effect of Varicella-zoster virus, the fourth type of herpesvirus, exists (Scully, 1982).

The pathogenesis of herpetic infections has been elucidated (Shillitoe and Silvermann, 1979). During a primary infection with *Herpesvirus hominis* type 1 or 2 in mature persons, the lesions are localized to the mucous membranes, but the virus travels centripetally along the sensory nerves towards the regional sensory ganglion. In a very small proportion of cases, the virus passes into the central nervous system causing encephalitis, but usually it remains latent in the ganglion for the lifetime of the individual. Recurring herpetic infections, *e.g.* herpes labialis, are due to the release of virus from latency followed by centrifugal passage along the sensory nerve to the epithelium. This reactivation may lead to recurrent herpetic lesions, asymptomatic shedding of virus or possibly malignant transformation.

A few anecdotal reports have suggested that *Herpesvirus hominis* might be implicated in oral carcinomata, particularly of the lip. However, there is little, if any, epidemiological support for such an association between herpes labialis and oral carcinoma (Shillitoe and Silvermann, 1979; Scully and Ward-Smooth, 1984).

Lehner *et al.* (1973a, b) reported that cell-mediated immune responses to *Herpesvirus hominis* type 1 are specifically elevated in patients with dysplastic leukoplakia, reaching levels seen otherwise only in patients with active primary or recurrent herpetic infection. The macrophage migration inhibition response to *Herpesvirus hominis* type 1 is also elevated in patients with dysplastic leukoplakia, as occurs during the convalescent phase of recurrent herpes infection.

Serological studies have revealed higher titres of serum immunoglobulins to *Herpesvirus hominis* non-virion antigens (*i.e.* not a structural component of the virus) in patients with oral carcinoma than in
controls (Hollinshead and Tarro, 1973; Tarro and Sabin, 1973). Silverman et al. (1976) correlated "tumour burden" with in vitro lymphocyte reactivity and immunoglobulins to herpesvirus "tumour-associated" antigens (HVH-TAA), in head and neck cancer patients. They found that clinical "tumour burden" impairs lymphocyte reactivity to phytohaemagglutinin and is associated with a high incidence of immunoglobulins to HVH-TAA in head and neck squamous cell carcinoma. Also found was a correlation between the immune defects in clinically cured patients and neoplasm extent prior to treatment. A study providing new evidence for an association between Herpesvirus hominis, heavy cigarette smoking and head and neck squamous cell carcinoma, and also arguing strongly for the presence of herpesvirus-induced antigens in mucosal surfaces, was reported by Smith et al. (1976). They showed that serum immunoglobulins, particularly IgA, to Herpesvirus hominis-induced antigens occur in a greater percentage of sera of patients with squamous cell carcinoma (61 per cent), patients previously treated for squamous cell carcinoma (56 per cent) and heavy cigarette smokers (57 per cent). Shillitoe et al. (1981) studied the relationship between neutralizing antibody to Herpesvirus hominis type 1 in the sera of patients with oral squamous cell carcinoma, leukoplakia oris and in smoking and non-smoking controls. Patients with untreated oral carcinoma have virus neutralizing titres similar to those of smoking controls, but those with later stage neoplasm have higher titres than those with earlier stage lesions. Longer survival times are associated with higher antibody titres to Herpesvirus hominis type 1 in patients who were "tumour-free" after treatment. The data are consistent with a role for Herpesvirus hominis type 1 in the pathogenesis of oral squamous cell carcinoma, suggesting that the neoplasm results from the interaction between the virus and tobacco smoke.

More recently, an enzyme-linked immunosorbent assay (ELISA) was used by Shillitoe et al. (1983) to measure the immunoglobulin class of antibody against Herpesvirus hominis type 1 in patients with oral squamous cell carcinoma. Patients with untreated oral squamous carcinoma show higher titres of serum IgM immunoglobulin to Herpesvirus hominis type 1 than patients with either acute or recurrent herpetic infections or age-matched control subjects. Patients who had been treated successfully for oral squamous carcinoma more than one year earlier did not have higher levels of IgM antibody to Herpesvirus hominis.
type 1. It was concluded that the results were consistent with the hypothesis that oral squamous cell carcinoma is associated with the expression of *Herpesvirus hominis* type 1 thymus-independent antigens that stimulate an IgM rather than an IgG immunoglobulin response.

