CHAPTER 5

CLINICAL FEATURES OF ORAL SQUAMOUS CELL CARCINOMA

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CHAPTER 5
CLINICAL FEATURES OF ORAL SQUAMOUS CELL CARCINOMA

The clinical features of oral squamous cell carcinoma and its histological variants will be discussed. Aspects of early asymptomatic lesions, presenting symptoms of late oral lesions, multiple primary carcinoma and intra-osseous carcinoma will be presented.

5.1 EARLY ASYMPTOMATIC LESIONS

The detection of early asymptomatic lesions of oral squamous carcinoma is of paramount importance in the treatment and prognosis of malignant oral neoplasia.

Most early oral squamous cell carcinoma is manifested as an asymptomatic, small (less than two centimetres in diameter) lesion with or without keratotic components (speckled erythroplakia) in the floor of the mouth, soft palate complex which includes the soft palate and oropharynx, and the ventrolateral tongue (Mashberg et al., 1973; Mashberg and Meyers, 1976; Mashberg, 1977; Mashberg and Garfinkel, 1978). If labial lesions are excluded the former three sites account for 97 per cent of all early erythroplakic lesions.

Early squamous cell carcinoma of the lower labial vermillion is characterized by a generalized variegated, erythroplakic and leukoplastic, "blotchy" appearance of the labial vermillion; generalized desiccated, atrophic appearance with focal areas of leukoplakia, persistent chapping with localized flaking and crusting; and indistinct "wandering" vermillion border (La Riviere and Pickett, 1979).

5.2 PRESENTING SYMPTOMS OF ORAL SQUAMOUS CARCINOMA

During the early stages, many oral squamous cell carcinomata are asymptomatic. However, advance disease may be present even when the history of symptoms is only recent, of a few weeks duration. Likewise, it is also apparent that many neoplasms present with symptoms sometimes years before the neoplasm becomes visible. The symptoms generally complained of are in the majority of cases minor in nature, e.g. ulceration, soreness, tumour (i.e., swelling or lump) and pain. In
approximately 7 per cent of cases, urgent symptoms such as dysphagia, difficulty in phonation and difficulty in opening the mouth may be present (Cooke and Tapper-Jones, 1977; Williams, 1981).

The nature of the symptoms varies according to the anatomical location of the neoplasm (Robertson and Hornibrook, 1982). For squamous carcinoma of the lips (labial vermillion and mucosa) the most common presenting symptoms are a non-healing, visible lesion (62 per cent of cases) followed by an initially painless lump or swelling (38 per cent of cases). For the buccal mucosa the presenting symptoms are an initially painless lump or swelling (60 per cent) and local pain, followed by a non-healing, visible lesion. For the alveolar process and hard palate the commonest presenting symptom is a non-healing, visible lesion (36 per cent). For the floor of the mouth the commonest presenting symptom is a non-healing, visible lesion followed by an initially painless lump or swelling. For the anterior two-thirds of the tongue the commonest presenting symptom is pain (40 per cent), then a non-healing visible lesion. In contrast to carcinoma of the anterior two-thirds of the tongue, local pain is a later feature of lesions of the floor of the mouth and alveolar processes. Also, nearly a quarter of the patients with carcinoma of the alveolar processes and hard palate will experience difficulty with the fitting of their dental prostheses.

The oropharynx, consisting of the posterior-third or post-sulcal tongue, vallecula, palatine tonsils, palatoglossal and palatopharyngeal arches, posterior oropharyngeal wall, and soft palate, is relatively inaccessible to inspection and palpation. Consequently, squamous carcinoma of the oropharynx presents late with local pain, often identified by the patient as a persistent "sore throat" in 35–39 per cent of cases (which is referred to the pinna in 24–35 per cent of cases as otalgia). Thirty-five per cent of patients with squamous cell carcinoma of the post-sulcal tongue and 45 per cent with carcinoma of the palatine tonsil present with cervical lymph node metastases.

5.3 SQUAMOUS CARCINOMA AT VARIOUS ORAL ANATOMICAL SITES

Squamous cell carcinoma at various oral anatomical sites exhibits different clinical features (Crawford, 1979; Shafer et al., 1974; Pindborg, 1980; Lucas, 1984).
5.3.1 Labial vermilion and labial mucosa

Squamous cell carcinoma of the labial vermilion and labial mucosa commonly affects the lower lip in 95 per cent or more of cases. It shows variation in clinical appearance depending on the duration of the lesion and the nature of the neoplasm. The lesion commences on the vermilion border, usually to one side of the midline, as a small area of hyperkeratosis, induration, ulceration or surface irregularity. It enlarges to create a small crateriform defect or ulcer with a rolled, everted margin, or produces an exophytic, proliferative papillary neoplasm. The papillary, exophytic lesion grows slowly and infiltrates deep tissues late, whereas the more common ulcerative lesion invades early. Local destruction of the entire lip, invasion of gingivae, cheeks and mandible may occur in untreated advanced cases (see Plate 6).

Squamous cell carcinoma of the lip is slow to metastasize; however, anaplastic lesions may metastasize early to ipsilateral submental or submandibular lymph nodes and then to the superior deep cervical lymph nodes. Contralateral metastases may occur if the lesion is in the midline. The frequency of primary lymph node metastases at final presentation varies from 2–10 per cent (Boddie et al., 1977). Neoplasms of the upper lip grow more quickly and metastasize earlier because of a better lymphatic drainage. Carcinomata of clinical size T1 (less than 2 cm) rarely metastasize whereas T2 (2 to 4 cm) and T3 (greater than 4 cm) metastasize just as frequently as squamous cell carcinoma of the floor of the mouth and tongue (Wurman et al., 1975).¹

Primary multiple carcinomata have been reported involving the labial vermilion and labial mucosa (Newell et al., 1974). Kerato-acanthoma needs to be considered in the differential diagnosis (Azaz and Lustman, 1974).

5.3.2 Buccal mucosa

In squamous cell carcinoma of the buccal mucosa there is commonly variation in the clinical appearance. It is more frequently located along or inferior to the plane of occlusion, more commonly posteriorly near the lower third molar tooth, although it is sometimes located at the labial

¹. The TNM system is commonly used in the clinicopathological staging of oral cancer. See Appendix.
commissures. It presents as a small, ulcerated, indurated, usually initially painless neoplasm most often associated with leukoplakia or very rarely with erythroplakia, which enlarges becoming easily traumatized and secondarily infected producing oedema, pain and trismus. It may also present as a small nodule that enlarges to form a verrucous neoplasm which ultimately ulcerates. Extension may occur to the mandibular sulcus, less frequently into the maxillary buccal sulcus, skin, cervical musculature, bone and soft palate.

The majority of squamous cell carcinomata of the buccal mucosa (68 per cent) are T2 or T3 lesions at presentation with 48 per cent showing metastatic regional lymphadenopathy and 19 per cent having disseminated neoplastic disease (Conley and Sadayama, 1973). Vegers et al. (1979) found 61 per cent of lesions to be T3 and 59 per cent exhibiting regional metastases. Most metastases are found localized in the submandibular and superior deep cervical lymph nodes.

The labial commissure, an arbitrary square area of side 1.5 centimetres, extending distally from the angulus oris, often shows a characteristic squamous carcinoma. This exophytic squamous cell carcinoma, which sometimes may be a verrucous carcinoma, is often preceded by a nodular (speckled) leukoplakia. The neoplasm spreads posteriorly to involve the remaining buccal mucosa, and anteriorly to involve the overlying epidermis. The presence of Candida hyphae is usually noted. Implicated in the aetiology of squamous cell carcinoma of the buccal mucosa are chronic hyperplastic candidiasis (candidal leukoplakia) (Cawson, 1969) and tobacco and betel nut chewing (Conley and Sadayama, 1973). These are particularly associated with exophytic, papillary squamous cell carcinoma and verrucous carcinoma of the buccal mucosa. The uses of Shamma² in Saudi Arabia is also implicated in the aetiology of squamous cell carcinoma of the buccal mucosa (Yousef and Hashash, 1983).

5.3.3 Floor of mouth

The floor of the mouth is the crescentic anatomical area limited by the mandibular alveolar process (gingiva and mucosa), the fraenum

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2. Shamma is a native form of oral snuff.
sublingualis posteriorly in the midline and the base of the palatoglossal arch posterolaterally.

Early lesions of squamous cell carcinoma in the floor of the mouth are innocuous—appearing erythroplakia and leukoplakia, especially sublingual keratosis (Mashberg and Garfinkel, 1978; Pogrel, 1979; Friedlander et al., 1982) (see Plate 7). The typical neoplastic lesion is an indurated ulcer of varying size mainly located anteriorly and to one side of the midline. The ulcer has a raised, indurated margin and a reddish-grey, indolent—appearing granular base which is ordinarily free of slough. Sometimes the ulcer is inconspicuous. Many lesions appear at presentation to be midline, either having originated there initially or spreading from either side of the midline. Most lesions present at advanced stages: T3 and T4 (70–78 per cent) (Kolson et al., 1971). The lesion may be verrucous or papillary, spreading superficially, but more commonly it is an ulcer or fissure deeply infiltrating into the submucosa (Sambrook, 1983). The initial symptoms of the advanced lesion are pain most commonly, followed by pain or swelling or both, painless leukoplakia or ulceration (Ballard et al., 1978).

Early extension into the lingual alveolar mucosa, mandible, tongue, cheek and epidermis can occur, as can extension to involve the submandibular and sublingual salivary glands (El-Domeri, 1979; Applebaum et al., 1980; Friedlander et al., 1982). With extension into the tongue some limitation of lingual motion and slurring of speech may also result.

Regional lymph node metastasis varies from 44–65 per cent of cases, involving the submandibular lymph nodes, often bilaterally, and the superior deep cervical lymph nodes (Nakissa et al., 1978; Ballard et al., 1978; Lindberg, 1972). Distant metastases are less frequent and occur late in the course of the disease. They involve the lungs, pleurae, liver, skeleton, spleen, kidneys, adrenal glands, diaphragm, periosteum and myocardium (Ballard et al., 1978; Merrick and Jensen, 1979).

Of all oral squamous cell carcinomata, the lesions involving the floor of the mouth are most frequently associated with multiple primary carcinomata, mainly other intra-oral, oropharyngeal, oesophageal and bronchial lesions (Ballard et al., 1978; Nakissa et al., 1978).
5.3.4 Tongue

Squamous cell carcinoma of the tongue is the most common intra-oral carcinoma comprising 30 per cent of cases. The anterior two-thirds comprise approximately 70 per cent of cases and the post-sulcal (base) tongue about 30 per cent of cases of lingual squamous carcinoma (Strong, 1979; Ildstad et al., 1983).

The sites of predilection of squamous cell carcinoma of the tongue are the lateral and ventral surfaces, most commonly the middle third. Very rarely lesions may be observed in the middle of the dorsum linguæ just anterior to the sulcus terminalis. Geographical differences in site of predilection have been noted especially in areas where the habit of reverse smoking, where there is a proportional increase in post-sulcal lingual lesions.

The vast majority of post-sulcal lingual squamous cell carcinoma (77 per cent) are advanced lesions: clinical stages III and IV, of which 47 per cent are stage IV. In contrast, pre-sulcal lingual lesions are early, clinical stage I and II. The difference is explained in part by anatomical factors including the pattern of innervation for nociception, lymphatic drainage and the relative inaccessibility of the base of the tongue (Ildstad et al., 1983; Strong, 1979).

Clinical manifestations are varied. They include: (1) exophytic, papillary lesion usually associated with ulceration; (2) deep ulceration or fissure with indurated margins infiltrating deeply into the underlying lingual submucosa and musculature; (3) leukoplakia; (4) asymptomatic erythroplakia or erythroleukoplakia (speckled erythroplakia). An incidence rate of 11 per cent for secondary primary carcinomata as compared with 22 per cent for floor of mouth lesions, suggesting mucosal "field cancerization" was noted by Ildstad et al. (1983) (see Plate 8).

Symptoms depend on the location of the carcinoma. In the anterior two-thirds of the tongue the chief complaint is a lump or mass, often painless, whereas in the post-sulcal lesions "sore throat" and dysphagia are usually the presenting symptoms. Regional lymphadenopathy may be the first sign of lingual squamous carcinoma.

Regional lymph node metastasis readily occurs to the superior deep cervical and submandibular lymph nodes, and less frequently to the submental, inferior deep cervical and posterior cervical lymph nodes.
There may be ipsilateral, bilateral or contralateral lymph node involvement.

In young patients, under forty years of age, there is a female preponderance with a female: male ratio of 3:1. The lingual squamous cell carcinomata are mainly T1 and T2 lesions at presentation. Metastases are less frequent (38 per cent) and the disease is less lethal than in older age groups (McGregor et al., 1983).

5.3.5 Palate

Squamous cell carcinoma of the soft and hard palate is an uncommon lesion comprising about 5 per cent of all oral squamous carcinomata, of which the soft palate is more commonly affected (4:1 ratio of soft palate to hard palate). Some geographical variation in site distribution is noted, especially in Sweden where the ratio of soft to hard palate squamous carcinoma is 5:4.

Palatal squamous cell carcinoma manifests as either a poorly defined, ulcerated, painful lesion on one side of the midline or as a papillary, exophytic neoplasm. It frequently crosses the midline and may extend laterally to include the lingual gingiva or posteriorly to involve the faucæ, rarely the uvula and nasopharynx. Squamous cell carcinoma of the hard palate may perforate the bone of the hard palate and extend into the nasal fossæ (see Plate 9).

The initial presenting symptoms are: palatal swelling, pain and ulceration. Anaplastic lesions are more commonly found with ulceration (Eneroth and Moberger, 1973).

Pathological changes are often diffused over the surface of the soft palate, varying from epithelial dysplasia, carcinoma in situ to superficial and deeply invasive squamous cell carcinoma. Second metachronous3 or synchronous primary carcinomata generally occur in 20–38 per cent of cases, although none were noted by Ildstad et al. (1984). The majority of squamous carcinomata of the hard palate present at early clinical stages (I and II), whereas for soft palatal lesions the majority at presentation are advanced lesions (clinical stages III and IV) with 36 per cent of cases being clinical stage IV (Ildstad et al., 1984).

3. Metachronous means composed of several parts formed or developed at various times.
Regional lymph node metastases occur in 20–45 per cent of cases, with bilateral involvement in 5–15 per cent of all cases (Jaques, 1979). Distant metastases develop in 9 per cent of soft palate and 14 per cent of hard palate squamous carcinomata during the course of the illness. The organs and tissues involved in order of frequency are: lung, right ventricle and bony skeleton (Ildstad et al., 1984).

In reverse smokers, squamous cell carcinoma of the hard palate usually develops as an ulcer lateral to the midline of the glandular zone (Ramulu and Reddy, 1972). In a sample of 79 reverse smokers from Colombia, 78 exhibited palatal changes including 16 squamous cell carcinoma, 4 of which were in the palate, the remainder at the base of the tongue, tonsillar fauces and adjacent pharyngeal mucosa (Morrow and Suarez, 1971).

Since squamous cell carcinoma accounts for only 56–83 per cent of palatal neoplasms, the others mainly minor salivary gland neoplasms, the latter must be considered in the differential diagnoses of palatal lesions (Eneroth et al., 1972).

5.3.6 Gingiva and alveolar ridge/process

Despite the different histological structure of the gingiva and the alveolar mucosa most investigators fail to make a distinction between squamous carcinoma at the two sites. Furthermore, obscure terms are also used such as "lower alveolus", "alveolar ridge" and "alveolar process". The alveolar ridge is the anatomical term denoting the edentulous alveolar process, whereas the alveolar process denotes the osseous process of either the maxillae or mandible.

Squamous cell carcinoma of the gingiva mainly involves the attached rather than free gingiva, especially of the mandible. The premolar and molar regions are more commonly affected (Wald and Calcaterra, 1983). Gingival lesions account for approximately 10 per cent of all intra-oral carcinomata. The diagnosis of gingival squamous cell carcinoma is unduly delayed because of its similarity to common benign inflammatory lesions of periodontal or endodontic origin (Torabinejad and Rick, 1980; Gallagher and Svirsky, 1984).

Clinically, gingival squamous cell carcinoma is manifested as erosion, ulceration or as an exophytic, granular, serpiginous verrucous tumour which may or may not be painful (see Plate 10). Wald and Calcaterra
(1983) found ulceration and pain in almost every case. Extension along the periodontal membrane with destruction of alveolar bone and loosening of teeth may also be present. There is also superficial pressure erosion of the alveolar process from an alveolar ridge lesion which proliferates as a flat, elongated ulceration that characteristically bleeds easily. The maxillary gingival lesions may invade the sinus maxillaris, palate or fauces. Extension into the floor of the mouth, cheek and mandible, which may later show pathological fracture, can also occur. The majority of squamous cell carcinomata of the alveolar process and gingiva present early as clinical stage I and II lesions. Second metachronous or synchronous primary carcinomata occur in 13 per cent of cases (Ildstad et al., 1984).

Regional lymph node metastasis is common, occurring in about 50 per cent of cases. Mandibular lesions metastasize to the submandibular and superior deep cervical lymph nodes. Maxillary lesions metastasize to the retropharyngeal and superior deep cervical lymph nodes. A higher incidence of metastasis (84 per cent) was found by Willén and Nathanson (1973). Distant metastases occur in 9 per cent of cases and involve the following tissues in order of frequency: lung, right ventricle, skeleton and liver (Ildstad et al., 1984).

5.3.7 Oropharynx

The oropharynx is the area including the mucosa of the soft palate, uvula, palatoglossal and palatopharyngeal arches, tonsillar fossa and palatine tonsil. A distinction needs to be made between "palatine arch" and "palatine tonsil" lesions because the biological behaviours are different. Carcinoma of the palatine tonsil, mainly squamous cell carcinoma, appears to be more closely related to carcinoma of the nasopharynx, having similar biological behaviour and response to treatment, e.g. being very sensitive to radiotherapy (Crawford et al., 1979; Healy et al., 1976).

Carcinoma of the palatine tonsil initially consists of a superficial granular ulcer on the tonsillar surface itself or in the groove between the tonsil and one of its faucial pillars. It may also arise in a tonsillar crypt remaining undetected and manifesting clinically as cystic, cervical lymph node metastases. As it enlarges, the lesion tends to fungate from, and erode, the surface of the palatine tonsil. Occasionally, it may, like a
tonsillar lymphoma, invade deeply and produce a bulky, submucosal mass with little or no surface ulceration. Subsequent invasion of contiguous structures occurs, usually sequentially, *viz*: palatoglossal and palatopharyngeal arches, alveolar processes and buccal mucosa. Only unilateral tonsillar squamous cell carcinoma has been observed (Crawford *et al.*, 1979), and multiple malignant primary lesions of distal body sites may occur in 16–22 per cent of cases. In comparison, non-Hodgkin's lymphoma occurs unilaterally in 85 per cent of cases and multiple primary malignant lesions occur in 7 per cent of cases.

Cervical lymph node metastasis in palatine tonsillar squamous carcinoma varies from 55 to 90 per cent, and may be bilateral in 15 per cent of cases. In non-Hodgkin's lymphoma, which comprises about 14 per cent of tonsillar neoplasms, the incidence of metastatic cervical lymphadenopathy varies from 65–100 per cent. Distant metastases occur usually late in tonsillar squamous carcinoma but early in non-Hodgkin's lymphoma (Crawford *et al.*, 1979).

Squamous cell carcinoma of the oropharynx, excluding the palatine tonsil, the retromolar trigone and the contiguous buccal mucosa collectively account for nearly 50 per cent of all oral and oropharyngeal carcinomata. The prognosis is poor with a 40–50 per cent mortality. Squamous carcinoma arising from the palatoglossal arch and retromolar region represent the posterior extension of the spectrum of carcinogenesis commonly seen in the oral cavity. Carcinomata of the palatopharyngeal arch and the palatine tonsil are less common, and widespread dysplastic changes of the oral cavity are not a prominent feature (Shumrick and Quenelle, 1979). Eighty-five to 98 per cent of cases are elderly males with a history of heavy consumption of tobacco and alcohol.

Clinically, early squamous cell carcinoma of the oropharynx appears as erythroplakia and is often misdiagnosed as an inflammatory lesion. Ill-defined neoplasm margins are common and a characteristic finding is the "multicentricity" of lesions with 17–35 per cent of cases having or developing another primary carcinoma during their life time. The most common late symptoms are "sore throat", "lump in throat", cervical "lump", dysphagia and otalgia, and local bleeding.

Metastasis to regional lymph nodes occur in approximately 60–70 per cent of cases. The superior deep cervical lymph nodes become uniformly involved. Anterior lesions secondarily involve the submandibular and middle deep cervical lymph nodes, with approximately 7 per cent
developing contralateral nodes. Posterior lesions secondarily involve the middle deep cervical more often than the submandibular nodes and are more likely to show widespread cervical lymph node involvement, i.e. posterior triangle nodes in 10 per cent of cases. Contralateral lymph node involvement occurs in 13–23 per cent of cases (Healy et al., 1976; Shumrick and Quenelle, 1979). Distant metastases are seen in about 7 per cent of anterior oropharyngeal lesions and rarely if effective local and regional control of the carcinoma has been achieved. Posterior lesions show a higher incidence of distant metastases irrespective of local and regional control. Distant metastases occur, in order of frequency, in the lungs, bone and liver (Shumrick and Quenelle, 1979).

5.4 VARIANTS OF SQUAMOUS CELL CARCINOMA

5.4.1 Verrucous carcinoma

Verrucous carcinoma accounts for about 5 per cent of oral squamous cell carcinoma. It is generally seen in elderly Caucasian males in the 60–70 years age group (Shafer et al., 1974a; Eversole and Papanicolaou, 1983; Slootweg and Müller, 1983; Lucas, 1984c), although McCoy and Waldron (1981) found a 60 per cent prevalence in females associated with the habit of "snuff dipping" and an equal sex distribution was observed in the Japanese by Mizuno et al. (1983).

The oral sites of predilection are: the buccal mucosa; alveolar mucosa or gingiva; and occasionally the palate and floor of mouth, and tongue (Shafer, et al., 1974; Eversole and Papanicolaou, 1983; Slootweg and Müller, 1983; Mizuno et al., 1983; Lucas, 1984a). Other sites commonly involved are: larynx; nasal mucosa; oesophagus; skin; glans penis; vagina; scrotum; and perineum (McCoy and Waldron, 1981; Lucas, 1984a). Verrucous carcinoma has also been reported arising in odontogenic cysts of the jaws (Enriquez et al., 1980). Of all possible sites the oral cavity is the most common site (Burns et al., 1976; Chenault, 1979).

Verrucous carcinoma characteristically manifests as a slow growing sore that becomes a small, verrucous mass which then rapidly enlarges and becomes an indolent, fungating tumour. This neoplasm is often associated with leukoplakia of the adjacent mucosa. Rugae-like folds with deep clefts between them are present. It is a sessile lesion (Shafer et al.,
1974; McCoy and Waldron, 1981; Chenault, 1979; Eversole and Papanicolaou, 1983; Shootweg and Müller, 1983). Buccal lesions become extensive in size before they invade the deep tissues. Mandibular alveolar mucosal or gingival lesions grow into the overlying soft tissue and rapidly become fixed to the periosteum, invading and destroying the mandible. Pain, difficulty in mastication and rarely bleeding are common symptoms. There is commonly a regional lymphadenitis secondary to an inflammatory response of the host to the neoplasm. In maxillary lesions, extensive involvement of the labial and buccal vestibuli, maxillary tuberosity, palate and sinus maxillaris may occur (Shafer et al., 1974; Bohmfalk and Zallen, 1982). Localized bone destruction may also be due to pressure resorption.

Multiple primary carcinomata may be present. Shootweg and Müller (1983) found 48 per cent of lesions associated with or co-existing with other mucosal lesions, viz: epithelial dysplasia (26 per cent); squamous cell carcinoma (37 per cent); and another verrucous carcinoma (22 per cent). The other lesions either formed part of the verrucous carcinoma or occurred separately. They suggested that the presence of these multiple primary lesions are to be interpreted as an expression of an ubiquitous premalignant and malignant change in the whole oral epithelium. The relative incidence of verrucous to squamous cell carcinoma was found to be 9 per cent, with a male predominance.

