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Animal models of intra-oral chemical carcinogenesis: A review

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Abstract. Early attempts to induce malignant oral tumours were largely unsuccessful, as the oral mucosa is considerably more resistant to the actions of chemical carcinogens than skin. The first consistent success came when strong carcinogens were applied to the hamster cheek pouch. This model remains the most popular for studies of intra-oral carcinogenesis and is discussed at length. The advantages and disadvantages are critically reviewed. The models of intra-oral carcinogenesis in extra-pouch sites in the hamster, mouse, rat and nonhuman primates are considered. The possible reasons for the relative resistance of oral mucosa to chemical carcinogens are discussed, particularly in relation to portals of entry and saliva. Finally, the relative merits of the more popular models of intra-oral carcinogenesis are briefly discussed.

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I. Historical introduction

Early attempts to produce experimental malignant oral tumours were largely unsuccessful, the oral mucosa being considerably more resistant to the actions of chemical carcinogens than skin. Bonne (1925) noticed that some mice whose skin had been painted with coal tar developed papillomas in their mouth and stomach, probably due to tar which had been ingested when the animals licked themselves (Woglum 1926). Later, Bonne (1927) reported three squamous cell carcinomas developing in the palates of 50 mice which had been treated with coal tar for over a year. Leukoplakia was described in the palates of rabbits which had been repeatedly exposed to cigarette smoke for prolonged periods (Roffo 1930). Oyama (1935) succeeded in producing lingual carcinomas in 2 out of 16 rabbits by injecting coal tar into the tongue (Fujita et al. 1973). Levy (1948), however, failed to produce any neoplastic or preneoplastic changes in the labial gingivae of mice painted with 20 methylcholanthrene for up to 16 weeks, and Kreshover (1952) found murine labial mucosa to be resistant to repeated applications of tobacco smoke.

The essential combination of a susceptible tissue and a potent carcinogen was discovered by Salley (1954) when he showed that appli-

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cations of one of several polycyclic hydrocarbons could induce squamous cell carcinomas in the thin lining mucosa of the hamster cheek pouch. Since this discovery malignant neoplasms have been produced in a variety of animals in several intra-oral sites. The experimental models described in the literature will be considered in detail.

II. Experimental chemical carcinogenesis in the hamster cheek pouch

1) Introduction

The anatomical and physiological features of the hamster cheek pouch were described by Keyes & Dale (1944). The first documented attempt to produce tumours in this site was by Wantland (1954) who sprayed and painted 20 methylcholanthrene, 1,2,5,6 di-benzanthracene and 2 acetyl-aminofluorine into the hamster cheek pouch for up to 6 weeks (Salley 1954). The only change detected was epithelial hyperplasia.

The first successful attempt to produce tumours in the pouch was by Salley (1954). He investigated the effects of the powerfully carcinogenic polycyclic hydrocarbons 9, 10 dimethyl-1, 2-benzanthracene (DMBA), 20 methylcholanthrene (20 MC) and 3,4 benzpyrene (3,4 BP) dissolved in either acetone or benzene. Each of these carcinogens was painted onto the pouch thrice weekly for 16 weeks and the animals were observed for an additional 9 weeks. During the first 2 weeks there was an inflammatory phase with necrosis and sloughing of the distal part of the pouch. This was followed by healing and shrinkage of the pouch. The mucosa subsequently passed through four histologically recognisable stages: hyperplasia; papilloma; carcinoma-in-situ and squamous cell carcinoma, with or without metastasis. DMBA in acetone was the most effective carcinogen and the first tumour appeared after 7 weeks of painting. 20 MC and 3,4 BP were less effective carcinogens; the latent period was long (16–25 weeks) and the tumour yield was low.

In a later communication Salley (1957a) described the early changes in carcinogen-treated cheek pouch in more detail. White patches resembling human oral leukoplakia developed after eight or nine applications of carcinogen. The wall of the pouch passed through four histologically distinct stages before the appearance of overt neoplasms, these being inflammation, degeneration, regeneration and hyperplasia.

2) Standardisation of the model

Attempts to standardise the experimental techniques used for hamster cheek-pouch carcinogenesis were made by Morris (1961). He showed that the pouch epithelium of old hamsters was more resistant to the action of DMBA than that of young animals. From the standpoint of ease of manipulation and tumour production, 5 weeks appeared to be the optimum age for starting studies on experimental carcinogenesis. A 0.5% solution of DMBA in acetone produced the maximum tumour yield with minimum latent period and no loss of animals. A 1.5% solution, however, was associated with high morbidity and mortality and a 0.1% solution was ineffective. A shorter latent period was required for tumour production when animals were painted three times a week as opposed to twice weekly, but sex of the animal and caging conditions did not affect the tumour yield.

