TUMOR INDUCTION THROUGH VARYING LENGTHS OF CARCINOGEN EXPOSURE

LI-MIN LIN, KENT A. HEIDEMAN* and PATRICK D. TOTO**

Squamous cell carcinomas and papillomas were induced in the buccal mucosa of the left cheek pouch of Golden Syrian Hamsters using 9,10-dimethyl-1,2-benzanthracene (DMBA). Applications of a 0.5 percent solution of DMBA in mineral oil were made biweekly. Treatment and observation was for a period of 12 weeks after which the cheek pouch was inverted, excised and histological slides were prepared from the tissue. Tumors and papillomas were observed in all groups painted seven through twelve weeks. Those painted for seven weeks demonstrated papillomas. The changes from seven weeks onward progressed from papillomas to carcinoma. The longer the groups were painted, the more severe were the lesions.

Key words: carcinogen, induction, DMBA

(Kaohsiung J Med Sci 2: 394–400, 1986)

A majority of oral cancer cases are epithelial in nature. Attempts to produce this type of cancer in hamster cheek pouches through the use of chemical carcinogens have not only been successfully tested but are being used widely in research today.

A review of the literature reveals numerous studies in which tumors have been induced through the use of carcinogens[1-3]. As early as 1815, Yamagiwa[4] discovered that skin cancer could be produced by tarring the ear of a rabbit. Since the isolation of the carcinogenic hydrocarbon, benzpyrene from tar in 1932, by Cook and Kennaway[1-3], thousands of different compounds have been studied for their carcinogenic effects.

Levy and Ring[2] successfully implanted crystalline 9,10-dimethyl-1,2-benzanthracene subgingivally in the jaws of hamsters producing malignant tumors of the connective tissue.

The dark-eared albino hamsters have been shown to produce visible tumors earlier than other strains and also have a shorter mean latent period. In addition, the latent periods appear more evenly distributed[5]. Studies have been discussed in various papers in relation to such aspects as the sex[6], age[7], method of application[6], numbers of hamster percentage[4], chemical carcinogen[8-11] and solvent of choice[12], optimal concentration of DMBA[2,7] and frequency of application per week[7] of the hamsters, etc.

Morris[6] reported that after five and one-half weeks (16 paintings of carcinogen), initial lesions were evidenced. After seven weeks or 14 paintings of a standardized amount of DMBA, initial lesions were also observed.

In the studies of Salley[8,12,13] and Morris[7] hamsters were treated with DMBA continuously until tumors were visible. This enabled the investigator to establish a latent period but the critical cellular transformations which were responsible for malignancy had occurred before the treatment was discontinued.

This study is concerned with the question of whether a critical duration of exposure of DMBA to the cheek pouch epithelium exists for the induction of tumors. At what point

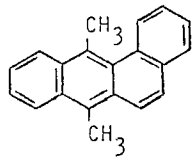
Oral Pathology and Diagnosis Department, School of Dentistry, Kaohsiung Medical College, Kaohsiung City 80731, Taiwan, Republic of China.
*Toquerville, Utah, U.S.A.
**Oral and General Pathology Department, School of Dentistry, Chicago Loyola University, U.S.A.

in time or stage of the carcinogenic induction process are the tissues irreversibly altered? If that brief period of change is detected, research efforts can be concentrated at this stage of development.

The question of malignant transformation following DMBA application at biweekly intervals has remained to be answered. During tumor induction, what minimal number of biweekly applications of 0.5 percent DMBA in mineral oil will lead to neoplasia within 12 weeks? This study is designed to answer this question.

MATERIALS AND METHODS

The carcinogen which was used in the tumor induction was 9, 10-dimethyl–1, 2-benzanthracene (DMBA).



This particular carcinogen has been used by Morris[6], Reiskin[5] and Salley[8] as well as many others[3,14].