Regional lymph node metastases do not occur or are very rare. However, regional lymphadenitis secondary to inflammatory response can occur (McCoy and Waldron, 1981; Bohmfalk and Zallen, 1982).

Shear and Pindborg (1980) suggest that a separate clinical entity, called verrucous hyperplasia, which may be clinically indistinguishable from verrucous carcinoma and may occur concurrently, needs to be considered in the differential diagnosis. Verrucous hyperplasia is associated clinically with leukoplakia and histologically with keratosis and epithelial dysplasia. It may develop from leukoplakia and in turn may develop into a verrucous carcinoma and/or squamous cell carcinoma. A similar lesion called proliferative verrucous leukoplakia, and constituting a continuum of hyperkeratotic disease, ranging from simply hyperkeratosis to verrucous and squamous carcinoma, has been described by Hansen et al. (1985). The value of adopting such a new term in an already controversial field of study remains to be seen. Although verrucous hyperplasia is regarded as a precancerous lesion, Shootweg and Müller
(1983) concluded that verrucous hyperplasia probably represents a morphological variant of verrucous carcinoma and is not a distinct clinical entity.

5.4.2 Spindle cell carcinoma

Spindle cell carcinoma is an histological variant of squamous cell carcinoma, more often found in elderly Caucasian males. It is found in the mamma; skin; oral mucosa; parotid salivary glands; oesophagus; bronchus; and cervix uteri (Lucas, 1984c). In oral tissues the lower lip is the commonest site, followed by the tongue; gingiva or alveolar mucosa; and occasionally the floor of the mouth; retromolar region; buccal mucosa; upper lip; and hard palate.

The principal symptoms are swelling and pain. Other complaints include: a non-healing ulcer; dysphagia; haemorrhage; and loosening of teeth (Ellis and Corio, 1980).

Clinically, the lesion is characterized by a polypoid, exophytic neoplasm with a short, broad peduncle, although sessile, nodular or endophytic neoplasms do occur. The size of the lesion varies from 0.5 to 5.0 centimetres in greatest dimension, with a mean of 2.0 cm. Most of these neoplasms are ulcerated with a firm, rubbery consistency and without central necrosis (Someren et al., 1976; Martin and Kahn, 1977; Ellis and Corio, 1980). Extensive invasion of contiguous structures occurs, viz.: palate; sinus maxillaris; temporomandibular joint; submandibular salivary gland; oropharynx; tongue; facial skin; cranial base; middle cranial fossa and hypophyseal fossa (Takagi and Ishikawa, 1982).

Regional lymph node metastasis readily occurs (26.5 per cent of cases) and distant metastases occur in 14.9 per cent of cases (Ellis and Corio, 1980). Distant metastases have been found in the lung, hilar lymph nodes and liver (Takagi and Ishikawa, 1982).

5.4.3 Adenoid squamous cell carcinoma

Adenoid squamous cell carcinoma is commonly found in the skin of the head and neck and occasionally of the extremities; labial vermilion; tongue; gingiva; and floor of mouth.
Cutaneous lesions clinically manifest as elevated nodules showing crusting, scaling or ulceration, sometimes with an elevated or rolled border (Shafer et al., 1974d).

Labial vermilion lesions more commonly affect the lower lip of elderly (over 50 years) males. Clinically, labial vermilion lesions are associated with solar keratosis. The lesion is non-specific, hyperkeratotic, ulcerated, granular or nodular varying in size from 0.2 to 1.8 cm. Pain is not a common feature. It is a slow growing, locally invasive lesion that seldom metastasizes (Jacoway et al., 1971; Tomich and Hutton, 1972; Weitzner, 1974). However, universal agreement on this point is lacking. Takagi et al. (1977) found no difference in metastatic propensity between adenoid squamous cell carcinoma and squamous cell carcinoma.

McLatchie et al. (1984) describes an adenoid squamous cell carcinoma that initially resembled a kerato-acanthoma of the skin of the cheek, both clinically and histopathologically. There was gross local destruction of the facial bones and calvarium, but no evidence of lymphatic spread. Local recurrence occurred, becoming locally aggressively invasive. Perineural lymphatic spread was also noted.

5.5 MULTIPLE PRIMARY CARCINOMA

Multiple primary malignant neoplasms of all kinds have been well documented in the literature with over 30 000 (sic) reported cases. The historical perspectives of such neoplasms have been reviewed by Moertel (1977). The criteria used to define multiple primary malignant neoplasms were enunciated originally by Warren and Gates (1932) as: (1) each of the "tumours" must present a definite picture of malignancy; (2) each must be distinct; and (3) the probability that one was a metastatic lesion from the other must be excluded.

Moertel (1977) has classified multiple primary neoplasms into three categories, viz.:

(1) neoplasms of multicentric origin involving (a) the same tissue and organ; (b) a common, contiguous tissue shared by different organs; and (c) the same tissue in bilaterally paired organs;
(2) neoplasms of different tissues or organs; and
(3) neoplasms of multicentric origin plus a lesion(s) of a different tissue or organ.
Multicentric lesions in contiguous epithelial surfaces have been observed for the lips, oral cavity, pharynx, larynx and oesophagus (Newell et al., 1974; Bharucha and Mehta, 1976; Newell and Krementz, 1977; Moertel, 1977; Wynder et al., 1977; Gill et al., 1979; Vrabec, 1979; Richardson, 1979; Levy, 1980; Quart and Yamane, 1983; Hordijk and De Jong, 1983).

Multicentric malignant neoplasms are considered by convention to be synchronous or metachronous, the former appearing simultaneously or within six months of diagnosis of the initial neoplasm, the latter appearing after six months but excluding recurrences of the index lesion, i.e. the initial lesion (Bharucha and Mehta, 1976; Gill et al., 1979; Richardson, 1979; Hordijk and De Jong, 1983).

Second upper respiratory and digestive tract squamous cell carcinomata have been shown by Tepperman and FitzPatrick (1981) to develop after treatment of floor of mouth squamous cell carcinoma at a constant incidence rate of 3.6 per cent per annum. Beyond the fifth year after treatment of squamous cell carcinoma of the floor of the mouth the relative survival rate of a patient without second carcinomata is a constant 35-40 per cent. Patients who later develop second neoplasms have an excess mortality for the same period of 5.2 per cent per annum which is attributable to the second squamous cell carcinoma. A constant and excessive (13-21 times normal) risk for developing new primary carcinomata exists in patients who survive a squamous carcinoma of the floor of the mouth. Distribution of new primary neoplasms occur mainly in the palatine tonsil, hypopharynx, larynx and oesophagus. New neoplasms arising distal to the oral cavity have a worse prognosis, with a 52 per cent 5-year crude survival rate compared with an 85 per cent rate for second neoplasms in the mouth. With the earlier diagnosis and treatment of the first neoplasm, second primary malignancies become a major threat to the prolonged survival of the individual.

5.6 INTRA-Osseous CARCINOMA

Intra-osseous carcinomata of the jaws are mainly due to invasion from squamous cell carcinomata of the adjacent structure, viz.: the gingiva, tongue or floor of the mouth. Much less often, intra-osseous carcinomata represent metastatic deposits from distant anatomical sites such as the mammae, thyroid gland and kidney. Rarely, intra-osseous
carcinomata are primary malignant neoplasms that may remain as wholly intra-osseous or may perforate the bone and invade the adjacent soft tissues. These primary neoplasms may arise from odontogenic cysts or from cell nests of odontogenic epithelium or vestigial embryonic epithelium from the fusion sites of embryonic processes (De Lathouwer and Verheest, 1974; Nithiananda, 1983; Lucas, 1984d; Lukinmaa et al., 1985).

Metastatic carcinoma to the oral soft tissues is rare (Lucas, 1984d). The principal sites of origin of oral metastatic carcinoma are: the lung, kidney, liver; other less common sites are the thyroid gland, trachea, skin, lymphatics, mamma, pancreas, testis and uterus. Soft tissue metastases appear most frequently in the gingivae, followed by the tongue and lips. Metastases to the jaws occur more frequently than in oral soft tissues, being more common in the mandible, particularly in the molar region. The commonest primary sites for intra-osseous metastases are the mamma, followed by the lung and kidney. Other primary sites include: stomach; colon; rectum; thyroid gland; testis; skin (melanocarcinoma); liver; pancreas; prostate gland; urinary bladder; ovary and uterus. Squamous cell carcinomata of the lip may also metastasize to the mandible via the mental foramen lymphatics. Parotid gland, nasal and tonsillar carcinomata also can metastasize to the mandible.

Metastases to the jaws from a variety of other malignant neoplasms is very rare, e.g.: osteosarcoma, chondrosarcoma, neuroblastoma, adrenal neuroblastoma, "medulloblastoma", ganglioneuroblastoma, clear cell sarcoma and retinoblastoma (Lucas, 1984a).

In a review of the Japanese literature by Nishimura et al. (1982) forty-one well documented metastatic neoplasms to the mouth and jaws were found. A female predilection was observed with a peak incidence occurring during the fourth to seventh decade. Unlike Caucasians, the most frequently encountered site of origin in the Japanese was the uterus, followed by the lung, kidney and stomach. Metastasis occurred in the gingiva (23 cases) and intra-osseously, mainly in the mandible in 16 cases, especially the body of the mandible. Common symptoms are swelling, pain, bleeding, paraesthesia and loosening of the teeth. Radiographic appearances are quite variable and non-specific, mainly radiolucent lesions. Histologically, adenocarcinomata showed the highest rate of metastasis followed by choriocarcinomata.
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The primary sites most commonly resulting in the invasion of bone by squamous cell carcinoma are: the gingiva and alveolar process (53 per cent); the tonsillar region including the retromolar area (31 per cent); and the floor of the mouth (16 per cent) (Payos, 1973; Whitehouse, 1976). In an histological analysis of 75 hemimandibulectomy specimens removed because of oral squamous cell carcinoma, Bhargava et al. (1970) found that 52 per cent of the neoplasms showed osseous infiltration. However, they point out that this high incidence may be due to the fact that all the cases studied were fairly advanced lesions necessitating hemimandibulectomy. Squamous and verrucous carcinomata exhibit the same frequency of osseous invasion, 53 and 50 per cent, respectively. Krishnamurthi et al. (1971) reported that 30 per cent of oral squamous cell carcinoma shows osseous invasion, radiographically.

Similarly, Larheim et al. (1971) reported that 33 per cent of all carcinomata (including salivary gland neoplasms) presented with bony destruction; carcinomata of the gingivo-alveolar mucosa, palate and floor of mouth comprising about 90 per cent of these neoplasms. Of this group, osteolytic lesions occur most frequently in maxillary gingival carcinomata (89 per cent), followed by palatal (58 per cent) and mandibular gingival lesions (49 per cent). The high incidence in the palate is a reflection of the minor salivary gland carcinomata at this site. Involvement of the maxillae frequently may include the sinus maxillaris and nasal fossae or both. The best routine radiographic projections for detecting intra-osseous neoplastic involvement are postero-anterior skull or conventional sinus projection, supplemented by hypocycloid tomography for the maxillae, while the mandible can be examined with panoramic radiography, and lateral oblique mandible and postero-anterior mandible views. Other important diagnostic tools include: computed tomography, bone scanning with radionuclides and magnetic resonance imaging (see Chapter 7 on Diagnosis).

Perineural invasion along the inferior alveolar nerve and its branches either proximally or distally with little or no clinical indication of involvement may occur (Southam, 1969; Bhargava et al., 1970). Pressure from invading squamous cell carcinoma may at the margins of the zone of invasion induce a reactive osteogenesis which histologically and radiographically resembles fibrous dysplasia of bone (Schwartz and Shklar, 1967). Intra-osseous carcinoma needs to be differentiated from osteomyelitis and osteoradionecrosis.
CHAPTER 6
PATHOLOGY OF ORAL SQUAMOUS CELL CARCINOMA

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CHAPTER 6
PATHOLOGY OF ORAL SQUAMOUS CELL CARCINOMA

The pathology and pathogenesis of oral squamous cell carcinoma and its variants, viz.: verrucous carcinoma, spindle cell carcinoma and adenoid squamous cell carcinoma will be reviewed. The main focus will be on the histopathology, histochemistry, cell kinetics, microradiographic and stereological studies and a brief account of the immunopathological of oral squamous cell carcinoma. Certain pertinent aspects of the general pathology of neoplasia and carcinogenesis have already been discussed.

6.1 HISTOPATHOLOGY

The role of histopathology in the diagnosis and prognosis of oral squamous cell carcinoma is of paramount importance, as will be discussed later. The majority of oral squamous cell carcinomata are readily diagnosable by well-established, logical, histopathological criteria on routine microscopic preparations. Most lesions are overtly carcinomata at presentation and are readily recognized as such. The major difficulty arises in determining what constitutes "premalignant" oral lesions. Methods for improving the objectivity and accuracy of diagnosis of premalignant lesions have been devised and include: multifactorial analyses, cytological and immunological investigations; biochemical and histochemical investigations; and epithelial cell kinetic studies (Johnson, 1976, 1977; for review see MacKenzie et al., 1980b).

At the cellular level squamous cell carcinomata show disturbances in the homeostatic mechanisms controlling cell division, maturation and aggregation. To these must also be added the host response and epithelial–mesenchymal interactions. Signs of incipient or overt neoplasia in stratified squamous epithelium include (Johnson, 1976, 1977):

1. cell division: abnormal mitoses, mitotic activity, hyperchromatism, nuclear–cytoplasmic ratio, nuclear pleomorphism, anisonucleosis, hyperplasia or atrophy;

2. cell maturation: keratosis/parakeratosis, dyskeratosis, irregular stratification; nucleolar alterations, nuclear–cytoplasmic ratio, cellular pleomorphism, hyperplasia or atrophy;
(3) cell aggregation: intercellular space, acantholysis, pseudopodia, invasion; and
(4) host stromal reactions: immune and inflammatory responses, vascularity, density of connective tissue fibres, ground substance density.

Oral squamous cell carcinoma exhibits considerable variation in histopathological features, varying from well–differentiated through to poorly–differentiated and anaplastic carcinomata. Generally oral squamous carcinomata tend to be moderately well–differentiated with some evidence of keratinization (Shafer et al., 1974). The well–differentiated lesion consists of sheets and nests of large neoplastic cells with large, hyperchromatic nuclei; mitoses (usually atypical); dyskeratosis resulting in epithelial or keratin "pearls"; and vagarious (erratic) pattern of invasion. In moderately well–differentiated lesions the resemblance to squamous epithelium is less pronounced. There is anisocytosis; greater members of mitoses; hyperchromatic nuclei; failure of keratinization, i.e. of differentiation of neoplastic keratinocytes. The poorly differentiated bear little resemblance to the cell of origin. There is anaplasia with a greater lack of cohesiveness and extremely vagarious patterns. Anaplastic lesions are very rare in the oral mucosa, metastasize early and extensively leading to an early death (see Plates 11, 12 and 13).

The distribution of oral squamous cell carcinoma according to site reveals that the labial vermilion, tongue, buccal mucosa and floor of mouth mostly exhibit well–differentiated lesions; the palate usually and the gingiva always exhibit well–differentiated lesions. The base of the tongue occasionally shows anaplastic carcinoma (Lucas, 1984c). In a more detailed analysis Fowler et al. (1980) found the following degree of differentiation of oral squamous cell carcinoma according to site in an Australian population (see Table 3). Of the total number of intra–oral squamous cell carcinomata 75 per cent are either well or moderately differentiated, with a higher proportion of females (52 per cent). Males have a higher incidence of poorly differentiated/anaplastic squamous cell carcinomata (29 per cent) than females (17 per cent).

The basement membrane in neoplasia is frequently regarded as having been breached with penetration and disruption by carcinoma. However, as pointed out by Lucas (1984) the basement membrane per se cannot be accurately delineated in ordinary haematoxylin–eosin histological sections and it seems that references to its integrity or


TABLE 3: Percentage distribution of degree of differentiation of oral squamous cell carcinoma according to site (after Fowler et al., 1980).

<table>
<thead>
<tr>
<th>Anatomical site</th>
<th>Well differentiated</th>
<th>Moderate</th>
<th>Poor/anaplastic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labial mucosa</td>
<td>83</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>Gingiva/floor of mouth</td>
<td>42</td>
<td>35</td>
<td>23</td>
</tr>
<tr>
<td>Tongue</td>
<td>41</td>
<td>37</td>
<td>22</td>
</tr>
<tr>
<td>Other unspecified intra-oral sites</td>
<td>45</td>
<td>35</td>
<td>20</td>
</tr>
</tbody>
</table>
discontinuity have been based more on the architecture of the epithelial-connective tissue junction than on the examination of a distinctive subepithelial structure. Ultrastructural studies (MacKinney and Singh, 1977; Chen and Harwick, 1977) and enzyme histochemical studies (Shklar, 1966) are needed to demonstrate changes occurring in the electron microscopic basal lamina structure.

The various degrees of differentiation of oral squamous cell carcinoma have led to the proposal of histological grading systems of the mode of invasion. Since Broders' introduction in 1925 of a mono-factorial grading system based upon the relative number of immature cells, several refinements and extensions have been proposed by Jakobsson et al. (1973). Eneroth and Moberger (1973), Willén and Nathanson (1973), Lund et al. (1974). The prognostic significance of the modified Jakobsson histological grading system employing eight parameters will be discussed in detail later. Most recently, Yamamoto et al. (1983a, b) have further modified the Jakobsson histological grading system of the mode of invasion of squamous cell carcinoma by subdividing Grade 4 (diffuse growth or invasion) into Grade 4C (diffuse invasion of cord-like type) and Grade 4D (diffuse invasion of diffuse type) so that the histological grades are:

Grade 1: well defined epithelial connective tissue margin;
Grade 2: cords, less marked borderline;
Grade 3: groups of cells, no distinct margin;
Grade 4: diffuse growth (or diffuse invasion);

4C: diffuse invasion of cord-like type;
4D: diffuse invasion of diffuse type.

These descriptions refer to the histological appearance of the modes of invasion of the neoplastic cells into the subjacent connective tissue as observed in haematoxylin-eosin stained histological sections.

Oral pigmented squamous cell carcinoma is a rare variant of squamous carcinoma which may be confused with a variety of pigmented lesions viz.: pigmented naevi and melanocarcinoma. Melanocyte colonization of oral squamous cell carcinoma has been reviewed by Dunlap and Tomich (1981) and Ide et al. (1981). Melanocytes or melanin pigmentation have been described in epidermal squamous cell carcinoma, breast adenocarcinoma, odontogenic neoplasms, salivary gland neoplasms: mucoepidermoid carcinoma, adenocarcinoma. The majority of oral squamous
carcinomata colonized by melanocytes occur in Negroes (Dunlap and Tomich, 1981), although Ide et al. (1981) noted one Mongoloid case. Clinically, lesions are pigmented, either uniformly or diffusely. Histologically, the lesions are moderately well differentiated squamous cell carcinoma showing pleomorphism, disordered stratification, abnormal mitoses and keratin "pearls". Melanocytes are interspersed among neoplastic keratinocytes, being more prominent in areas of intra-epithelial lateral invasion than in deeply invasive areas. The dendritic shapes of the melanocytes are clearly demonstrable by their abundant contents of melanin granules. The stroma around the neoplastic nests show numerous melanophores and intense chronic inflammatory cell infiltration. No hyperpigmentation of melanin occurs in the contiguous epithelium. Ide et al. (1981) consider the presence of melanocytes in squamous cell carcinoma as a reactive phenomenon stimulated by the presence of neoplasia. This view of melanocytic colonization and melanin production is shared by Dunlap and Tomich (1981) although the stimulus for "melanocytic hyperplasia" in squamous cell carcinoma is unknown and its significance, if any, is also unknown.

6.1.1 Histopathology of verrucous carcinoma

Verrucous carcinoma is a distinctive type of squamous cell carcinoma. It is a slow growing, papillary exophytic carcinoma with localized osseous destruction and a very limited propensity to metastasize (McCoy and Waldron, 1981; Bohmfalk and Zallen, 1982; Eversole and Papanicolaou, 1983; Slootweg and Müller, 1983; Lucas, 1984c).

Histological characteristics include (McCoy and Waldron, 1981; Bohmfalk and Zallen, 1982; Mizuno et al., 1983; Medina, 1984; Lucas, 1984a):

(1) verrucous, densely para- or orthokeratinized squamous epithelium;

(2) sharply circumscribed deep margin;

(3) bulbous, well-oriented acanthotic rete processes, often with a cryptic core of parakeratin invaginating the surface: "keratin plugs";

(4) "pushing" broad-front, advancing margin;
(5) the normal epithelium at the edge of the lesion is bent back upon itself by the continued growth of the neoplastic epithelium;
(6) the presence of epithelial "pearls" and small cysts;
(7) mitoses and cellular atypia, mainly in basilar and parabasilar cells, is rare; and
(8) always the presence of heavy chronic inflammatory cell infiltrate in the stroma.

Verrucous carcinoma is often associated with other lesions, mainly epithelial dysplasia and squamous cell carcinoma forming part of the verrucous carcinoma or occurring as separate lesions (Slootweg and Müller, 1983). Regional lymphadenitis secondary to an inflammatory response to neoplasia may also be present (Bohm Falk and Zallen, 1982).

Verrucous carcinoma needs to be distinguished histologically from chronic hyperplastic candidiasis, verrucous hyperplasia (Shear and Pindborg, 1980; Eversole and Papanicolaou, 1983; Slootweg and Müller, 1983), pseudo-epitheliomatous hyperplasia, kerato-akanthoma and well-differentiated squamous cell carcinoma (Lucas, 1984a) (see Plates 14-17).

6.1.2 Histopathology of adenoid squamous cell carcinoma

Adenoid squamous cell carcinoma is a variant of squamous cell carcinoma. Synonyms include adeno-akanthoma and pseudoglandular squamous cell carcinoma (Shafer et al., 1974; Lucas 1984a). The preferred term is adenoid squamous cell carcinoma because it emphasizes a positive characteristic histopathological feature (Tomich and Hutton, 1972). It is generally a neoplastic lesion of the epidermis of the head and neck, but occasionally may involve the skin of the extremities; labial vermillion (Jacoway et al., 1971; Tomich and Hutton, 1972, Weitzner, 1974); tongue and gingiva (Takagi et al., 1977); and floor of mouth (Gerughty et al., 1968). Clinically, labial lesions can present as ulceration, hyperkeratosis or an exophytic neoplasm. Adenoid squamous cell carcinoma can manifest initially as kerato-akanthoma, both clinically and histologically (McLatchie et al., 1984).

Histopathologically, labial lesions are similar to cutaneous ones with the exception that the former are not anatomically associated with pilosebaceous structures. They exhibit proliferation of surface dysplastic epithelium into the corium with lateral and deep extensions showing
characteristic solid and tubular ductal structures. These duct-like structures are lined with a layer of cuboidal cells often containing or enclosing acantholytic or dyskeratotic cells. They are the result of central acantholysis of the proliferating neoplasm producing cystic spaces (Shafer et al., 1974; Tomich and Hutton, 1972; Lucas, 1984a). The cuboidal cells exhibit pleomorphic nuclei and dense eosinophilic cytoplasm (Takagi et al., 1977). There is heavy chronic inflammatory cell infiltrate in the corium which nearly always shows the basophilic degeneration typical of solar damage (Jacoway et al., 1971). Histochromically there is no evidence of epithelial mucins, sialomucins or glycogen in the labial lesions, whereas in cutaneous lesions there is evidence of hyaluronic acid inter-epithelially and intra-luminally, plus glycogen in various areas especially superficially (Johnson and Helwig, 1966).

Adenoid squamous cell carcinoma rarely metastasizes, although Takagi et al. (1977) found no evidence of a diminished propensity for metastasis. Recurrences are common following local excision and there is evidence of perineural lymphatic infiltration in recurrent lesions (Takagi et al., 1977). Weitzner (1974) was unable to find any lymph node metastases or deaths resulting from the neoplasm for periods of 10 to 72 months, and suggested that adenoid squamous cell carcinoma had a good prognosis.

