Tumor Induction Through Varying Lengths of Carcinogen Exposure

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TUMOR INDUCTION THROUGH VARYING LENGTHS OF CARCINOGEN EXPOSURE

by

Kent A. Heideman

A Thesis Submitted to the Faculty of the Graduate School of Loyola University of Chicago in Partial Fulfillment of the Requirements for the Degree of Master of Science

April

1978
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VITA

The author, Kent A. Heideman, is the son of Orin D. and Dixie K. Heideman. He was born in Hurricane, Utah on February 11, 1950.

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In September of 1976 he entered the graduate school of Loyola University of Chicago to study for the degree of Master of Science in Oral Biology.
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CHAPTER I

INTRODUCTION

A majority of oral cancer is 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.

In this study 9,10-dimethyl-1,2-benzanthracene (DMBA) was used as the chemical carcinogen in the tumor induction process. The Golden Syrian Hamster, its pouches lined with squamous cell epithelium, is the animal of choice to be used in this study.

The progress of the induced tumors begins with erythema and ulceration followed by repair. Later, increased mitotic activity, hyperkeratosis with acanthosis, papilloma and finally squamous cell carcinoma develop in the stages of the lesion.

Tumors develop in all animals with good uniformity of response and no loss of animals due to
toxicity. Histopathologic studies of biopsy tissue removed from the pouch after 12 weeks of exposure satisfy all microscopic criteria for malignancy. This uniform carcinogenic response of the hamster cheek pouch affords an excellent opportunity for many studies.

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 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 hamster pouch is a good model because of its similarities to human oral tissue. The same type of erythema and leukoplakia observed in the hamster is seen in humans. People who are in daily contact with hydrocarbons such as wildcatters demonstrate comparable skin changes. Hyperkeratotic, pigmented plaques appear on their arms and hands. The pouch is lined with stratified squamous epithelium which has neither follicles nor glands. It responds
vigorously to the carcinogen as mentioned above, and has its own control in that only one of the two pouches is exposed to the chemical. The other is not exposed. Its rich blood supply assures both adequate nourishment and oxygen. This circulation is responsible for the tissue's ability to maintain a constant temperature under conditions of extreme environmental variability. The anatomic characteristics of the pouch afford easy access and observation.
CHAPTER II

LITERATURE REVIEW

A review of the literature reveals numerous studies in which tumors have been induced through the use of carcinogens. As early as 1915 Yamagiwa 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, thousands of different compounds have been studied for their carcinogenic effects.

In 1950, Levy and Ring successfully implanted crystalline 9,10-dimethyl-1,2-benzanthracene subgingivally in the jaws of hamsters producing malignant tumors of connective tissue. Four to five months following implantation tumors developed in eight of the ten animals treated.

Again in 1950 Levy, Gorlin, and Gottsegen investigated the mucous membrane and skin surfaces
of the lower lip of mice, age two months. A 0.6 percent solution of DMBA with benzene as a solvent was applied. The changes in the mucous membrane following sacrifice were increased amounts of surface keratin and an increase in the size and number of granules in the stratum granulosum with an extension of some granules into the stratum spinosum. Slight hyperplasia of the epithelial cells was noticed with the proliferation being most marked in the stratum germinativum. Hydropic changes occurred in the cells of the stratum spinosum and stratum granulosum. Thickening of lamina propria occurred because of edema and a fine fibrinous precipitate.

Salley (1954) commenced a study to determine the susceptibility of the hamster cheek pouch epithelium to the action of chemical carcinogenic compounds and to determine the carcinogen of choice for subsequent investigations of initiation, development and metabolism of induced oral carcinoma. He experimented with three carcinogens; 9,10-dimethyl-1,2-benzanthracene, 20-methylcholanthrene, and 3,4-benzpyrene. In addition to this, he determined to find the better solvent, acetone or benzene, in which the
carcinogen was to be dissolved. A 0.5 percent solution was used in each group. Histologic sections were made from various parts of the body in determining the results. Histologically, four degrees of change were seen: hyperplasia, benign papilloma, squamous cell carcinoma in situ (preinvasive), and squamous cell carcinoma with local invasion and metastasis.

As far as choice of chemical carcinogen, the 9,10-dimethyl-1,2-benzanthracene was obviously the most potent when used in acetone. All survivors showed squamous cell carcinoma with metastasis. The next in order of potency was the same compound in a benzene solution. However, this solution was not satisfactory because of the high mortality observed as the study progressed.