More evidence for an association between *Herpesvirus hominis* and oral carcinoma has been provided by the demonstration in oral squamous cell carcinoma of possible *Herpesvirus hominis* nucleic acid (Eglin et al., 1983; Scully and Ward-Booth, 1984). Biopsy specimens from patients with oral squamous cell carcinoma were examined by *in situ* hybridization for evidence of RNA complementary to *Herpesvirus hominis* type 1 and type 2, and *Adenovirus* type 2. The principle of hybridization is based on the fact that *Herpesvirus hominis* RNA is synthesized in an infected cell on a template of viral DNA. Multiple copies of RNA can be produced from a single viral DNA strand facilitating the detection of HVH-RNA. It is possible to detect HVH-RNA by incubating the tissue with an exogenous radio-labelled HVH-DNA probe which, if it binds, is said to have "hybridized" with the HVH-RNA. Using this method it was found that RNA complementary to *Herpesvirus hominis* type 1 and 2 occurs in 66 per cent of squamous carcinomata and in 33 per cent of non-malignant lesions from other patients (Eglin et al., 1983). In a subsequent study with internally paired controls, RNA complementary to *Herpesvirus hominis* was found in 53 per cent of carcinoma biopsy specimens but in none of the normal mucosa from the same patients. Although these results do not specifically implicate *Herpesvirus hominis* type 1 rather than type 2, they provide "... the strongest evidence yet available ..." for an association between *Herpesvirus hominis* and oral carcinoma, but they do not constitute unequivocal evidence for viral oncogenesis since the technique may detect a closely related gene other than the *Herpesvirus hominis* (Scully and Ward-Booth, 1984). Intact virion has not been detected in neoplastic tissue or cultured from oral carcinoma (Shillitoe and Silverman, 1979; Eglin et al., 1983). However, this does not negate a viral aetiology since herpesvirus might still be incorporated in the host genome.

Animal experimentation studies have not as yet demonstrated a direct carcinogenic effect of *Herpesvirus hominis in vivo*, but a co-carcinogenic effect has been shown by Hirsch et al. (1983), who found that rats exposed to oral snuff alone or in combinations with herpes simplex infection have a higher incidence of neoplasia and "tumour-like"
conditions than control rats exposed only to *Herpesvirus hominis* type 1. The incidence of squamous cell carcinoma is significantly (p < 0.05) higher in rats exposed to snuff or *Herpesvirus hominis* type 1 and snuff in combinations than in control animals. Any ultimate hypothesis concerning the aetiology of oral squamous cell carcinoma must take into account the known relationship of the disease to alcohol and tobacco consumption and its possible interaction with viral and chemical agents which may potentiate the carcinogenic effects of each (Shillitoe and Silverman, 1979). Other viruses have been implicated as possible aetiological factors in oral carcinogenesis such as human papillomavirus (Syriänen et al., 1983; Löning et al., 1984). However, the evidence is as yet elementary and unconvincing.

4.8 ULTRAVIOLET LIGHT AND LABIAL CARCINOMA

Many risk factors have been associated with squamous cell carcinoma of the labial vermillion. These include: tobacco smoking (especially pipe smoking); actinic irradiation; rural residence (especially in low parallels of latitude); "out-door" occupations, including fishing; inflammatory processes; herpes simplex infection; thermal burns due to actinic irradiation or pipe stem; and endogenous factors: elderly male Caucasians with fair, ruddy complexions (Anderson, 1971; Loré et al., 1979; Lindquist, 1979; La Riviere and Pickett, 1979; Decker and Goldstein, 1982; Preston–Martin et al., 1982).

A large body of epidemiological evidence suggests an association between labial vermillion squamous cell carcinoma and prolonged, intense exposure to actinic irradiation. Outdoor occupations such as farming and rural residence, especially in low parallels of latitude, seem to support the hypothesis that ultraviolet light is an aetiological factor. Sixty to 80 per cent of labial squamous cell carcinoma occur in elderly, fair-skinned male Caucasians who work outdoors. Ninety-five per cent of lesions occur on the lower lip, often in conjunction with labial solar keratoses. Twenty-nine per cent of patients with multiple primary labial carcinomata have associated cutaneous carcinoma, known to be caused by actinic irradiation (Decker and Goldstein, 1982).