The pathogenesis of adenoid squamous cell carcinoma is unknown. In cutaneous and labial vermillion lesions actinic keratosis with acantholysis is considered a prerequisite for neoplasia (Tomich and Hutton, 1972; Weitzner, 1974; Takagi et al., 1977; Lucas, 1984a; McLatchie et al., 1984). It is not known whether adenoid squamous cell carcinoma arises as a new, distinct carcinoma from the usual squamous cell carcinoma or is a single "metamorphic differentiation" of an existing squamous cell carcinoma.

In the differential diagnosis it needs to be emphasized that unlike kerato-akanthoma there is no "shouldering" of the margin but a gradual transition from normal keratinized epithelium through dysplasia to early acantholysis of the neoplastic cells. Also considerable keratin may be present but not keratin "plugs" of kerato-akanthoma. There is also a superficial ulceration, acantholysis near the surface, glandular-like differentiation and penetration of the dermis beyond the pilosebaceous apparatus.
6.1.3  Histopathology of spindle cell carcinoma

Spindle cell carcinoma is a rare variant of squamous cell carcinoma chiefly occurring on the labial vermilion, and occasionally at other intra-oral sites, parotid gland, mammae, skin, oesophagus, bronchus and cervix uteri (Somerren et al., 1976; Ellis and Corio, 1980; Lucas, 1984c). Spindle cell carcinoma of the oral cavity mainly occurs on the lower labial vermilion, followed by the tongue, gingiva and alveolar ridge. Occasionally it is found at the floor of the mouth, retromolar region, buccal mucosa, upper lip and hard palate. There is a slight male predilection with a mean age of 51 years for males and 67 years for females (Ellis and Corio, 1980).

Microscopically, spindle cell carcinoma is characterized by three histomorphological patterns: fasciculated; myxomatous; and streaming; with variable mitotic activity, pleomorphism, benign and atypical multinucleated giant cells, inflammation, vasculitis and infiltration of subjacent structures, such as skeletal muscle, bone, salivary glands and nerves. Nearly all lesions exhibit an extensively ulcerated surface covered with an eosinophilic fibrous exudate with prominent vascularity immediately subjacent. The bulk of the macroscopically fleshy, polypoid, exophytic or endophytic lesion is composed of spindle-shaped, fusiform cells separated by delicate strands of connective tissue. These cells are enlarged, round or polygonal with oval or fusiform nuclei and abundant, finely granular, eosinophilic cytoplasm. The nuclei are variable in size, shape and are vesicular with prominent nucleoli. Foci of necrosis and inflammation, dysplasia and poorly to well differentiated squamous cell carcinoma with keratin "pearls" and intracellular bridges are scattered throughout the lesion. The dysplastic epithelium usually composed a minor portion of the neoplasm and is found mostly within the stalk or periphery of the lesion. At the periphery of the infiltrating squamous islands transitional zones from squamous cells to spindle cells are present.

The histomorphological patterns include:

(1) fasciculated pattern: most common, composed of highly cellular groups of elongated bipolar cells in parallel alignment with the fasciculi interwoven. The cell nuclei are elongated, elliptical and vesicular, each containing one or more nucleoli. A variable number of round cells with more abundant cytoplasm and round vesicular nuclei are scattered among the elongated cells;
(2) myxomatous pattern produced by spindle and stellate cells with prominent intercellular spaces. The nuclei are more spherical but still vesicular with conspicuous nucleoli; and

(3) streaming pattern composed of moderately cellular groups of spindle cells and pleomorphic cells arranged in randomly oriented cords and sheets without any notable intercellular oedema or interweaving fasciculi.

Both benign-appearing, multinucleated, foreign body giant cells and atypical neoplastic giant cells with multiple pleomorphic nuclei are present within the spindle component of these neoplasms. Acute and chronic inflammatory cell infiltrates, mild to moderate in intensity, are frequently dispersed throughout the spindle cell elements. Occasionally osteoid and chondroid material have been reported within the spindle cell component (Ellis and Corio, 1980; Someren et al., 1976). Histologically, reticulin fibres encircling the neoplastic cells and forming an alveolar pattern have been described (Martin and Khan, 1977; Takagi and Ishikawa, 1982).

Metastases may be composed of spindle cell elements only, squamous cell carcinomatous and spindle cell "sarcomatous" elements together (Ellis and Corio, 1980; Takagi and Ishikawa, 1982).

The pathogenesis of spindle cell carcinoma is problematical. These biphasic neoplasms of the oral cavity have been suggested to represent:

(1) a squamous cell carcinoma associated with an atypical, benign, exuberant, reactive connective tissue process: "pseudosarcoma" (Lane, 1957); (2) a combination or collision growth of a carcinoma and a sarcoma: "carcinosarcoma" (Martin and Khan, 1977; Harris, 1982); or (3) a squamous cell carcinoma with a peculiar spindle cell anaplasia: "spindle cell carcinoma". Prognosis and treatment depend upon the concept of pathogenesis adopted. Evidence of local and distant metastasis of the spindle cell element and an overall mortality rate of 42 per cent would seem to preclude an interpretation of a benign reactive process. Features arguing against carcinosarcoma include: (1) the presence of transitional areas between basal squamous epithelial cells and spindle cells seen histologically and electron microscopically (Battifora, 1976); (2) lymphatic spread was the most frequent metastatic pathway, the predominate route of carcinomata; (3) the average age at occurrence correlated well with that for oral squamous cell carcinoma; and (4) the simultaneous occurrence of epithelial and mesenchymal malignant neoplasms at the same site would seem to be highly unlikely. Electron microscopic studies have
proved contradictory with several studies supporting the epithelial histopathogenesis of spindle cells (Lichtiger et al., 1970; Someren et al., 1976), whilst others have been interpreted as supporting a mesenchymal histopathogenesis (Goeliner et al., 1973; Martin and Khan, 1977). However, Battifora (1976) has shown that the ultrastructural epithelial characteristics of spindle cells can apparently transform gradually to mesenchymal characteristics. Circumstantial evidence favours the concept that these biphasic oral neoplasms represent a variant of squamous cell carcinoma and thus the term spindle cell carcinoma is to be preferred.

The prognosis of spindle cell carcinoma is much the same as for oral squamous cell carcinoma, i.e. generally poor (Ellis and Corio, 1980).

6.2 ULTRASTRUCTURAL FEATURES OF ORAL SQUAMOUS CELL CARCINOMA

Ultrastructural investigations including transmission and scanning electron microscopy, and stereological studies of oral squamous cell carcinoma have been limited in numbers. The ultrastructural features of oral epithelial dysplasia and squamous cell carcinoma can be summarized as the following (Seifert and Burkhardt, 1977; Chen and Harwick, 1977; McKinney and Singh, 1977; Kocher et al., 1981; Kandarkar et al., 1981; Ince et al., 1982; Löning and Burkhardt, 1982; Hong-cai et al., 1982; White and Gohari, 1985):

1. cellular abnormalities: cellular enlargement; nuclear enlargement; irregular shape of nucleus: ovoid with corrugated or folded or pitted nucleoplasmic membrane; malalignment of basal cells along the basal lamina; increase in filament bundles in basal cells; abnormal chromatic distribution;

2. cytoplasmic organelle abnormalities: increase in number of irregularly shaped, small or large mitochondria; decrease in mitochondrial cristae; decrease in centrioles and microtubules; increase in polyribosomes and rough endoplasmic reticulum;

3. abnormalities in cell maturation: dyskeratosis; migration of basal cells into superficial strata; delayed or arrested cell maturation; decrease or absence of keratohyalin granules and membrane-coating granules;
(4) abnormalities of basal lamina: dissolution; discontinuity; duplication or lamination; atypical basal lamina structural material; incomplete development of hemidesmosomes; membrane bound cytoplasmic protrusions or microvilli: "epithelial pseudopodia" penetrating through the basal lamina;

(5) cell membrane alterations: spongiosis: widening of intercellular spaces with proteinaceous débris; cytoplasmic protrusions; cytoplasmic constriction; convolutions of cell membrane aspects related to other epithelial cells; decrease in the number of nexi (gap junctions);

(6) desmosomal abnormalities: decrease in number; shortening in length; predominantly of simple form; lack of intermediate zona densa; decrease in plaque formation; lack of tonofibrillar insertion; complete loss or lack of outline; presence of intracytoplasmic maculae adherentes (desmosomes);

(7) atypical tonofibrils: clumping, perinuclear spirals or whorls; and

(8) abnormalities of submucosa: phagocytosis of dyskeratotic cells by macrophages or foreign body giant cells, sometimes neoplastic keratinocytes in stratum basale and in invasive neoplastic nests; excessive inflammatory cell infiltrate, predominantly T lymphocytes; irregular collagen fibres; increase in collagen; desmoplasia: the formation of excessive dense fibrous connective tissue stroma; dilated lymphatic spaces.

The ovoid nuclear bodies described by Chen and Harwick (1977) are composed of concentrically arranged fine filaments with electron-dense granules scattered in the central position.

The ultrastructural changes in the basal cell-stromal interface undergoing neoplastic transformation have been investigated by McKinney and Singh (1977) and White and Gohari (1985). The basal lamina complex is composed of hemidesmosomes, lamina lucida, lamina densa, and associated filamentous and fibrillar elements. In neoplasia it exhibits disruption, discontinuity, duplication and separation of lamina densa and a decrease in the number of hemidesmosomes. Membrane bound cytoplasmic protrusions, "pseudopodia" of various size and shape, extending through the epithelial-connective tissue junction into the lamina propria have been described in experimentally-induced carcinomata, human premalignant lesions and squamous cell and basal cell carcinomata. However, they are not specific for malignant lesions, having been described in non-neoplastic
conditions such as pemphigus, pemphigoid, lichen planus and chronic periodontitis. These cytoplasmic protrusions are associated with a zone of peripheral microfilaments which may be responsible for mediating an increase in cellular motility by contractile proteins, e.g. actin, actinin, myosin, meromyosin (Gabbiani et al., 1976). The loss of the lamina densa may be a prerequisite for this form of micro-invasion, with cellular invasion being further facilitated by the destruction of collagen fibrils in the immediate subjacent lamina propria (Tarpey and White, 1984).

An hypothesis for basal lamina breakdown in oral squamous cell carcinoma has been proposed by McKinney and Singh (1977). Advanced epithelial dysplasia or carcinoma in situ may produce excessive quantities of collagenases or collagenase-like enzymes in the endoplasmic reticulum and released by exocytosis into the intercellular spaces between the basal cells. These intercellular spaces are slightly increased in width because hyaluronidase has reduced or removed the cell glycocalyx composed of glycosaminoglycans. Additional collagenase may be released into the intercellular spaces from migratory leukocytes in the epithelium. These proteases may permeate through the intercellular spaces and eventually hydrolyze the basal lamina and underlying stromal collagen and anchoring fibrils. As basal lamina interruptions and stromal electron-lucent areas develop, the basal cells develop cytoplasmic protrusions into the surrounding lamina propria. These events mark the conversion of an intra-epithelial carcinoma into an invasive neoplastic lesion: squamous cell carcinoma (see Figure 5).

Stereological intersection counting techniques of electron micrographs of basal cells provide estimates of the proportion of total basal cell membrane in contact with connective tissue in control, hyperplastic, dysplastic and neoplastic lesions (White and Gohari, 1985). Significantly elevated values occur in epithelial dysplasia and squamous carcinoma. The changes in this parameters are considered to reflect an increased motility in the transforming basal cell prior to and concomitant with cellular invasion, and may prove to be of value as a structural indicator of malignant transformation. Stereological point-counting methods have been used to determine the volumetric alterations in collagen from the lamina propria of hamster buccal pouch mucosa during experimental oral carcinogenesis (Tarpey and White, 1984). The volume densities of collagen present in a unit volume of extracellular lamina propria decrease progressively and significantly in 7,12-dimethylbenz-(a)-

A. Golgi apparatus.
B. Rough endoplasmic reticulum.
C. Discharge of vacuoles into widened intercellular space.
D. Collagenases disrupt basal lamina.
E. Clear areas formed by breakdown of lamina propria.
F. Pseudopodia of neoplastic cells.
G. Hemidesmosomes.
H. Anchoring fibres.
I. Collagen fibres.
anthracene (DMBA) treated tissues when compared to normal untreated oral mucosa. Values from the inflammatory controls are comparable with those from the dysplastic stage of carcinogenesis. Although the mechanisms responsible for these decreases in collagen volume density are unknown, it is believed that contributory factors might include collagen degradation by enzymes originating in either the epithelium or the cells of the inflammatory infiltrate, dilution of collagen produced by inflammatory oedema or alterations in the synthetic capabilities of fibroblasts.

An increased nuclear-cytoplasmic ratio is one of the cytological features of epithelia dysplasia taken into account in the histopathological assessment of the malignant potential of a lesion (Smith and Pindborg, 1969; Kramer et al., 1970a, b; Franklin and Smith, 1980). Attempts to quantify alterations in nuclear-cytoplasmic ratio at the ultrastructural level during experimental carcinogenesis in hamster buccal pouch epithelium were carried out using stereological point-counting techniques by White and Gohari (1981). The results indicate that progressive decreases in the ratio occur in all pathological stages during differentiation, i.e. between basal and granular layer cells. During carcinogenesis there is a tendency for the ratios to decrease in each cell stratum but no significant differences are detected between normal and premalignant lesions. Consequently, it was concluded that nuclear-cytoplasmic ratio is not a useful ultrastructural discriminant for the detection of premalignancy.

An electron microscopic and morphometric study of the distribution of desmosomes (maculae adherentes) and nexi (gap junctions) in precarcinomatous and carcinomatous lesions of squamous epithelia revealed a significant decrease in the proportion of cell surface occupied by nexi and desmosomes in oral squamous cell carcinoma (Kocher et al., 1981). Moreover, the distribution of desmosomes appears irregular in squamous cell carcinoma: desmosomes are relative numerous in the central part and sparse at the periphery of the invading neoplasm islets where cells are in direct contact with the surrounding connective tissue. In general, neoplastic desmosomes are connected to a smaller number of tonofilaments than desmosomes of normal oral epithelium. These results support the assumption that invasive neoplastic cells are mechanically independent from other cells of the same neoplasm, i.e. there is loss of adhesion. The drastic decrease in gap junctions (nexi) in neoplastic and some
preneoplastic lesions may play a role in the aberrant behaviour of
neoplastic cells since nexi have been shown to be the sites of chemical
and electrotonic coupling of cells in epithelia. It is probable that the
development of a contractile apparatus, a lack of mechanical adhesion and
a lack of chemical and electrotonic coupling play an important role in
determining the invasive behaviour of neoplastic cells.

Scanning electron microscopy of the oral mucosal lesions reveals
certain characteristic features of oral squamous cell carcinoma (Reichert
and Althoff, 1979). These include: areas of desquamation and
invaginations; neoplastic epithelial cells forming nests surrounded by fine
stroma; surface cells exhibiting polymorphism and varying degrees of
differentiation: ortho- or parakeratinization; sometimes the surface is
composed of bizarre lamellary patterns suggesting irregular keratinization;
cell margins showing incomplete steps; cell surface with microvilli, pits
and microridges; and cells showing pleomorphism.

6.2.1. Ultrastructural features of verrucous carcinoma

Ultrastructurally, verrucous carcinoma exhibits the features of a well
differentiated squamous cell carcinoma (Prioleau et al., 1980). There are
numerous maculae adherentes and interdigitating microvilli; focally
keratinocytes with flat borders and abundant tonofilaments distributed in
the periphery of cytoplasm leaving a tonofilament-free perinuclear halo;
large number of free ribosomes alternating with a moderate amount of
rough and smooth mitochondria; large numbers of glycogen granules in
most keratinocytes; and nuclei are oval with irregular envelopes, slight
peripheral chromatin clumping, one or two prominent nucleoli and the
occasional nuclear body.

The epithelial–stromal junction is a site of major changes viz.:
marked duplication of the basal lamina with an increase in density and
inferior displacement of the original anchoring filaments, new slender
filaments attached to more recently formed basal lamina, focal areas of
disruption and grouping of hemidesmosomes; occasionally the presence of
round, electron-dense membrane-bound organelles of uncertain nature
(150 nm in diameter) in the cytoplasm of basal cells; apoptotic
keratinocytes with karyorrhexis and densely packed cytoplasm in neoplasm
and adjacent stroma; and the presence of lymphocytes and eosinophils in
basal portion but no melanocytes or Langerhans' cells. The focal
collections of hemidesmosomes suggest a probable association with a
tendency not to invade, for invasion by keratinocytes of basal cell
carcinomata have been correlated with the relative absence of
hemidesmosomes. The round or oval, electron-dense intracytoplasmic
membrane-bound bodies seen in the basal cell might represent zymogen
granules for collagenases as seen in Bowen's disease and basal cell
carcinoma.

6.2.2 Ultrastructural features of spindle cell carcinoma

Ultrastructural features of spindle cell carcinoma have been reported
by Someren et al. (1976); Battifora (1976); Martin and Kahn (1977); and
Harris (1982). These studies prove to be contradictory in providing
evidence for the pathogenesis of spindle cell carcinoma.

Ultrastructural studies supporting the mesenchymal pathogenesis,
such as Martin and Khan (1977), reveal the following ultrastructural
features characteristic of fibroblasts: (1) spindle cells showing abundant,
short branching profiles of rough endoplasmic reticulum, some dilated and
filled with electron-dense granular material; (2) cell membranes of
adjacent spindle cells are closely applied or separated by bundles of
collagen fibres; (3) few attachment sites present, but no tonofilament
bundles or well formed desmosomes; (4) the occasional fine filaments and
lipid droplets in the cytoplasm; and (5) elongated, irregular nuclei with
multiple nucleoli.

Studies supporting the epithelial pathogenesis of spindle cell
carcinoma such as Someren et al. (1976) and Battifora (1976) reveal the
following ultrastructural features: essentially there exist two basic cell
types, viz.: (1) an elongated spindle cell with an abundant cytoplasm;
large amounts of rough endoplasmic reticulum with dilated cisternae
containing finely granular or fibrillar material; sparse, oval or round
mitochondria; large nuclei and finely distributed chromatin with moderate
degree of marginal condensation; multiple, large and sharply demarcated
nucleoli; nuclear membrane showing numerous invaginations; occasional
poorly defined desmosomes; tonofilament bundles interspersed between
organelles although no discernible attachment to desmosomes; both
desmosomes and tonofilaments markedly diminished in numbers; and most
cells separated by intercellular matrix containing collagen fibrils and some
short elastic fibres; and (2) less frequently observed small, irregularly
shaped cell with endoplasmic reticulum of varying sizes of cisternae; irregular clumps of collagen fibres in the vicinity of the cells which are regarded as reactive fibroblasts (Lichtiger et al., 1970; Goellner et al., 1973). In addition Battifora (1976) found some cells with cell membranes usually separated by fine filaments resembling actin myofilaments and haphazardly dispersed intracytoplasmic coarse tonofilbrils and the presence of poorly developed or well developed tonofilaments associated with desmosomes. A gradual transition to typical squamous cells was noted in epidermal spindle cell carcinoma suggesting that the pseudo-sarcomatous component originates from "mesenchymal metaplasia" of squamous cells which also elaborate collagen. The presence in the same cell of tonofilaments and myofilaments seems to suggest that it is exhibiting both epithelial and mesenchymal properties concurrently.

An alternative explanation to the hypothesis of simple transformation or "metaplasia" of squamous to mesenchymal cells might lie in the phenomenon of cell fusion (Harris, 1982). Fusion between squamous cells and fibroblasts or myofibroblasts would result in cells with the properties of both cell types (supra vide). This might further explain the apparent discrepancy between the histologically "aggressive-looking" spindle cells of human spindle cell carcinomata and the infrequency with which they appear to metastasize. The observations presented by Harris (1982) suggest that the rather rigid distinction made between epithelial and mesenchymal neoplasm, with the notable exception of pleomorphic salivary adenomata, may not always be valid.

6.3 HISTOCHEMISTRY OF ORAL SQUAMOUS CARCINOMA

The extensive literature on the histochemistry of malignant neoplasia is often contradictory and difficult to interpret. Biochemical and histochemical changes in oral premalignancy have been reviewed by Johnson et al. (1980). At present there are no reliable histochemical or biochemical methods for testing the malignant potential of human oral mucosal lesions.

Some difficulty arises from a failure to recognize which are the "key" or "rate limiting" enzymes of the metabolic pathways under investigation. However, certain generalizations are possible, especially in relation to respiratory enzymes (Johnson, 1977). An increase in the activity of enzymes of anaerobic glycolysis and a decrease in
mitochondrial enzyme activity is one characteristic feature of malignant epithelium. Raised activities of lactate dehydrogenase (LDH) and a concomitant decrease of succinate dehydrogenase (SDH) activity have been repeatedly shown (Cabrini, 1973). Neoplastic cells show an increased anabolic activity for the synthesis of lipoproteins and nucleic acids necessary to sustain the increased rates of cell proliferation. The pentose phosphate pathway provides ribose for the synthesis of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) and hence for protein and nucleic acid synthesis. Ribose is also a major source of reduced nicotinamide adenine dinucleotide phosphate (NADPH), a necessary requirement of biosynthesis. Glucose-6-phosphate dehydrogenase (G6PD), the "key" enzyme of the pathway has been shown to show an increased activity in oral dysplastic lesions but not in benign hyperplastic and neoplastic lesions (Evans et al., 1983). However, no pattern of activity that could aid the diagnosis of premalignant lesions was conclusively discerned. The very low levels of G6PD in neoplastic lesions was difficult to explain, but increased cell death, variations in blood supply and necrosis may be contributing factors. An increase in acid phosphatase activity in basal epithelial cells and in stromal macrophages has been shown in experimental oral carcinogenesis (Smith, 1972). The amount of collagenase released from established oral carcinomata apparently correlates with survival time (Abramson et al., 1975). Recently Chomette et al. (1985), in an histo-enzymological study of the levels of activities of oxidative enzymes, diphorases, acid phosphatases and naphthol esterases, in a number of lesions found, in severe epithelial dysplasia, carcinoma in situ and squamous cell carcinoma, numerous variations of activities of oxidative enzymes, esterases and acid phosphatases from one cell to the other. However, the histo-enzymological variations observed were not sufficiently characteristic to contribute to the histological diagnosis.

Histochemical staining techniques can be used to corroborate ultrastructural observations. For example, Gomori's stain for reticulin fibres has been used to demonstrate disruption of the basement membrane, Dane's method for keratin to demonstrate dyskeratosis and periodic acid-Schiff method (PAS) for glycogen to demonstrate glycogen in keratin pearls (Chen and Harwick, 1977). Ultrastructural histochemical techniques, e.g. silver methenamine, have been used to demonstrate plasma membrane, membrane-coating granules, tonofilaments, intracytoplasmic
desmosome clusters and desmosomes in dysplastic leukoplakia (Kandarkar et al., 1981).

6.4 CELL KINETIC STUDIES

Cell kinetic studies in oral preneoplasia and neoplasia have been reviewed by Scragg and Johnson (1980, 1982). Cell kinetic studies constitute only one of a number of branches of investigations in which consistent differences are detectable between normal and abnormal tissues. Other areas of investigation include:

1. changes in histological parameters measured by stereological techniques, e.g.: nuclear–cytoplasmic ratios, nuclear densities, Interface ratios and volume-to-interface area ratios (Franklin and Craig, 1978a, b; Franklin et al., 1980; White and Gohari, 1981, 1985; Keszler and Cabrini, 1983);

2. changes in cell membrane markers, e.g. disappearance of blood group antigens A and B from oral epithelial cells in leukoplakia and squamous cell carcinoma (Dabelsteen and Pindborg, 1973; Dabelsteen et al., 1975; George et al., 1980; Dabelsteen, 1980) and expression of carcino-embryonic antigen (Silverman et al., 1976; Toto, 1979);

3. alterations in cell karyotypes (Bazopoulou-Kyrkanidou et al., 1983);

4. enzyme histochemistry (Cabrini, 1973; Johnson et al., 1980; Evans et al., 1983; Chomette et al., 1985);

5. changes in the levels of cyclic nucleotides and polyamines;

6. elaboration of "tumour angiogenesis factor" (Folkman et al., 1971); and

7. systemic immunodepression.

