〔Original〕 Matsumoto Shigaku, 9 : 174~182, 1983
key words : lingual tumors — DMBA — hamster — jet injection

Experimental Production of Lingual Tumor by Jet Injection of 9, 10-dimethyl 1, 2-benzanthracene

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Summary

Ninety-six male golden hamsters, 3 months of age, were injected with a 0.25% solution of 9, 10-dimethyl 1, 2-benzanthracene in acetone into the tongue at weekly intervals using a Panjet injector (Panjet®: Wright, G. E.). Thirty hamsters surviving for six months or longer after initiation of the carcinogen injection were subjected to observation.

By the 11th week of experiment, the lingual mucosa became slightly hypertrophic and whitish in patches. Around the 24th week, nearly 80% of the animals were found to have formation of nodules in the lingual mucosa. After the 35th week of treatment the nodules grew in size of showed a papillary growth in occasinal animals. Histopathologically, there were seen dysplasia (11 animals), dysplasia with epithelial down growth (7 animals), papilloma (7 animals), carcinoma (3 animals), hemangioma (1 animal), neurilemmoma (1 animal).

Introduction

Malignant growths induced experimentally by injection of any carcinogenic substance are usually nonepithelial, as has been reported in the literature. As is also the case with tumors of the tongue, there have been very few reports[2,15,17] of the development of lingual carcinoma by local injection of carcinogen, and as yet no report of the development of lingual hemangioma or neurilemmoma.

The present report deals with the development of hemangioma and neurilemoma of the tongue, as well as epithelial tumors (papillomas, exophytic low grade squamous cell carcinoma), in hamsters injected locally with a carcinogen. Injections were administered using a jet injector designed to eject a fine spray of solution via its nozzle at such speed as to penetrate the surface layer of lingual tissue (Panjet®: Wright, G. E.).

Experimental procedure

Ninety-six, three-month-old male golden hamsters were injected with a 0.25% solution of 9, 10

(accepted on November 4, 1983)

-dimethyl 1, 2-benzanthracene (DMBA) in acetone into the right lateral border, middle third of the tongue at weekly intervals using a Panjet injector (Fig. 1). The amount of the carcinogen solution administered at each injection was between 0.012 and 0.029ml.

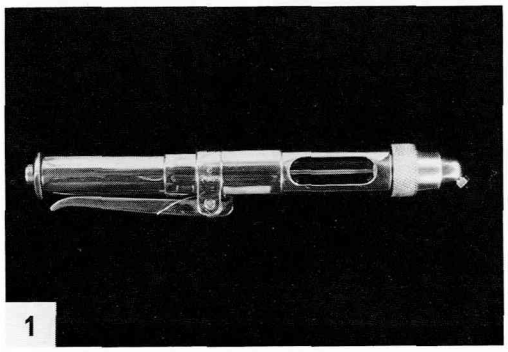

Fig. 1. Jet injector

Thirty hamsters surviving for six months or longer after initiation of the carcinogen injections were subjected to observation for development of tumors, and the observation was completed 393 days after the injections were initiated. Histopathologic examination was made on all animals which died spontaneously during the observation period.

Results

Macroscopic findings:

Nearly all of the animals developed ulceration of the mucosa at the injection site 3 days after local injection of 0.25% DMBA in acetone with a Panjet injector (Fig. 2). In only a few animals, petechiae were also noted at the same site. These local reactions to injection became progressively less conspicuous with increasing cumulative number of doses given.

After 11 weeks of experiment, ulceration was no longer manifest at the injection site in any of the animals and the mucosa of this region became slightly hypertrophic and whitish in patches. After about 13 weeks of experiment, several small areas of mucosal elevation were noted to have arisen in the lesions, and as the treatment with DMBA progressed to the 24th or 25th week, nearly