Five weeks old Golden Syrian Hamsters were obtained from the Engel Laboratory in Indiana, U.S.A. The hamsters were divided into 13 groups of three each. All animals were housed in wirecages with six animals per cage. They were fed tap water and a commercial diet. A 0.5 percent mineral oil solution of 9. 10-dimethyl–1, 2-benzanthracene (DMBA) was applied twice weekly with a No. 4 camel's hair brush to the right cheek pouch of all animals except group 13. Group 13 was painted twice weekly with mineral oil for a period of 12 weeks served as a control. The left pouch of all animals in groups (1 through 12) also served as a control.

In the application of the carcinogen, the pouch was extended by the intraoral insertion of a mounted wire loop and the buccal mucosa was brushed with the solution.

At the end of each week, one group of three animals was omitted from the painting procedure. Daily grossly changes of pouches were recorded and after 12 weeks all animals were sacrificed and the number of tumors formation in the pouches were recorded. The animals were killed by a lethal dose of diethyl-ether. Pouches were removed from each animal by inverting, extending with forceps and then excising. The tissue was then immersed in formalin.

After fixation in formalin, the tissue was dehydrated in ascending alcohols, cleared in xylene and embedded in paraffin. Sections were cut at six microns and stained with hematoxylin and eosin. All sections were then examined histologically to determine tumor production as well as gradation.

RESULTS

Gross examination of the pouches revealed that the progress of the induced tumors began with erythema and ulceration followed by repair. Later there was increased mitotic activity, hyperkeratosis with acanthosis, papilloma and finally squamous cell carcinoma developed in the stage of the lesion.

Tumors developed in all animals with good uniformity of response and no loss of animals in this study. Histopathologic studies of biopsy tissue removed from the pouches, after 12 weeks of exposure satisfy all microscopic criteria for malignancy.

A summarization of the results obtained in this investigation are compiled in Table 1. As can be seen, cancerous growths were seen in animals from seven weeks through twelve weeks. All growths occureed in the right cheek pouch of the hamsters. The left pouch,

Table 1. Chart of DMBA Paintings and Results

*Group (weeks)	6	7	8	9	10	11	12
Percent with tumors	0	33	66	33	100	100	100
Mean diameter of tumor (mm)	–	<2.5	2.5	4	4	6	9

* Group number corresponds with number of weeks of painting with DMBA.

which served as the control, demonstrated no abnormal growth. A gross and histological view of the cheek pouches from the control group is seen in Figure 1 & 2.

The control specimen in Figure 1 exhibits good vascularization (clearly visible vessels), a smooth surface and no apparent pathological changes. However, in the pouch painted with DMBA for seven weeks (Fig. 3) close examination reveals an irregular surface, a whitish granular appearance and a rubbery consistency. This marked change in color, texture and consistency is indicative of an abonrmal change.

A tissue sample from a pouch treated with DMBA twice weekly for seven weeks is seen in Figure 4. The reddish or inflammatory appearance and the granular texture are easily observed. The mucosa appeared thickened, with an irregular surface and various papilomatous growth. The underlying connective tissue showed no observable disturbances. Histological examination revealed hyperkeratosis, acanthosis, mild dyskeratosis and a few infiltrated lymphocytes. In addition, deep invaginations of keratin or keratin plugs are prominent in the small papilloma observed.

In the tissue which was exposed to the carcinogen for a period of 11 weeks, tumors of increased size, accompanied by a darkening of color, and a very irregular surface with small areas of ulceration were observed. At this stage the animal presented dramatic changes in physical appearance with loss of hair and a generally weakened condition.

Histologically the tumor demonstrates tumor cell invasion into the underlying fibrous connective tissue.

After 12 weeks of exposure, the hamster cheek pouch showed a large tumor mass with a multiple ulcerative, purulent lesion as seen