At the 33rd General Meeting of the International Association for Dental Research, Salley (1955) discussed the effects of mineral oil as a solvent for DMBA. The differences were studied by using the animals cheek pouch as an in vivo microscopic spread preparation so that the early stages of tumor initiation could be observed. The carcin-
ogen dissolved in mineral oil produced a higher incidence of tumors and a lower toxicity. It appears that the mineral oil acted as a co-carcinogen. Less tissue deformity was also observed.

Morris (1956) at the 34th General Meeting of the International Association for Dental Research reported his study of establishing the best carcinogenic effect of four different concentrations of DMBA. He desired to establish a procedure whereby the oral epithelium of the Syrian Hamster could be stimulated to undergo a change from the normal to the malignant in a minimum period of time. He also wished to produce a submaximal response of the epithelium. In this study he utilized four different concentrations of 9,10-dimethyl-1,2-benzanthracene: 1.5 percent, 0.5 percent, 0.1 percent and 0.05 percent, in a mineral solvent.

The animals were divided into four groups with each receiving an application of carcinogen three times weekly. Early changes in the epithelium were observed and a discrete change recorded as an initial lesion. Tumors were biopsied in an attempt to determine earliest onset of malignancy. The
animals receiving the 1.5 percent solution exhibited an extreme reaction to the carcinogen after the second application. The mild erythema and necrosis of the cheek pouch mucosa, commonly seen during the first week of painting in animals receiving the 0.5 percent concentration of the carcinogen was very severe in the 1.5 percent group. In some of the animals the pouches never healed and eight of the 15 animals died before development of the initial lesion. In contrast to this, the animals in the 0.1 percent group exhibited only mild erythema of the mucosa. The animals in the 0.05 percent group were without observable change. All in all, the 0.5 percent concentration proved to be the best.

Salley (1957) studied the progressive changes in oral epithelium before neoplastic transformation took place. He points out that earlier studies of epithelial carcinogenesis on skin stress the importance of the carcinogenic process to the hair follicles and sebaceous glands. Earlier opinions were that the structures degenerated in the early stages of the process only to regenerate and become hyperplastic, forming the centers of the induced tumors.
Salley indicates that there are no accessory or other structures in the hamster cheek pouch which could play a role in the carcinogenic process. He reported gross observations of inflammation and edema after the initial and second to third applications of DMBA. After eight or nine treatments, there were raised, whitish lesions on the painted epithelium similar to leukoplakia in human oral mucosa. The first evidence of a tumor was seen in a hamster which had been treated with 19 applications of DMBA. From this point on, all pouches exhibited papillomatous growths and several were allowed to grow until they reached sizes in excess of one centimeter in diameter. His results indicate histologically that the oral epithelium passes through four stages before neoplastic transformation: inflammation, degeneration characterized by necrosis, regeneration, and hyperplasia. Of interest was the observation by Salley that the inflammation after the first few treatments was never again present even though the tissue was continually being irritated with frequent applications of DMBA. It was suggested that the cells had become acclimated to the irritant in the relatively short
period of one week. Experimentally induced benign tumors will disappear in some species of animals if treatment is terminated. However, Salley discontinued treatment after the first appearance of benign neoplasms without any sign of regression.

Morris (1961) saw the necessity to standardize such factors as the dosage, the mode and length of administration of the agent, the site of application, the age and the sex. He carried out three separate experiments in solving some of the above factors. His efforts were to determine whether the age of the animal at the time of the initial exposure to a carcinogen affects the response of the cheek pouch mucosa; the response of the cheek pouch to different concentrations of a carcinogen; and whether the frequency of application of a carcinogen affects the response of the cheek pouch. In determining the ideal age for hamsters used for experimental chemical carcinogenesis, he divided 45 hamsters into littermated groups. All animals received a 0.5 percent mineral solution of DMBA. The painting was begun on the first group when they were three weeks old; the second group when they were six weeks old;
the third group when they were nine weeks old; and the fourth group when they were 18 months old.

In his second experiment Morris (1961) was to determine the optimal concentration for rapid production of malignant tumors. Having done this part of the experiment earlier, Morris (1956), he merely reported his findings from that study. From the results of the above two experiments and an additional third study he determined that the tissues of the cheek pouches of the older hamsters are more resistant to the carcinogenic stimuli than those of the younger animals. The ideal age appeared to be about five weeks although there was no difference between the response seen between the ages of three and nine weeks. As far as the optimal concentration, the 0.5 percent DMBA solution produced the most rapid response of the tissue. This concentration produced the maximum tumor response with minimum latent period and no loss of animals due to toxicity. He determined that a shorter latent period is required for tumor development in animals exposed to DMBA three times per week than those receiving the carcinogen twice weekly. However, a smaller total dose is required to produce
tumors in all animals when the DMBA is applied twice weekly than when given three times weekly.