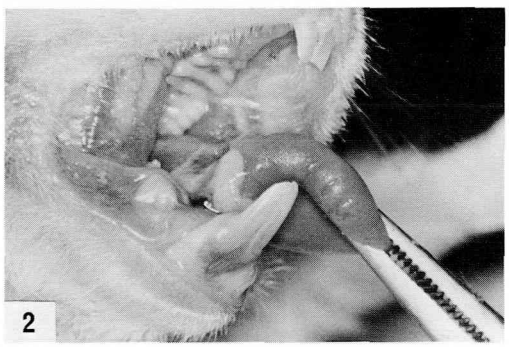

Fig. 2. Ulcer of the tongue, as seen 3 days after local injection of DMBA

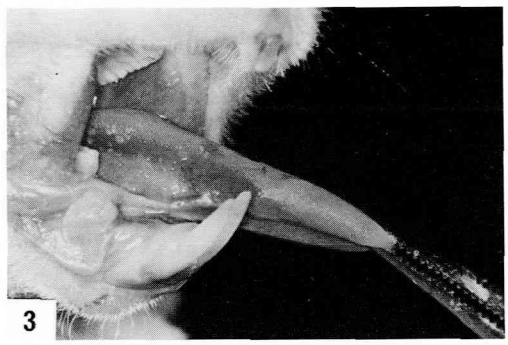

Fig. 3. Tongue of animal during 17th week showing multiple small nodular growths

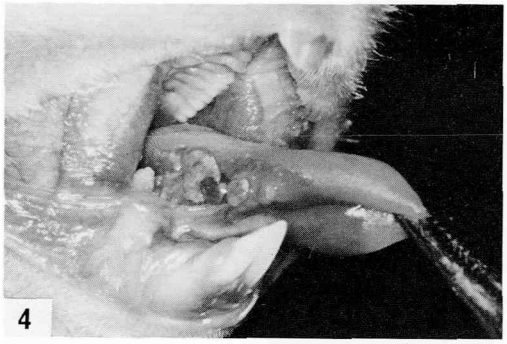

Fig. 4. Tongue of animal during 35th week showing large papillary growth

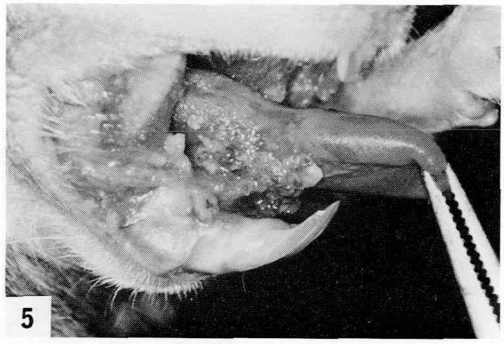

Fig. 5. Tongue of animal during 55th week showing large papillary spreading tumor

80% of the animals were found to have formation of nodules while the remainder had leukoplakias in the lingual mucosa (Fig. 3). After the 35th week of treatment, the nodules grew in size or showed a papillary growth in occasional animals (Fig. 4). These findings became increasingly apparent after the 40th week, the tumorous growth extending so far as to involve the floor of the mouth (Fig. 5).

Histopathologic findings :

Histologically, there was mucosal dysplasia at the injection site in 11 animals. In these cases it was epithelial dysplasia with narrower pegs and a somewhat more pronounced tendency to keratinization as compared to the mucosa on the normal side (Fig. 6). The mucosa at the injection site presented microscopic features of papillary growth in some animals.

There were 7 animals in which further extensions of the peg-shaped proliferation into the connective tissue were noted. In cases with these findings, exophytic papillary growth was rather inconspicuous ; the peg-shaped extensions with nuclear hyperchromasia and "uprising" of basal cells were found (Fig. 7).

Most of the lesions observed grossly as an overt papillary growth of mucosa were seen microscopically as a dendriform proliferation of keratinized epithelium characteristic of papilloma (Fig. 8), and this finding was seen in 7 animals.

Furthermore, 3 cases presented findings of exophytic low grade squamous cell carcinoma of the

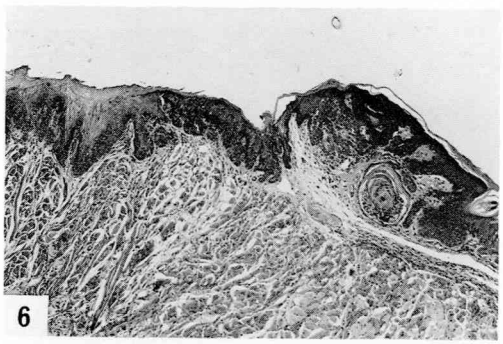

Fig. 6. Dysplastic epithelium with keratotic crypt (Hematoxylin and eosin stain. Magnification, ×52)