Morris & Reiskin (1966) investigated the response of cheek-pouch mucosa to varying lengths of carcinogen exposure to determine whether a critical duration of exposure to the carcinogen was necessary for tumour induction. They stated that painting the pouch three times a week for 4 weeks resulted in tumours developing in all animals, but that painting for

less than 3 weeks failed to produce any tumours. They thought this finding implied that the changes responsible for subsequent malignant transformation followed an exposure of 3–4 weeks. At this stage no gross or histological features indicative of future malignant transformation could be detected. Having made this statement, the authors went on to describe tumours developing in animals which had been exposed to carcinogen for only 1 week (three paintings). This ambiguity makes interpretation of the study difficult; much more work is required on minimal dose experiments.

MacDonald (1978) discussed how the response to carcinogen could be more effectively localised. Animals painted with a 0.25% solution of DMBA had significantly fewer tumours developing outside the treated area of mucosa than hamsters painted with a 0.5% solution of carcinogen. There was, however, no significant difference in the yield of tumours within the experimental areas between the groups painted with these concentrations of carcinogen.

3) Metastasis

Although Salley (1954) reported frequent cervical lymph node metastases in his original paper, he did not mention metastases in later papers, and indeed most subsequent workers have failed to demonstrate metastases despite thorough searches of regional lymph nodes and internal organs. Rwomushana et al. (1970) reported one case of cervical lymph node metastasis out of 562 carcinogen-treated hamsters. Recently, however, Craig (1977) has shown that cervical lymph node metastases can be produced by extending the duration of the tumour-bearing period. This was accomplished by removing exophytic tumours while they were small, which allowed the development of more deeply invasive endophytic tumours.

4) Solvents

DMBA has been dissolved in a variety of solvents which have been found to influence the latent period, morbidity, mortality, distribution and yield of tumours. Salley (1954) found that acetone was less toxic and was associated with a lower mortality and shorter latent period than benzene. Heavy mineral oil solvent reduced the latent period for tumour production from 7 weeks (as seen with DMBA in acetone) to 4½ weeks (Salley 1955, 1957a). This reduction in latent period was probably due to increased tissue penetration of carcinogen rather than a true co-carcinogenic action (Berenblum & Shubik 1947, Salaman & Roe 1964). As Stormby & Wallenius (1964) claimed that mineral oil caused digestive tract disturbances, some workers have preferred to use liquid paraffin (Smith 1968, Franklin 1977, Ferguson & Smillie 1979). Attempts to localise and prolong the carcinogenic effect have been made by incorporating DMBA into an adhesive vehicle (Orabase®) (Renstrup et al. 1962). Dimethyl sulphoxide (DMSO), a solvent which readily penetrates the skin, has been said to reduce significantly the period for tumour production (Dachi et al. 1967, Shteyer & Lalonde 1974) and increase the tumour yield (Elzay 1967). Some workers, however, have produced contradictory results using DMSO solvent. Siegel & Shklar (1969) and Shklar et al. (1969), for example, found that animals treated with DMBA in DMSO developed fewer neoplasms than animals treated with DMBA dissolved in heavy mineral oil.

5) Electron microscopy

Relatively few investigators have studied changes in carcinogen-treated cheek pouch by electron microscopy. Listgarten et al. (1963) demonstrated widening of intercellular spaces within 2 days of DMBA application but also

observed similar changes when a noncarcinogenic irritant was used. Carcinoma cells showed clumping of tonofibrils at the cytoplasmic periphery. Bulbous epithelial cell pseudopodia which project through the lamina densa have been described in preneoplastic lesions of the cheek pouch (Woods & Smith 1969a, 1970, MacDonald 1973, McKinney & Singh 1977). These pseudopodia do not appear to be specific to carcinogen-treated mucosa, being produced also by applications of 4-hydroxyanisole (Woods & Smith 1969b), a chemical having no known carcinogenic activity. The pseudopodia produced by 4-hydroxyanisole, however, regress soon after applications of the chemical are stopped, whereas those produced by DMBA persist following cessation of painting.