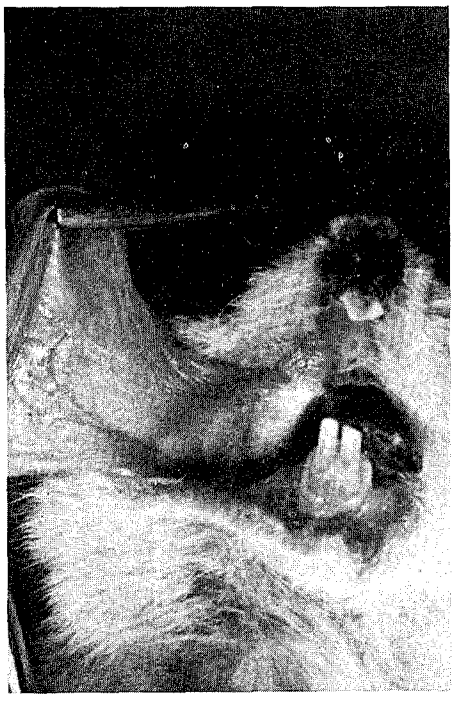


Fig. 1. The control (unpainted) hamster cheek pouch with good vascularization, and smooth mucosal surface is clear.

Fig. 2. Histologic section from a control pouch. The thin keratin layer is characterized on the smooth epithelium. Note the flattened rete ridge with the distinct basal cell layer and unremarkable submucosal connective tissues. (H & E Stain x 40)

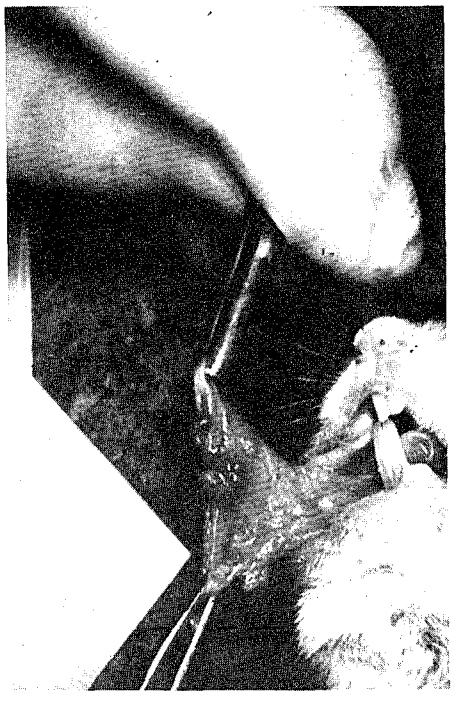


Fig. 3. A tissue sample from a pouch treated with DMBA twice weekly for seven weeks. The reddish, inflammatory papillomatous appearance is obvious in the granular texture.

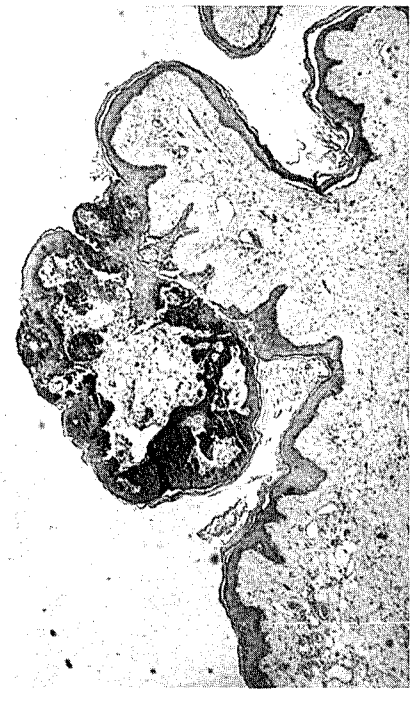


Fig. 4. Mild dyskeratosis and deep keratin plugs are seen after exposure to DMBA for seven weeks. (H & E Stain x 20)