In the same study Morris (1961) investigated the "dripping-brush" method and a "wiped-brush" method in which the brush was wiped once against the side of the container before the DMBA was introduced into the hamster cheek pouch. Average amounts of solution delivered were 47.7 and 31.3 mg. by the dripping- and wiped-brush methods respectively. No differences could be demonstrated when both methods were compared.

In the above studies Morris (1961) also determined that the sex of the animals under the conditions to which they were exposed had no relation to the observed responses. The conditions of caging in which three or four animals were housed together revealed no apparent effect on the experimental results.

Morris and Reiskin (1965) discussed whether a critical duration of exposure of cheek pouch cells to the carcinogen exists for the induction of tumors. They exposed the cheek pouches of adult hamsters for varied amounts of time using 7,12-dimethylbenz(a)an-
thracene as the chemical carcinogen. The animals were divided into 13 groups and painted three times weekly. At the end of each week one group was omitted from the painting procedure. After 21 weeks all animals were sacrificed and the number of tumors contained in both pouches of each animal was recorded. After the 21 week period all animals painted for three weeks or more exhibited tumors.

Reiskin and Berry (1968) induced tumors in the cheek pouches of three strains of hamsters using DMBA as the chemical carcinogen. Animals from two strains were designated by coat color as cream and golden. The third strain was Dark-Eared Albinos (DEA). DMBA was applied until all animals treated developed tumors. Visible tumors developed first in the DEA hamsters with a mean latent period of 7.3 ± 2.8 weeks which was significantly different from the other two groups. Tumor histology was similar for all three groups. Tumor growth was rapid and three growth patterns were observed in all three strains: (a) most tumors grew continuously but with changing rates; (b) a few tumors grew continuously with constant rates; and (c) a few tumors went through periods
of regression although growth was always resumed.

Thilagaratnam and Main (1972) studied the cell cycle characteristics in the hamster cheek pouch epithelium under normal conditions, in hyperplasia, which was induced by DMBA treatment for five weeks, in pre-neoplastic epithelium after 12 weeks of DMBA applications and in DMBA induced squamous cell carcinoma. The individual cell cycle consists of four phases; $G_1$, the interval between mitosis and DNA synthesis; $S$, during which duplication of DNA occurs; $G_2$, the interval between cessation of DNA synthesis and onset of mitosis; and finally, mitosis itself in its four phases of prophase, metaphase, anaphase and telophase. Vinblastine sulphate was used as a mitostatic agent to measure mitotic rate, and tritiated ($H^3$) thymidine autoradiography using the pulse chase method was employed to estimate the duration of cell cycle and its phases.

Thilagaratnam and Main (1972) compiled in the table below the durations of the cell cycle and its phases in hours in the cheek pouch.
The percentages show the reduction in time between the normal epithelium and the squamous cell carcinoma. It was demonstrated that all phases of the cycle were progressively reduced during the stages of carcinoma induction. The drastic reduction in $T_c$ is mainly due to the great reduction of $T_{gl}$.

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

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 (1961), Reiskin (1968), and Salley (1952) as well as many others.

The normal hamster pouch wall consists of four distinct layers. The surface is stratified squamous epithelium two to four layers thick, the second layer being dense fibrous connective tissue. The third layer is a thin band of longitudinal muscle fibers and the fourth layer is loose areolar connective tissue.

Studies have shown that only one type of
malignant change occurs, a squamous cell carcinoma produced by the DMBA in the pouch. Mineral oil was chosen as the solvent because of its nonvolatile characteristics. It also causes less tissue deformity and distributes in the intercellular spaces of the basal epithelial cell layer.

Five week old Golden Syrian Hamsters were obtained from the Engel Laboratory in Indiana. The hamsters were divided into 13 groups of three each. All animals were housed in wire cages 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. The left pouch of all animals in groups one through 12 also served as a control.

The wiped-brush method was used 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. After 12 weeks all animals were sacrificed and the number of tumors in the pouches recorded. The length and width of each tumor was also recorded. The animals were sacrificed by a lethal dose of diethyl ether. Pouches were removed from each animal by everting and 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.
CHAPTER IV

RESULTS

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 12 weeks. All growths occurred in the right cheek pouch of the hamsters. The left pouch, which served as the control, demonstrated no abnormal growths. A cheek pouch from the control group is pictured in Figure 1.