6) Histochemistry

Changes in enzyme activity during cheek-pouch carcinogenesis have been described. Alteration in the activity of several hydrolases and dehydrogenases was demonstrated by Mori et al. (1962) in experimentally-produced carcinomas. They found a striking increase in alkaline phosphatase activity in the stratum spinosum, especially in relationship to inflamed areas. They speculated that the increased enzyme activity was a result of inflammatory and regenerative changes in the carcinogen-damaged tissues. A progressive increase in the alkaline phosphatase activity in the basal and prickle cell layers of carcinogen-treated cheek pouch was found by Luthra et al. (1969). Smith (1972) showed increased acid phosphatase activity in premalignant cheek-pouch lesions. This activity was seen as fine droplets in the basal cells, and similar droplets were seen in human oral leukoplakia. Smith (1972) suggested that these changes were due to alterations in lysosomes. A further important observation in this experiment was that in some instances the changes described preceded the development of epithelial dysplasia. Shklar (1965) found that lactic dehydrogenase activity was increased and succinic dehydrogenase activity decreased in carcinomas as compared with normal pouch. This observation suggested an alteration from aerobic to anaerobic respiration during carcinogenesis.

7) Cell kinetics

Cell cycle characteristics have been studied in the cheek-pouch model. Reiskin & Berry (1968) and Thilagaratnam & Main (1972) showed that in DMBA-induced cheek-pouch carcinogenesis the cell cycle time was shorter than normal and the cell proliferation rate was higher. Thilagaratnam & Main (1972) found that the reduction in cell cycle time was progressive with advancing stages of carcinogenesis. All phases of the cell cycle were reduced, especially G1 which was shortened by about 90%.

8) Co-carcinogenesis

Berenblum (1954) has described the concept that carcinogenesis is made up of at least two clearly defined stages. In the initiating stage cells are irreversibly converted into tumour cells but remain latent or dormant. Subsequent treatment of such cells with an agent, which itself has no carcinogenic properties, causes them to undergo progressive growth. This process is called promotion. In addition, some agents augment the action of dilute carcinogens when applied simultaneously and are called co-carcinogens (Berenblum 1970).

The hamster cheek-pouch model has been used to examine a variety of carcinogenic, co-carcinogenic and promoting agents thought to be associated with human oral carcinogenesis. In addition, the influence of several systemic factors on the formation of oral neoplasms has been investigated and some

interesting observations have been made.

Croton oil has been shown to exert a co-carcinogenic action with DMBA in cheek-pouch carcinogenesis in old hamsters by Silberman & Shklar (1963). However, in young animals this combination resulted in a retardation of carcinogenesis. Silberman & Shklar (1963) thought the retardation was due to the severe inflammatory reaction following croton oil applications to young animals in some way inhibiting the direct carcinogenic action of DMBA.

Croton oil is perhaps an unwise choice of promoting agent in the hamster cheek pouch as hamster skin has been shown to be refractory to its action (Homburger 1969). Further investigations into this area are clearly required.

Alcohol has been found to act locally with DMBA as a co-carcinogen and promoting agent, reducing the latent period for tumour development and producing more aggressive tumours (Elzay 1966). Henefer (1966) studied the influence of 30% alcohol as the animals' sole source of fluid on DMBA-induced cheek-pouch carcinogenesis. Although he found no significant difference in tumour latency or incidence when compared with water-receiving controls, the number of tumour-bearing animals was small. Using a larger series of animals Freedman & Shklar (1978) showed that 10% alcohol in the drinking water enhanced the effect of DMBA applied to the pouch. Dysplastic and neoplastic lesions developed more rapidly, grew to a larger size and were more deeply invasive and anaplastic than in the control group.

Dachi (1962) showed that Tween 60 (polyoxyethylene sorbitan monostearate), a powerful promoter in the skin, acted co-carcinogenically with DMBA in the cheek pouch. Tween 80 (polyoxyethylene sorbitan monooleate), however, appeared to have no influence on DMBA-induced cheek-pouch carcinogenesis (Sabes et al. 1959).

Many experiments on co-carcinogenesis have been less than ideal as the optimum tumour-producing concentration of DMBA was used. This would tend to mask any weak co-carcinogenic action of the agents being examined.

The cheek-pouch model has been used to examine the effects of whole cigarette smoke and cigarette smoke condensates (Kreshover & Salley 1957, Tabah et al. 1957, Moore & Miller 1958, Kendrick 1964). No tumours resulted from these experiments. However, Elzay (1969) reported that whole cigarette smoke acted as a promoting agent but not as a co-carcinogen in DMBA-induced cheek-pouch carcinogenesis and was a more potent promoter than alcohol. Snuff and chewing tobacco (Peacock & Brawley 1959) and various ingredients of betel quid (Dunham & Herrold 1962) have also failed to produce neoplastic changes in the pouch. Dunham et al. (1966) reported that repeated applications of calcium hydroxide to the pouch produced inflammatory hyperplasia with occasional areas of epithelial atypia. Chang (1966) found that while separate applications of extracts of betel nut or slaked lime to the pouch produced hyperplastic and hyperkeratotic lesions, when these agents were applied together a few papillomas resulted.