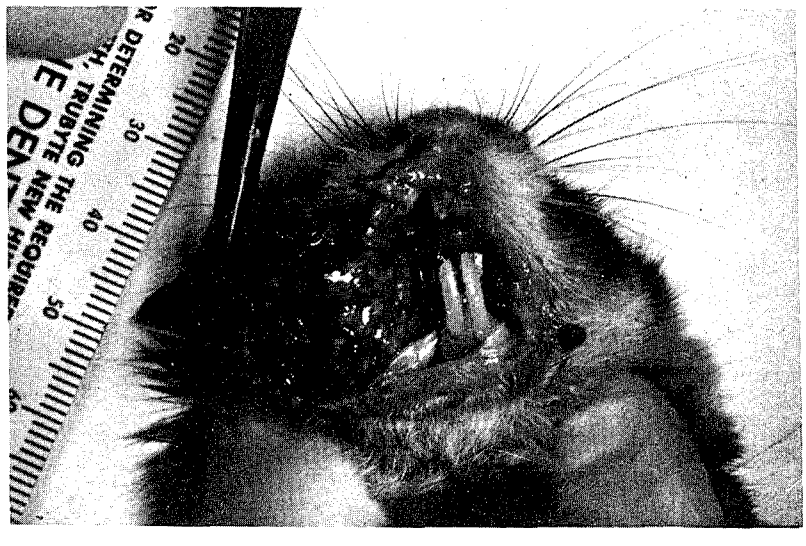


Fig. 5. This animal had been exposed to the DMBA for 12 weeks. A large tumor mass with a multiple ulcerative purulent is evident.

in Figure 5.

The microscopic examination revealed destruction of invaded underlying tissue by tumor cells as shown in Figure 6. Moderate lymphocytic infiltration was observed.

In addition, tumor cells had invaded the muscle. These cells demonstrated hyperchromatism, pleomorphism, disorientation and abnormal mitosis.

DISCUSSION

The hamster pouch, because of its similarities to human oral tissue, is suitable for use in the study of lesions produced through chemical carcinogenesis. The anatomic characteristics of pouches afford easy access and observation. The similarities of carcinoma symptoms such as erythema and leukoplakia make the hamster invaluable as a model.

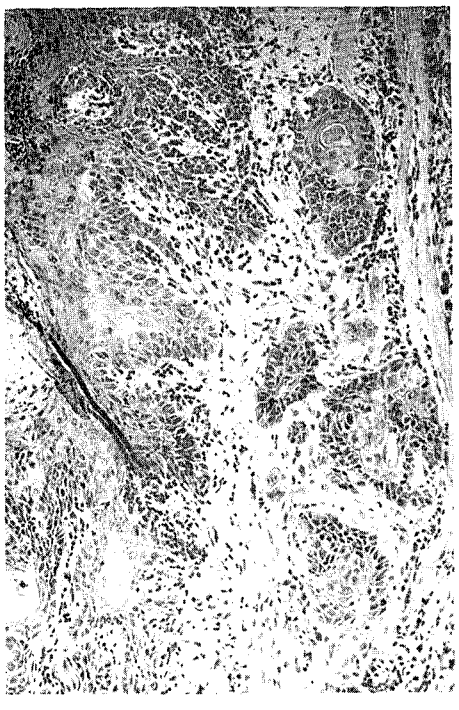


Fig. 6. The histological picture reveals a discontinuation of epithelium which has been destroyed by tumor cells. There is moderate lymphocytic infiltration and tumor cell invasion of surrounding muscle cells. (H & E Stain × 100)

From the results obtained in this study it can be shown that the irreversible changes took place between the sixth and seventh week of painting. However, it must be noted that after 7 weeks only 33 percent of the animals exhibited hyperkeratotic characteristics, whereas the remaining animals appeared normal. Of those animals exposed to the carcinogen for eight weeks, 66 percent exhibited premalignant symptoms. Of the nine weeks group, 33 percent demonstrated tumors. In the period from 10 to 12 weeks, 100 percent tumor production was exhibited. No sound explanation can be presented for this irregularity at this time, other than the possibility that Golden Syrian Hamsters may not exhibit quite the uniformity of results as other strains such as the Dark-Eared Albino Hamster[5].

Previous studies indicated that an even shorter tumor response might have been elicited had the subsequent experimental observation time-span been longer. Morris[15] painted a group of 13 animals three times in one week and discontinued painting them. At the end of 21 weeks four animals had developed tumors.

The development of initial inflammation within the first couple of weeks was also reported by others[3,13,14], with regeneration thereafter. Of the animals which eventually developed tumors, no evidence of regression was observed. In fact, there was dramatic tumor growth, discoloration, hyperchromatism, pleomorphism and necrosis.