A comparison of a control pouch versus one painted with DMBA for seven weeks is shown in Figure 2. The control specimen in both Figure 1 and Figure 2 exhibit good vascularization (clearly visible vessels), a smooth surface and no apparent pathological changes. Contrasted to this is the treated pouch in Figure 2. 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 abnormal changes although the retrogressive changes seen in malignant tumors such as hemorrhage, infarction and infection which may cause a rapid increase in growth are not yet evident.

A histological section from a control cheek pouch can be seen in Figure 6. The unremarkable epithelium and submucosa clearly represent normal conditions. Note the flattened rete ridges and the distinct basal cell layer. Normal conditions are further characterized by the very thin keratin layer on the surface epithelium.

A tissue sample from a pouch treated with DMBA twice weekly for seven weeks is seen in Figure 3. The reddish or inflammatory appearance and the granular texture are easily observed. The mucosa appears thickened with an irregular surface. Total time elapsed from onset of the experiment is 12 weeks as in all animals.

A histologic picture of hamster cheek pouch epithelium exposed to DMBA for seven weeks is seen in Figure 7 under low power on the microscope. Hyperkeratosis and acanthosis are evident with the
underlying connective tissue showing no observable disturbances.

Figure 8 reveals 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 middle of the picture. Abnormal sequences in cellular maturation results in the keratinization of cells before they reach the epithelial surface.

A hamster cheek pouch exposed to DMBA for ten weeks can be seen in Figure 9. An enlarged papilloma with invasive squamous cell carcinoma is seen under low power. Tumor cell islands, keratin pearls, individual cell keratinization, severe dyskeratosis and submucosa edema from the destruction of the normal structure of the muscle are clearly shown in Figure 10.

A sample of tissue which was exposed to the carcinogen for a period of 11 weeks is seen in Figure 4. An increase in the size of the tumor is noted with a darker color change and a very irregular surface with small areas of ulceration. At this stage the animal presented dramatic changes in physical
appearances with loss of hair and a general weakened condition.

A section of cheek pouch in Figure 11 which was exposed to the carcinogen for 11 weeks demonstrates tumor cell invasion into the underlying fibrous connective tissue.

A large tumor mass with a multiple ulcerative, purulent lesion is seen in Figure 5. This animal had been exposed to the DMBA for 12 weeks.

The discontinuation of epithelium which has been destroyed by tumor cells which have invaded the underlying tissue is shown in Figure 12. Moderate lymphocytic infiltration is observed. The tissue was exposed to DMBA treatment for 12 weeks.

Tissue also exposed to the carcinogen for 12 weeks is viewed under high power in Figure 13. Tumor cells which have invaded the muscle can be seen. These cells demonstrate hyperchromatism, pleomorphism, disorientation and abnormal mitosis.
CHAPTER V

DISCUSSION

The responsibility of research in the field of oral cancer lies on the shoulders of those in the dental profession. In retrospect, it can be seen that vast areas of questions have been adequately investigated and the possibility now exists to examine experimentally the relationship of many specific variables of neoplastic transformation. Of utmost importance is the correlation of the results of successive experiments in order that the experimental lesion can be obtained repeatedly with uniformity of results. The standardization of many important variables will be summarized in the following pages.

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

The questions that arise are, which strain of hamster will yield the best results, and, is sex a significant variable? 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. As far as the sex of the animals, no difference could be distinguished between male and female even though the female hamsters gain more weight than do the males. It has been established also that numbers of three, four, five or six hamsters per cage have little bearing on the results obtained during experimentation.

Age of the hamster has been shown to be an important factor with five weeks being established as the optimal age at the onset of experimentation. Younger animals experience too high of a mortality rate and older animals yield poorer responses to the chemical carcinogen employed.

The method of application of the chemical carcinogen can be done by two methods, both of which
deliver approximately equal amounts of carcinogen. They are the wiped-brush method in which the brush used to apply the carcinogen is wiped once on the side of the container before the carcinogen is applied to the hamster pouch, or a dripping-brush method in which the brush is not wiped on the container before using.

In reference to the chemical carcinogen of choice, 9,10-dimethyl-1,2-benzanthracene has been standardized as the chemical of best response. The optimal concentration of DMBA in a mineral oil solvent is 0.5 percent. As mentioned, Salley (1955), mineral oil is the best solvent yielding the most uniform response with low toxicity.

The frequency of application per week is determined by the investigator himself. Applications three times weekly yield a shorter latent period but a smaller total dose is required when animals are painted twice weekly. This has been reported by Morris (1961) with similar results achieved by Blum, Grady, and Kirby-Smith (1942) using ultraviolet light on the skin of mice.