British and Scandinavian ancestry with fair, ruddy complexions and long lifetime exposure to the sun in excess of 50 000 hours with frequent, acute, painful sunburn have been shown to be positively
correlated with higher risk of developing carcinoma of the skin and lower lip (La Riviere and Pickett, 1979). Negroes have a very low risk of developing labial squamous cell carcinoma because of the increased amount and more even distribution of melanin pigment (not number of melanocytes) in response to sunlight affords protection from actinic damage (Anderson, 1971; La Riviere and Pickett, 1979).

In hereditary diseases such as albinism and xeroderma pigmentosum, the skin and labial vermilion, lacking melanin, are extremely sensitive to sunburn and susceptible to actinic carcinogenesis on minimal exposure to sunlight (Anderson, 1971; Kraemer et al., 1982; Yagi and Prabhu, 1983).

Actinic radiation (wavelength less than 320 nm) has insufficient energy to produce ionization in tissues. Its absorption causes activation of molecules and initiation of photochemical reactions in nucleic acids and proteins responsible for cell injury and death in the stratum germinativum epidermidis. The long-term effect of actinic radiation on skin is to accelerate senile degeneration of the lamina propria becoming acellular and amorphous with the concomitant loss of elastic fibres and vascular components (La Riviere and Pickett, 1979).

The mechanism of actinic radiation carcinogenesis is best elucidated by studying hereditary disorders, such as xeroderma pigmentosum, which exhibit clinically ultraviolet light hypersensitivity, cellular hyper-sensitivity to ultraviolet radiation and to some chemical carcinogens, and defective DNA repair (Kraemer et al., 1982). Cells cultured from xeroderma pigmentosum (XP) patients are hypersensitive to the lethal effects of ultraviolet light. Most XP cells are defective in an early stage of DNA repair of ultraviolet light-induced damage (Royer-Pokora and Haseltine, 1984). The rate of removal, i.e. "excision" of pyrimidine dimers in non-dividing cultures of normal and XP cells was studied by Kantor and Hull (1984). In general, an abnormal sensitivity of non-dividing cells to ultraviolet light is associated with a reduced dimer-excision rate. The regulation of DNA repair in serum-stimulated xeroderma pigmentosum cells reveals that each of the three XP complementation groups examined fail to increase their capacity for nucleotide excision repair above the basal levels. These results suggest that there may be a relationship between the sensitivity of XP cells from each complementation group to specific DNA damaging agents and their inability to regulate nucleotide excision repair during cell stimulation (Gupta and Sirover, 1984). A genetic model for some cases of excision-deficient xeroderma pigmentosum
was recently proposed by Lambert and Lambert (1985). The trait, i.e. XP, is expressed if and only if the individual is homozygous or hemizygous for defective alleles at more than one of a specific set of loci.

It has been suggested that the relative immunity of women to the development of labial squamous cell is attributed to the use of lipstick, which serves as a sun-screen, and less outdoor exposure reducing the cumulative effects of actinic damage (Wurman et al., 1975; Preston-Martin et al., 1982). Lipstick and pigmented labial coverings act as a sun-screen in addition to maintaining a pliant, non-keratotic condition of the lips.

Evidence which fails to indicate an expected correlation between labial and skin squamous cell carcinoma, arguing against a primary association with actinic radiation exposure, has been provided by Szpak et al. (1977) and Lindqvist (1979). The incidence of cutaneous, but not labial, carcinoma increases linearly with decreasing latitude. The highest incidence of labial squamous cell carcinoma among countries with accurate cancer registration occurs in Canada, but this high value is related to the unexplained prevalence of the disease among Newfoundland fishermen with an incidence of 106.8 per 100 000 population per year (Spitzer et al., 1975). Lindqvist (1979) found substantial differences with respect to various epidemiological parameters between labial and cutaneous carcinomata. The age-incidence pattern of labial carcinoma is closer to the pattern of oral carcinoma than to that of cutaneous carcinoma. There is a distinct decrease with age in the male-female ratio suggesting an occupationally-bound extra risk factor for working-age males. A negative correlation between the amount of total solar radiation energy and the risk of labial carcinoma in Finland, together with the differences in the geographic distribution of labial and epidermal carcinomata, make it improbable that solar radiation is a major risk factor for labial squamous cell carcinoma. A negative geographic correlation exists between the age-adjusted incidence rate of labial carcinoma and that of carcinomata of the colon and prostate gland. In addition, a highly negative correlation exists between incidence rate and the median income per caput (p < 0.001) indicating that low socio-economic status is associated with a higher risk of labial squamous carcinoma (Lindqvist and Teppo, 1978).