Of great importance was the more than moderate lymphocytic infiltration in the treated pouch. This suggests, contrary to Park and Good[3,16], that the hamster pouch is not a privileged site.

The critical number of consecutive paintings required for tumor induction in a 12 weeks period is seven weeks of biweekly paintings with 0.5 percent DMBA in mineral oil.

The longer the carcinogen is applied, the greater is the size and number of tumors at the end of the 12 weeks period.

The DMBA-induced tumors were squamous cell carcinoma type with excellent histologic uniformity. Those hamsters painted for six weeks could not be classified as

abnormal.

REFERENCES

1. Levy BM, Gorlin R, Gottsegen R: A histologic study of the reaction of skin and mucous membrane to a single topical application of 9, 10-dimethyl–1, 2-benzanthracene. J Dent Res **29**: 679, 1950.
2. Levy BM, Ring JR: Experimental production of jaw tumors in hamsters. Oral Surg Oral Med and Oral Path **3**: 362, 1950.
3. Meng CL: Experimental carcinogenesis. Bull Dept Dent NDMC **12**: 18–34, 1982.
4. Yamagiwa R, Itchikawa K, Verhandl D: Jap Path Ges 1914. Quoted by Berenblum I: Advances in Cancer Res 2, 1954.
5. Reiskin AB, Berry RJ: Cell proliferation and carcinogenesis in the hamster cheek pouch. Cancer Res **28**: 898–905, 1968.
6. Morris AL: Factors influencing experimental carcinogenesis in the hamster cheek pouch. J Dent Res **40**: 3–15, 1961.
7. Morris AL: Carcinogenic response of hamster cheek pouch epithelium to four concentrations of 9, 10-dimethyl–1, 2-benzanthracene. 34th gen meeting Intern Assoc Dent Res 56, 1956.
8. Salley JJ: Experimental carcinogenesis in the cheek pouch of the Syrian hamster. J Dent Res **33**: 253, 1954.
9. Shklar G: Experimental oral pathology in the Syrian hamster. Progr Exp. tumor Res **16**: 518–538, 1972.
10. Renstrup G, Smulow JB, Glickman I: Effect of chronic mechanical irritation on chemically induced carcinogenesis in the hamster cheek pouch. J Am Dent Assoc **64**: 770–771, 1962.
11. Eisenberg E: Neoplasia following cessation of DMBA application to hamster buccal pouch. J Dent Res **56**: 1430, 1977.
12. Salley JJ: Effect of mineral oil as a solvent for 9, 10-dimethyl–1, 2-benzanthracene. J Dent Res **34**: 723, 1955.
13. Salley JJ: Histologic changes in the hamster cheek pouch during early hydrocarbon carcinogenesis. J Dent Res **36**: 48–55, 1957.
14. Thilagaratnam CN, Main JH: Changes in cell cycle characteristics in hamster cheek pouch epithelium during treatment with DMBA. J Oral Path **1**: 89–102, 1972.
15. Morris AL, Reiskin AB: Hamster cheek pouch response to varying lengths of carcinogen exposure. J Dent Res **44**: 664–667, 1965.
16. Park BH, Good RA: Principles of modern immunobiology. 232, 1972.

倉鼠口腔頰囊袋病變與誘癌劑塗擦時間長短之關係

林立民 KENT A. HEIDEMAN PATRICK D. TOTO

倉鼠口腔頰囊袋塗抹誘癌劑0.5%之二苯甲蒽(9.10-dimethyl-1.2-benzanthracene, DMBA)，每週二次連續到十二週之久。在第七週觀察時，臨床及組織切片上均顯有乳突瘤的形成，而七週到十二週之間，倉鼠口腔頰囊袋的變化漸由乳突瘤轉變爲嚴重增生之癌前變化，最後轉變爲惡性扁平鱗狀細胞上皮癌。誘癌劑的塗擦時間愈長，病變形成的癌愈爲顯著。

高雄醫學院 牙醫學系

*Toquerville, Utah, U.S.A.

**Oral and General Pathology Department, School of Dentistry, Chicago Loyola University, U.S.A.