The results of the experiment reported here
corroborate these findings. Morris (1961) reports that after five and one-half weeks, 16 paintings of carcinogen, initial lesions were demonstrated. After seven weeks and 14 paintings, which delivered the same amount of DMBA, initial lesions were also observed.

In other studies reviewed, Salley (1954), Morris (1956), Salley (1957), 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.

From the results obtained in this study it can be shown that the irreversible change took place between the sixth and seventh week of painting when observed for a total of 12 weeks. However, it must be noted that 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 week group, 33 percent demonstrated tumors; with all animals from
10 weeks to 12 weeks exhibiting 100 percent tumor production. No sound explanation can be presented for this irregularity at this time other than the fact that Golden Syrian Hamsters may not exhibit quite the uniformity of results as other strains such as the Dark-Eared Albino Hamster.

An even shorter tumor response might have been elicited had the experimental observation period been longer. Morris (1965) 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 hamsters developed the initial inflammation reported by others within the first couple of weeks with regeneration thereafter. Of the animals which eventually developed tumors, no evidence of regression was observed. In fact, the opposite was demonstrated with 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, that the hamster pouch is not a privileged site.
CHAPTER VI

SUMMARY

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 everted, excised and histological slides prepared from the tissue. Tumors and papillomas were observed in all groups painted seven through 12 weeks. Those painted for seven weeks demonstrated papillomas. The changes from seven weeks onward progressed from papilloma to malignant tumors. The longer the groups were painted, the more severe were the lesions.
CHAPTER VII

CONCLUSIONS

The following conclusions can be made from this investigation:

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

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

3. The DMBA tumors induced were squamous cell carcinoma type with excellent histologic uniformity.

4. Those hamsters painted for six weeks could not be classified as abnormal.
CHAPTER VIII

BIBLIOGRAPHY


FIGURE 1

Demonstrated here is the control or unpainted hamster cheek pouch. Good vascularization, a smooth surface, and normal pink color is clearly seen.
FIGURE 2

This shows a comparison of a control pouch versus one treated with DMBA for seven weeks. The control on the right clearly shows vessels, a smooth surface and good pink color. Note the whitish, granular appearance of the treated pouch.
FIGURE 3

Seen above is a tissue sample from a pouch treated with DMBA twice weekly for seven weeks. The reddish, inflammatory appearance is obvious as is the granular texture.
This tissue was exposed to the carcinogen for a period of 11 weeks. A fairly large tumor is seen along with an irregular, granular surface. Small areas of ulceration can be seen.
This animal had been exposed to the DMBA for 12 weeks. A large tumor mass with a multiple ulcerative, purulent lesion is evident.
This represents a section from a control pouch. The thin keratin layer is characterized on the smooth epithelium. Note the flattened rete ridge cells and the distinct basal cell layer.
FIGURE 7

This hamster cheek pouch epithelium exposed to DMBA for seven weeks shows hyperkeratosis and acanthosis under low power.
FIGURE 8

Mild dyskeratosis and deep keratin plugs are seen under high power. Exposure to DMBA was for seven weeks.
A section of hamster cheek pouch exposed to the carcinogen for 10 weeks is shown. It reveals an enlarged papillary, invasive squamous carcinoma.
FIGURE 10

Keratin pearls, tumor cell islands and basal cell hyperplasia can be seen under high power. Exposure to DMBA was 10 weeks.
FIGURE 11

This section of cheek pouch which was exposed to the carcinogen for 11 weeks demonstrates tumor cell invasion into the underlying fibrous connective tissue, and a reactive lymphocytic infiltration in the lamina propria.
The histological picture reveals a discontinuation of epithelium which is destroyed by tumor cells. There is moderate lymphocytic infiltration. Exposure to DMBA was for 12 weeks.
FIGURE 13

Viewed under high power the tumor cells demonstrate invasion to surrounding muscle cells. Cheek pouch was exposed to the carcinogen for 12 weeks.
Table 1

Tumor Size and Frequency

<table>
<thead>
<tr>
<th>*Group (weeks)</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent with tumors</td>
<td>0</td>
<td>33</td>
<td>66</td>
<td>33</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Mean diameter of largest tumor (mm)</td>
<td>-</td>
<td>-</td>
<td>2.5</td>
<td>4</td>
<td>4</td>
<td>6</td>
<td>9</td>
</tr>
</tbody>
</table>

*Group number corresponds with number of weeks of painting with DMBA.
The thesis submitted by Kent A. Heideman has been read and approved by the following committee:

Dr. Li-Min Lin, D.D.S., M.S.
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The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval by the Committee with reference to content and form.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science in Oral Biology.

April 22, 1978

Li-Min Lin, D.D.S., M.S.
Director