Chronic mechanical irritation is another of the many factors implicated in the aetiology of human oral malignancy (Hoback 1946, Watanabe 1970), and the effects of such irritation on the development of carcinomas have been studied in the cheek pouch. Renstrup et al. (1962) showed that chronic mechanical irritation from a stainless steel wire ligated around a molar tooth and projecting into the pouch did not produce any tumours. However, this irritation decreased the latent period but not the final tumour yield when used in combination with DMBA applications. Shteyer & Lalonde (1974), using a similar experimental model to that of Renstrup et al.

(1962), failed to demonstrate any enhancing effect of chronic mechanical irritation. Shklar (1968) showed that manipulation and incision of carcinogen-treated cheek pouch did not influence tumour yield, latency or spread.

MacDonald & Pospisil (1981) compared the effects of DMBA applied to normal hamster pouches and pouches treated previously by cryoprobe. There were no differences in the tumour yield, latency, size or differentiation. However, when the pouches were painted with DMBA for 8 weeks and then subjected to cryotherapy, larger and more anaplastic tumours developed (MacDonald, personal communication).

9) Nutritional status

The effects of the nutritional status of the hamster on cheek-pouch carcinogenesis have received little attention. A higher incidence of tumours in vitamin A-deficient animals was reported by Rowe & Gorlin (1959). These workers also showed that hamsters on a restricted calorie diet had a lower incidence of tumours than those fed *ad libitum* or on a vitamin A-deficient diet. In contrast, local applications of vitamin A palmitate had a potentiating effect on DMBA-induced cheek-pouch carcinogenesis when the vitamin was applied simultaneously with the carcinogen (Levij & Polliack 1968), before DMBA treatment (Levij et al. 1969) or following DMBA treatment (Polliack & Levij 1969). Salley et al. (1962) showed that the latent period for tumour development in chronically thiamine-deficient hamsters treated with DMBA was significantly shorter than that for the control group. Dietary zinc excess was found to cause inhibition of cheek-pouch carcinogenesis by Poswillo & Cohen (1971), but Edwards (1976) failed to show a distinct inhibitory effect of zinc on this form of carcinogenesis.

10) Hormones

The influence of several hormones on hamster cheek-pouch carcinogenesis has been studied. A higher incidence of tumours has been reported in DMBA-treated cheek pouches of castrated hamsters receiving systemic oestrogens by Polliack et al. (1969). They suggested that this enhancement resulted from either an oestrogen-induced increase in permeability of cell membranes or a co-carcinogenic effect of the reaction between DMBA and the mitogenic action of oestrogen. Applications of cortisone before DMBA painting were shown to increase the incidence of tumours in the pouch (Sabes et al. 1963). Siegal & Shklar (1969) found that topically applied triamcinolone inhibited DMBA/DMSO cheek-pouch carcinogenesis but there was no difference between pouches treated with DMBA alone and a combination of DMBA and triamcinolone. Conflicting results have also been reported on the action of systemically administered steroids on cheek-pouch carcinogenesis. For example, Shklar (1967) reported more rapid tumour development in cortisone-treated animals and the development of larger and more deeply invasive neoplasms. Smith (1967), however, found no evidence that systemically administered cortisone produced earlier invasion or more aggressive tumours. The effects of cortisone may be due to its influence on lymphocytes and the immune response. Indeed, immunosuppression by antilymphocyte serum (Woods 1969, Giunta & Shklar 1971) greatly enhanced tumour induction in the cheek-pouch model. Similarly, the latent period was decreased and more anaplastic tumours developed after systemic administration of the antimetabolic drug methotrexate (Shklar et al. 1966). Stimulation of the immune system by BCG delays chemical carcinogenesis in the cheek-pouch model (Giunta et al. 1974). A similar effect can be produced by systemically ad-

ministered Levamisole® (Eisenberg & Shklar 1977), an agent thought to enhance cell-mediated immunity (Churchill & David 1973).

11) Discussion of the cheek-pouch model

The use of the cheek-pouch model in studies on 'intra-oral' carcinogenesis has been criticised by a number of investigators; their objections, together with personal observations, will be discussed.