It is now recognized that all forms of tobacco use, not just the chronic chemical and thermo-mechanical irritation of pipe smoking,
increase the risk of labial vermilion squamous cell carcinoma (Loré et al., 1979; Decker and Goldstein, 1982). In a descriptive epidemiological study in Los Angeles Preston-Martin et al. (1982) found labial squamous cell carcinoma to be a disease predominantly of Caucasian males with a male-female ratio of 9.8 for the lower lip and 1.0 for the upper lip. Tobacco usage is responsible for 60 per cent of male labial carcinomata, and the incidence rates after substraction of the proportion of the incidence due to tobacco use are more than three times greater in males than in females. To account for this distribution it was hypothesized that exposure to ultraviolet irradiation, wind and cold resulting in chapping are important aetiological factors, and that the use of labial coverings by females serve as protection against the cumulative effects of climate.

Carcinoma of the labial vermilion is much more common in Irish peasant women and Negresses, who smoke pipes, than in non-pipe smoking women. Likewise, 50 per cent of Swedish women with labial squamous cell carcinoma are pipe smokers (Molnár et al., 1974). However, the association of pipe smoking and labial carcinomata may be the result of case-control disparities in age, residence, occupation, or combinations of these and related factors (Keller, 1970). Furthermore, the statistical correlation of residence, nativity, outdoor occupations and age are stronger overall than that for tobacco, smoked or unsmoked, and may be more decisive than the use of tobacco in the occurrence of labial carcinomata.

Poor oral hygiene, alcohol, hepatic cirrhosis and syphilis do not appear to be as important risk factors for labial squamous cell carcinoma as for other intra-oral carcinomata, although it is recognized that poorly fitting dental prostheses, sharp and jagged teeth and chronic periodontitis may cause persistent irritation on the lip (Decker and Goldstein, 1982). Caron (1980) reported an association between herpes labialis and labial squamous cell carcinoma suggesting a possible aetiological role for Herpesvirus hominis. As yet there is little hard evidence to support such a contention.

4.9 MISCELLANEOUS RISK FACTORS

Miscellaneous risk factors of oral carcinogenesis include socio-economic factors, including specific occupational hazards; diet; and nutrition (Smith, 1977; Wynder and Stellman, 1977).
4.9.1 Socio-Economic Factors

Socio-economic status: a measure of income, education and occupation may produce reliable measures of knowledge about health care reflecting either recognition of signs of disease or stage of disease at diagnosis. Socio-economic status may reflect exposure to carcinogens due to occupation or "life-style". The suspected role of social stress in the aetiology of physiopathological conditions such as coronary heart disease, peptic ulcers and colitis, is well documented. "Life-style" and social class have been implicated in studies of breast and cervical cancer as sources of increased risk. With neoplasia the elucidation of the social environment of high-risk groups may help in the identification of carcinogens that are part of the prevailing social norms and therefore are difficult to eliminate, such as the use of alcohol and tobacco.

A statistically significant inverse relationship between mortality from oral cancer and socio-economic status exists (Blot and Fraumeni, 1977). A strong relationship between socio-economic status and tobacco-related cancer is reflected by the smoking habits of different groups (Wynder and Stellman, 1977). Tobacco-related cancer of the oral cavity, larynx, and oesophagus, afflicts lower socio-economic groups of males because of their lower cessation rate and lesser preference for low-tar cigarettes and greater consumption of alcohol with its associated nutritional deficiencies.