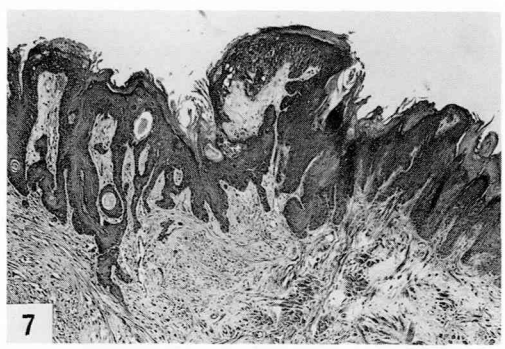

Fig. 7. Dysplastic epithelium with some sign of epithelial downgrowth (Hematoxylin and eosin stain. Magnification, ×65)

Fig. 8. Papilloma of tongue (Hematoxylin and eosin stain. Magnification, ×65)

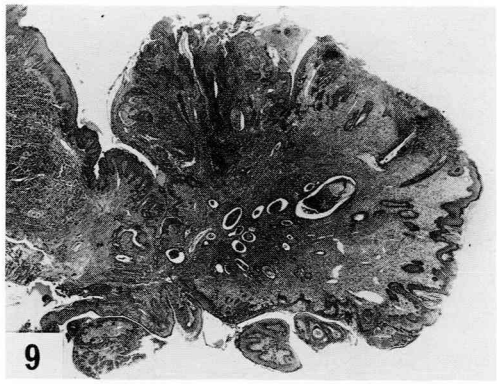

Fig. 9. Low-power view of low grade squamous cell carcinoma of tongue in DMBA injection animal during 39th week. The epithelium shows papillary proliferation, the surface of which is hyperkeratinized. In other areas of the epithelium are seen a growth advancing in the form of pegs and hyperchromasia of the nuclei of basal cells. (Hematoxylin and eosin stain. Magnification, ×16)

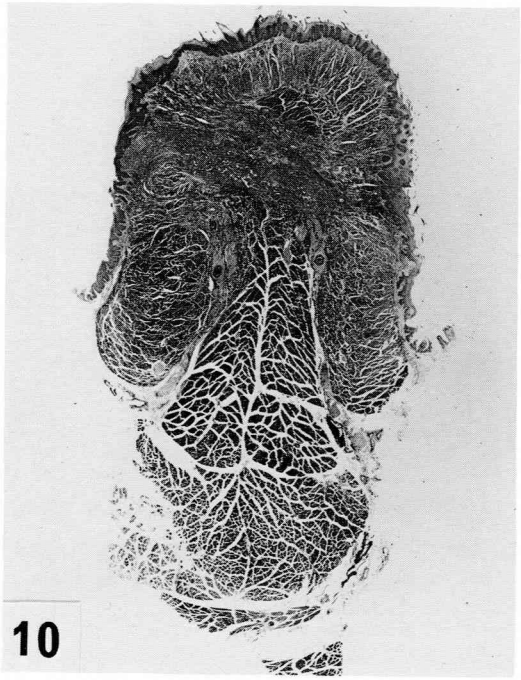

Fig.10. Low-power view of hemangioma of tongue in DMBA injection animal during 39th week (Hematoxylin and eosin stain. Magnification, ×10)