A major objection to the cheek pouch is that the truly intra-oral nature of this tissue is debatable. Kolas (1955) stated that the cheek pouch could not be considered to be representative of the oral cavity proper as it was not subjected to the same environmental influences as the rest of the mouth. However, the pouch appears to be equally susceptible to carcinogenesis over the whole of its surface, including the area adjacent to the oral cavity proper, which is presumably subjected to the same environmental influences. Stormby & Wallenius (1964) also objected to the cheek pouch on the basis of major anatomical and histological differences between it and other oral mucosae. Salley (1957b) drew attention to the fact that the cheek pouch is derived embryologically from the primitive buccal cavity and maintained that the histology of the pouch, apart from the absence of adnexal structures, is identical to the rest of the oral cavity. However, this assertion is incorrect. In addition to being considerably thinner than the rest of the oral mucosa and having a unique submucosal connective tissue (Walker et al. 1970), the cheek pouch has a single layer of progenitor cells (Eveson & MacDonald 1978). In the oral mucosa proper there is a suprabasal dividing cell population.

Smith (1968) found that the histochemical features of human and cheek pouch premalignant lesions were closely similar and considered that objections to the use of the pouch in regard to the intra-oral nature were unimportant. In the standard model of cheek-pouch carcinogenesis, in which 0.5% solution of DMBA is used (Morris 1961), neoplastic transformation is very rapid. Homburger (1972) has drawn attention to the possibility that many of the so-called features of premalignancy described in such models could be epiphenomena related to a hyperplasiogenic or irritant action of the carcinogen. From the quantitative studies reported by Eveson & MacDonald (1978) it would appear that much of the hyperkeratosis seen in the early stages of carcinogen treatment is a reversible reaction and probably related to an irritant action of the carcinogen.

A further objection to the cheek pouch is that it is a site of immunological privilege. For example, it will accept heterografts of both normal and neoplastic tissues (Billingham et al. 1960, Williams et al. 1971). The relationship between this unusual characteristic and the development and behaviour of chemically-induced neoplasms is not well understood, but Smith (1968) suggested that this property could in part be responsible for the high degree of susceptibility of the pouch to chemical carcinogens. The effects of immunosuppressive or immunostimulatory agents on chemically-induced tumours show that the degree of immunologic privilege in the cheek pouch is relative and restricted to the afferent limb of the cell-mediated immune response. Contact hypersensitivity reactions can be induced in the cheek pouch after skin applications of dinitrochlorobenzene (DNCB), a simple allergen which is a nonspecific stimulator of T lymphocytes (Mohammad & Mincer 1976). However, stimulation of cell-mediated immunity in the cheek pouch by local applications of DNCB does not alter the latent period or tumour yield in DMBA-induced carcinogenesis (Murphy & Giunta 1978).

Finally, a somewhat mundane but no less

valid objection to the cheek pouch is the technical difficulty of handling the tissue. The pouch mucosa is very thin and has a gelatinous, sticky, submucosal layer and this can make the taking of biopsy specimens and the trimming of blocks very difficult.

The standard hamster cheek-pouch model cannot be considered to be an 'ideal' model of intra-oral carcinogenesis. The cheek pouch model has considerable potential as a model of 'epithelial' carcinogenesis, especially in relation to epithelio-mesenchymal interactions, due to its relatively simple morphology and the absence of adnexal structures, and for the evaluation of suspected carcinogenic and co-carcinogenic agents.

III. Models of intra-oral carcinogenesis in extra-pouch sites

1) Hamster

The oral mucosa proper of the hamster is considerably more resistant to the action of chemical carcinogens than the cheek pouch. In his initial experiments on cheek-pouch carcinogenesis, Salley (1954) recorded carcinomas occasionally developing in the palate, buccal mucosa, tongue, oesophagus and stomach, presumably as a result of overflow of carcinogen into and beyond the oral cavity. In a later study, Salley & Kreshover (1959) found that thrice weekly painting of the palates of hamsters with DMBA resulted in carcinomas developing in 54% of the animals after 16 weeks. When a similar technique was applied to hamster gingiva, only 10% of the experimental animals developed carcinomas (Al-Ani & Shklar 1966) but several papillomas and numerous areas of dysplasia were present. Mesrobian & Shklar (1969) applied powdered DMBA to hamster gingiva once weekly and held the carcinogen in place by cyanoacrylate tissue adhesive. All the experimental animals developed squamous cell carcinomas by 20 weeks. Dachi (1967) induced lingual carcinomas in 4 out of 15 hamsters by applying DMBA in DMSO. However, larger and more anaplastic tumours developed in adjacent skin and oral mucosa and these lesions were frequently responsible for premature deaths. A more successful model of lingual carcinoma in the hamster was developed by Fujita et al. (1973a, 1973b). They applied DMBA to the tongue after first scratching and ulcerating the area with a root-canal barbed broach. There were regional variations in the susceptibility of the lingual mucosa to the carcinogen but when the lateral border was treated in this manner, all the experimental animals developed invasive neoplasms between 13 and 25 weeks.