4.9.2 Occupation

Few studies have been made of the occupational components of oral carcinogenesis. A survey of the geographical variations by county in the United States of America (Fraumeni, 1977; Blot and Fraumeni, 1977) revealed that mortality is elevated in counties with leather, paper and chemical industries for males and for females in counties with apparel and textile manufacturing. Excess mortality remains even when demographic factors and alcohol and tobacco consumption are accounted for. Urban-rural factors indicate sharply elevated mortality among urban males, regardless of race, and among Caucasian rural females in the southeastern United States of America. Wahl (1976) found in India that an increased risk of oral carcinoma occurs in unskilled workers, agricultural rent receivers and money lenders, and businessmen. Wynder and Stellman
(1977) found that skilled or clerical, semi-skilled and unskilled male workers accounted for 90 per cent of oral carcinoma patients.

Industries implicated as associated with increased risk of oral carcinoma include: leather and leather products industry, paper, chemical, textiles, farming, fishing and electronics industries. Commercial fishing as a risk factor in carcinoma of the lip in Newfoundland was shown by Spitzer et al. (1975). Compared to the rate of lip carcinoma of the male population of Newfoundland (27.0 per 100 000), the Newfoundland fishermen have an average annual incidence of 106.8 per 100 000. The age-adjusted relative risk for labial carcinoma is 1.65, i.e. fishermen have a 65 per cent higher probability of developing carcinoma than other males of comparable age. The age-standardized relative risk is 4.4 (p < 0.001) by cohort analysis. When assessed with adjustment simultaneously for age, "outdooriness" and pipe smoking, the risk factor for fishing is 1.5 (p < 0.05; Mantel-Haenszel chi-square = 3.989). Additional analyses did not show relations between labial carcinoma and the following factors: alcohol consumption, recurrent mouth lesions or herpes labialis; dental condition; common dietary patterns; or frequent ingestion of food at a very high temperature. The use of tobacco (considered together and including cigarettes, pipe and chewing tobacco) is not important as a risk factor; however, for pipe smoking alone the risk factor is 1.50 (p < 0.05). The use of the mouth as a third hand to handle tar-coated nets seems to protect fishermen, rendering them less than half as likely to acquire carcinoma of the lip than those engaged in the occupation who employ other techniques. No other work activities on work conditions of fishermen explored as possible risk factors gave any evidence of positive associations suggesting no independent contribution to the neoplasm. Neither was a specific carcinogen identified.

Cancer risks associated with employment in the leather and leather products industry at Buffalo, New York (Découfle, 1979), revealed significantly increased risks of urinary bladder carcinoma, lymphoma, oral, pharyngeal and laryngeal carcinomata in males with a history of employment in plants manufacturing shoes, gloves, belts, handbags and luggage, and shoemakers or shoe repairers which cannot be explained by differences in smoking habits. A review of the processes involved in leather manufacture reveals a large exposure to a number of potentially carcinogenic chemicals (Découfle, 1979). Exposure in the preliminary processes are mainly to sulphides, sulphates and aliphatic amines
(diethylamine). During the tanning processes exposure to sodium dichromate, chromic sulphate, tannic acid, tannin extracts, alum, methanal, zirconium and synthetic organic tannages (phenol aldehyde, melamine urea, styrene maleic anhydride). Exposure during the final finishing procedures include tar, coal dyes (aniline dyes including azo dyes), aromatic amines (2-naphthylamine), benzidine dyes, finishing pigments made from azo dyes [auramine, magenta (fuchsine)], benzene, nickle chloride and nickle sulphate. All these chemicals have been shown to be carcinogenic in laboratory animals.

A high incidence in both mortality and morbidity of oral and pharyngeal carcinoma among textile workers in England and Wales was noted by the Office of Population Censuses and Surveys (1972). Moss and Lee (1974) found a 77 per cent excess of deaths from oral and pharyngeal cancers in male textile workers, except for weavers and knitters, compared with the male population of England and Wales. The excess appeared greatest in wool fibre preparers. However, they advised caution about this conclusion which was based on a single set of numerators and compared with various denominators, adding that information on the mortality of female textile workers and on mortality in both sexes was incomplete. Moreover, occupations according to death certificates are not always the best indicators of occupational history throughout working life. A morbidity survey of oral and pharyngeal cancer in the two main textile regions on cotton and wool respectively (Whitaker et al., 1979) repudiate the findings of Moss and Lee (1974). The association between oral cancer and the type of textile work occurs. The only significant excess of textile workers with oral and pharyngeal cancer found was for females in the Northwest, a cotton area, possibly indicating a chance finding. A review of the occupational histories of women in the rural southeastern United States of America by Winn et al. (1981) showed no increased risk of oral and pharyngeal cancer among textile workers, after controlling for oral snuff use which is an endemic habit in the industry.

