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Studies on Taiwan betel quid carcinogenicity in hamster cheek pouch

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The hamster cheek pouch was employed in this study to elucidate the role of Taiwan betel quid on oral carcinogenesis. One hundred and sixty male Syrian golden hamsters were divided into eight experimental groups and their respective controls, each composed of ten animals. The incidence of tumor in cheek pouches was recorded after short-term(12 weeks) or mid-term(24 weeks) insertion of betel quid subsequent to a graded period(2 weeks, 4 weeks, or 6 weeks) of topical application of 0.5% DMBA(7,12-dimethyl-benz[a] anthracene) three times a week. The tumor rate was significantly higher in the group using DMBA as an initiator for 4 weeks and betel quid as a promotor for 24 weeks as compared with that in the DMBA control group. Treatment of betel quid alone for mid-term(36 weeks) or long-term(52 weeks), or 0.1% DMBA applied for 10 weeks after prior betel quid treatment did not lead to any tumor growth. The results indicated that the Taiwan betel quid was probably a promoting factor in oral carcinogenesis, rather than an initiator or a significant carcinogen.

Key words: betel quid, promotion, hamster cheek pouch, oral carcinoma, carcinogenesis.
檳榔，口腔癌，促癌作用，金田鼠頰囊袋.

Health care workers have long noticed a close correlation between betel quid chewing and the occurrence of oral cancer. This habit alone or in combination with tobacco chewing has been condemned as the major etiologies of oral cancer in certain south Asian countries[1-5]. In Taiwan, epidemiological investigations show high prevalence rates of betel chewing among oral cancer patients, being ranged from 32.1% to as high as 86.2%[5-9]. Also, the incidence rate of buccal carcinoma is commonly reported as high as that in other countries where the betel nut chewing habit prevails[2,5,10]. Interestingly, the overall incidence of oral cancer(I.C.D.140-145) is estimated to constitute only 3% in all human cancers in Taiwan, much lower than that of the south Asian countries. The age-standardized incidence rates of oral cancer per 100,000 people are 3.55 in males and 1.3 in females[10], lower than that in Hong Kong Chinese who do not practice this habit[11]. These paradoxical findings implicate the necessity of an advanced investigation of the carcinogenicity of Taiwan betel quid.

It is not yet determined whether betel quid or its various components are capable of inducing tumor by experiments. Some animal experiments show positive results[12-15], while others deny its significance[16-19]. It must be emphasized that these studies have employed a great variety of betel quid compositions and preparations derived from different geographical regions. However, little is known about the Taiwan betel quid in this respect. Huang[20] drew attention to the "co-carcinogenic" effect of Taiwan betel quid by applying its extracts and DMBA

(9,10-dimethyl-1, 2-benz[a]anthracene) concurrently in hamster cheek pouches and noticing a higher tumor yield. However, the sample size is too small to validate the hypothesis statistically.

In the present studies, we attempted to apply the concept of two-phase carcinogenesis to elucidate the roles that betel quid play in oral cancer causation. This theory was first introduced by Berenblum and shubik in 1947[21]. It is postulated that carcinogenesis consists of a specific and irreversible phase, in which normal cells are converted to latent tumor cells, and a promoting phase in which the genetically altered cells are stimulated to become morphologic tumor. In this study, the hamster cheek pouch was selected as the animal model to test the Taiwan betel quid in terms of its carcinogenic potential *per se* and its tumor promoting effect following DMBA treatment. The relatively large pouch in hamster facilitated the insertion and storage of quid inside for testing. DMBA was painted over the cheek pouches according to the model established by Salley[22] and Shklar[23].

Animals

One hundred and sixty male Syrian golden hamsters, aged about six to eight weeks, were purchased from the Animal Center of National Taiwan University. They were fed with standard purina laboratory pellets and water *ad libitum*.

Betel quid

The same brand of Taiwan betel quid was purchased in Tainan City for the entire study. It consisted of a bisected fresh green areca nut(including the husk) sandwiched with a spike of betel vine and brown paste containing slaked lime and catechu. In our study, the betel quid was divided into four pieces, weighing approximately 1.5 grams. Each piece included all the basic parts of the quid. It was then inserted into the right cheek pouch with a tissue forcep and retained herein by an external wire (#28) collar twisted around the animal's neck. The betel quid piece was renewed twice a week until the end of the betel quid treatment period.