Cell kinetic investigations of head and neck squamous cell carcinoma in humans have produced more equivocal results than from experimental animal studies (Scragg and Johnson, 1982). Accurate data on cell cycle parameters are available for only a few human oral carcinomata. Heterogeneity within the cell population is reported to contribute to the wide distribution of cell cycle times observed among squamous carcinomata. A high cell loss factor (80–90 per cent of cell birth rate) might also contribute to tissue heterogeneity.
The measurement of the DNA content in head and neck squamous cell carcinoma also indicate a high degree of heterogeneity. The DNA distribution pattern of neoplastic cells can be determined by cytophotometry (Cabrini, 1973; Nervi et al., 1980; Müller et al., 1981; Doseva et al., 1984) and flow cytometry (Frentz et al., 1985). The resulting histograms contain cytogenetic information (ploidy stages of neoplasms and corresponding to the mean number of chromosomes) and information on the proliferative activity (S-phase cell pool of the cell cycle). Such studies, it is suggested by Müller et al. (1981), can be carried out relatively quickly and economically on a routine basis in the clinical setting. Individual cytobiological parameters demonstrating the in vivo state can be obtained for each patient prior to the commencement of treatment, providing information on the radiosensitivity of neoplastic cells.

Precise counting of chromosome numbers and a full karyotype analysis may also be performed (Scruggs and Johnson, 1982). A karyotype is the sum of the specific characteristics of a cell nucleus including chromosome number, form, size and points of spindle fibre attachment. The chromosome complement of neoplastic cells is very often abnormal. Chromosome aberrations are non-random, but tend instead to cluster in twelve autosomal chromosomes of the 24 chromosome types of the human karyotype. This non-random involvement of chromosomes in neoplasia has been interpreted as being a reflection of the propensity of certain chromosomes to respond more frequently to neoplasia or, alternatively, that the selectively involved chromosomes carry genetic material of importance to normal development and/or proliferation of cells. Subsequently the importance of the affected genetic material may be enhanced by secondary chromosomal events, which are reflected in the chromosome aberrations in cells of the developed neoplasms.

Bazopoulou-Kyrkanidou et al. (1982) showed that squamous cell carcinomata of the oral cavity consist of populations of cells with abnormal karyotypes, exhibiting both numerical and structural chromosome changes, e.g. the modal population of neoplastic cells frequently is triploid or diploid and rarely tetraploid or pentaploid; and in banded chromosomes structural aberrations include short and long term deletions with random translocation to other chromosomes, long arm duplications and an excess of the centromeric region.
Epithelial cell proliferation rates, viz.: DNA synthesis time (Tₐ); labelling index (L.I.); and cell cycle time (Tₑ) using in vivo (Bresciani et al., 1974) and in vitro (Sakuma, 1980) double labelling methods have been determined. The absolute values of the cell cycle parameters are open to criticism depending on the method used.

Despite the voluminous literature on mitotic activity in neoplasms, and the significance placed in the histopathological diagnosis of neoplasia, it has been shown by Johnson (1976) and Kramer et al. (1970a, b) using multifactorial statistical analysis of the histological features that mitotic activity is insignificant, especially as a prognostic indicator. These studies highlight the importance of considering as many histological features as possible in making accurate prognostications, rather than emphasizing one variable alone.

6.5 PATTERNS AND MECHANISMS OF BONE INVASION

The histopathological evaluation of bone invasion in squamous carcinoma of the buccal mucosa was studied by Bhargava et al. (1970). Fifty-two per cent of advanced squamous cell carcinoma show infiltration of bone by neoplastic cells. Of these lesions 56 per cent of grade I and II (Broders' classification) exhibit infiltration compared with 60 per cent of grade III. Osteomyelitis is not significantly related to neoplastic infiltration of bone, being found in 64 per cent of mandibles infiltrated and 53 per cent of those not infiltrated with carcinoma.

Squamous carcinomata of the head and neck spread to bone by two routes: (1) haematogenous metastases to distinct parts of the skeleton are observed occasionally clinically, but are more usually occult; and (2) direct invasion of local bones of the skull is more commonly seen clinically especially of squamous carcinoma of the buccal cavity, paranasal sinuses and nasopharynx. Infiltration of metaplastic bone is also a common feature of neoplasms spreading into the osteocartilaginous laryngeal framework.

Patterns and mechanisms of bone invasion by squamous cell carcinoma of the head and neck were investigated by studying the surgical pathology of resected specimens, in established cell lines and in xenografts by means of an in vitro model of osteolysis and biochemical estimations of osteolytic prostaglandins (Carter et al., 1983; Tsao et al., 1983). The overall incidence of direct bone invasion from squamous cell
carcinoma of the head and neck is 28 per cent. Oral squamous carcinoma (18/61 cases) resulting in bone invasion, the mandible is involved in 17/18 cases. Detailed surgical pathology has shown that these squamous carcinomata invade the contiguous skeletal or metaplastic bone principally through an indirect process: the normal osteoclasts of the host are activated and erode bone in front of the advancing neoplasm's edge. The osteoclastic response then declines and, in the second phase, neoplastic cells move forward onto the bone surface and take over the destructive process. The osteoclastic response is a striking histological feature of invaded bone during the early stages, though it is not "tumour-specific". The osteolysis by the osteoclasts and neoplastic cells correlates well with the radiolucent lesions seen radiographically. Some local osteoblastic proliferation, particularly in the vicinity of the slowly infiltrating neoplasms, is present, which incidentally provides the pathological basis for scintigraphic scanning. Perineural spread is also important at some sites. Access by way of periosteal lymphatics is of doubtful significance.

Mechanisms of bone invasion have been determined from freshly excised neoplasms, established neoplastic cell lines and xenografts (Tsao et al., 1983). The following results emerge: (1) fresh neoplasms regularly resorb bone in vitro: activity is consistently, but not completely reduced by indomethacin (an inhibitor of prostaglandin synthesis). The neoplasms release PGE₂ in amounts sufficient to account for about 50 per cent of the bone resorption observed. Small amounts of non-prostaglandin (indomethacin-resistant) osteolytic factors are also produced; (2) control non-neoplastic tissues show a variable capacity to resorb bone in vitro: PGE₂ levels in these tissues may be related to their content of inflammatory cells; (3) neoplasm cell lines also resorb bone in vitro, but for most lines activity is not significantly blocked by indomethacin and PGE₂ levels are generally insufficient to account for the osteolysis observed. Non-prostaglandin osteolytic factors (osteolysis) thus predominate; and (4) xenografts of squamous cell carcinoma also show osteolytic activity in vitro. Bone resorption by xenografts is uninhibited by Indomethacin and only small amounts of osteolytic prostaglandins (PGE₂ and PGF₂α) are detected, reflecting in part the fact that the xenografts have an heterologous (i.e. immune-derived) stroma. It was concluded that most squamous carcinomata of the head and neck are osteolytic in vitro and release a mixture of prostaglandin and non-prostaglandin osteoclastic stimulating factors. These factors are derived
from both neoplastic and stromal elements, and are "tumour-associated" rather than "tumour-specific". In vitro bone resorption and prostaglandin release does not correlate with the pathological features of the neoplasm: site, size, degree of differentiation, presence or absence of clinical bone invasion; or with post-operative survival.

It is suggested by Carter et al. (1983) that the anti-prostaglandin drugs alone are unlikely to be effective in controlling bone invasion, although once the unknown non-prostaglandin osteolysins are identified, it may be feasible to block the osteolysins pharmacologically and thus impede the metastatic growth of malignant neoplasms, irrespective of origin, within local or distant parts of the skeleton. Interestingly, Cornwall et al. (1983) describe the use of ibuprofen, an inhibitor of prostaglandin synthesis, to significantly inhibit the development of chemically-induced squamous cell carcinoma of the hamster buccal pouch. The possibility exists that the ibuprofen might not only delay neoplasia but may actually prevent it if the carcinogen used was less active. Similar results have been demonstrated with aspirin and indomethacin by Perkins and Shklar (1982).

Studies designed by Karmali et al. (1984) to measure neoplastic tissue prostaglandin content (ng/g wet tissue), as well as production in vitro by squamous cell carcinoma microsomes (ng/mg protein/10 min.) reveal that squamous cell carcinoma of the head and neck contain large amounts of 5-eicosanoids in the order: PGE₂ > PGE₁ > 6-keto-PGF₁α > PGF₂α > TXB₂. Amounts of all but PGF₂α were higher in large neoplasms (> 3cm in diameter). However, the capacity of neoplasm microsomes to synthesize prostaglandins in vitro is inversely related to neoplasm size. These results suggest a defect in the removal of eicosanoids from large neoplasms. The physiological significance of this increase in the local levels of eicosanoids remains to be determined. However, a role in bone invasion can be envisaged because PGE₂ and PGF₂α are known osteolysins.

6.6 METASTASIS OF ORAL SQUAMOUS CELL CARCINOMA

Oral squamous cell carcinoma characteristicly metastasizes mainly via the lymphatics and less frequently via the blood stream and perineurally.
6.6.1 Lymphatic metastasis

Lymphatic metastasis of oral squamous cell carcinoma occurs via two mechanisms (Kissane, 1985b):

(1) metastatic embolization in which neoplastic cells embolize to the peripheral sinus of the regional lymph node where they multiply and eventually invade the lymphoid pulp and develop a supporting stroma; and

(2) lymphatic permeation: the continuous growth of neoplastic tissue within the lymphatic vessels usually accounting for local spread.

The precise role of lymph nodes in neoplasia remains unclear; however, they may represent an initial barrier, filter or destructive mechanism which is eventually overcome by the intensity or duration of neoplastic cell embolization.

Metastases to regional lymph nodes from oral squamous cell carcinoma show varying incidence rates according to site, size and histopathological grading of the neoplasm. Generally the principal lymph nodes involved in metastasis are the submandibular, superficial and deep cervical and occasionally the submental, pre-auricular, posterior auricular and supraclavicular (Di Troia, 1972; Shafer et al., 1974). For the incidence of lymph node metastasis by site of primary neoplasm and site of lymph node see Table 4. Carcinoma was found by Shingaki et al. (1985) to have had extended beyond the lymph node capsule in 30 per cent of positive lymph nodes and 52 per cent of cases had lymph nodes with extra-nodal spread. Lymph node metastases were present in 94 per cent of symptomatic, intra-oral squamous cell carcinomata in an Australian population (Fowler et al., 1980), 56 per cent of which had only one area involved while 38 per cent had multiple areas involved.

The pattern of flow of lymph rather than the detailed anatomy of the lymphatics per se is of greater importance in the spread of metastases (Sharpe, 1981). Lower labial vermillion and mucosal lesions show a low metastatic rate, the majority metastasizing to the submandibular and superior deep cervical lymph nodes. Lateral labial lesions first metastasize to the ipsilateral submandibular and submental lymph nodes, whereas central lesions may show bilateral lymphadenopathy. Nodal metastases of the superior lip are more diverse, and may initially affect the parotid, pre-auricular, facial, as well as the submandibular
TABLE 4: Incidence of lymph node metastasis (Shingaki *et al.*, 1985).

<table>
<thead>
<tr>
<th>Incidence (per cent)</th>
</tr>
</thead>
</table>

(1) By anatomical site

<table>
<thead>
<tr>
<th>Site</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oropharynx</td>
<td>75.0</td>
</tr>
<tr>
<td>Sinus maxillaris</td>
<td>52.9</td>
</tr>
<tr>
<td>Oral Cavity</td>
<td>40.5</td>
</tr>
</tbody>
</table>

(2) By site of lymph node

<table>
<thead>
<tr>
<th>Site</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Submandibular</td>
<td>36.5</td>
</tr>
<tr>
<td>Superior deep cervical</td>
<td>31.7</td>
</tr>
<tr>
<td>Middle deep cervical</td>
<td>7.9</td>
</tr>
<tr>
<td>Submental</td>
<td>6.3</td>
</tr>
<tr>
<td>Lower deep cervical</td>
<td>3.1</td>
</tr>
</tbody>
</table>
lymph nodes. The incidence of lymphadenopathy, both on admission and after treatment of the primary labial lesion, varies from 2-3 per cent and is inversely correlated with the degree of histological differentiation (Wurman et al., 1975). For example, Brown et al. (1976) found 11 per cent of well-differentiated lesions had or developed lymph node metastasis compared with 100 per cent of poorly differentiated lesions.

Buccal mucosal lesions metastasize in approximately 50 per cent of cases to the submandibular and superior deep cervical lymph nodes. Gingival lesions metastasize in greater than 50 per cent of cases to the submandibular and superior deep cervical lymph nodes. Floor of mouth lesions metastasize in 50-76 per cent of cases to the submandibular and superior deep cervical lymph nodes. Palatal lesions metastasize in 30 per cent of cases for the hard palate and in 60 per cent for the soft palate (Sharpe, 1981).

Anterior lingual lesions metastasize early in 30-50 per cent of cases to the submandibular, superior deep cervical and inferior deep cervical lymph nodes. Posterior lingual lesions have a higher metastatic rate of 62-91 per cent. Ipsilateral, bilateral or contralateral metastases are observed (Shafer et al., 1984; Martis et al., 1979; Crawford et al., 1979; Lucas, 1984c). Lingual lymph nodes are occasionally found along the course of some lymph vessels between the tongue and the cervical regional lymph nodes. These lingual lymph nodes may become the sites of metastases from lingual squamous cell carcinoma (Ozeki et al., 1985). Lingual lymph nodes are paralingual, intermediate lymph nodes appearing occasionally along the course of effluent lymphatic vessels from the lingual mucosa and muscle layer to the cervical lymph nodes. They are divided into two groups: (1) lateral lingual located either on the lateral aspect of the m. genioglossus or m. hyoglossus along the lingual blood vessels; and (2) median lingual nodes located on the central collecting lymphatics that run towards the floor of the tongue along the septum linguae.

For oropharyngeal squamous cell carcinoma the metastatic rate is high, between 60 and 80 per cent, to the superior deep cervical lymph nodes, especially initially the jugulodigastric node. Secondary involvement includes the submandibular and middle deep cervical nodes and rarely the submental or superior posterior cervical lymph nodes for anterior lesions. Posterior lesions secondarily involve the middle deep cervical more than the submandibular and show more widespread cervical nodal involvement.
including the posterior cervical triangle in 10 per cent of cases. Contralateral lymphadenopathy occurs in 23 per cent of posterior lesions and 7 per cent of anterior lesions (Shumrick and Quenelle, 1979).

Contralateral metastases occur in three ways (Di Troia, 1972): (1) by crossing afferent lymphatic vessels; (2) by actual spread over the midline via efferent lymphatics after regional lymph nodes become extensively involved producing collateral lymphatic flow; or (3) as in certain anatomical areas where there is no definite midline and the superficial lymphatic capillaries form a rich plexus with the capacity to direct flow to either side. The presence of contralateral nodal involvement confers a very poor prognosis. Contralateral metastases from lesions of the gingivae and buccal mucosa are rare. The incidence of contralateral metastasis from the tongue is 4 per cent for the anterior two-thirds to 25 per cent for the post-sulcal part. Apical lingual lesions become bilateral problems only when there is extension to the floor of the mouth where "cross-spread" is thought to occur via the submental nodes or by directly crossing collecting vessels in the floor of the mouth. When contralateral involvement of the floor of the mouth lesions occurs (17 per cent of cases), the predominant pathway is via the superior deep cervical with the submandibular involved two-thirds as frequently.

6.6.2 Trans-capsular or extracapsular metastasis

Trans-capsular or extracapsular spread of metastatic squamous cell carcinoma from cervical lymph nodes is a well recognized phenomenon with important clinical and prognostic implications (Carter et al., 1985; Johnson et al., 1985). Two aspects still remain confused, viz.: the reported incidence is variable; and there is no agreement regarding morphological extent. Undefined it can cover a range of changes from microscopic breaks in the lymph node capsule to gross invasion of the neoplasm into local tissues.

The incidence, extent and selected clinicopathological correlations of trans-capsular spread from metastases in the cervical lymph nodes was investigated by Carter et al. (1985) in 210 specimens obtained from 203 patients with squamous cell carcinoma of the head and neck by radical neck dissections. Trans-capsular spread was detected in 137 of 159 (86 per cent) positive specimens and classified as macroscopic in 74 (54 per
cent) and microscopic in 63 (46 per cent). Macroscopic trans-capsular
spread is more frequently associated with large nodal masses, greater than
3 cm in diameter (69 per cent), but also in some specimens with smaller
lymph nodes, less than 3 cm in diameter (39 per cent). Anatomical
structures most commonly invaded by macroscopic spread from nodal
metastases are skeletal muscle and the tunica adventitia of the internal
jugular vein. The occurrence and extent of trans-capsular spread is
correlated with three clinical events: (1) the subsequent development of
neoplasm in the ipsilateral neck; (2) its time of appearance; and (3) the
concurrent relapse of neoplasm at the primary site. Macroscopic trans-
capsular infiltration is associated with a high incidence (44 per cent) of
recurrence in ipsilateral neck, particularly within 12 months of surgery.
Microscopic trans-capsular growth is associated with a lower incidence (25
per cent) of recurrence but this difference is statistically insignificant.
Similar recurrence figures (32 per cent) occur in the minority of patients
whose modal disease is intracapsular at the time of neck dissection. No
correlations exist between the incidence and extent of trans-capsular
spread and the degree of differentiation of the nodal metastases or their
anatomical locations in the neck; or with the site and degree of
differentiation of the primary neoplasm.

The process whereby metastatic neoplastic cells break through the
nodal capsule is unclear. Mechanical disruption by an expanding neoplasm
is the most obvious suggestion but unlikely to operate in small lymph
nodes only partly replaced by neoplasm. An alternative explanation for
intracapsular growth is the spread from neoplastic emboli initially lodged
in capsular or juxtacapsular lymphatic vessels. There may be focal
destruction of capsular collagen by Type I collagenase synthesized by
both the neoplasm and the stroma since the incidence of trans-capsular
spread is not reduced in the often fibrotic neck dissections from
previously irradiated patients (Carter et al., 1985).

Some investigations (Johnson et al., 1985) have argued that trans-
capsular spread of metastatic neoplasm is the single most significant
determinant for predicting recurrent cervical neoplastic disease, but the
contribution of other local factors such as the number and size of
involved lymph nodes, their anatomical level or levels, and pre-operative
fixation are still being debated. Noone et al. (1974) found a poorer
prognosis in patients with palpable neoplastic lymphadenopathy; early
recurrence of neoplasm; extracapsular spread of metastasis; microscopic
evidence of desmoplasia and necrosis of lymph node; and confirmed nodal metastases. The rate of recurrence is very high (80 per cent) in patients having metastatic lymph nodes with histological evidence of extra-nodal spread (Shingaki et al., 1985). This local recurrence in the neck after radical neck dissection may result from metastasis from persistent or recurrent lesions at the primary site; dissemination of neoplastic cells during surgery; and incomplete removal due to the presence of lymph nodes with extra-nodal metastasis to lymph nodes outside the field of dissection. Treatment failure in cervical lesions is responsible for the recurrence in most cases.

Cervical lymph node metastasis of occult primary neoplasia needs to be considered in any adult who has asymmetric enlargement of one or more cervical lymph nodes. Spiro et al. (1983) found that of 132 patients treated for metastatic carcinoma of the cervical lymph nodes, with no apparent primary neoplasm, 60 per cent were metastatic squamous cell carcinoma, 22 per cent adenocarcinoma, 10 per cent anaplastic carcinoma and 8 per cent melanocarcinoma. The hypopharynx, rather than the nasopharynx, is more often the involved site when the primary neoplasm can be identified.

6.6.3 Distant metastasis

Distant metastases of head and neck squamous cell carcinoma are generally regarded to be infrequent below the clavicles, a generalization no longer considered valid (O'Brien et al., 1971; Merrick and Jensen, 1979; Papac, 1984; Lucas, 1984c). The clinical incidence of 10–30 per cent is generally not reliable because autopsy studies yield an increase about twice as great, i.e. about 50 per cent.

O'Brien et al. (1971) in an autopsy study of patients who died from squamous cell carcinoma of the head and neck found distant metastases in 46.7 per cent of cases. Sites showing the greatest tendency to metastasize are: the larynx (including the epiglottis, 63 per cent); hypopharynx (50 per cent); floor of mouth (64 per cent); base of tongue (39 per cent); lateral margin of tongue (27 per cent); palatine tonsil (27 per cent) and pharynx (17 per cent). Papac (1984) in a clinical and autopsy assessment found 30.7 per cent of cases of head and neck squamous cell carcinoma to have distant metastases. The incidence of metastasis varied according to site with the greatest tendency to
metastasize from the larynx (excluding the true vocal cords), hypopharynx, tonsillar fossa, oropharynx, tongue and floor of mouth.

The sites of metastases from head and neck squamous cell carcinoma include: lung; liver; bone; and less frequently gastro-intestinal tract; brain; skin; kidney; hilar and paratracheal lymph nodes; heart and adrenal glands; and thyroid gland (Papac, 1984).

The occurrence of distant metastases is related to primary neoplasm site, clinical stage at presentation and development of infectious complications during the course of the neoplastic disease (Papac, 1984). Locally extensive (T3 and T4) lesions are more likely to metastasize, as are lesions with a lymph nodal status of N1 and N3. Patients who develop metastases have a much lower incidence of infectious complications than patients who do not develop distant metastases (p < 0.005). It is suggested that infectious complications provide some stimulation of host immune mechanisms, with consequent inhibition of neoplasm dissemination. However, the duration of survival is unrelated to the development of distant metastases and there is no evidence to suggest that lethal infectious complications such as wound sepsis, sinusitis and pneumoniae reduce the potential for metastasis. Additionally, there is no indication that therapeutic modalities have any impact on the development of metastases.

With regard to oral squamous cell carcinoma visceral metastases are generally uncommon and practically never occur in the early stages of neoplasia. The sites with the greatest frequency to metastasize are the palatine tonsil, oropharynx, tongue and floor of mouth (Papac, 1984; Lucas, 1984a). Merrick and Jensen (1979) describe a rare, fulminant, well differentiated squamous cell carcinoma of the floor of the mouth of a young male (24 years old) with widespread metastases including some in the myocardium resulting in death five months after the initial symptoms. At autopsy, residual squamous cell carcinoma was present in the floor of the mouth and there were massive metastases in the right cervical lymph nodes, lungs, pleurae, sternum, vertebrae, os pubis, myocardium, liver, spleen, kidneys, adrenal glands, diaphragm and peritoneum. Additional findings included pericardial effusion, left-sided bronchopneumonia, calcium deposits compatible with nephrocalcinosis and fibrin deposits compatible with disseminated intravascular coagulation. The metastases showed a moderately-to-highly differentiated squamous cell carcinoma. This case demonstrates two important features: (1) squamous cell
carcinoma in the young is more virulent; and (2) that widespread metastases can result leading to death.

6.6.4 Perineural spread

Although the infiltration of peripheral nerves by carcinoma has been known for about 150 years, it has only recently been appreciated that the more common basal and squamous cell carcinomata along with the adenoid cystic carcinoma of the salivary glands and head and neck often spread via the peripheral nerves (Carter et al., 1983; Morris and Joffe, 1983). The most commonly affected cranial nerve is the trigeminal, followed by the facial and less commonly the greater auricular and oculomotor nerves. The early diagnosis of perineural spread of facial cutaneous carcinomata depends on the recognition of the triad of clinical features (Morris and Joffe, 1983): (1) presence or history of facial epidermal carcinomata; (2) initial symptoms and signs confined to superficial terminal branches of the trigeminal and facial nerves; and (3) the evolution of symptoms and signs being best explained on the basis of a lesion spreading centrally along the trigeminal or facial nerves involving branches of these nerves in the sequence in which they arise. In those patients with trigeminal involvement pain takes precedence over anaesthesia, paraesthesia or formication. Specialized radiological investigations may prove unhelpful in the diagnosis of perineural invasion, although modern computerized tomography may be potentially useful (Norman et al., 1981).