Marefat & Shklar (1977) and Eveson (1979), using similar models to that of Fujita et al. (1973a, 1973b), showed that gross ulceration is not a necessary precursor of malignancy in this model. The ultrastructural changes in the hamster tongue model appear to be similar to those described in the cheek pouch (Marefat et al. 1979). Recently Shklar et al. (1980) have shown that retinoic acid can inhibit carcinogenesis in the hamster tongue. Attempts to produce tumours in the hamster ventral lingual mucosa using the water-soluble carcinogen 4-nitroquinoline N-oxide (4NQO) were unsuccessful (Eveson & MacDonald 1977).

An entirely different approach to the study of intra-oral carcinogenesis has been the use of systemically administered carcinogens. For example, Herrold (1966) showed that hyperkeratosis of the oral mucosa developed in hamsters which had received N methylnitrosourea (NMU) parenterally. Edwards (1978) extended this work and showed that intragastric administration of NMU led to the development of oral premalignant lesions, papillomas and squamous cell carcinomas. He believed that these lesions were unlikely to be due to local contamination with NMU but

valid objection to the cheek pouch is the technical difficulty of handling the tissue. The pouch mucosa is very thin and has a gelatinous, sticky, submucosal layer and this can make the taking of biopsy specimens and the trimming of blocks very difficult.

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suggested that minute doses in saliva might have a topical effect. The use of NMU given either systemically or topically has received relatively little attention, and further study may show this agent to be of value in investigations of intra-oral carcinogenesis.

2) Mouse

The production of oral tumours in mice has proved difficult. Van Prohaska et al. (1939) reported one carcinoma developing in the oral mucosa of mice which had been fed with 20 MC in olive oil over a period of 6 months. No tumours were produced by painting mouse gingiva with 20 MC for up to 16 weeks (Levy 1948) but when 20 MC was injected under the dorsal mucosa of their tongues, malignant epithelial tumours developed (Levy 1958). Goldhaber (1957) found that when salivary glands were removed from mice 20 MC could induce carcinomas in the labial mucosa. Carcinomas of murine buccal mucosa were produced by Protzel et al. (1964) using 3, 4 BP as the carcinogen. When the carcinogen was applied to animals in which severe liver damage had been induced the frequency of tumours was increased and the latent period decreased. Labial carcinomas were produced in nearly half the experimental animals when mice were painted with 4NQO for prolonged periods (Fujino et al. 1965). The tumour yield rose to nearly 80% when, in addition to applications of 4NQO, a metal wire inserted between the lower incisors was used to injure continually the labial mucosa. Five per cent of animals also developed lingual carcinomas.

3) Rat

Initial attempts to induce oral tumours in rats also met with limited success. Wallenius (1966) showed that only about 30% of experimental animals developed oral squamous cell carcinomas after painting with DMBA for 16 months. When a similar method of carcinogen application was used in desalivated rats, malignant tumours developed in all animals by 11 months. Attempts to produce lingual neoplasms in the rat were unsuccessful even when DMBA was bonded to the tongue with cyanoacrylate tissue adhesive (Giunta & Shklar 1972). When the water-soluble carcinogen 4NQO was used, however, palatal carcinomas were produced in all experimental animals by seven months (Wallenius & Lekholm 1973a). In addition 75% of animals developed carcinomas on the dorsum of the tongue and 20% showed carcinomas of gingiva or stomach.

The rat-palate model using either DMBA in combination with antisialagogues or 4NQO in propylene glycol is becoming increasingly popular in studies of intra-oral carcinogenesis. Heyden (1974) investigated glucose 6-phosphate dehydrogenase activity in the palatal epithelium of DMBA-treated animals. He showed the development of enzymatic reactions in epithelium before the development of morphologic dysplasia. An increase in chromosomal abnormalities (deviations) has been demonstrated in the DMBA and 4NQO model and a close correlation was found between such changes and the simultaneous presence of dysplasia and aberrant glucose 6-phosphate dehydrogenase activity (Wallenius et al. 1975). In addition it was found that nonrandom chromosomal abnormalities induced by DMBA differed from those induced by 4NQO.

Lekholm et al. (1975) showed focal loss of lipid in the advanced stages of carcinogenesis induced by 4NQO. An attempt to assess the role of alteration in the fatty acid pattern of the oral epithelium in relation to carcinogenesis was made by Lekholm & Wallenius (1976). They studied the effects of 4NQO application to the palates of rats in which liver damage had been induced by essential fatty acid deprivation or carbon tetra-

chloride-induced cirrhosis. Although the initial reaction of the oral mucosa to the carcinogen appeared to be most severe in the animals with essential fatty acid deficiency, carcinomas developed earlier in the cirrhotic group. It was concluded from this experiment that there was no correlation between the extent of the disturbance in lipid metabolism and reduced resistance of the oral mucosa to topically applied carcinogen. Recently Wallenius et al. (1979) showed that a zinc-supplemented diet accelerated and a zinc-deficient diet retarded the development of 4NQO-induced carcinomas of the rat palate.