Carcinogen

DMBA(7,12-dimethylbenz[a]anthracene)was purchased from Sigma Chemical Ltd (U.S.A.), dissolved in heavy mineral oil(Sigma), and prepared with concentrations of 0.5% and 0.1% seperately. It was painted on the hamster cheek pouches with a #4 camel brush three times per week according to our design and the technique used by Jin[24].

Experimental designs

The animals were divided into 8 experimental and 8 control groups, each composed of 10 animals. The right cheek pouches were painted with 0.5% DMBA for 2 weeks in Groups I and II, for 4 weeks in Groups III and IV, and for 6 weeks in Groups V and VI. The experimental animals received subsequent betel quid insertion for 12 weeks in Groups Ia, IIIa and Va, and for 24 weeks in Groups IIa, IVa, and VIa. Then they were left untreated for another 6 weeks before being sacrificed. The subgroups Ib-VIb received no additional treatment and were sacrificed approximately the same time as the corresponding experimental animals. In reverse, the animals were tested with betel quid first for 36 weeks in Group VII and for 52 weeks in Group VIII. Then 0.1% DMBA was applied over the cheek pouch subsequently for 10 weeks in Groups VIIa and VIIIa animals, whereas no further treatment was performed on the Groups VIIb and VIIIb.

The left cheek pouches of all animals remained untreated and served as the built-in control. There was a time-lapse of about one week between the application of DMBA and betel quid in all experimental group animals.

Autopsy

At the end of the schedules, the animals were sacrificed by inhalation of an overdose of ethyl ether. Both right and left cheek pouches were excised, examined grossly for evidence of pathosis, and then photographed. Tumors, if present, were measured with a ruler in millimeters and recorded. The pouches were then fixed in 10% neutral buffered formalin, sectioned in paraffin, and stained with hematoxylin and eosin for light microscopic study.

Statistical analysis

The difference in tumor rate (number of animals bearing dysplasia or carcinoma/number of survived animals) between the experimental groups and the corresponding control groups was evaluated by the Student's t-test (two-tailed). Statistical significance was indicated when $p<0.05$.

RESULTS

Gross observations

The left cheek pouches of all animals and the right cheek pouches of Group I, Group IIb, Group VII, and Group VIII animals appeared normal, with no visible tumors (Figure 1). Other groups showed tumor growth in variable rates and numbers (Figure 2). The tumors ranged in diameter from one to 21 mm, and there were one to five tumors per pouch. Most of them were exophytic.

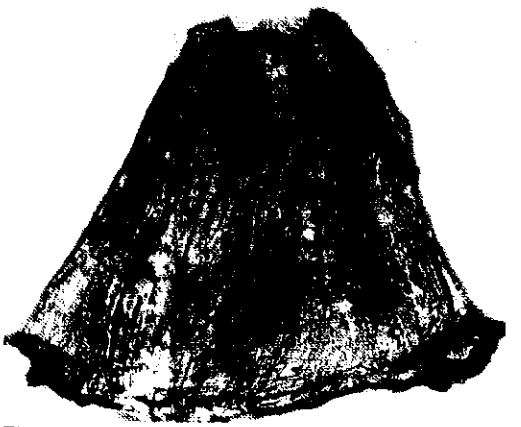

Figure 1. Hamster cheek pouch in Group II animal following 2-week DMBA application. No tumor was seen by gross examination.

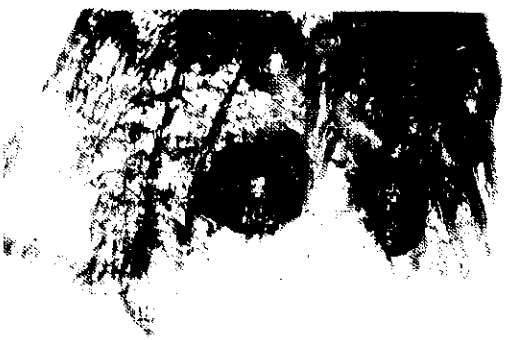

Figure 2. Hamster cheek pouch in Group IVa animal following 4-week DMBA plus 24-week betel quid application, note multiple exophytic tumors on cheek pouch.