Infiltrating carcinomata frequently invade perineural spaces which provide a route for further dissemination, notably intracranially. The perineural spaces were originally regarded as lymphatics but now have been shown to be separate from any part of the organized lymphatic system (Larson et al., 1966; Rodin et al., 1967).

Perineural spread has been demonstrated histologically in 36 per cent of surgical resection specimens of head and neck squamous cell carcinoma and in 90 per cent of a necropsy series. Perineural infiltration is observed mostly in the vicinity of carcinomata arising in the buccal cavity (50 per cent of cases), and at all sites it is most commonly encountered near neoplasms greater than 2.5 cm in diameter. Perineural spread near cervical lymph node metastases is, by contrast, uncommon in the surgical series (Carter et al., 1983).
The basic histopathological changes of perineural infiltration involve
the characteristic concentration of neoplastic cells at the margin of the
nerve showing only limited extension inwards into the nerve bundle.
However, the neoplastic cells may track proximally or distally within the
perineural spaces. Distant spread for greater than two centimetres is
unusual, and interval sampling of involved nerves in necropsy material
indicates that most perineural neoplastic cells are confined to the distal
one centimetre of the affected nerve. Infiltrated nerve trunks may show
minor degenerative changes with varying degrees of myelin and axonal
degeneration, probably of anoxic origin and segmental infarction.
Immunohistochemical changes, demonstrated using monoclonal antibodies
against epitopes (antigenic determinants) within axonal and myelin
components of human neural tissue, regularly reveal fine structural axonal
and myelin changes (Carter et al., 1983).

An unusual case of a 70 year old male with squamous cell carcinoma
of the lip presenting with mandibular metastasis and multiple cranial
nerve palsies has been recently described by Banerjee and Gottschalk
(1984). The patient presented with right mental neuropathy, ipsilateral
progressive involvement of all three divisions of trigeminal nerve, facial
and vestibulocochlear nerves and complete ophthalmoplegia. Although
repeated studies of computed tomography (CT) scan of the head and
cerebral angiography were negative, positive cerebrospinal fluid (CSF)
cytology, high CSF protein and low CSF glucose strongly favoured
meningeal involvement at the brain stem level. Although haematogenous
dissemination from the primary labial squamous cell carcinoma to the
mandible and brainstem could not be ruled out, extension through the
perineural space via the trigeminal nerve was considered a speculative
possibility.

6.7 IMMUNOLOGICAL ASPECTS OF ORAL SQUAMOUS CARCINOMA

A review of the immunological aspects of malignant neoplasia of the
head and neck, with particular reference to oral neoplasia, has been
reported by Scully (1982a). The concept of immunological surveillance,
which proposed that the host recognizes a neoplasm as foreign and then
mounts a protective immune response against the neoplasm, is now
considered to be an oversimplification. The immune system may influence
the development of neoplasia, but whether a neoplasm develops because of
a failure of recognition mechanisms or because of a breakdown of the immune response remains unclear. Evidence for the influence of the immune response on malignant disease includes: (1) increased incidence of neoplasia with ageing; (2) increased incidence of neoplasia in primary immunodeficiencies, especially involving the lymphoid tissue, although there is no evidence of a predilection to oral squamous cell carcinoma; and (3) increased neoplasia in secondary immunodeficiencies, especially following renal transplantation, although no particular increase in oral squamous cell carcinoma has been noted.

6.7.1 Stromal and lymph nodal response

The stromal reaction under oral epithelial dysplasia and squamous cell carcinoma involves a predominantly mononuclear cell infiltrate distributed within the epithelium and neoplasm. The cell infiltrate consists of leukocytes, lymphocytes, plasmacytes, mast cells, macrophages and giant cells. Other aspects of the stromal reaction include the presence of Russell bodies, basal lamina defects and collagen fibre degeneration. There is a positive correlation between the total number of lymphocytic and plasmacytic infiltration and the grade of epithelial dysplasia. There is also a correlation between the cellular stromal reaction and differentiation of the neoplasm; with a highly differentiated lesion, e.g. verrucous carcinoma, having heavier infiltration, whereas there is a complete absence of infiltrate around deeply infiltrative and advancing front of squamous cell carcinoma. Lack of differentiation, poor lymphocytic response and mucosal change in stroma are signs of poor prognosis (Johnson, 1976; Seifert and Burkhardt, 1977).

The cell phenotypes of the stromal response in human oral squamous cell carcinoma have been identified more precisely by means of monoclonal antibodies (Löning et al., 1983; Hiratsuka et al., 1984a, b). In squamous cell carcinoma there is an increase in the numbers of T lymphocyte subpopulations: suppressor/cytotoxic, helper/inducer subtypes, macrophages and Langerhans’ cells. The vast majority of lymphocyte infiltrates in oral squamous cell carcinoma are T lymphocytes, with infiltration in the peripheral region of the neoplasm being more prominent than that in the stroma among the neoplastic nests. T cells surround the neoplastic nests, and frequently produce massive perivascular
accumulations. The grade of T lymphocyte infiltration: slight, moderate, marked, is correlated well with the size of the neoplasm and is more marked in patients without cervical lymph node metastasis. There is also a significant correlation between the grade of T cell infiltration at the periphery of the invading neoplastic mass in the initial biopsy specimens and the clinical neoplasm regression rates with bleomycin anti-neoplastic chemotherapy, but there is no such correlation with surgically resected specimens. This suggests that T lymphocytes might inhibit the development and dissemination of neoplastic cells and that T cell infiltration correlates with the clinical course or prognosis of oral squamous cell carcinoma. The degree of B cell infiltration is generally weak and negligible in the stroma and in the peripheral regions of the neoplasm. Macrophage infiltration is also less significant, being distributed predominantly in the stroma or in the superficial necrotic tissues.

The pathological significance of Langerhans' cells in oral squamous cell carcinoma has been studied by their positive reaction to S100 protein, a bovine brain extract, using peroxidase-anti-peroxidase (PAP) staining method (Kurihara and Hashimoto, 1984). Langerhans' cells show a suprabasal, intra epithelial distribution. They are less frequently located in neoplastic nests in central or superficial areas than in peripheral or deep areas. A positive correlation between the frequency of Langerhans' cells and the degree of differentiation, and lymphocyte infiltration exists. Langerhans' cells are more frequent in carcinomata in early stages of stromal invasion than in advanced stages. An intimate relationship between Langerhans' cells and lymphocytes is suggested by their manner of distribution in clusters consisting of Langerhans' cells and lymphocytes in the epithelium adjacent to the neoplasm, neoplastic stroma, nests and subjacent connective tissue in order of decreasing frequency. These findings suggest some immunological role of Langerhans' cells in oral squamous carcinoma, although the details remain to be determined.

The giant cell stromal reaction in squamous cell carcinomata has been studied electron microscopically and ultrahistochemically by Burkhardt et al. (1976), and Burkhardt and Gebbers (1977). These studies have demonstrated unequivocally that giant cells in the stroma of squamous carcinomata are not neoplastic giant cells but are macrophage polykaryons formed by the fusion of macrophages of monocytic origin. Avid phagocytic activity of multinucleated giant cells in bleomycin-treated
carcinomata was demonstrated. These cells phagocytize devitalized, keratinized neoplastic cells by a unique form of phagocytosis and digestion. The mode of intracellular ingestion is unusual in that no distinct digestive vacuoles or formation of phagolysosomes occurs. The enveloping plasma membrane of the giant cell is apparently "melted down" partly or completely and high concentrations of hydrolases are built up around the keratinized neoplastic cells. The hydrolases are not membrane limited at this stage, and the surrounding cytoplasm shows a fine granular area which is free of organelles, analogous to the "clear zone" or hyalin rim of osteoclasts. This zone is interpreted as a seal protecting the rest of the cell against autolysis. This mode of digestion can be considered as a combination of extracellular and membrane-bound intracellular digestion showing many parallels to the highly specialized activities of osteoclasts.

The resorptive activity in the stromal reaction of squamous cell carcinomata is a morphologically and functionally complex process of cooperation of macrophages. This again is only a part of the stromal reaction during neoplasm regression, induced by bleomycin chemotherapy, which is further characterized by immunological cellular interactions and cytotoxic activity of macrophages and lymphocytes, the appearance of stimulated plasma cells, concomitant nonspecific inflammatory infiltrate and changes in the connective tissue (Burkhardt and Gebbers, 1976).

Human oral squamous cell carcinoma has been shown to produce colony stimulating activity and granulocytosis, accounting for a marked granulocytosis of unknown origin observed in some patients with malignant neoplasia (Okabe et al., 1978; Sato et al., 1979).

Eosinophil infiltration in kerato–acanthoma and squamous cell carcinoma of the skin was studied by Lowe et al. (1984). Eosinophil infiltration is more commonly found in well–differentiated, keratinizing squamous cell carcinoma. It is unrelated to the size, site or aetiology of the lesions. The pattern of tissue eosinophilia in late cases of squamous cell carcinoma is more extensive than for early lesions. The presence of eosinophilia in cases of squamous cell carcinoma may be related to the production of an "eosinophilochemotactic factor" reported in squamous carcinoma at other sites. Although eosinophilia per se is not a diagnostic feature it should be added to the list of criteria which help to distinguish between kerato–acanthoma and squamous cell carcinoma.
Regional lymph nodes have been classified into four morphological and immunological patterns (Noone et al., 1974; Jansa and Pastrnák, 1980; Ring et al., 1985):

(1) lymphocyte predominance or sinus histiocytosis, which supposedly reflects the active response of the thymus-dependent (T) cells linked to cellular immunity;

(2) germinal centre predominance, which indicates an active response in the thymus-dependent germinal centres related to humoral immunity;

(3) unstimulated, reflecting no discernible response; and

(4) lymphocyte depletion, referring to a lymph node with a paucity of lymphocytes.

A statistically significant prognostic value of regional lymph node patterns of immune response has been demonstrated. Lymphocyte predominance or sinus histiocytosis pattern has a better prognosis than either germinal centre predominance or unstimulated patterns. These correlations are independent of the clinical stage and metastatic nodal status.

Studies of cell populations of regional lymph nodes in patients with head and neck malignant neoplasia have suggested an increase in the nodal content of B lymphocytes. However, the nodal lymphocytes appear to be unable to mediate cytolysis of immunoglobulin-coated target cells suggesting there are changes in T lymphocyte subpopulations (Scully, 1982a).

6.7.2 Immunological changes

The immunological changes detected in patients with head and neck malignant neoplasia include (Scully, 1982a):

(1) cell-mediated immunity: impairment of delayed hypersensitivity reactions, reduced T lymphocyte numbers, reduced T lymphocyte responses to mitogens and some antigens, immune complexes or suppressor cells may be implicated;

(2) humoral immunity: increases in serum glycoproteins, serum carcino-embryonic antigen, serum beta-2 macroglobulin and loss of blood group iso-antigens A and H from neoplastic cells; and

(3) other humoral factors: increase in serum IgA and IgE.
Depression in cell-mediated immune response is the most obvious immunological change associated with head and neck neoplasia. Although it is difficult to establish whether the defect in cell-mediated immunity is primary or secondary to neoplasia, the former is favoured since cellular responses remain depressed after surgical treatment. However, exogenous factors, viz.: alcohol, especially with hepatic disease; malnutrition; smoking; chemotherapy; anaesthesia, surgery and radiotherapy may decrease the immune responsiveness of patients with malignant neoplasia. Anergy to antigens including recall antigens: antigens which host has been previously exposed to; is a feature particularly of advanced malignant neoplasia of the head and neck, appearing earlier in carcinoma than in sarcoma or melanocarcinoma. Leukocyte function, i.e. a decrease in lymphokine production and inhibition of leukocyte adherence has been shown to be impaired in head and neck cancer.

T lymphocyte function (as assessed by the lymphoproliferative response: lymphocyte transformation to T lymphocyte mitogens such as phytohaemagglutinin and concanavalin A) is depressed, as is lymphocyte transformation in response to Herpesvirus hominis type 1 and Candida albicans antigens (Lehner et al., 1973a,b; Shillitoe and Silverman, 1979).

Changes in humoral immunity are less profound and less well defined than are defects of cell-mediated immunity. Also, since immunoglobulin synthesis may be T lymphocyte dependent, any defects in synthesis may be explicable in terms of defective cell-mediated immunity. A pronounced humoral immune reaction with local accumulation of plasmacytes may occur in dysplastic leukoplakia and oral squamous cell carcinoma (Löning and Burkhardt, 1979). The local immune homeostasis of the oral mucosa with premalignant and malignant lesions varies with the degree of dysplasia, differentiation of the neoplasm and therapy. Plasmacyte density increases with the grade of epithelial dysplasia and is proportional to the degree of differentiation and the amount of keratin in carcinomata. Plasma cell response decreases following radiotherapy but remains unchanged following bleomycin therapy. The distribution of IgA and IgG in the epithelium reveals a localization throughout all epithelial strata in leukoplakias with dysplasia and carcinomata, indicating a leakage of locally synthesized immunoglobulins through an altered oral mucosa.

Increased serum concentrations of IgA and IgE, with normal levels of IgG, IgM and IgD have been described in oral squamous cell carcinomata (Scully, 1982b). These raised levels of IgA and IgE may reflect the
changes in cell-mediated immunity, the production of both immunoglobulins being related to T lymphocyte activity. Humoral immune responses may enhance neoplasia by the production of blocking factors in the serum, which can either be immunoglobulins that block the recognition by the host of neoplasm-associated antigens, or immune complexes that might block cell-mediated immune responses. Other humoral factors that might influence cell-mediated immune responses include: (1) several immune-reactive proteins (IRP), particularly certain serum glycoproteins, e.g. haptoglobin, alpha-1 acid glycoproteins and alpha-1 antitrypsin; the serum levels of which are inversely correlated with anergy to dinitrochlorobenzene (DNCB); and (2) impaired lymphoproliferative responses to phytohaemagglutinin (PHA). The levels of other proteins, such as pre-albumin and alpha-2 HS glycoprotein correlate directly with both humoral and cell-mediated immune responses. While humoral factors may suppress cell-mediated immune responses, there are also various suppressor leukocytes that may regulate cell-mediated immune responses in head and neck malignant neoplasia (Scully, 1982a).

A mathematical model of the macrophage–T lymphocyte interactions that generate an anti-neoplastic immune response has been recently described by De Boer et al. (1985). The model specifies that induction of cytotoxic T lymphocytes and antigen presentation (opsonization) by macrophages leads to the activation of helper T cells and the production of lymphoid factors which induce cytotoxic macrophages, T lymphoblastosis and an inflammatory response. The model shows that helper T cells play a crucial role in neoplasm behaviour: neoplasms that differ minimally in antigenicity, i.e. helper reactivity, can differ markedly in rejectability. Immunization was found to yield protection against neoplasm doses that would otherwise be lethal, because it increases the number of helper T cells. The magnitude of the cytotoxic effector cell response depends on the time at which the helper T cells become activated: early helper activity steeply increases the magnitude of the immune response. The type of cytotoxic effector cells that eradicates the neoplasm depends on neoplasm antigenicity: low antigenic neoplasms are attacked mainly by macrophages, whereas large, highly antigenic lesions are eradicated by cytotoxic T lymphocytes only.

The effects of specific anti-neoplastic responses such as natural killer cells, cytotoxic T lymphocytes, activated macrophages and immunoglobulins are most relevant in the immunological control of
neoplasia by the host. Interleukin-2, a lymphokine (also called T cell growth factor), can generate T lymphocytes and other accessory cells cytotoxic for neoplastic cells, thereby potentiating the immune regression of solid head and neck neoplasia. Hargett et al. (1985) found that the depressed T cell proliferative response to phytohaemagglutinin commonly observed in head and neck cancer is not explicable by impaired interleukin-2 synthesis. Other explanations may include abnormalities in lymphocyte receptors for interleukin-2 or transferrin or possibly depressed transferrin levels in the serum. However, it should be noted that the peripheral blood lymphocytes used to assay interleukin-2 activity may not necessarily reflect levels of production in tissues or lymph nodes.

Marked reduction in random migration and chemotaxis of monocytes occurs in squamous cell carcinoma of the head and neck (Walter and Danielson, 1985). It is suggested that the results indicate that a monocyte defect in neoplasia either affects binding of all chemotaxins to all classes of receptors or affects some subsequent common activation step. Although the full significance of depressed monocyte chemotaxis in human neoplasia is not known, two general consequences are likely, viz.: (1) decreased monocyte chemotaxis may occur prior to neoplasm initiation or during the active growth phase; and (2) decreased host resistance to microbial infection may result which may, in part, account for the high incidence of mortality caused by bacterial infection in terminal cancer patients.

Natural killer (NK) cells comprise an heterogeneous population of effector cells, both functionally and phenotypically distinct from B lymphocytes and mature antigen-sensitive T lymphocytes, with the capacity to spontaneously lyse target cells of widely different tissue provenance in a genetically unrestricted manner. Consequently, NK cells have been widely implicated in immunosurveillance against neoplastic and virus-infected cells, as well as in the homeostasis of haematopoetic differentiation and regulation of immune function (Bishop et al., 1984; Saljo et al., 1984; Kimber and Moore, 1985). In common with cytotoxic T lymphocytes, the cytolytic mechanism may be resolved into several discrete stages: (1) target cell recognition by NK cells following transient contact of some undefined membrane structures; (2) induction of NK cells with elaboration of a factor or factors which trigger the cytotoxic process; and (3) disruption of the target cell membrane integrity involving the formation of ultrastructurally discrete lesions. Target cell recognition
appears to involve several chemical entities, e.g. glycoproteins and glycolipids, while susceptibility is also influenced by a multiplicity of factors operative at post-recognition stages of the cytolyis.

Natural killer cell-mediated cytolyis is subject to various regulatory influences. Potentiating effects of interferon (IFN-alpha, beta, gamma) and interleukin-2, products of activated T lymphocytes, indicate a possible pathway by which adaptive immune responses may augment natural cytotoxicity under local physiological conditions (Kimber and Moore, 1985). IFN appears to modulate natural cytotoxic function by intervention at several stages, e.g. IFN can directly increase the numbers of active NK cells with the generation of cytotoxic activity in target-binding by non-lytic lymphocytes and can enhance the kinetics of cytolyis by mature NK cells and possibly increase their capacity to recycle and interact with multiple target cells. Other immunological functions of IFN in head and neck cancer have been observed (Sato et al., 1984). Interleukin-2, an immuno-regulatory lymphokine central to the clonal expansion of T lymphocytes, has been shown to enhance NK cell activity and proliferation, although it is unclear whether it is itself sufficient for the potentiation of cytolyis or acts solely through the induction of interferon (Kimber and Moore, 1985). Negative regulation is mediated by certain prostaglandins (PGE1, PGE2, PGD2) and a variety of cell types including macrophages, granulocytes and thymocytes, as well as subsets of peripheral blood lymphocytes. The mechanism of action, specificity and physiological relevance of such putative suppressor cells has yet to be defined.

Natural killer cells may have a key role in the control of metastases as results indicate that depression of NK activity induced by chemotherapy results in the promotion of metastatic disease (Saljo et al., 1984). NK cells have also been shown to recognize Herpesvirus hominis type 1 glycoproteins: cell surface components. There is a positive correlation between the expression of viral glycoproteins gB and gC and susceptibility of Herpesvirus hominis type 1 infected target cells to NK cell activity. Moreover, mutations affecting glycoprotein epitopes defined by monoclonal antibodies also influence the interaction between NK effector cells and viral-infected target cells, suggesting that viral antigenic sites recognized by NK cells overlap with those recognized by anti-Herpesvirus hominis type 1 antibodies (Bishop et al., 1984).
6.7.3 Immunological changes associated with viruses

There exists some evidence of the involvement of *Herpesvirus hominis* type 1 (HVH-1, also called HSV-1: herpes simplex virus type 1) in head and neck and possibly oral squamous cell carcinoma (Hollinshead and Tarro, 1973; Tarro and Sabin, 1973). Serum immunoglobulins to non-virion antigens (not structural components of the virion) of HVH are shown to be specifically increased in head and neck cancer. Soluble membrane antigens from labial carcinoma react with this immunoglobulin implying immunological identity of the "tumour-associated antigen" with the non-virion antigen.

Lehner *et al.* (1973a, b) reported that cell-mediated immune responses to HVH-1 are specifically depressed in patients with a leukoplakia without epithelial dysplasia. When epithelial dysplasia is present the lymphocyte transformation response is specifically elevated, reaching levels seen otherwise only in patients with an active primary or recurrent herpetic infection. The macrophage migration inhibition responses to HVH-1 is also elevated in patients with epithelial dysplasia.

Viral-specific humoral immunity to *Herpesvirus hominis*-induced antigens in head and neck squamous cell carcinoma has been described by Smith *et al.* (1976). Titres of serum IgA specific for HVH-1-induced antigens are increased in squamous cell carcinoma, but the titre appears to be independent of the total serum IgA levels, indicating that the rise in serum IgA titre cannot wholly be accounted for by *Herpesvirus hominis* immunoglobulins; e.g. a high titre may also be present in heavy cigarette smokers. It has been suggested that these findings provide new evidence for an association between *Herpesvirus hominis*, heavy cigarette consumption and head and neck squamous cell carcinoma.

Recently, Shillitoe *et al.* (1984) found that oral squamous cell carcinoma is associated with a significantly higher IgM response with two peaks, at 4 and 48 hours after infection with *Herpesvirus hominis* type 1 antigen and virion, as well as an IgA response with peaks at 8 and 48 hours. These results suggest the existence of at least two different HVH-1 antigens associated with oral squamous cell carcinoma. Both are late antigens, one is recognized by IgA and the other by IgM immunoglobulin. Further evidence corroborating the fact that oral squamous cell carcinoma is associated with the expression of HVH-1 antigens that stimulate IgM rather than IgG immunoglobulin response has
been provided by Shillitoe et al. (1982) and an enzyme–linked immunoabsorbent assay (ELISA) method (Shillitoe et al., 1983). The data are considered consistent with a role for both *Herpesvirus hominis* type 1 and smoking in oral carcinogenesis. The detection of RNA complementary to *Herpesvirus hominis* in 53 per cent of squamous cell carcinoma compared to none in normal oral mucosa, although not specifically implicating HVH–1 rather than HVH–2, provides some of the strongest evidence yet available for an association between *Herpesvirus hominis* type 1 or 2 and oral squamous cell carcinoma according to Eglin et al. (1983) and Scully and Ward-Booth (1984).

6.7.4 Tumour–associated antigens

Among the changes associated with neoplasia are changes in cellular antigens, including the re-appearance of foetal antigens (oncofoetal antigens), the appearance of new antigens ("tumour–associated transplantation antigens", TATA) and loss of some antigens such as some HLA antigens and blood group iso–antigens (Old, 1981).

Carcino-embryonic antigen (CEA), an oncofoetal antigen, serum levels are increased in head and neck squamous cell carcinoma, especially in cigarette smokers. However, CEA assay is not sufficiently specific to be of diagnostic value (Silverman et al., 1976; Toto, 1979).

"Tumour–associated transplantation antigens" have received very little attention in head and neck malignant neoplasia studies. Beta–2 microglobulin, a low molecular weight constituent of cell surface histocompatibility antigens (HLA antigens), is released in small quantities into the serum normally and in increased amounts in patients with oral premalignant lesions and carcinoma (Scully, 1982a). This increased release to beta–2 microglobulin may reflect an immune response to the neoplasm or changes in cell recognition associated with neoplasia.