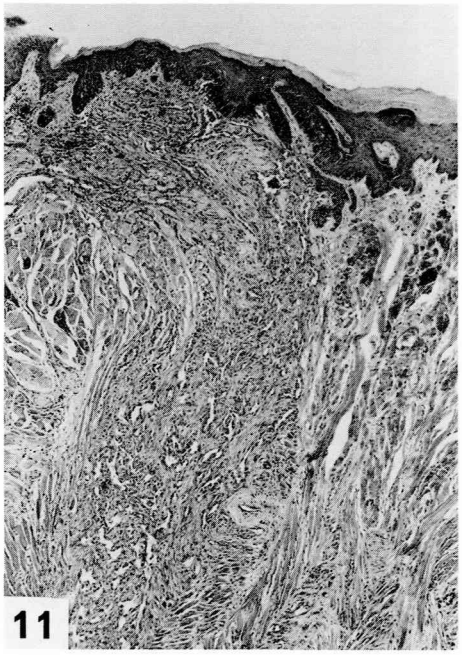

Fig. 11. Medium-power view of specimen in Fig. 10. Long, slender cords of endothelial proliferation are seen from immediately beneath the mucosal epithelium downward, involving bundles of striated muscle fibers in occasional areas. In this tissue are seen many small lumina, most of which being vacant, while some contain erythrocytes. There is little or no round cell infiltration. In the areas of epithelium adjacent to the tumor are seen peg-shaped extensions, nuclear hyperchromasia and the so-called "uprising" of basilar cells. These findings indicate the tissue to be in a precancerous state. (Hematoxylin and eosin stain. Magnification. ×52)

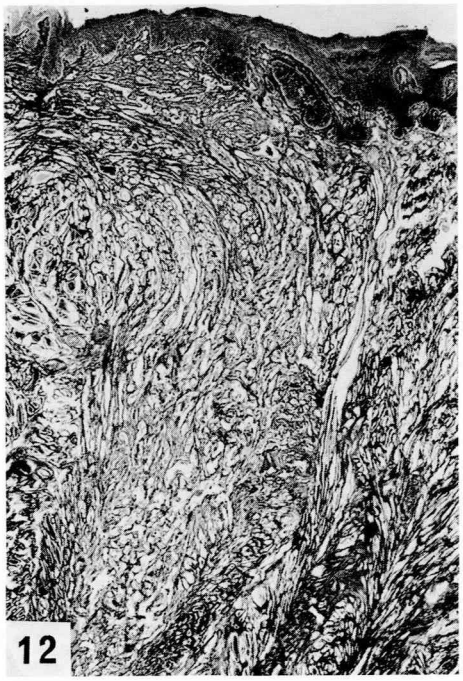

Fig. 12. Medium-power view of specimen in Fig. 10. (Elastica-van Gieson stain. Magnification, ×52)

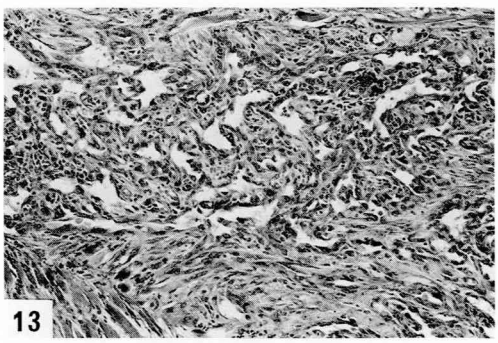

Fig. 13. High-power view of specimen in Fig. 10. (Hematoxylin and eosin stain. Magnification, ×160)

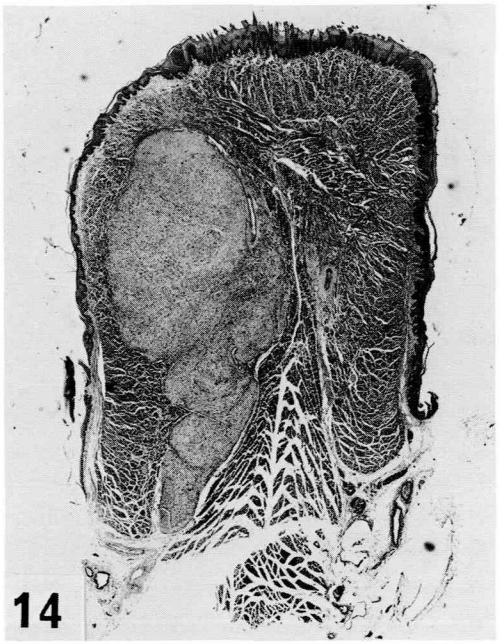

Fig. 14. Low-power view of neurilemmoma (Antoni type B) of tongue in DMBA injection animal during 39th week. (Hematoxylin and eosin stain. Magnification, ×12)