Microscopic observations

The right cheek pouches of Group I to Group VI animals demonstrated areas of normal epithelium, dysplasia, carcinoma *in situ*, papillary carcinoma, or invasive carcinoma (Figures 3 and 4). The carcinomas appeared to be moderately-to well-differentiated. Occasionally, cancer invasion into muscular layer was seen. But it seemed not to relate to the tumor size, nor did it relate to betel quid treatment. The incidences of dysplasis and carcinoma in hamster cheek pouches in different groups are depicted

Figure 3. Light micrograph of buccal pouch in Figure 1 showing normal mucosa. (H & E. X31)

in Table 1. The tumor rates in Group IVa (4 wk DMBA + 24 wk BQ) and Group Va (6 wk DMBA + 12 wk BQ) were significantly higher ($p<0.05$ and $p<0.01$, respectively) than those in Group IVb (4 wk DMBA only) and Group Vb (6 wk DMBA only), respectively. The carcinoma rate was significantly higher($p<0.01$) in Group Va (6 wk DMBA plus subsequent 12 wk BQ) than in Group Vb (6 wk DMBA). The right cheek pouches of Group VII(36 wk BQ with or without subsequent 10 wk DMBA) and Group VIII(52 wk BQ with or without subsequent 10 wk DMBA) animals showed foci of hyperkeratosis only with no tumor formation(Table 2). The left pouches of all animals appeared to be normal.

Figure 4. Light micrograph of buccal pouch in Figure 2 showing a well-differentiated squamous cell carcinoma with invasion into the underlying connective tissue. (H & E. X80)

DISCUSSION

In this study, continous exposure to Taiwan betel quid alone for 52 weeks did not result in any detectable tumor growth or dysplastic change in animals. This was in contrast to the findings of Ranadive *et al.*[12] who showed that non-tobacco-containing betel quids do induce tumors in hamster cheek pouches. Hamner[18], on the other hand, applied betel quids on baboon's buccal mucosa and found a few foci of cellular atypia but no tumor. These conflicting results may in part be attributed to the regional disparities in the constituents of betel quids or the processing techniques. Awang[25] demonstrated a wide variation in the arecoline contents among some commercial betel quids produced from different regions and found it is closely related to the regional prevalence of oral leukoplakia. Other studies use extracts of betel quids or its ingredients for tests[13,14,20,26]. We believe that using the original form of quids gives the closest profile of its total effect on carcinogenesis in human. For instance, the mechanical irritation on the mucosa exerted by the quid is a desirable effect, because this mimicked the oral condition of human in practice.

We demonstrated that the Taiwan betel quid was capable of enhancing tumor formation, whereas no tumor was yielded in animals using betel quid as an initiator. The results inferred that the betel quid chewed in this region was not a significant carcinogen, if it was at all. Instead, it acted to promote carcinogenesis. Few studies have been done with respect to the tumor promoting effect of the betel quid or its constituents. Stich *et al.*[27] introduced areca nut extracts to the

Table 1. Incidence of dysplasia or carcinoma in hamster cheek pouch treated with 0.5% DMBA alone or followed by betel quid insertion

Group No.	Treatment	Effective animal No.	No. of animals with dysplasia or carcinoma(%)	No. of animals with carcinoma(%)
Ia	2wk DMBA+12wk BQ	9	6 (66.7%)	0
Ib	2wk DMBA	10	3 (30%)	0
IIa	2wk DMBA+24wk BQ	8	5 (62.5%)	1 (12.5%)
IIb	2wk DMBA	10	3 (30%)	0
IIIa	4wk DMBA+12wk BQ	10	5 (50%)	1 (10%)
IIIb	4wk DMBA	10	5 (50%)	1 (10%)
IVa	4wk DMBA+24wk BQ	9	8*(88.9%)	6*(66.7%)
IVb	4wk DMBA	9	3 (33.3%)	1 (11.1%)
Va	6wk DMBA+12wk BQ	7	7 (100%)	7**(100%)
Vb	6wk DMBA	9	8 (88.9%)	1 (11.1%)
VIa	6wk DMBA+24wk BQ	10	9 (90%)	9 (90%)
VIb	6wk DMBA	10	10 (100%)	7 (70%)

BQ=betel quid.
* Signficantly different from Group IVb($p<0.05$).
**Signficantly different from Group Vb($p<0.01$).