Cellular antigens that may be lost in oral squamous cell carcinoma include blood group iso–antigens A and B (Dabelsteen and Fulling, 1971; Dabelsteen and Pindborg, 1973; Liu et al., 1974; Dabelsteen et al., 1975; George et al., 1980; Schaumburg–Lever et al., 1984) and receptors for the lectin *Ricinus communis*, which specifically binds to beta–D–galactopyranosyl residues of cell surface glycoproteins and glycolipids (Dabelsteen and MacKenzie, 1978). These findings imply changes in carbohydrate moieties of the plasma membranes of oral neoplastic cells.
Recent studies have demonstrated that the number of epidermal growth factor (EGF)\(^1\) receptors is elevated in squamous cell carcinoma cells in tissue culture and in biopsy specimens of human squamous carcinomata of the lung, detected with a murine monoclonal antibody, EGF-R1, which binds specifically to the receptor (Hendler and Ozanne, 1984). The increases in receptor ranged from two-and-a-half to five fold that of normal skin, and occur in all bronchial squamous carcinomata and cutaneous squamous cell carcinomata of the head and neck; while 7/8 adenocarcinomata, all small cell and 4/8 undifferentiated bronchial carcinomata exhibit negligible amounts of EGF receptor. It was suggested that EGF receptor may be an excellent marker for squamous cell carcinomata.

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1. Epidermal growth factor stimulates both cell proliferation and keratinization (Cohen and Carpenter, 1975).
CHAPTER 7

DIAGNOSIS OF ORAL SQUAMOUS CARCINOMA

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CHAPTER 7

DIAGNOSIS OF ORAL SQUAMOUS CARCINOMA

The diagnosis of overtly symptomatic oral squamous cell carcinoma is not difficult. The clinical signs; ulceration, induration, fungation, fixation, bleeding and pain, are readily obvious. Cervical lymphadenopathy or extra-oral swellings lead to the examination of the oral cavity, oropharynx, nasopharynx and hypopharynx for the overt primary malignant lesion. Greater difficulty is encountered with the diagnosis of early asymptomatic oral squamous cell carcinomata.

Although the oral cavity and oropharynx are easily accessible to examination, 60 per cent of oral malignancies present late as well advanced lesions. Consequently, the surgical morbidity and mortality remain high, with a five-year survival rate of about 35 per cent (American Cancer Society, 1976). By comparison, skin and labial vermilion squamous cell carcinomata have survival rates of 90 per cent (Mashberg and Garfinkel, 1978). This emphasizes the importance of the detection of early malignant lesions, establishing a definitive diagnosis and implementing appropriate treatment at the earliest stage to enhance the prognosis.

Since the patient is unable to discern the insidious neoplastic changes in the oral mucosal membrane, it is incumbent upon the dental surgeon or medical practitioner to recognize these early changes and thereby reduce the morbidity and mortality significantly. This can be achieved by adopting the following diagnostic procedures:

(1) identification of early asymptomatic lesions;
(2) initial evaluation of suspected lesions: history, physical examination, photographic and/or diagrammatic recording of the lesion, elimination of predisposing factors and re-evaluation in 10–14 days;
(3) toluidine blue vital staining of suspected lesions;
(4) exfoliative cytology especially of ulcerative lesions; acridine binding of exfoliated cells;
(5) biopsy and histopathological examination for a definitive diagnosis; and
(6) evaluation of histopathologically proven neoplasia: complete medical history, physical examination, routine radiographic examination and laboratory tests, computed tomography, radio-nuclide scans, liver-spleen scans, panendoscopy and clinical staging.

Consultations are obtained from all the various services that may contribute to the patient's care, including: surgery, radiotherapy, chemotherapy, dental service, dietetics, psychiatry, speech therapy, social services, rehabilitation medicine and the clergy.

New diagnostic, or potentially diagnostic techniques are constantly being developed and an update of some of these techniques relevant to oral malignant neoplasia will be discussed later in this chapter.

7.1 DIAGNOSIS OF EARLY ORAL SQUAMOUS CELL CARCINOMA

Epidemiological studies have confirmed that the factors relevant to the prognosis of oral squamous cell carcinoma include: evidence of distant metastases; clinically positive, fixed regional lymphadenopathy; patient's age over 70 years; neoplasm size greater than four centimetres in diameter; and deep neoplasm infiltration (Platz et al., 1983, 1985). Less accurately, the TNM classifications system (U.I.C.C., 1978; A.J.C., 1978) and the STNMP system (Rapidis et al., 1977) have shown that clinical stage I lesions have a better prognosis than more advanced lesions. Therefore, it is quite obvious that there is a need to diagnose oral malignancy early. This is not a new concept, but it has only recently received new impetus.

Mashberg et al. (1973) and Mashberg and Garfinkel (1978) have stressed the importance of the early detection of asymptomatic oral squamous cell carcinoma. They argue that the main factor in the failure to detect early, asymptomatic, easily treatable malignant lesions is related to the propagation of ambiguous and inaccurate criteria for the diagnosis of malignant and "potentially" malignant oral mucosal lesions. There are misconceptions concerning oral neoplasia regarding its aetiology and pathogenesis. Undue emphasis is placed on the importance of recognizing "precancerous" leukoplakia oris and symptomatic squamous carcinoma, with a concomitant neglect in identification of asymptomatic and premalignant and malignant lesions. They conclude that persistent asymptomatic erythroplakia, rather than leukoplakia, in high risk sites of the oral
mucosa is the earliest and primary sign of oral carcinoma. An asymptomatic, small (less than or equal to two centimetres) erythroplakic lesion with or without keratotic components (speckled erythroplakia) in the floor of the mouth, soft palate complex and ventrolateral tongue, if its duration is 14 days or longer, should be considered carcinoma in situ or squamous cell carcinoma unless proven otherwise by biopsy. With the early recognition and treatment of these carcinomata there should result a decrease in surgical morbidity and an increase in survival rates.

A thorough oral examination of all patients, but especially of high risk patients, e.g. elderly males who are heavy smokers and alcohol consumers, is mandatory for the early detection of oral squamous carcinoma and should be incorporated as a regular clinical procedure for all dental patients (Mashberg et al., 1973; Mashberg and Garfinkel, 1978; Robertson and Hornibrook, 1982; Carl and Sako, 1982; Orlan, 1983). Oral cancer mass screening has been advocated by Maldonado (1979); but it is really not feasible because it is not cost-effective. However, as a public education exercise it may be worthwhile. Atterbury (1979) advocates patient self-examination of para-oral tissues for the detection of early oral neoplasia. He asserts that "... with relatively little instruction ..." patients can learn to detect early signs and symptoms of oral neoplasia, thereby assuring timely, accurate pathological diagnosis and specific early treatment resulting in better prognosis. Programmes to educate the community and relevant health professionals have been conducted by Hall et al. (1980), inter alios. Hall et al. (1980) found that only by making dental practitioners aware of the immediate rewards (unfortunately pecuniary) could they be stimulated to integrate a programme of early detection into daily clinical practice.

In an attempt to determine the reasons for the delay in the recognition of oral cancer Cooke and Tapper-Jones (1977) studied the case histories of fifty oral squamous cell carcinoma patients. The period of time between the earliest onset of symptoms and the seeking of professional advice was 2–6 months for 65 years of age or below. The main reason for the delay was lack of pain. A further delay of 7–14 days to 1–2 months occurred between the time of the patient's first complaint and confirmation of diagnosis, which often reflects the need for a second biopsy. A further period of 8–28 days occurred between confirmation of diagnosis and the start of definitive treatment, reflecting the need for consultation by various allied departments. A survey of all known cases
of "carcinoma" of the oral cavity and oropharynx in a population of half a million Yorkishmen showed that the late diagnosis in the majority of cases is inevitable (Williams, 1981). There is no evidence of undue delay by the patient, once symptoms present, or by the medical and dental practitioner or by the hospital responsible for patient referral. Williams concludes that only a small minority of patients (18 per cent) would have been influenced by a vigorous cancer education programme and that the poor prognosis of this disease can only be improved by more effective treatment of the advanced lesion, since its late diagnosis is an inevitable occurrence, a view not shared by other workers.

If one accepts the claims by Mashaberg and Garfinkel (1978) that persistent asymptomatic erythroplakia, rather than leukoplakia, in high risk sites of the oral cavity is the earliest and primary sign of oral squamous cell carcinoma, then the early recognition of such lesions is of paramount importance. The identification of such asymptomatic lesions is facilitated by drying the oral mucosa with sterile gauze. In this manner the lesions appear more granular. Palpation is rarely of diagnostic significance because the lesions rarely are ulcerated, raised or indurated. The erythroplastic areas may contain patchy or speckled keratin, i.e. appear as erythroleukoplakia or speckled erythroplakia. A granular, elevated or speckled surface in an erythroplakic area should arouse suspicion of the neoplastic nature of the lesion. Early lesions are often amenable to aggressive local treatment with minimal morbidity and optimal survival.

Squamous cell carcinoma of the lower labial vermilion may be unsuspected and undiagnosed in its early clinical stages and may appear in occult and non-characteristic forms (La Riviere and Pickett, 1979). A review of the literature reveals that most of the accumulated data on squamous cell carcinoma of the lower lip is from frank, established malignant lesions, typically described as "... fungating, crusting, pustular lesion ..." or "... irregular ulcer with firm raised borders and an indurated base ...". Early lesions of the lip are not as characteristic and noticeable as the more advanced forms. Most labial lesions with foci of squamous cell carcinoma, severe epithelial dysplasia, or both, are characterized by the following clinical signs:

(1) generalized variegated, red and white, blotchy appearance of the labial vermilion;
(2) generalized dry, atrophic appearance with focal areas of leukoplakia;
(3) persistent chapping with localized flaking and crusting; and
(4) indistinct, "wandering" vermilion border.

7.2 INITIAL EVALUATION OF SUSPECTED MALIGNANT LESION

The initial evaluation of any oral lesion begins with a medical and dental history followed by a physical examination, both extra-oral and intra-oral (Killey et al., 1975; Moore, 1976; Brightman, 1984a). The art of history taking has evolved over centuries. It is probably the most important singular step in the diagnosis of a medical or surgical condition. It should be a planned conversation designed to enable the patient to communicate his symptoms, signs, emotions and anxieties to the clinician.

Medical questionnaires are to be deplored if simply given expeditiously as a time-saving method. They are not a substitute for a proper, personally obtained history. The assignment of the responsibility of history taking to an auxiliary is highly undesirable. It depreciates the importance of this diagnostic procedure. A completely taken case history usually leads the clinician to a correct diagnosis before he even begins the actual physical examination of the patient.

On completion of the case history a detailed examination of the extra-oral and intra-oral structures should be performed in a logical and systematic manner. Standard methods of performing a physical examination are to be found in surgical and medical textbooks on diagnosis (Davis and Bolin, 1977; Clain, 1980; Bouchier and Morris, 1982; MacLeod, 1981, 1983; Browse, 1983; Swash and Mason, 1984). In oral medicine and oral surgery an abbreviated physical examination technique is commonly employed (Killey et al., 1975; Brightman, 1984a). The order of the examination is a matter of individual preference, but it is more effective if a systematic, routine examination procedure is adopted.

Palpation of the lymph nodes of the head and neck is an essential part of every examination. Infections, reactive hyperplasia secondary to neoplasia, and neoplasia, both primary and metastatic can be detected.

The accuracy of the clinical assessment of cervical lymphadenopathy is reported to be between 70 and 80 per cent for the deep cervical lymph nodes, falling to 44 per cent for submental and submandibular nodes
(Cady and Catlin, 1969). Furthermore, the palpability of a lymph node depends upon its location and consistency, e.g. in the cervical region the lower limit of palpability is 5 mm in diameter in the superficial area and 10 mm in a deeper area (Di Troia, 1972). The determination of a "fixed" lymph node, defined as one that is immobile and fixed to the underlying structures, as opposed to "freely mobile" or "reduced mobility" nodes is related to the size of the lymph node involved (Spiro et al., 1974). Large metastatic lymph nodes (greater than or equal to 6 cm) exhibit greater number of fixation (25 per cent) and reduced mobility (65 per cent) than smaller metastatically involved lymph nodes (less than 3 cm).

Whilst examining the lymph nodes specific attention should be given to size, location, consistency and mobility. Lymphadenopathy due to metastatic carcinoma is generally described as "rock hard" in consistency, whereas lymphadenopathy due to Hodgkin's disease is described as "rubbery hard" (Killey et al., 1975). Soft, freely mobile and tender lymph nodes suggest an inflammatory reaction. Fibrotic lymph nodes from previous episodes of oral pharyngeal infections may prove difficult to distinguish from neoplastically involved lymph nodes.

Careful scrutiny and palpation of all areas of the oral cavity and oropharynx, especially the posterior-third or base of the tongue, is required (Atterbury, 1979; Lumerman et al., 1982; Shugar et al., 1982; Orlan, 1983). Routine screening for squamous cell carcinoma of the base of the tongue in high risk patients, i.e. elderly males with histories of excessive consumption of tobacco and alcohol, is highly desirable because of the poor prognosis: less than 15 per cent of cases survive for five years. A technique for the routine screening of squamous cell carcinoma of the base of the tongue, sweeping the index finger under constant gentle compression, is described by Shugar et al. (1982). The late diagnosis of squamous cell carcinoma of the base of the tongue has been attributed to the relative lack of regional "pain" fibres, the often infiltrative rather than ulcerative nature of the lesion and the inaccessibility of the area to examination leading to a more advanced lesion at diagnosis (Du Pont et al., 1978).

All lesions found during examination should be described in detail with attention to location, size, colour, texture and other significant physical characteristics. Photographs, especially colour transparencies, often prove invaluable for subsequent comparisons. Diagrams are useful if
completed accurately as described by Roed-Petersen and Renstrup (1969) and Jolly (1976b).

Following the case history and examination a provisional or differential diagnosis is made. All potentially noxious habits should be identified and attempts made to alter or abolish them. Probable sources of irritation should be reduced or eliminated whenever possible. An observation appointment for the re-evaluation of a suspected malignant lesion should be scheduled 10 to 14 days later. This interval of time permits the resolution of inflammatory lesions. Lesions persisting beyond the observation period without apparent cause should be considered neoplastic until proven otherwise by histopathological examination (Brightman, 1984d).

7.3 TOLUIDINE BLUE VITAL STAINING

Tolonium chloride (toluidine blue 0)1 application has been established as an efficacious diagnostic adjunct in the detection of asymptomatic oral squamous cell carcinoma (Poswillo, 1975; Mashberg, 1980, 1981, 1983; Silverman et al., 1984), both when applied topically or when used as a rinse for screening of high risk patients.

7.3.1 Chemistry

Tolonium chloride (toluidine blue 0) is a basic, thiazine, metachromatic dye. Its chemical name is 3-amino-7-dimethylamino-2-methylphenazathionium chloride or 3-amino-7-dimethylamino-2-methylphenothiazin-5-ium chloride (Reynolds, 1982; Windholz, 1983).

7.3.2 Topical application

The topical application of tolonium chloride has been extensively studied by Mashberg since 1973 as an in vivo diagnostic adjunct in the detection of asymptomatic oral squamous cell carcinoma. Following a carefully taken history and clinical examination suspected early neoplastic lesions are re-evaluated after 10 to 14 days using toluidine blue 0 staining. This time interval allows the resolution of inflammatory lesions,

1. Either term is correct and shall be used interchangeably here.
thereby decreasing the number of false positives, i.e. under-diagnosis, of 6.7 per cent and a false positive rate, i.e. over-diagnosis of 8.5 per cent. Combining clinical criteria and toluidine blue 0 staining, both diagnostic modalities reduce the false negative rate to 1.9 per cent.

Using a slightly modified technique Silverman et al. (1984) showed an overall accuracy of toluidine blue 0 uptake of 91 per cent. In both dysplastic and malignant lesions the false negatives were 2 per cent.

Although the interpretation of the staining characteristics of early carcinoma is more objective than are clinical impressions before staining, some degree of familiarity with stain interpretation is desirable. If an entire lesion or a portion of a lesion stains dark blue in a solid or stippled fashion, neoplasia must be considered. An occasional circumscribed, light blue equivocal stain must also be considered positive unless proven otherwise by biopsy. Normal tissue will not absorb the stain; however, small areas of intense stain mechanically retained may be observed. These may be removed with gentle swabbing with ethanoic acid. Large areas of excess stain may accumulate on the dorsum linguæ, areas coated with surface débris or keratin, and gingival sulci. Occasionally a film of stain from the dorsum linguæ may be transferred to portions of the soft palate as a result of deglutition: the stain is not well circumscribed but appears diffuse and amorphous. A light blue film may also be observed over a large area of mucosa as a result of saliva tinged with dye (Mashberg, 1980, 1983). The area of stain in a neoplastic lesion is variable depending on the nature of the lesion's surface. For example, an entirely erythroplastic lesion, with granular or atrophic mucosa stains uniformly dark blue, whereas a speckled erythroplastic lesion stains in a speckled or patchy manner: the stain not penetrating areas of keratosis. Erythema due to inflammation and lesions with limited epithelial dysplasia stain variably: the stain does not appear to penetrate unless there is mucosal atrophy or a granular surface. Since the pathological diagnoses of the degrees of cellular atypia and epithelial dysplasia are frequently equivocal, a correlation between staining and pathological diagnosis is difficult. Consequently, the results of staining dysplastic lesions, not yet frankly neoplastic, are of questionable value.

Small areas of intense stippled blue stain are considered much more significant than broad diffuse areas of light blue. It should be noted that such small areas, which may represent carcinoma in situ or squamous cell carcinoma may not be detected histologically because the area in question
may be missed in the mechanical preparation of the histological slide. In such cases further sectioning of the block is indicated to elicit the neoplasm (Mashberg, 1980). Although the use of toluidine blue staining is not advocated for obvious symptomatic carcinomata, exophytic lesions with minimal necrosis stain fairly well a deep royal blue of varying degree depending on the amount of débris and/or keratin present. Large extensive carcinomata with ulcerations stain poorly, if at all. The stain is not successful in determining the peripheral extent of neoplastic lesions since non-atrophic, keratinized or submucosal portions of the neoplasm do not stain. Tolonium chloride is useful, however, in staining areas of "field cancerization" peripheral to the central symptomatic neoplasm.

The mechanism of toluidine blue staining in vivo remains unknown, and all explanations are regarded as speculation by Silverman et al. (1984). Tolonium chloride selectively stains polyanionic acid tissue components such as sulphate, carboxylate, and phosphate radicals, deoxyribonucleic acid and ribonucleic acid. Since toluidine blue 0 is regarded as a nuclear stain, selective dye uptake by dysplastic and neoplastic cells that contain quantitatively more nucleic acids than normal tissue is considered plausible (Lundgren et al., 1979). In addition, studies have demonstrated a greater affinity of basic dyes, such as tolonium chloride, for neoplasm ribonucleic acid (Lepage et al., 1975), and binding to nucleohistones and DNA (Miura and Ohba, 1967). Microscopic tissue-binding sites of toluidine blue from frozen sections reveal in some cells a blue reaction outlining cytoplasmic and nuclear borders, but only in the outer two or three cell surface layers (Silverman et al., 1984).

7.3.3 Toluidine blue rinse

The use of tolonium chloride rinse is advocated as a screening agent for the discovery of asymptomatic oral squamous cell carcinoma in high risk patients that are undetected by routine soft tissue examination (Mashberg, 1983). The target population consists of those persons who are moderate to heavy consumers of alcoholic beverages and cigarettes. If the technique is adopted on a large scale, it is anticipated that increased numbers of carcinomata will be discovered with a concomitant increase in survival rates and a decrease in post-treatment morbidity. If a lesion is found with the tolonium chloride rinse, all inflammatory and irritating factors should be eliminated and the area subsequently stained
by means of a rinse or topical application 10 to 14 days later. A positive stain at this occasion necessitates a biopsy and histological examination.

When compared to the results of topical application, tolonium chloride rinse for the screening of high-risk patients shows a false negative rate of 11.1 per cent and false positive rate of 9.2 per cent compared to 2.5 per cent and 12.0 per cent for topical application (Mashberg, 1983). These false positive values are significantly much lower than for clinical impression alone (28.5 per cent).

Tolonium chloride rinse offers a diagnostic mechanism for the recognition of small, asymptomatic carcinomata, 2 cm or less in diameter that remain undetected because of their clinically innocuous appearance. It is also a screening modality for the identification of patients at risk of developing second primary carcinomata, either synchronous or metachronous, in the upper aerodigestive tract (Mashberg, 1983).

7.3.4 Conclusion

In conclusion, toluidine blue 0 staining, either topical application or rinse, is an efficacious diagnostic adjunct in the detection of asymptomatic oral squamous cell carcinoma. It appears to offer an immediate, feasible, diagnostic "control" over the subjective impression of the clinician. Its value lies in the reinforcement of clinical impression, the control over clinical false negatives, reduction of false positives, and the possibility of discovering second primary lesions. It is a very simple and expeditious "office" procedure that does not require an intermediary in the diagnosis as does exfoliative cytology (infra vide). Consequently, this should make oral exfoliative cytology redundant. All persistent lesions that stain positively need to be biopsied and examined histopathologically for a definitive diagnosis (Mashberg, 1980, 1983; Silverman et al., 1984).

7.4 ORAL EXFOLIATIVE CYTOLOGY

Cytology is the scientific discipline dealing with the morphological and/or chemical characteristics of individual cells or cell parts. Exfoliative cytology is a cytodiagnostic technique examining the morphological characteristics of exfoliated or superficial cells that have been removed off mucous membrane. It is chiefly used in the
cytopathological diagnosis of bullous oral mucosal lesions, periodontal, preneoplastic and neoplastic lesions (Bánóczy, 1976). Other uses include sex chromatin evaluation and predicting the response to anti-neoplastic radiotherapy (Folsom et al., 1972).

An historical review of cytodiagnosis is provided by Christopherson (1983). Cytology for the diagnosis of malignant neoplasia was initiated by Martin and Ellis in 1930, who developed aspiration biopsy using an 18-gauge needle and staining the smear with haematoxylin and eosin stains. They were primarily concerned with the cytological diagnosis of cancer rather than the early detection of neoplasia. Papanicolaou is credited as the first to utilize exfoliative cytology for the detection of cancer: the now famous Papanicolaou cervico-vaginal smear.

A review of oral exfoliative cytology in the detection of oral malignant neoplasia by Folsom et al. (1972) reveals that:

1. oral exfoliative cytology is not a substitute for biopsy;
2. it has the potential for early detection of malignant lesions;
3. it is a useful adjunct in the evaluation of visible oral mucosal lesions;
4. its value is limited if cancer is clinically suspected, but a combined cytology–biopsy approach may be of value; and
5. it may be useful for repeated follow-up examination to indicate an appropriate site for biopsy.

However, cytological examination of oral lesions does not constitute a definitive diagnosis. The false negative rate of oral exfoliative cytological examination varies from 9–31 per cent. The reliability of the procedure varies from site to site, apparently depending to some extent on the degree of keratinization of the epithelium, whilst the accuracy of the procedure ranges from 69 to 100 per cent.

Even though the reports documenting the reliability of cytodiagnosis of oral neoplasia are inconclusive, they do argue against its value in the detection of premalignant lesions (Blozis, 1972). The use of exfoliative cytology in diagnosing malignant transformation in oral preneoplastic lesions is controversial. Both good and poor results have been obtained by different groups of investigators. The generally poor results are explicable if one considers that exfoliative cytology is a surface sampling technique, rather than a pooling of exfoliated cells. It is not representative of the deeper strata of a lesion. Unfortunately, premalignant and neoplastic changes are located in or near the stratum
basale, an area not readily sampled. Unless the surface of the lesion is eroded, ulcerated or relatively atrophic, exfoliative cytology appears to be of marginal value.

Bánóczy and Rigó (1976), *inter alia*, have established the importance of exfoliative cytology in the diagnosis of malignancy in leukoplakia erosiva. Furthermore, Bánóczy (1976) suggests that the main value of oral exfoliative cytology lies as a diagnostic adjunct in the diagnosis of early oral squamous cell carcinoma with ulcerated surfaces and dysplastic leukoplakia erosiva. In these early cases the early diagnosis and indication for a biopsy is of great importance. In advanced ulcerated carcinomata the effectiveness of exfoliative cytology is diminished by the greater possibility of clinically correct diagnosis and worse prognosis. Also, in the course of serial examinations, clinically unsuspected early oral squamous cell carcinoma might be detected by exfoliative cytology, and in extensive lesions the site of biopsy might be selected providing a better sample.