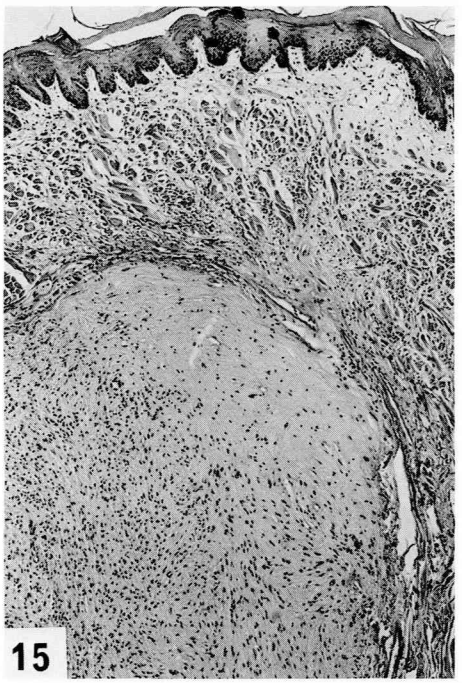

Fig. 15. Medium-power view of specimen in Fig. 14. There is nodular proliferation of cells in the striated muscle. These cells are spindle-shaped and their nuclei spheroidal and of various sizes. Under high power magnification, structures resembling cross sections of nerve fiber bundles are noted in areas surrounding the tumor mass. The tumor is fairly well demarcated though occasionally involving bundles of muscle fibers. Sections with van Gieson stain revealed a remarkable paucity of collagen fibers among tumor cells. (Hematoxylin and eosin stain. Magnification, ×52)

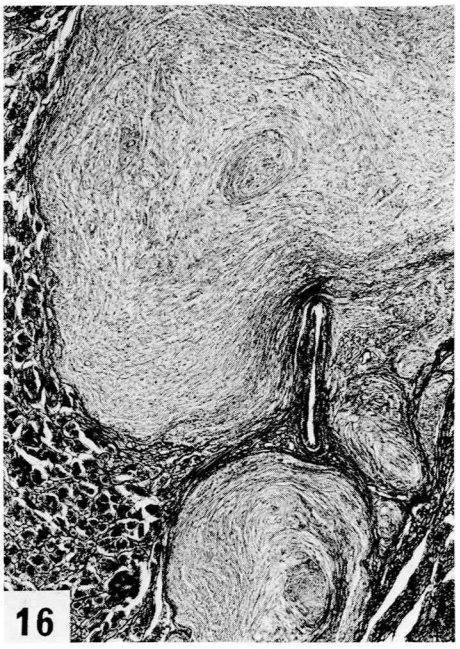

Fig. 16. Medium-power view of specimen in Fig. 14 (Elastica-van Gieson stain. Magnification, ×52)

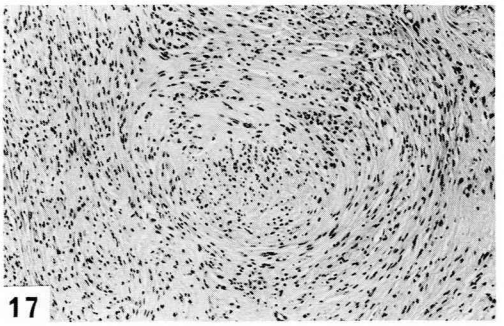

Fig. 17. High-power view of specimen in Fig. 14 (Hematoxylin and eosin stain. Magnification, ×130)

tongue. The epithelium of the tongue showed papillary proliferation, the surface of which was hyperkeratinized. In other areas of the epithelium, a growth advancing in the form of pegs and hyperchromasia of the nuclei of basal cells was seen (Fig. 9).

In one animal, there was histopathologic evidence of capillary hemangioma characterized by long, slender cords of endothelial proliferation from directly beneath the mucosal epithelium downwards into underlying tissues (Figs. 10, 11, 12, and 13). Neurilemmoma with microscopic cross-sections of nerve fiber bundles in the striated muscle was seen in another animal (Figs. 14, 15, 16, and 17).

Discussion

There have been several reports of attempts to produce tumorous growth of the tongue by topical application to the lingual mucosa, [5.7.8.9.10.13.16.18.19] injection into the submucosa [2.6.11.15.17] or oral administration of carcinogenic substances [12]. However, reports of experimental production of tumor by injection of such substance into the tongue as yet are few. Haga [11] (1913) observed atypical epithelial proliferation in the tongue of domestic rabbits injected with a solution (0.5 g) of scarlet red in olive oil into the lingual margin, and Bullock and Rohdenburg [1] (1918) demonstrated an atypical proliferation of lingual glands in rats treated by injections of scarlet red in olive oil into the tongue. However, neither attempt achieved formation of a tumor.