Table 2. Incidence of dysplasia or carcinoma in hamster cheek pouch treated with betel quid insertion alone or followed by 0.1% DMBA application

Group No.	Treatment	Effective animal No.	No. of animals with dysplasia	No. of animals with carcinoma
VIIa	36wk BQ+10wk DMBA	6	0	0
VIIb	36wk BQ	10	0	0
VIIIa	52wk BQ+10wk DMBA	8	0	0
VIIIb	52wk BQ	9	0	0

BQ=betel quid.

bovine papillomavirus transformation assay and found an enhanced formation of transformed foci, indicating a promoting effect. Tanaka *et al*[28]. fed the ACI rats with betel nut in diet and noticed an enhancing effect on oral carcinogenesis. Since the rodent bioassays are accepted worldwide as valid models to predict carcinogens in human, sometimes even with similar organ specificity at comparable dose[29,30], it seems realistic to assume the betel quid to possess the same capability in promoting oral carcinogenesis in human. It was noteworthy that the exposure time of betel quid capable of promoting tumor formation in this animal experiment was attainable in human practice. The duration of 24 week application of betel quid was calculated to be comparable to that of an addicted chewer who chewed 40 quids per day for eight years, assuming an average chewing time of 20 minutes for each quid.

It was of particular interest when we compared the results of our study with the epidemiological database on oral cancer in Taiwan. The fact that the incidence rate of oral cancer in Taiwan is comparable to that in those countries which do not practice this habit was parallel to our finding that Taiwan betel quid was not a significant carcinogen *per se*. Somehow, other factors such as background ionizing radiation, exogenous or

endogenous carcinogens, certain viruses, or spontaneous mutation could bring about the very step of tumor initiation. Thus, if betel quid chewing is a quite effective way of tumor promotion, it will not be surprising to see the oral cancer patients in Taiwan carried a higher prevalence rate of betel quid chewing, as emphasized by many authors[6-9]. Such a model would seem to be an oversimplification of what must be a complex process. Nonetheless, it suggests an attractive explanation for our epidemiological findings.

Recent advances in knowledge of oncogenes have added ' substantial evidence to the " two-phase " or " multistage " concept of oncogenesis. It is currently accepted that cancer arises from normal cells through a complicated evolution including multiple genetic alterations, and probably aberration at the epigenetic level in gene expression and differentiaton[31]. Accordingly, initiators denote to carcinogens and almost invariably, to direct or indirect mutagens. Promotors, on the other hand, are not necessarily carcinogens or mutagens. They are effective only after the administration of initiators and must be repeatedly applied both within a given time frame and above a certain threshold dose. How the promotors exert their effects are even less clear. A number of *in vitro* studies assume the action to lie in their ability to inhibit metabolic cooperation' between cells[32,33].

In this study, a two-week application of DMBA plus a subsequent 24-week treatment of betel quid was found to induce carcinoma in one animal. The animals which received a two-week painting of DMBA plus subsequent betel quid insertion also showed higher occurrence rates of dysplasia than their DMBA control animals. However, the tumor-promoting activity was most obvious in Group IVa in which DMBA application for 4 weeks was followed by betel quid treatment for 24 weeks. Although 6 weeks of DMBA application with subsequent betel quid treatment in Group Va did elicit a marked rise in cancer rate as compared with its DMBA control, the statistical significance was overwhelmed when we pooled the number of cancer and precancerous lesions together. Thus, it seems that the duration of DMBA treatment is critical for the promoting effect of betel quid to show up. We have adopted the timetable in Group IV as the working protocol in our further studies.

In conclusion, the results of this study indicated that the Taiwan betel quid was probably a tumor promotor or an enhancer, rather than a significant carcinogen. Futher experiments are now being undertaken to distinguish the effects of its various components on oral cancinogenesis.

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REFERENCES

1. Awang MN. Betel quid and oral carcinogenesis. Sing Med J, 29:589-593, 1988.
2. Sanghri LD. Cancer epidemiology: the Indian scene. J Cancer Res Clin Oncol, 99:10-14, 1981.
3. A WHO meeting: Control of oral cancer in developing countries. Bull WHO, 62:817-830, 1984.
4. Jayant K, Deo MG. Oral cancer and cultural practices in relation to betel quid and tobacco chewing and smoking. Cancer Detect Prev, 9:207-213, 1986.
5. Kwan HW. A statistical study on oral carcinomas in Taiwan with emphasis on the relationship with betel nut chewing: A preliminary report. J Formosan Med Assoc, 75:497-505,1976.