The effectiveness of exfoliative cytological method is influenced by the site and type of lesion (Blozis, 1972; Bánóczy, 1976). A rather poor degree of reliability is found in specimens taken from lesions of the labial vermilion and attached gingiva, both keratinized areas of oral mucosa membrane. Extremely keratotic lesions are poor samples because only anucleate squamae are obtained and are not of diagnostic value. Extensive surface necrosis, well differentiated squamous cell carcinoma and verrucous carcinoma are not suitable for cytodiagnosis.

### 7.4.1 Rationale and indications

The rationale of the exfoliative cytological technique developed by Papanicolaou is based on two principles, *viz.*: (1) individual neoplastic cells often can be diagnosed as such microscopically by their large size, pleomorphism, increased nuclear–cytoplasmic ratio, nuclear hyperchromasia, prominent nucleoli and abnormal mitoses; and (2) neoplastic cells exfoliate more readily than normal cells, possibly because of their lowered cohesiveness as a result of either a lowered calcium content or a decrease in the number of zonulae occludentes (tight junctions) (Kissane, 1985b).
Indications for exfoliative cytology include (Folsom et al., 1972; Blozis, 1972; Bánóczy, 1976; Erozan, 1979; Sapp, 1979; Christopherson, 1983; Owings, 1984):

1. Screening procedure, especially for occult primary lesions of the head and neck;
2. Adjunct to biopsy in the examination of large lesions;
3. As follow-up procedure of radiotherapy patients for the detection of recurrences;
4. As a compromise for patients who refuse a biopsy;
5. In debilitated patients or those with haemorrhagic diatheses which constitute a poor surgical risk;
6. In the diagnosis of vesiculo-bullous lesions and some anaemias; and
7. In rapid cytological examination of surgical specimens: "imprint cytology".

7.4.2 Cytodiagnostic techniques

There are several cytodiagnostic techniques advocated and used by clinicians and pathologists. These include: cytological smear; smear curettage; sputum collection and washings; aspiration; fine-needle aspiration; imprint cytology; and acridine stain techniques.

Cytological smear. Direct sampling of the lesion by surface abrasion using a single layer of gauze wrapped around a gloved finger, a wooden spatula, a brush or a curette has been described by Erozan (1979) and Sapp (1979).

The cytological smear report can be any of the following: unsatisfactory; negative; atypical; suspicious; highly suggestive; or positive. A report of "unsatisfactory" means insufficient cells were sampled or that the type of lesion is unsuitable for cytodiagnosis. The lesion should be reconsidered for excisional or incisional biopsy. A "negative" report indicates that superficial cells sampled do not exhibit any morphological features of malignant neoplasia. The possibility still remains that deeper cells are abnormal, especially in keratinized leukoplakias. A report of "atypical" indicates the cells contain abnormal features. Many factors other than neoplasia may cause these aberrations, e.g. bacterial, viral and mycotic infections or inflammation caused by
chemical and physical agents. Reports of "suspicious", "highly suggestive" and "positive" of malignancy indicate the necessity for biopsy to establish the definitive diagnosis and institute the appropriate therapy.

**Smear curettage.** A modification of the exfoliative cytological smear is "smear curettage" described by Dumbach *et al.* (1981). It employs a sharpened spoon curette or excavator to sample the lesion. It is particularly efficient in investigating small lesions.

**Sputum collection and washings.** These methods are chiefly employed in the detection of lower respiratory lesions (Erozan, 1979). Sputum washings have been used for screening purposes, but their yield is lower than direct sampling techniques.

**Aspiration techniques.** These techniques employing a cell aspirator (Zetterwall cell aspirator) have been devised to increase the quantity of the specimen in order to enhance the reliability of the cytological diagnosis (Sigurdson and Willén, 1979a, b). Whereas a wooden spatula collects superficial epithelial cells, the cell aspirator collects cells from most epithelial strata and in a sharply circumscribed portion of the mucosal surface. No subepithelial damage occurs with the use of either method.

Quantitatively the cell aspirator produces significantly better results both with regard to the total number of cells obtained and to the number of epithelial cells in different stages of differentiation (Sigurdson and Willén, 1979b). However, the number of cells obtained is dependent on the surface structure and the size of the lesion, *e.g.* the cell aspirator obtains 50 per cent more cells from leukoplakias, increasing the reliability of the cytological diagnosis. Qualitatively the cell aspirator collects epithelial cells which are better preserved morphologically. However, a large number of compact cell clusters are also collected reducing the diagnostic accuracy.

**Fine-needle aspiration cytology.** Fine-needle aspiration cytology has been practised for over fifty years with varying enthusiasm (Christopherson, 1983). The technique was introduced in 1930 by Martin and Ellis for the assessment of the presence of metastatic lymphadenopathy. The technique was first used by Coley, Sharp and Ellis in 1931 in differentiating inflammatory from neoplastic osseous lesions, in differentiating metastatic carcinoma from primary osseous neoplasms, and in confirming the presence of osseous metastases. Despite these indications, there is only limited acceptance of the technique among head
and neck surgeons for three major reasons (Feldman et al., 1983): (1) there is an apparent perception that the reliability of fine-needle aspiration is insufficient to allow major clinical decisions to be based solely on aspiration without histological, i.e. biopsy, confirmation; (2) fear that the technique may "seed" needle tracks with neoplastic cells; and (3) that an experienced cytopathologist must be available to interpret specimens.

However, Feldman et al. (1983) argue that if its reliability and utility in head and neck surgical oncology were well established, fine-needle aspiration cytology would provide an alternative diagnostic technique to the danger of open cervical dissection and the inaccuracies (false-negatives and false-positives of 20–30 per cent) of relying on clinical acumen alone. They suggest that fine-needle aspiration cytology is a highly accurate, safe, predictive and valuable technique in the management of squamous cell carcinoma of the head and neck, providing useful information in resolving the problem of a cervical lymph node metastasis from an occult primary after negative panendoscopy and blind biopsies; in verifying early recurrence; and in distinguishing post-irradiation oedema from recurrence.

Cellular evaluation of head and neck aspirates are reported in one of four categories (Feldman et al., 1983): unsatisfactory; negative; suspicious; or positive for malignancy. A report that a specimen is "unsatisfactory" provides no clinical information. It is not synonymous with "negative". A "negative" specimen, though carrying considerable weight, does not exclude neoplasia. If such a report is at variance with the clinical impression, biopsy is warranted. A "suspicious" specimen requires biopsy because 60 per cent of aspirates prove to be positive on histopathological examination. A "positive" fine-needle aspirate is regarded in many institutions as being as definitive as a tissue biopsy specimen, especially for mammary carcinoma.

It should be appreciated that fine-needle aspiration technique differs from needle aspiration biopsy using a 14 gauge needle to obtain a tissue core for histological examination, infra vide (Feldman, et al., 1983; Christopherson, 1983). Fine-needle aspiration cytology in contrast to needle aspiration biopsy is not associated with "tumour implants" along the needle track. It is also not associated with a sampling error caused by a large bone (14 gauge) needle pushing aside a small, non-fixed lesion during its single pass. The only theoretical disadvantage of fine-needle
aspiration is the possibility of haematogenous spread of neoplastic emboli with the resultant increase in distant metastases and higher mortality rate. The demonstrated clinical advantages of avoiding open cervical biopsy, greater accuracy in clinical staging, more precise therapeutic planning and earlier detection of recurrences outweigh this theoretical disadvantage.

**Imprint cytology.** This method has been shown to be a useful adjunct to the use of frozen sections by Owings (1984), which yields more diagnostic information than either method alone. Diagnostic accuracy is putatively good, with demonstration of better cytological detail than that obtained with frozen sections.

The procedure is simple. It involves sectioning the specimen tissue and lightly touching with a glass slide the cut surface. Lesions that are sparsely cellular or that contain abundant fibrous tissue can be scraped gently with a clean knife blade to increase the yield of cells. The "imprint" is then fixed immediately or allowed to dry and stained appropriately.

Diagnostic errors in the interpretation of cytological specimens are generally of two types: (1) those resulting from the failure to seek or observe diagnostic features in the slides; and (2) those resulting from the misinterpretation of the features observed. Cytological preparations obtained from surgical specimens by imprint cytology can be processed rapidly and inexpensively within minutes of receipt of the fresh tissue and unlike other cytological techniques allow the elucidation of the architectural features of the tissue.

**Acridine staining.** Acridine staining is a method which indicates the DNA content of populations of desquamated buccal cells by measuring the amount of acriflavine, a basic acridine dye, they bind. Acriflavine is a yellow acridine dye, C₉H₆N₂Cl obtained by methylation of proflavine red crystals or usually in admixture with proflavine as a deep orange powder. It is often used as an antiseptic in its hydrochloride reddish brown salt (Roth *et al.*, 1972).

The rationale for developing a cancer screening method which depends only upon the solitary, non-specific parameter of DNA content is based on the fact that the cells desquamating from the upper strata of the buccal epithelium are non-replicating. Thus an increase in the rate of mitosis, the most common non-neoplastic cause for an increase in DNA content in a cell population, is eliminated as a spurious factor. By
measuring DNA only, emphasis is given to profound aberrations of cell replication: polyploidy,\textsuperscript{2} polyteny\textsuperscript{3} and endoreduplication in cells; which bear a strong correlation with neoplasia.\textsuperscript{4} Comparisons of the frequency distributions for uptake of acriflavine by 50 000-cell samples reveal sufficient deviation from control groups by cancer group to suggest clinical utility of the method as a health screening programme.

7.5 BIOPSY AND HISTOPATHOLOGICAL EXAMINATION

The definitive diagnosis of oral neoplasia depends on biopsy: the removal of tissue from a living organism for the purposes of histological examination and diagnosis. There are two types of biopsy, \textit{viz.} (1) excisional biopsy; and (2) incisional biopsy. Excisional biopsy is the total excision of a small lesion for microscopic study. It is to be preferred if the size of the lesion is such that it can be removed along with a margin of normal tissue, approximately 10–15 mm wide, and the wound can be closed for primary healing. Incisional biopsy is the excision of a small representative part of the lesion including some normal tissue for microscopic examination. It is useful for large lesions (Shafer et al., 1974f).

There are at least six different biopsy techniques commonly in use, \textit{viz.}:

(1) excision by scalpel;
(2) electrosurgical excision (electrocautery);
(3) carbon dioxide surgical laser biopsy;
(4) forceps or punch biopsy (drill biopsy);
(5) aspiration biopsy \textit{via} wide-bore needle; and
(6) exfoliation cytology.

7.5.1 Indications

Biopsy examination is used to confirm a clinical suspicion of malignant neoplasia and to evaluate the non-neoplastic lesions such as chronic inflammatory hyperplasia, granulomata, erosive lichen planus and

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2. Polyploidy: the state of having more than two full sets of homologous chromosomes.
3. Polyteny: reduplication of chromonemata without separation into distinct daughter chromosomes.
cysts. Although biopsy is mainly important in establishing the diagnosis, it also assists in determining clinical staging and prognosis, the type of treatment and the evaluation of the progress and response of such treatment by serial biopsies (Howe, 1971; Sapp, 1979; Brightman, 1984b).

To aid in the histopathological diagnosis complete details of case history, examination and results of tests should accompany the biopsy specimen to the pathology laboratory. Data should include the patient’s age; sex; site of lesion; clinical features such as size, colour, texture, consistency; presence of pain or tenderness; mobility of lesion; functional difficulties such as limitation of lingual movements; and the presence of regional lymphadenopathy. The duration of the illness; clinical diagnosis (provisional or differential); and the treatment given, particularly if it may affect the histological appearances of the specimen, should be included. Also, additional information, viz.: radiographs and laboratory tests: haematological, biochemical, microbiological; may be useful to the pathologist.

It is important to obtain an adequate, representative surgical specimen, usually greater than 10 mm x 5 mm in size. Accurate selection of the site of the specimen which will provide the best diagnostic potential needs to be accomplished. An area of macroscopically normal tissue needs to be included and areas of necrosis must be avoided. Crushing, tearing or burning of the specimen must be avoided.

To enable the correct orientation and interpretation of the clinical features of the lesion by the pathologist in the laboratory a simple sketch outlining the main features of the lesion and the exact site from which the surgical specimen was obtained, is highly desirable. Jolly (1976b) has developed a standard oral chart suitable for recording the clinical features of lesions. It is based on the topographical classification of the oral mucosa developed by Roed-Petersen and Renstrup (1969). The lesions are sketched pre-operatively and their various features outlined and annotated on the chart. The precise biopsy site is drawn in at the end of the operation and the chart sent to the laboratory together with the specimen and clinical history. A suture placed at the normal mucosal end facilitates the correct orientation of the surgical specimen. A dye, e.g. Castellani’s Paint (U.S.N.F.), that forms an indelible marker that remains visible throughout the entire processing of the biopsy specimen further enhances the recognition of the clinically impressive features of the lesion by the pathologist (Jolly, 1976b). Castellani's Paint is applied
with a fine sterile mapping pen to the dried mucosa outlining the key features of the area to be removed with the corresponding details shown on the oral chart. The dye is also useful for placing orientation marks on the specimen, effectively replacing the conventional suture. The dye does not seem to interfere with routine haematoxylin-eosin staining of surgical specimens.

7.5.2 Biopsy techniques

Excision by scalpel. Excisional biopsy is indicated when the lesion is relatively small (less than one centimetre), sessile or pedunculated, circumscribed and freely movable, and located above the mucosa or subjacent to it. It is contra-indicated if the lesion is large and diffuse, or is part of the surface projection of a larger, more extensive submucosal lesion. If total removal with a margin of macroscopically normal tissue cannot be obtained easily an incisional biopsy is indicated.

To avoid three dimensional distortion of the surgical specimen it should be placed immediately after removal from the mouth on a small piece of thin, flat, permeable cardboard. The specimens are placed on the cardboard with the mucosal surface facing upwards, gently wiped free of blood with a saline-moistened gauge before being placed into the fixative (Jolly, 1976b). The specimen remains on the cardboard during fixation and is best trimmed to produce the "cutting surface" whilst still attached to the cardboard. This will maximize the diagnostic value of the surgical specimen by allowing histological sections to be cut normal to the epithelial surface.

For excisional and incisional biopsy with a scalpel a similar technique is employed. An elliptical incision is preferred although some surgeons employ a triangular wedge of tissue with the apex at the centre of the mass and the base in the adjacent normal tissue (Howe, 1971; Oringer, 1982).

Electrosurgical excision. Although universally regarded as an inferior method of obtaining surgical specimens for histopathological examination there are some biased advocates of electrosurgical biopsy, one of whom happens to be the founder of the American Academy of Dental Electrosurgery, Oringer (1982). Electrocautery results in fulguration, i.e. electodesiccation. An electrothermal burn results in coagulation necrosis
surrounded by an areola of erythema. Microscopically there is microvesiculation and separation of the epithelium with trans-epithelial coagulation of the corium (Kisseane, 1985f). The nuclei are pyknotic, elongated and aligned in parallel or palisading fashion, so-called "nuclear streaming". There is peripheral ischaemic necrosis, i.e. infarction. Healing of such a wound, especially by secondary intention, will always result in scar tissue formation. Also considerable damage to the surgical specimen can be envisaged rendering it less than ideal for histopathological examination.

Carbon dioxide surgical laser excision. The carbon dioxide surgical laser is regarded by many as the ideal surgical technique for oral and soft tissue lesions (Pecaro and Garehime, 1983; Fisher and Frame, 1984; Frame, 1984). It is a precise means of eliminating dysplastic and neoplastic epithelial lesions of the oral mucosa. It can be used in two ways in the oral cavity: (1) excision of soft tissue lesions in a manner similar to the cutting diathermy or scalpel; and (2) vaporization of the surface mucosa.

Healing after laser surgery differs from that of healing by secondary intention, being related to the manner of tissue destruction in a laser wound. Unlike electrocautery, the laser wound is not a burn but rather due to an almost instantaneous vaporization of intra-cellular fluid with the disintegration of the cell structure (Hall et al., 1971; Hall, 1971). This pattern of cell destruction probably occurs without the release of effective chemical mediators of acute inflammation. Any cell coming in contact with the laser beam is destroyed and the temperature is sufficiently high to denature cell protein. Shedding of possibly neoplastic cells or immunologically active cell particles is unlikely during laser surgery. There is also a minimal degree of wound contraction, probably due to the small number of myofibroblasts present. The surgical specimen excised shows minimum damage and can be processed accordingly for histopathological examination (Fisher and Frame, 1984).

Forceps biopsy. The use of biopsy forceps rather than a scalpel may offer certain advantages, especially in the posterior oral cavity or in relatively inaccessible areas such as the uvula or retromolar trigone. The functional portion of the instrument consists of a cupped beak and a cutting beak. The tissue to be biopsied is grasped firmly and removed with a rapid twisting and pulling movement. Care must be exercised when biopsy forceps are used on movable tissue such as soft palate, uvula
and floor of mouth to ensure an adequate depth of tissue and to prevent stripping of large areas of the mucosa (Brightman, 1984d).

**Punch biopsy.** Punch biopsy of oral tissues is to be avoided, since only parts of a lesion that project above the level of the surrounding tissue can be obtained without forcing the punch into the lesion. The resulting specimen is usually small and difficult to orientate for "blocking" (Jolly, 1976b; Sapp, 1979). Drill biopsy can be obtained using a modified Ellis biopsy drill which fits into a straight dental handpiece. The instrument can be used to obtain cylindrical specimens 12 mm long and 1.4 mm in diameter. It is useful for obtaining biopsy specimens from bony dystrophies and central osseous lesions. To be of any use, the lesion must be of uniform appearance, not very large, and adjacent normal tissue not required. It does not offer any advantage over other biopsy techniques. The resultant tissue specimen is small, frequently superficial and fragmented making an accurate histopathological interpretation difficult (Howe, 1971b; Sapp, 1979).

**Aspiration biopsy.** Aspiration biopsy using a wide-bore, 14–18 gauge needle was developed by Martin and Ellis in 1930 for the cytological diagnosis of neoplasia (Christopherson, 1983). It differs from fine-needle aspiration cytology, ut supra (Feldman et al., 1983). It is used to obtain a tissue core for histopathological examination and to ascertain the nature of cystic and fluctuant soft tissue or intra-osseous lesions. It is performed by inserting a large gauge needle into the lesion to the desired depth and exerting sufficient negative pressure on the plunger of the syringe to obtain a sample (Sapp, 1979). Interpretation of various clinical results indicate that the inability to aspirate is associated with a solid lesion; aspiration of air may indicate the sinus maxillaris or a solitary bone cyst; aspiration of purulent exudate indicates either an abscess or an infected cyst; aspiration of keratin denotes an odontogenic keratocyst; straw coloured aspirate containing cholesterol crystals denotes a periodontal or dentigerous cyst; aspiration of blood may denote an haemangiomata or blood vessel; and the inspissated material aspirated with extreme difficulty denotes a dermoid cyst (Killey et al., 1975).

The main disadvantages of aspiration biopsy are the increase in neoplasm sampling error as the 14 gauge solid needle may push aside the mass in its single pass yielding a negative diagnosis; and the possibility of "seeding" neoplastic cells into deeper tissues (Feldman et al., 1983).
7.5.3 Biopsy error

According to Jolly (1976b) most misdiagnoses of a lesion stem from sampling error in the surgery or the laboratory: either a failure to find the most abnormal part or a failure to define the true extent of a lesion. It is generally accepted that there are many factors which minimize biopsy error. These include: biopsy of suspected malignant neoplastic lesions by a specialist with appropriate training; excisional biopsy in preference to incisional whenever feasible; the shortest possible delay between incisional biopsy and definitive treatment; avoidance of unnecessary manipulation of lesions, injection into neoplasm and penetration of underlying periosteum; and gentle handling of lesion and surgical specimen. To these established methods can be added the correct site of biopsy yielding a representative specimen with the highest diagnostic value; proper, adequate historical and clinical information passed on to the pathologist to aid in diagnosis; correct management of specimen avoiding distortion by placing on flat, thin, permeable cardboard prior to fixing and processing; careful and accurate "cutting up" or trimming of specimen by the pathologist, not a pathology technician, and the use of "step" sections at intervals through the specimen to yield additional diagnostic information.

The correct biopsy site is that part of the lesion which will give the most important information about the lesion. It is important that the correct site must be included in the surgical specimen and that it must be identified and examined by the pathologist. Areas of erythroplakia and speckled erythroplakia are likely to show histological evidence of epithelial dysplasia, carcinoma in situ or squamous cell carcinoma (Mashberg, 1978).

The risk of metastasis resulting from biopsy procedures needs to be kept in mind especially since blood vessels and lymphatics are opened during incision of lesions. However, the importance of obtaining a definitive diagnosis by histopathological means outweighs this risk, especially if manipulation and forceful palpation of suspected malignancies are avoided (Jolly, 1976b). Furthermore, to avoid the necessity of repeat biopsies the pathologist must be provided with enough information from the surgeon to enable a correct diagnosis to be made (ut supra).
In trimming the surgical specimen on the microtome to produce the cutting surface, a variable and unpredictable amount of tissue is lost before the first usable sections can be obtained. Thus the most valuable part of the biopsy specimen may be lost in its preparation. Consequently, the selection and trimming of the cutting surface is one of the most critical steps in the entire biopsy procedure (Jolly, 1976b). The selection of the plane of cutting is of critical importance in the case of excision biopsies in establishing whether or not the line of excision is clear of the lesion. For excisional biopsies the plane of sectioning must be parallel to the short axis of the elliptical specimen and parallel to the long axis for incisional biopsies.

Step sections at intervals through the specimen can be used to explore the third dimension of heterogeneous lesions (Jolly, 1976b). The interval may be varied depending upon the heterogeneity and size of the specimen and step sections may be regulated from all or only selected parts of the specimen. Additional diagnostic information from 17–30 per cent can be achieved by using step sections, which can be significant in determining treatment and prognosis.

A margin of normal tissue, no less than 5 mm, when excising the smallest lesion suspected of being a squamous cell carcinoma is obligatory because of the subepithelial spread of carcinoma. An elliptical block of tissue with a rectangular cross-section ensures an even margin of normal tissue (Jolly, 1976b). When the histopathological examination reveals neoplasm close to the line of excision the likelihood of residual neoplasm in the mouth must be recognized. When neoplasm is seen adjacent to the line of excision, it must be assumed that incomplete excision has occurred.

The histopathological evaluation of the surgical specimen is essentially subjective, as has been noted previously. A wide range of histopathological diagnoses can be made, including:

1. benign lesions: parakeratosis, orthokeratosis, hyperkeratosis, acanthosis, pseudo-epitheliomatous hyperplasia, acute and chronic inflammation;

2. epithelial dysplasia: mild, moderate, severe and carcinoma in situ; and

3. squamous cell carcinoma and variants: verrucous carcinoma, spindle cell carcinoma, adenoid squamous cell carcinoma; and rarely basal cell carcinoma and melanocarcinoma.
7.6 NEW DIAGNOSTIC TECHNIQUES

Several new, potentially diagnostic techniques are currently under investigation for the diagnosis and assessment of oral squamous cell carcinoma. These include:

1. ultrasonography;
2. immunohistochemical techniques;
3. monoclonal antibody techniques;
4. radio-immunological techniques using radiolabelled antibodies; and
5. electron microscopy.

7.6.1 Ultrasoundography

The use of ultrasonography in the detection of neoplasia, both primary and metastatic, in the head and neck has been recently reported by Gooding (1980), Ishikawa et al. (1983) and Bruneton et al. (1984). The development of high-resolution real-time equipment has increased the role of ultrasound in the exploration of small body parts.

Grey-scale ultrasonography has been shown to improve visualization of space-occupying lesions and calculi of the parotid salivary gland (Gooding, 1980; Wittich et al., 1985). Ultrasonography provides clinically useful information by precisely outlining neoplasm borders or by detecting multiple or bilateral lesions.

The clinical applicability of grey-scale ultrasonography to oral and cervical space-occupying lesions was investigated by Ishikawa et al. (1983) in detail. The differential diagnosis of head and neck tumours, i.e. masses, is based on four major ultrasonographic parameters identified, viz.:

1. ultrasound pattern: solid or cystic (sonolucent);
2. boundary echo: smooth or irregular;
3. internal echo: homogeneous or heterogeneous; and
4. posterior wall echo: enhancement, intermediate or attenuation.