In 1935, Oyama [17] first described the development of squamous cell carcinoma in the tongue of domestic rabbits by injections of coal tar into ulcers that had been induced by mechanical stimulation of the mucosa via a metal ring fixed on a molar tooth. Later, Levy [15] (1958) observed malignant changes at 16 weeks in the tongue of mice receiving lingual submucosal injections of about 0.01ml of an aqueous suspension of 0.3mg methylcholoathrene. Carcinoma was evident in 3 of 18 mice sacrificed at 64 weeks after injection and there was a noticeably increased incidence of granular cell myoblastoma in the treated group as compared to controls, according to the report. Unfortunately, however, the report did not provide histopathologic findings of the tumors.

In recent years, Chino and Kameyama [2] (1965) have succeeded in inducing the development of carcinoma of the tongue in 2 of 20 mice injected with 0.05ml of propylene glycol containing 0.15mg of 4-nitroquinoline 1-oxide into the tongue.

In the present study, papillomatous growths and low grade squamous cell carcinoma developed in the tongue following submucosal injections of the carcinogen by means of a fine, high-speed spray of solution using a high pressure jet injector, thus obviating the insertion of a needle. It is of profound interest that hemangioma and neurilemmoma of the tongue developed in these animals.

An exhaustive search of the literature has failed to reveal any report of experimental induction of hemangioma or neurilemmoma in the oral cavity. However, in a study described by Kawauchi [14] (1973), hemangioma developed in 2 rats, neurofibroma in 3, lymphangioma in 2, and angiosarcoma and lymphangioma in 1 of 26 rats with N-methyl-N'-nitro-N-nitrosoguanidine crystals inserted into an artificial pouch prepared in the lower lip. In view of the fact that neither papilloma nor carcinoma developed in that series, the investigator interpreted the development of such peculiar types of tumors (hemangioma and neurofibroma) as being probably due to marked actions of the carcinogen causing ulceration of the mucosa lining the pouch, thereby permitting its direct actions upon the underlying mesenchymal tissue. Although preliminary experiments had shown that a solution injected via the jet injector primarily reached an area adjacent to the basement membrane

of the epithelium, it is conceivable by analogy, that the injected material might have gained direct entrance to the underlying mesenchyme in some of the animals of the present series, thus leading to the development of mesencymal growths. Moreover, there were seen abnormal overgrowths of the neurobundles in several animals (Fig. 18) so the neurilemmoma observed in this experiment was probably not congenital.

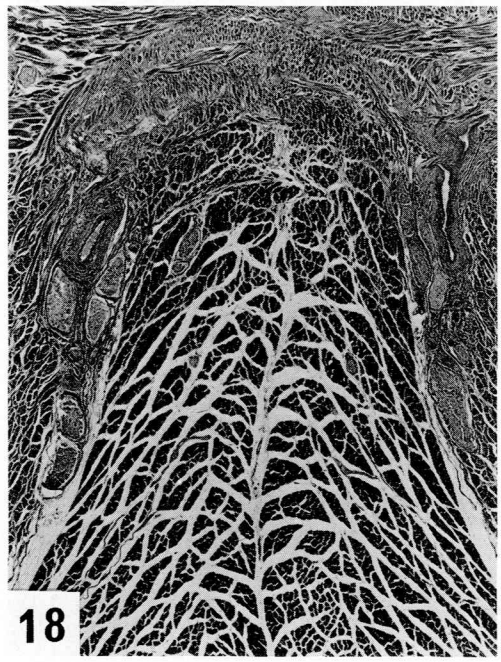

Fig. 18. Low-power view of specimen. Abnormal overgrowths of the neurobundles are apparent. (Hematoxylin and eosin stain. Magnification, ×25)

In any case, the present investigation could not encompass any attempt at clarification of the pathogenetic process of these tumors, which will be the subject of ensuing studies.

The authors wish to express their gratitude to Dr. Shigeo Eda, Professor of Oral Pathology, Matsumoto Dental College, for making valuable suggestions.

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