6. Chang KM. Betel nut chewing and mouth cancer in Taiwan. First report: survey of disposition of mouth cancer in Taiwan. J Formosan Med Assoc, 63:437-448, 1964.
7. Chen CH, Lin CZ, Chang P. A statistical study on 234 cases of oral carcinoma. J Laryngol Society ROC, 19:20-25, 1984.
8. Chen CH. An epidemiological study of oral squamous cell carcinoma in southern Taiwan.. J Formosan Dent Assoc, 10:268-274, 1987.
9. Yang CS, Lin DL, Lin SN, Won YK. Evaluation of treatment of oral cancer patients in Changhwa Christian Hospital. Chin J Oral Maxillofac Surg. 2: abstract from the 6th annual meeting of R.O.C. Association of Oral and Maxillofacial Surgeons. Chin J OMS, 3:A-19(024), 1992.
10. Tsang CH, Chiu CH. An epidemiological study of oral cancer in Taiwan. Chin Dent J, 9:104-115, 1990.
11. Wu PC, Pang SW, Chan KW, Lai CC. Statistical and epidemiological analysis of oral tumors in the Hong Kong Chinese. J Oral Pathol, 15: 98-102, 1986.
12. Ranadive KJ, Ranadive SN, Shirapurkar NM, Grothoskar SV. Betel quid chewing and oral cancer: experimental studies on hamsters. Int J Cancer, 24:835-843, 1979.
13. Suri K, Goldman HM, Wells H. Carcinogenic effect of a Dimethyl Suphoxide extract of betel nut on the mucosa of the hamster buccal pouch. Nature, 230:383-384, 1971.
14. Ranadive KJ, Gothoskar SV, Rao AR, Tezabwalla BU, Ambayl RY. Experimental studies on betel nut and tobacco carcinogenicity, Int J Cancer, 17:469-476, 1976.
15. Bhide SV, Shivapurkar NM, Gothoskar SV, Ranadive KJ. Carcinogenicity of betel quid ingredients: Feeding mice with aqueous extract and the polyphenol fraction of betel nut. Br J Cancer, 40:922-926, 1979.
16. Dunham LJ, Herrold KM. Failure to produce tumors in the hamster cheek pouch exposure to ingredients of betel quid: Histopathologic change in the pouch and other organs by exposure to known carcinogens. J Nat Cancer Inst, 29:1047-1067, 1964.
17. Eisen MJ. Betel chewing among natives of the Southwest Pacific Islands. Lack of carcinogenic action. Cancer Res, 6:139-141, 1946.
18. Hamner JE. Betel quid inducement of epithelial atypia in the buccal mucosa of baboons. Cancer, 30:1001-1005, 1972.
19. Weerapradist W, Boonpuknavig V. Effect of betel nut on the buccal mucosa of the hamster cheek pouch: comparative histological study of the Thai and Indian betel nut and Di-Methyl Benzanthracene(DMBA). J Dent Assoc Thai, 33: 53-67, 1983.
20. Huang YL. A study on the effect of betel quids ingredients in DMBA-induced carcinoma of hamster cheek pouch. M.S.D. Thesis, Kaoshiung Medical College, Kaoshiung, Taiwan, 1989.
21. Berenblum I, Shubik P. A new qualitative approach to the study of the stages of chemical carcinogenesis in the mouse's skin. Br J Cancer, 1:383-391, 1947.
22. Salley JJ. Experimental carcinogenesis in the cheek pouch of the Syrian hamster. J Dent Res, 35:253-262, 1954.
23. Shklar G. Experimental oral pathology in the Syrian hamster. Progr Exp Tumor Res, 16:518-538, 1972.
24. Jin YT, Lin LM. Lectin binding patterns in squamous epithelium in experimentally induced hamster buccal pouch carcinoma. J Oral Pathol Med, 18:446-450, 1989.
25. Awang MN. Estimation of arecoline contents in the commercial areca nut and its relation to the oral precancerous lesion. Sing Med J, 27: 317-324, 1986.
26. Rao AR. Modifying influences of betel quid ingredients on B(a)P-induced carcinogenesis in the buccal pouch of hamster. Int J Cancer, 33: 581-586, 1984.
27. Stich HF, Tsang SS. Promoting activity of betel quid ingredients and their inhibition by retinol. Cancer Letters, 45:71-77, 1989.
28. Tanaka T, Kuniyasu T, Shima H, Sugie S, Mori H, Takahashi M, Hirono I. Carcinogenicity of betel quid III. Enhancement of 4-nitroquinoline-1-oxide and N-2-fluorenylacetamide-induced carcinogensis in rats by subsequent administration of betel nut. J Natl Cancer Inst, 77:777-781, 1986.
29. Perra FP, Rall DP. Carcinogenesis mechanisms. The debate continues. Science, 252:903-904, 1991.
30. Weinstein IB. Mitogenesis is only one factor in carcinogenesis. Science, 251:387-388, 1991.
31. Lawley PB. Concepts of carcinogenesis. In " Biology of carcinogensis " , Waring MJ and Ponder BA ed, MTP Press Ltd, Lancaster, pp.1-22, 1987.
32. Yotti LP, Chang CC, Trosko JE. Elimination of metabolic cooperation in Chinese hamster cells by a tumor promotor. Science, 206:1089, 1979.
33. Chang CC, Trosko JE, Warren ST. In vitro assay for tumor promotors and anti-promotors. J Environ Pathol Toxicol, 2:43, 1978.