An increase in overall cancer mortality for electrical and electronic workers was found in the latest decennial supplement to the mortality statistics of England and Wales (Office of Population Censuses and Surveys, 1978). A higher than expected morbidity for oral cancer was also found. A cohort study in Sweden (Vågerö and Olin, 1983) assessing the relative risks of cancer in persons employed in the electronics or electrical manufacturing industry, revealed a slightly higher total
incidence of cancer (at all sites) in this industry than in the general working population, for males as well as females, e.g. 1.15 for males, 1.08 for females. The age-adjusted relative risks for the oral cavity are 1.28 for males and 1.20 for females; for the pharynx 2.00, and for the respiratory tract including the lungs 1.52. When adjusted for age, region (i.e. county of residence) and social class, blue collar workers in the electronics industry have twice the risk of getting pharyngeal cancer compared with the working population in general. No such excess risk is associated with oral cancer. Risk estimates for workers more unqualified and machine-bound preoccupied with assembly-type jobs, however, reveal a 2.98 relative risk for oral cancer when age and regionally adjusted.

In considering the interpretation of these results three methodological problems should be noted. Firstly, studying the electronics industry as a whole presents various work environments and potential work hazards which may be obscured in the data: an estimated slight excess risk, referring to the industry as a whole, could reflect some hazardous practice of a more severe type in some part of the industry. Secondly, categories in the study were formed on the basis of where people worked at the time of the 1960 census, rather than on the basis of their entire work history: this would tend to dilute a causal relationship and underestimate relative risk. Thirdly, mis-classifications of the branch of industry or occupation could occur, tending to underestimate the relative risk.

4.9.3 Diet and Nutrition

Much attention has been given by researchers to the association between diet and nutrition and neoplasia, deduced from epidemiological and limited experimental studies (Kissane, 1986a). In an analysis of any association consideration must be given to the following: (1) the possibility that food additives, as well as nutrients, may act as carcinogens, co-carcinogens, promoters or a combination of these; and (2) nutritional imbalances or deficiencies resulting in biochemical abnormalities, which in turn promote neoplasia. Food contaminants implicated in neoplasia include mycotoxins (e.g. aflatoxin) and nitrosamines in charred parts of broiled meat and fish. Deficiencies in iron, iodine, riboflavin, vitamin A, pyridoxine and choline have also been implicated in various types of neoplasia. High dietary fat intake has been
implicated in breast cancer. Several inhibitory dietary components have also been described, *viz.* anti-oxidant food additives, vitamin A and selenium.

The importance of dietary and nutritional factors in the aetiology of oral squamous cell carcinoma is difficult to determine. No significant data have been presented to support the speculation that nutritional conditions, including deficiencies, predispose the individual to oral squamous cell carcinoma (Smith, 1979). No relationship between oral squamous cell carcinoma and the temperature of coffee, tea or other foods exists (Graham *et al.*, 1977). Neither is there any observed relationship between any particular food group including meats, fats and those containing vitamins A and C, method of food preparation or utilization of fats. No differences occur between cases and controls in the extent to which they eat irregularly, use purgatives, or ingest spices and condiments.