Ultrasonography is a safe, reliable examination method in diagnosing soft-tissue masses of oral and cervical areas. It delineates the present location and internal structure of the lesion. Most benign neoplasms, e.g. pleomorphic adenoma, lipoma, papillary cystadenoma lymphomatosis (Warthin's tumour), and haemangioma are round, oval or knobby hypo-
echoic masses with smooth boundary echoes and homogeneous internal echoes. All are solid except for lipoma which produces a cystic pattern. Malignant neoplasms are typically solid, irregular and heterogeneous with attenuation of the posterior wall echo. Cysts, e.g. ranulae and branchial cyst, are visualized as having smooth boundary echoes and an echo-free region of sonolucence suggestive of a benign condition, plus posterior wall enhancement.

A comparative study by Bruneton et al. (1984) on the value of ultrasonography in the detection of metastasis to cervical lymph nodes from head and neck primary cancers revealed that ultrasound examination is more sensitive than clinical examination alone. High-resolution, real-time ultrasonography is of primary value in providing information on the anatomical nature of metastatic lymphadenopathy including the detection of subclinical lymph node involvement, volumetric evaluation of clinically evident lymph nodes, and determination of vascular connections, in particular internal jugular venous thromboses. Furthermore, ultrasonography is an excellent surveillance technique allowing the evaluation of the efficacy of anti-neoplastic chemotherapy or radiotherapy, especially in patients in whom radiotherapy has caused thickening of cervical soft tissues.

Another comparative study by Hauenstein et al. (1981) on computerized ultrasonography of squamous cell carcinoma of the floor of the mouth, tongue and regional lymphadenopathy indicates that ultrasonography can only serve as a supplementary method of examination providing therapy-related decisions. Furthermore, a diagnosis based on ultrasonography with or without computer tomography remains problematical, especially since neoplasm specific diagnostic signs are lacking with either method.

7.6.2 Immunohistochemical techniques

The diagnostic efficiency of undifferentiated, anaplastic neoplasms by the surgical pathologist can be enhanced by the use of immunohistochemical immunofluorescence (IMF) and immunoperoxidase (IMP) which employ polyclonal and monoclonal antibodies (Baumal et al., 1984).

Polyclonal antibodies have been developed against intermediate-sized (10 nm diameter) filaments forming the major cytoskeletal elements
in nearly all cells. Five protein subclasses of intermediate filaments (IFs) of pathological interest include:

1. cytokeratins (CK): are characteristic of true epithelia;
2. neurofilaments: found in most, but not all, neurons;
3. glial fibrillary acidic (GFA) protein: occurs in astrocytic derivatives;
4. desmin: is a marker of most myogenic differentiations; and
5. vimentin (Vi): is typical of mesenchymal cells and is also found with one of the other IF types in certain non-epithelial cells and in many cultural epithelial cells (Espinoza and Azar, 1982; Debus et al., 1984; Kahn et al., 1984; Miettinen et al., 1984).

Polyclonal experiments have revealed that intermediate filament typing can distinguish the major human neoplasm groups, yielding additional information in cases that are difficult to diagnose with conventional histological stains.

Monoclonal antibodies raised against actin, neuroblastoma, HLA-DR, T lymphocytes - Leu series and T lymphocytes - OKT series have been used for immunohistochemical diagnosis of anaplastic neoplasia (Löning et al., 1983; Baumal et al., 1984; Hiratsuka et al., 1984a, b).

Other histochemical studies have been developed using antibodies against involucrin (Murphy et al., 1984; Said et al., 1984); flaggrin (Klein-Szanto et al., 1984; Itoiz et al., 1985); tumour antigens (Woodhouse et al., 1985; Karcher, 1984; Wang, 1984); Herpesvirus hominis type 1 (Shillito et al., 1982, 1983, 1984); epithelial membrane antigen (Sloane and Ormerod, 1981); epidermal growth factor receptors (Hendler and Ozanne, 1984). Leukocyte adherence inhibition assay (LAI assay) has also been suggested as being valuable in the diagnosis and monitoring of anti-neoplastic immunity of oral squamous cell carcinoma (Kövesi and Pekete, 1982; Prabha et al., 1984). The demonstration of loss of the iso-antigens of blood groups A, B and H and its diagnostic significance has been well established since the work of Davidsohn and Ni (1972).

Intermediate filaments. The use of intermediate filament antibodies in the diagnosis and classification of neoplasia has been reviewed by Miettinen et al. (1984). Immunohistochemistry of intermediate filaments (IFs) is a new and important way of evaluating epithelial, mesenchymal, muscle, glial or neural differentiation in neoplasms. It is based on the stable cell-type specific expression of IF proteins in normal and
neoplastic tissues, *i.e.* when cells undergo neoplastic change they usually continue to produce the filaments characteristic of their normal cell of origin. A scheme for the use of immunohistochemical immunofluorescence (IMF) and immunoperoxidase (IMP), employing polyclonal and monoclonal antibodies, to increase diagnostic precision in assessing anaplastic neoplasms has been described by Baumal *et al.* (1984).

Cytokeratin can be demonstrated in different types of carcinomata, *viz.*: transitional cell carcinoma; squamous cell carcinoma; adenocarcinoma; and also mesothelioma (Baumal *et al.*, 1984). Espinoza and Azar (1982) found that all squamous cell carcinomata, regardless of site are keratin-positive stained. The keratin-positivity is positively correlated with the presence of ultrastructural intermediate-sized filaments arranged in loose or dense bundles in the cytoplasm of neoplastic epithelial cells and macula adherens (desmosome) type of intercellular junctions readily seen in all squamous cell carcinomata, regardless of the degree of differentiation, in basal cell carcinomata and in sebaceous carcinomata. Kahn *et al.* (1984) showed that the epithelial nature of anaplastic and poorly differentiated neoplasms, characterized under light microscopy as large and polygonal, spindle or small, round cells, can be confirmed by immunohistochemistry using anti-cytokeratin antibodies (ECK and MBCK) and immunoperoxidase staining. Baumal *et al.* (1984) and Kahn *et al.* (1984) suggest that both ultrastructural and immunohistochemical studies using immunoglobulins to cytokeratins should be performed in assessing poorly differentiated and anaplastic neoplasms.

Cytokeratin-positive neoplasms can be further evaluated by using monoclonal anticytokeratin immunoglobulins (Debus *et al.*, 1984). Monoclonal antibodies, if appropriately characterized, offer a readily exchangeable reagent of known and reproducible properties (Köhler and Milstein, 1975). Also, gel electrophoretic studies of different epithelia have revealed complex patterns of cytokeratin polypeptides that show characteristic patterns of distribution and expression. In normal human epithelia and carcinomata, nineteen different cytokeratins have been identified and catalogued by Moll *et al.* (1982). The use of an appropriate collection of cytokeratin antibodies with different specificities allows both the distinguishing of different types of carcinomata and the further subdivision of carcinomata in relation to their histological origin.

**Involucrin.** Immunohistochemical studies using antibodies against involucrin have been described by Murphy *et al.* (1984) and Said *et al.*
(1984). Involucrin is a recently recognized structural component of mature squamous epithelial cells. It is a precursor of the cross-linked envelope protein of human stratum corneum and its appearance in the superior strata of the epidermis is a function of the normal differentiation of the keratinocyte. Immunoperoxidase staining reveals four patterns of reactivity: (1) diffuse intracellular staining typical of keratinocytes of the upper third of normal epidermis and epidermal hyperplasia and benign neoplasia; (2) staining at cell borders, seen principally in benign epidermal neoplasms; (3) patchy staining characteristic of squamous cell carcinoma in situ; and (4) absence of staining in benign and neoplastic "... basaloid epithelium ...". Said et al. (1984) also found that basal cell carcinoma is negative for involucrin while squamous cell carcinoma stained strongly positive and squamous carcinoma in situ reveals an increased staining of "dyskeratotic" cells at all levels in the epithelium. Abnormal staining patterns are also seen in non-neoplastic epidermis adjacent to carcinomata. Both these studies suggest immunohistochemical staining for involucrin may be useful in identifying dysplastic and neoplastic squamous epithelia, as well as in furthering our understanding of the altered maturation and kinetics of proliferative processes afflicting keratinocytes.

Filaggrin. The immunohistochemical detection of filaggrin in preneoplastic and neoplastic lesions of human oral mucosa has been described by Itoiz et al. (1985) and Klein-Szanto et al. (1984). Filaggrin is a histidine rich basic protein first isolated and characterized in 1977 and formerly named "stratum corneum basic protein" or "histidine rich basic protein". The existence of a phosphorylated precursor of filaggrin in keratohyalin granules and its interaction with keratin intermediate filaments has led to the hypothesis that after secretion from keratohyalin granules, filaggrin forms the supporting matrix of keratin filaments needed to complete the differentiation of keratinized epithelial cells. Filaggrin has been detected by immunohistochemical methods in the strata granulosum and corneum of normal epidermis and oral mucous membranes, and of kerato-acanthomata. All epidermal squamous cell carcinomata exhibit an absence or marked reduction in filaggrin positivity. Consequently, filaggrin detection is a potentially useful aid in the differential diagnosis of squamous cell carcinoma and kerato-acanthoma.

Using the peroxidase-antiperoxidase (PAP) technique for the detection of filaggrin in surgical specimens, Itoiz et al. (1985) found that
the stratum granulosum of normal orthokeratinized epithelium is always positive, whereas the stratum corneum is negative. Parakeratinized and non-keratinized epithelia stain less than orthokeratinized epithelium. In leukoplakia and verrucous carcinoma, the reaction is irregular in both strata granulosum and corneum. Carcinoma in situ shows a virtually negative reaction. Squamous cell carcinoma shows a very weak or negative reaction with positive reaction found in some keratin pearls of well-differentiated areas. The immunohistochemical demonstration of altered filaggrin patterns of reaction is in accordance with the finding of keratohyalin granules of irregular shape and distribution in human leukoplakia. The homogeneous reaction of the stratum granulosum and the positivity of the stratum corneum indicates that the differentiation process in hyperkeratotic lesions produces a less mature keratinized substance chemically or structurally different from normal.

The failure to demonstrate filaggrin in the surface layers of all stratified squamous epithelia has prompted several hypotheses. Dale et al. (1978) postulated that either the protein is lost at the time of terminal maturation or is chemically masked, or the compact nature of the stratum corneum prevents access of antibodies during the immunohistochemical procedure. Scott and Harding (1981) have shown that complete proteolysis of filaggrin takes place under normal circumstances in the epithelium, and suggest that this degradation could be related to the process of complete orthokeratinization, the lack of which would permit the detection of filaggrin in surface strata of incompletely keratinized or non-keratinized epithelia, such as those of normal buccal mucosa, and of some oral leukoplasias. Preneoplastic and neoplastic lesions of oral epithelia usually exhibit simultaneous alterations of keratinization and cell maturity. The decrease or absence in filaggrin in these lesions suggests that the distribution pattern of this protein could be used as a marker of differentiation, of atypia in leukoplakia, and of oral epithelial neoplasms.

Tumour antigens. "Tumour antigen" studies of oral carcinomata have proved unrewarding. Karcher (1984) carried out a tissue polypeptide antigen test (TPA) in patients with maxillofacial neoplasms before and after surgery. No conclusion could be drawn regarding the importance of this test in maxillofacial neoplasms.

A novel in vitro technique for investigating cell-mediated immunity and a new specific method for cancer diagnosis was described by Wang
(1984). The "active" T lymphocyte was adapted into an in vitro assay, active E-rosette-forming test (ARFT), for response to human "tumour antigens". Tumour antigen incubated with peripheral blood lymphocytes from patients with corresponding cancer, produce a significant increase in the ability of the lymphocyte to function as an active rosette-forming cell (ARFC) when compared with lymphocytes cultured without antigen (p < 0.001). The assay appears to be specific for the antigen corresponding to the cancer of the given patient, e.g. gastric carcinoma patients have gastric "tumour antigens" which increase ARFC numbers, but produce no increase in ARFC of mammary carcinoma patients. No interference by the HLA complex occurs in this assay. More recently, the immunohistochemical detection of "Ca antigen" in normal and neoplastic human tissue using Ca 1 monoclonal antibody has been described (Woodhouse et al., 1985). The monoclonal antibody revealed a widespread distribution of Ca antigen in carcinomata and normal tissues, diminishing its diagnostic usefulness as a potential "tumour marker".

Epithelial membrane antigen. The distribution of epithelial membrane antigen (EMA) in normal and neoplastic tissues and its value in diagnosis has been described by Sloane and Ormerod (1981). Although normal squamous epithelium stains negatively for EMA, the contiguous epithelium in inflammation and neoplasia is stained positive. Increased staining is also observed in metaplasia, epithelial dysplasia of the colon and mammae and neoplasia: adenocarcinoma, transitional cell carcinoma, anaplastic carcinoma, squamous cell carcinoma, mesothelioma, synovial sarcoma, teratoma and nephroblastoma. The staining of neoplasms is related to both their histogenesis and degree of differentiation, being found only in lesions of surface epithelial or mesothelial origin and being more consistently present in well and moderately differentiated neoplasms. The distinction of anaplastic carcinomata from lymphomata and the identification of spindle cell carcinoma and of minute metastatic breast carcinoma in hepatic and haemopoietic tissues are useful applications of EMA indirect immunoperoxidase method. The data suggest that the antiserum used identifies a single surface component expressed on a variety of epithelial cell surfaces, although the possibility cannot be ruled out that a family of very closely related molecules exists which may be delineated by more specific anti-sera. The localization of epithelial membrane antigen to surface and luminal membranes in the normal state suggests that it may have a protective function and the increased
quantities of EMA observed in inflammatory and neoplastic states indicate that increase production may represent a response of epithelial cells to injury. This synthesis of EMA may be related to intercellular contact: poor cell contact having been reported in malignant neoplasia.

Epidermal growth factor. A recent study by Hendler and Ozanne (1984) suggests that epidermal growth factor (EGF) receptors may provide an "... excellent marker for epidermoid malignancies ...". Epidermal growth factor (EGF) promotes the growth of cultural benign and malignant neoplastic cells. All squamous cell carcinomata including bronchogenic squamous carcinoma and head and neck squamous carcinomata exhibit increased levels of EGF receptor. The use of a murine monoclonal antibody, EGF-R1, which binds specifically to the receptor and does not bind significantly to normal pulmonary tissues or non-epidermoid neoplasms, indicates a potentially useful diagnostic technique.

Leukocyte adherence inhibition assay. Another potentially useful diagnostic technique is the leukocyte adherence inhibition assay (LAI). Kövesi and Fekete (1982) suggest that leukocyte adherence inhibition assay performed with encephalitogenic factor can be used as an immunodiagnostic tool in oral squamous cell carcinoma. LAI assay measures the reduced capacity of leukocytes to adhere to a glass surface as a result of incubation with solid neoplasm extracts. A good correlation exists between percentage LAI and clinical stages of neoplasm, using encephalitogenic factor, with an accuracy of 100 per cent. More recently, the usefulness of leukocyte adherence inhibition assay in monitoring anti-neoplasm immunity in oral squamous cell carcinoma was established by Prabha et al. (1984). Sixty-seven per cent of oral squamous cell carcinoma patients show a high degree of leukocyte adherence inhibition in the presence of oral cancer extract. The test is highly specific and the leukocytes of oral squamous carcinoma patients show significant inhibition only in the presence of oral cancer extract.


Davidsohn and Ni (1970) and Davidsohn (1972) have described a technique for the detection of iso-antigens A, B and H in tissue cells
using mixed cell agglutination reaction (MCAR) or specific red cell adherence reaction (SRCA). Several conclusions were drawn from the study results, *viz.*: (1) the loss of any of the three iso-antigens A, B and H is an early indication of carcinoma; (2) a negative SRCA test indicates a loss of iso-antigens and favours the diagnosis of carcinoma; (3) a negative reaction in a primary carcinoma suggests the possibility or probability or even presence of metastases; (4) a positive reaction in a primary carcinoma makes the presence of metastasis extremely unlikely; and (5) SRCA may be useful in the early diagnosis and prognosis of carcinomata that normally contain the three antigens. Also, the loss of blood iso-antigens is not an all or none phenomenon, rather there is a progressive loss of iso-antigens in the course of neoplastic transformation. This loss of iso-antigens precedes the formation of distant metastases. It is interpreted as evidence of physiological "de-differentiation" analogous to the morphological de-differentiation of anaplasia.

Recently, Schaumburg-Lever *et al.* (1984) studied the distribution of A, B and H blood group antigens by means of peroxidase-anti-peroxidase technique. In normal skin the epidermis of persons of blood group O show H antigen throughout; of blood group A, H and A antigens; and of blood group B, H and B antigens. This is in contrast with Davidsohn's (1970) findings in which the strata malpighii et basale were negative for iso-antigens. In lesions of solar keratosis, half of the cases of Bowen's disease, and eighty per cent of squamous cell carcinomata, no iso-antigens occur. In the benign lesions examined, the antigens of A, B and H blood groups are always present, although in verrucae the staining is confined to upper strata of the epidermis.

The demonstration of A and B iso-antigens in normal oral mucous membrane (Dabelsteen and Fulling, 1971) and oral squamous cell carcinoma (Dabelsteen and Pindborg, 1971) using immunoperoxidase and immunofluorescence staining methods reveals that in normal oral stratified squamous epithelium A and B antigens are demonstrable except in stratum basale. A positive correlation between the degree of anaplasia and the absence of antigens exists. Blood group antigens disappear or decrease in amount in oral squamous cell carcinoma as well as in contiguous atypical epithelia, suggesting that the change in antigen pattern at the cell surface is a neoplasm-associated change which may precede neoplastic development.
The specificity of the specific red cell adherence test (SRCA) in the immunological differentiation of neoplastic oral epithelium from lichen planus and kerato-acanthoma has been studied by George (1977). Normal and non-neoplastic oral epithelium show a positive adherence, whilst neoplastic oral epithelium shows a negative adherence of erythrocytes to tissue, due to the loss of tissue iso-antigens A, B and H on the neoplasms. The results of the study demonstrate the specificity of this adherence, with a 98 per cent correct diagnosis in the original blind study. Using fluorescene-labelled anti-human immunoglobulins to react with antibodies to the A or B iso-antigens and the fluorescene-labelled lectin *Lotus tetragonobolus* specific for the residue L-fucose of H antigen, George *et al.* (1980) determined that the "complete" blood-group glycoproteins are not detectable on the neoplastic oral epithelial cell surface. No variation of altered antigenicity between either well or poorly differentiated oral squamous cell carcinoma was demonstrable (George, 1982). According to Coon and Weinstein (1982) the relationship between ABH antigens and the precursor specificities described by George *et al.* (1980) are oversimplifications. ABH antigens may be components of glycolipids and glycoproteins with N-linked oligosaccharide chains as well as the O-linked chains illustrated by George. Furthermore, the O-linked chains with identical internal structures are components of many glycoproteins that do not bear ABH determinants.

**Micronucleus assay.** Micronucleus assay has been applied to exfoliated cells of high cancer risk groups; tobacco smokers, tobacco chewers and alcohol consumers, in order to detect genotoxicity in the buccal mucosa and to quantify the synergistic effect of the mixture of carcinogens released from the chewing of tobacco and betel nut (Stich *et al.*, 1982; Stich and Rosin, 1983). It combines all the advantages of *in vitro* short term assays with those using intact organisms with all their defence mechanisms. Micronucleus formation is a marker of the extent of chromosome breakage, which during mitosis, fails to become incorporated in the main nucleus. The significance of chromosome aberrations in neoplastic transformation is unknown; however, micronucleus formation seems 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.
Micronucleus formation is relevant to cancer risk only if the DNA breakage that it reflects, or some related process, is commonly involved in at least one of the rate-determining "stages" of carcinogenesis in the oral mucosa (Stich et al., 1984). Evidence purposed to support this supposition includes the "striking" parallels between the cases of micronucleus formation and the etiology of oral cancer, viz.: both conditions are affected strongly and synergistically by tobacco and alcohol usage (Stich and Rosin, 1983); and both are affected by x-irradiation and by betel quid usage (Stich et al., 1982; Stich et al., 1983).

7.6.3 Monoclonal antibody techniques

The immunological response to any foreign antigen is polyclonal: many different clones of B lymphocytes are stimulated to produce immunoglobulins. Monoclonal antibodies occur naturally in patients with multiple myeloma where neoplastic transformation occurs in a clone of B lymphocytes resulting in large quantities of identical immunoglobulins to be produced. In 1975 Köhler and Milstein constructed an hybrid myeloma (hybridoma) which produced a monoclonal antibody directed against a specified antigen. By this method monoclonal antibodies preferentially reacting to certain neoplasm types have been produced using three systems: mouse, rat and human (Sikora, 1982). Once established, human-human hybridomata show an apparent preferential loss of chromosomes, common in rodent-human hybrids, enabling stable hybrids to be established. Lymphocytes from cancer patients can be collected from peripheral blood, spleen, draining lymph nodes or "intratumourally". These are fused with an established myeloma culture line to produce hybridomata. Screening and cloning of many fusion products to find suitable immunoglobulins specific for an human neoplastic cell surface involves: (1) radio-immunoassay, both indirect and direct; (2) immunohistology, immunofluorescence or immunoperoxidase; and (3) cytotoxicity, complement and K cells.

Monoclonal antibodies can be raised against a wide variety of human neoplasms including: colorectal carcinoma (anti-carcinoembryonic antigen, CEA); melanoma; mammary carcinoma; lymphomata; leukaemias; glioma; neuroblastoma; sarcoma; bronchiogenic carcinoma; bladder, prostate and testicular neoplasms. Recently, monoclonal antibodies have been developed against squamous cell carcinoma including oral squamous
carcinoma (Carey et al., 1983; Debus et al., 1984; Pickering and Misra, 1984; Eskinazi et al., 1985; Woodhouse et al., 1985).

The use of monoclonal antibodies in the diagnosis of epithelial premalignant lesions has been described by Dabeisteen et al. (1983) and in the immunohistological analysis of lymphocyte subpopulations and T cell subsets in human oral squamous cell carcinoma (Hiratsuka et al., 1984a,b).

Autologous serological typing, using neoplastic cell lines and serum from the same individual together with extensive absorption analysis to determine the range and frequency of occurrence of antigens, has made it possible to identify human "tumour" antigens that are sufficiently immunogenic to elicit an immune response (Carey et al., 1983). It is suggested by Eskinazi et al. (1985) that the use of monoclonal antibodies against squamous cell carcinoma could lead to more thorough systemic exploration of patients, allowing for an earlier detection of other primary neoplasms and therefore significantly improving the prognosis. Other tests found useful for the serodiagnosis of neoplasia include: the micro–enzyme–linked immuno–absorbent assay (ELISA); and an ELISA inhibition test using monoclonal antibodies (Katz et al., 1985a). The ELISA inhibition assay may be useful for detecting culture supernatants reactive against neoplasm–associated serum antigens.

The use of monoclonal antibodies against cytokeratins associated with squamous cell carcinoma has been studied by Pickering and Misra (1984) and Debus et al. (1984). Human monoclonal antibodies, five IgM (λ) antibodies, stain the cytoplasm of autologous and allogeneic squamous carcinoma cells. All five IgM monoclonal antibodies stain all strata of normal epidermis but most intensely the stratum corneum. Two of the five hybridoma antibodies can recognize an antigenic determinant common to all intermediate filament proteins. Debus et al. (1984) described cytokeratin typing of human carcinomata by murine monoclonal antibodies (CK1, CK2, CK3 and CK4) which recognize a single human cytokeratin polypeptide (human cytokeratin No. 18) present in simple but not in stratified squamous epithelia; and with the monoclonal antibody KG8.13 and guinea pig ker A antibodies, both of which recognize a variety of cytokeratins common to all epithelial cell types. Basal cell carcinoma, cloacogenic carcinoma and squamous cell carcinoma of the epidermis, tongue and oesophagus appear negative with CK1–CK4 but positive with KG8.13 and ker A antibodies.