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台灣市售檳榔在金田鼠頰囊袋之致癌性研究

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嚼檳榔是否致癌迄今無定論，台灣嚼檳人口估計約二百萬，但有關台灣檳榔之致癌能力研究卻不多。本實驗以金田鼠頰囊袋爲動物模型，160隻金田鼠分成八組〔含實驗組及控制組〕，第一至第六組動物先接受0.5%DMBA一周三次的局部塗抹，第一及第二組，第三及第四組，第五及第六組分別塗抹二、四、六星期後，再以市售檳榔塊置入實驗組動物的頰囊袋內12或24星期，檳榔一星期換新二次，結果發現有放入檳榔的動物比沒有檳榔者在合併使用DMBA(carcinogen)下似有較多的細胞分化不全或癌形成，其中以DMBA塗抹四星期再放入檳榔24星期組與DMBA控制組比較有統計學上明顯的差異($p<0.05$)。第七及第八組動物則先放入檳榔36星期或52星期，再以0.1%DMBA塗抹實驗組動物10星期，結果實驗組及控制組動物均無癌或癌前期變化出現。此動物實驗顯示，台灣市售檳榔本身並無明顯之致癌力，卻能促進口腔癌的形成。

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The left cheek pouches of all animals remained untreated and served as the built-in control. There was a time-lapse of about one week between the application of DMBA and betel quid in all experimental group animals.

Autopsy

At the end of the schedules, the animals were sacrificed by inhalation of an overdose of ethyl ether. Both right and left cheek pouches were excised, examined grossly for evidence of pathosis, and then photographed. Tumors, if present, were measured with a ruler in millimeters and recorded. The pouches were then fixed in 10% neutral buffered formalin, sectioned in paraffin, and stained with hematoxylin and eosin for light microscopic study.

Statistical analysis

The difference in tumor rate (number of animals bearing dysplasia or carcinoma/number of survived animals) between the experimental groups and the corresponding control groups was evaluated by the Student's t-test (two-tailed). Statistical significance was indicated when $p<0.05$.

RESULTS

Gross observations

The left cheek pouches of all animals and the right cheek pouches of Group I, Group IIb, Group VII, and Group VIII animals appeared normal, with no visible tumors (Figure 1). Other groups showed tumor growth in variable rates and numbers (Figure 2). The tumors ranged in diameter from one to 21 mm, and there were one to five tumors per pouch. Most of them were exophytic.

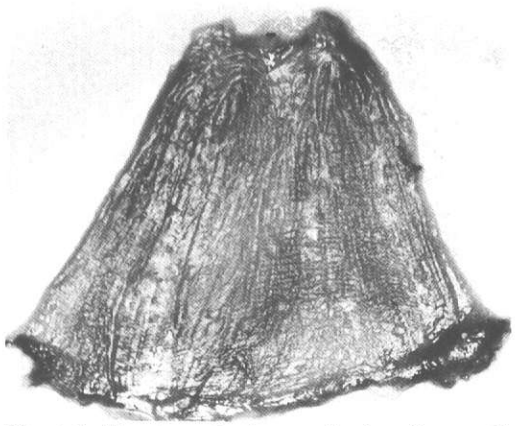

Figure 1. Hamster cheek pouch in Group II animal following 2-week DMBA application. No tumor was seen by gross examination.