The Japanese workers Yamamura et al. (1975) attempted to prolong the action of chemical carcinogens by implanting them into surgically-created caecal pouches in the rat lip. The carcinogens investigated were DMBA, crystalline 20 MC and crystalline N-methyl-n-nitro-nitrosoguanidine. These carcinogens induced a variety of neoplasms including squamous cell papillomas, carcinomas, neurofibromas, lymphangiomas, haemangiomas and haemangiosarcomas. This wide variety of neoplasms, whilst being interesting as an observation, would make the model system of limited value in a study of squamous cell carcinoma.

4) Primates

Primates appear to be very resistant to the actions of known chemical carcinogens in most sites (Kent 1960), and there is no satisfactory simian model of oral carcinogenesis. One monkey developed a carcinoma of the tongue after a radioactive source was implanted into the maxillary antrum (Melnikov 1963). Cohen & Smith (1967) painted monkey oral mucosa bi-weekly with DMBA for nearly 4 years without finding any changes indicative of transformation to malignancy. When tobacco paste was inserted into surgically-created pouches in monkey cheek, there were minimal histological changes apart from "ballooning" of epithelial cells (Cohen & Smith 1967), a feature previously described in human oral epithelium following exposure to snuff (Pindborg & Renstrup 1963), chewing tobacco (Zegarelli et al. 1961) and components of betel quid (Pindborg et al. 1964). However, when the application of tobacco to these cheek pouches was repeated at intervals for up to 3 years, epithelial atypia developed (Cohen et al. 1971). Hamner (1972) showed that moderate to severe epithelial atypia could be induced in protein-deficient baboons by implanting betel quid with tobacco into surgically-created buccal mucosal pouches.

IV. Resistance of the oral mucosa to chemical carcinogens

The oral mucosa, apart from the hamster cheek pouch, is much more resistant to the action of chemical carcinogens than skin (Levy 1948, Levy et al. 1950, Kreshover 1952, Kolas 1955). The most frequent explanations for the differences in susceptibility are the influence of saliva in the mouth and the presence of 'portals of entry' in the skin formed by sebaceous glands and hair follicles.

Kolas (1955) attempted to demonstrate an anti-carcinogenic action of saliva by applying saliva to the ears of mice before painting with 20 MC. He found no protective effects. Further attempts to show a protective action of saliva by extirpating the major salivary glands failed to have any effect in either mice (Goldhaber et al. 1956) or hamsters (Kreshover & Salley 1957). However, the degree of desalivation achieved in these experiments was only partial as the minor salivary glands were left intact. Wallenius has undertaken a number of careful experiments to determine the role of saliva in carcinogenesis. Stormby & Wallenius (1964) found that hamsters with reduced salivation following

surgical removal of the major salivary glands developed more neoplastic lesions after intra-oral carcinogen applications than the controls, but the difference was not statistically significant. In 1966 Wallenius showed that when the cheek skin of rats was transplanted into the buccal mucosa and then painted with carcinogen no changes were found in the transplanted skin after 11 months, whereas in its normal situation tumours developed within 6 months. Wallenius (1966) then applied carcinogen to rats' oral mucosa after surgical removal of the major salivary glands and repeated injections of an antisialagogue. Xerostomic animals developed buccal carcinomas after 11 months of carcinogen application, whereas none of the normal controls and only a third of animals with reduced salivation developed tumours. Wallenius believed the protective action of saliva was due to the formation of a moist mucous barrier rather than a diluting action as proposed by Kreshover & Salley (1957). Wallenius & Lekholm (1973a) produced high yields of malignant tumours in rat palate and tongue in less than 7 months using the water-soluble carcinogen 4NQO in animals with intact salivation. They attempted to explain the increased efficiency of 4NQO over DMBA in producing oral tumours in the rat by suggesting that the salivary layer was protective against DMBA, which is fat-soluble, but not against the water-soluble 4NQO. This supposition appeared to be verified by *in-vitro* experiments (Wallenius & Lekholm 1973b). However, Eveson & MacDonald (1977) found hamster tongue to be relatively refractory to the action of 4NQO. This suggested that a difference in the susceptibility of a species or site, rather than a fundamental difference between the reaction of fat-soluble and water-soluble carcinogens and the salivary layer, accounted for the efficiency of 4NQO in producing oral tumours in the rat.