However, Graham (1980), in a review of dietary factors in the aetiology of oral cancer concluded that some epidemiological and experimental animal studies provide evidence for a reduction in the risk of several gastro-intestinal neoplasms associated with green and yellow vegetable intake. The risk reduction of colonic cancer, especially, may be related to one or more properties of these vegetables, *viz.* dietary fibre, vitamins A, C or E, or their arylhydrocarbon hydroxylase (AHH) activity induced by indoles in these products which may detoxify potential carcinogens. Preston-Martin *et al.* (1982), suggest that factors associated with low socio-economic status, such as poor dentition and nutritional deficiencies may help explain the higher rates of oral and pharyngeal squamous cell carcinoma observed in lower socio-economic groups and the decline in incidence rates among bourgeois Caucasians with a high utilization of dental health care. In a case-control interview study of women in North Carolina, Winn *et al.* (1984) studied the role of diet in the aetiology of oral and pharyngeal carcinoma. An inverse association between oral and pharyngeal cancer and the intake of fruits and vegetables and breads and cereals occurs which cannot be attributed to an association with general nutritional status. After controlling for demographic characteristics, tobacco and alcohol use, relative weight and intake for other food groups, the statistically significant relative risks of 0.65 for moderate and 0.52 for high consumption of fruits and vegetables were found.
The apparent protective effect noted for high consumption of total fruits and vegetables is consistent with several biological mechanisms. Fruit and vegetables are the primary source of beta-carotene, which is metabolized to retinol (vitamin A) in humans. Evidence exists suggesting that retinoids and vitamin A-containing foods, as well as beta-carotene itself, reduce the risk of oral and pharyngeal cancer. However, Winn et al. (1984) found that retinol, which is present in substantial amounts in milk, cheese and eggs, is only very weakly and inconsistently associated with a reduced risk of cancer. Vitamin C (ascorbate), also found almost exclusively in fruits and vegetables, has been proposed as an anti-neoplastic agent because it inhibits the formation of nitrosamines from amines or amides and nitrite.

The inverse association between the intake of breads and cereals and cancer risk is close in magnitude to that for fruits and vegetables, and appears independent of the effect of other food groups. The effect is limited to whole-grain breads and cereals. One hypothesis that may account for the apparent protective effect of both fruits and vegetables, and breads and cereals is that the bulk and fibre of these foods may cleanse the mouth and pharynx of ingested carcinogens (Winn et al., 1984).

Nutritional deficiencies are commonly associated with alcoholism (McCoy et al., 1980). Carcinomata of the head and neck also seem to occur most commonly in persons who do not eat nutritionally balanced diets. Nutritional deficiencies arising from either under-nutrition and/or as a direct consequence of alcohol intake (impaired absorption or enhanced elimination) may play a role in head and neck carcinogenesis.

Several problems need to be considered in the interpretation of case-control investigations of nutrition and neoplasia (Winn et al., 1984). Firstly, the differences between cases and controls may be a consequence of the disease process in cases, e.g. malignant neoplasia in general and oral and pharyngeal carcinoma in particular, may interfere with the patient's ability to eat, leading to dietary disturbances. Secondly, when a hospital control group is used, there is a possibility that the patients' conditions in the control group may have influenced their diet, or conversely dietary factors may have contributed to the disease occurrence in some members. Thirdly, the validity of the diet history method of data collection. Fourthly, the value of interviews conducted with the next of kin rather than with the subject themselves. Finally,
FIGURE 4. Diagram showing the inter-relationships of factors associated with oral squamous cell carcinoma (Modified from Binnie et al., 1983).
misclassification of dietary habits due to deficiencies in the method or recall of the subjects is likely to be random, i.e. similar for cases and controls, if the study population was unaware of the specific suspicions about links between diet and oral cancer.

4.10 SUMMARY

The most commonly implicated risk factors in oral carcinogenesis are tobacco usage, excessive alcohol consumption, sideropenic dysphagia, chronic hyperplastic candidiasis and Herpesvirus hominis. All these factors exhibit an interrelationship with at least one other factor (Binnie et al., 1983). Despite the importance of determining the influence of individual factors as carcinogens, promoters, or initiators, the combination and interrelationship of these factors may prove to be more informative about populations at risk (see Figure 4).

Many carcinogens and mutagens are inactive until metabolized. This activation can be accomplished by certain tissue enzymes or by bacteria. Tannenbaum et al. (1978) have shown that oral bacteria can convert amines to carcinogenic nitrosamines. Oral bacteria and fungi, or salivary enzymes of oral cancer patients might be particularly effective in activating carcinogens.

Outlines for future research in the study of oral carcinogenesis should include, according to Binnie et al. (1983), the following research objectives:

(1) to discover if patients with oral cancer have greater intrinsic ability to activate pre-carcinogens into carcinogens than do normal individuals;

(2) to identify accurately the carcinogenic substances and combination of substances which may be responsible for oral cancer;

(3) to determine the exact role of the two micro-organisms which may be associated with the aetiology of oral cancer, i.e. Herpesvirus hominis and Candida albicans; and

(4) to develop valid animal models of human oral squamous cell carcinoma.