Although there is a good deal of evidence that saliva exerts some protective influence in oral carcinogenesis, the importance of portals of entry is much less clear. There is little doubt that adnexal structures play an important role in chemical carcinogenesis in skin. Lacassagne & Latarjet (1946) painted 20 MC onto areas of mouse skin in which appendages were absent due to scarring or UV light irradiation and found these areas to be resistant to tumour induction. Suntzeff et al. (1947) also failed to induce neoplasms in the skin of newborn mice, which have immature sebaceous glands and hair follicles. The skin appendages may aid retention of the carcinogen. This is supported by Simpson & Cramer (1943), who showed by using fluorescence microscopy that 20 MC accumulated in sebaceous glands after a single application of carcinogen.

Despite the apparent importance of portals of entry in cutaneous carcinogenesis, their possible role in intra-oral carcinogenesis is equivocal. Levy (1958) found that when 20 MC was injected into the submucosa of mouse tongue there was a striking increase in the frequency of tumours developing in the overlying epithelium when compared with superficial painting of the carcinogen. This observation appeared to support a role of portals of entry in intra-oral carcinogenesis. However, the oral tissue most susceptible to the action of chemical carcinogens is the hamster cheek pouch which contains no appendages (Salley 1961a). Studies utilizing the fluorescent properties of some polycyclic hydrocarbons (Salley 1961b) and ^{14}C labelled DMBA (Meskin & Woolfrey 1964) have shown that in the cheek pouch carcinogens pass through apparently intact epithelium and are retained in the lamina propria. In the palate, Salley (1961b) showed that carcinogen could be detected in the lamina propria before the minor mucous glands. Goldhaber (1957) suggested that ulceration of mucosa allowed penetration of carcinogen, but this was not supported by the work of Morris (1961), who showed that very

low concentrations of DMBA could produce neoplasms in hamster check pouch without any macroscopical evidence of ulceration, a finding confirmed by MacDonald (1973) and Eveson & MacDonald (1978). Homburger (1969) has suggested that microscopical defects are formed in the mucosa during mastication and these cannot be ruled out as potential portals of entry for chemical carcinogens. Listgarten et al. (1963) considered that the intercellular oedema induced by carcinogen painting could facilitate penetration of the carcinogen.

V. Conclusions

The hamster cheek-pouch and tongue models using DMBA and the rat palate model using 4NQO appear to be the most suitable models of oral carcinogenesis at this time. Each model has advantages and disadvantages and none can be considered ideal.

Although the properties of any experimental model depend on the information which the investigator is seeking, certain characteristics are desirable. The site chosen should, if possible, have similar histology to the equivalent human site. This should include such features as the pattern of keratinization and the presence of a suprabasal dividing cell population. The only model which fully satisfies this requirement is the rat palate. It is an advantage if the neoplastic changes are reasonably well localised so that the animals do not die prematurely from tumours developing outside the experimental area. Careful attention to the technique of application and the use of an appropriate concentration of carcinogen gives good tumour localisation in the cheek-pouch model (MacDonald 1978). In the hamster-tongue model and the rat-palate model tumours frequently develop outside the experimental area but are only rarely the cause of premature deaths. In most of the experimental models of intra-oral carcinogenesis carcinomas develop from papillomas, a situation which is rare in humans. The fact that the carcinomas induced in the rat-palate model are endophytic, ulcerative lesions is thus a distinct advantage. In some circumstances it is useful if there is not a complete transformation of premalignant to malignant lesions, as this allows the correlation of early histological changes with subsequent malignant transformation. This is clearly of considerable importance in relation to the histological assessment of premalignant lesions in humans. Animal models may help to show which of the individual histological features described as epithelial atypia are reactive and basically epiphenomena and which, if any, are genuine markers of subsequent malignant change. Comparison between epithelial hyperplasia induced by carcinogens and noncarcinogenic irritants such as mechanical trauma (Eveson & MacDonald 1981) or chemical agents (Craig & Franklin 1977, Franklin & Craig 1978) may help to shed some light on this contentious area of diagnostic histopathology.

Finally, it is pertinent to add that much of the voluminous literature on experimental intra-oral carcinogenesis has been highly empirical and has contributed little to our understanding of the causation, development or control of oral cancer in man. The models described merely form a baseline of data. Further studies are now needed using objective criteria and more precise investigative tools such as stereology, autoradiography, histochemistry and immunohistochemistry. In addition, the lack of adnexal structures in certain areas of the oral mucosa would make this tissue extremely useful for studying the role of epithelio-mesenchymal interactions during carcinogenesis. Such studies might yield fundamental information of importance to the general field of oncology.

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