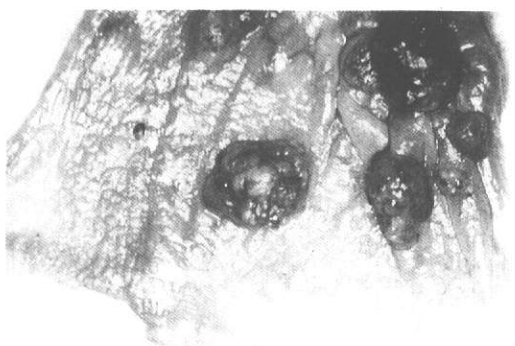

Figure 2. Hamster cheek pouch in Group IVa animal following 4-week DMBA plus 24-week betel quid application, note multiple exophytic tumors on cheek pouch.

Microscopic observations

The right cheek pouches of Group I to Group VI animals demonstrated areas of normal epithelium, dysplasia, carcinoma *in situ*, papillary carcinoma, or invasive carcinoma (Figures 3 and 4). The carcinomas appeared to be moderately-to well-differentiated. Occasionally, cancer invasion into muscular layer was seen. But it seemed not to relate to the tumor size, nor did it relate to betel quid treatment. The incidences of dysplasis and carcinoma in hamster cheek pouches in different groups are depicted

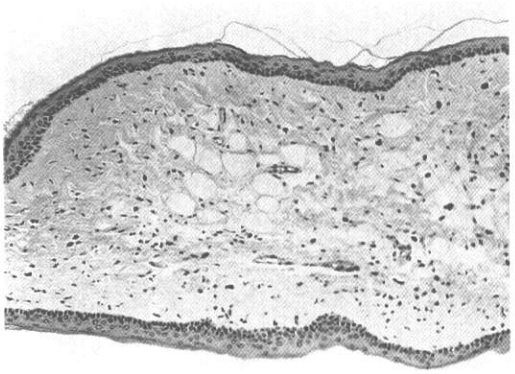

Figure 3. Light micrograph of buccal pouch in Figure 1 showing normal mucosa. (H & E, X31)

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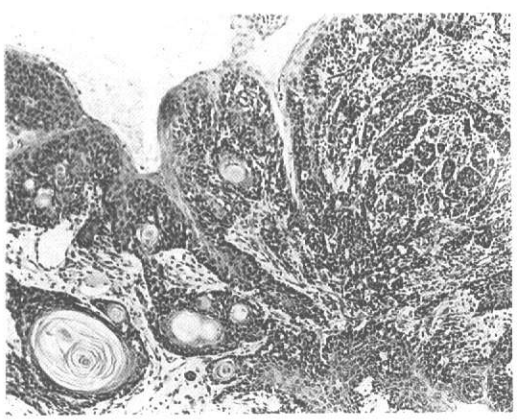

Figure 4. Light micrograph of buccal pouch in Figure 2 showing a well-differentiated squamous cell carcinoma with invasion into the underlying connective tissue. (H & E, X80)

DISCUSSION

In this study, continous exposure to Taiwan betel quid alone for 52 weeks did not result in any detectable tumor growth or dysplastic change in animals. This was in contrast to the findings of Ranadive *et al.*[12] who showed that non-tobacco-containing betel quids do induce tumors in hamster cheek pouches. Hamner[18], on the other hand, applied betel quids on baboon's buccal mucosa and found a few foci of cellular atypia but no tumor. These conflicting results may in part be attributed to the regional disparities in the constituents of betel quids or the processing techniques. Awang[25] demonstrated a wide variation in the arecoline contents among some commercial betel quids produced from different regions and found it is closely related to the regional prevalence of oral leukoplakia. Other studies use extracts of betel quids or its ingredients for tests[13,14,20,26]. We believe that using the original form of quids gives the closest profile of its total effect on carcinogenesis in human. For instance, the mechanical irritation on the mucosa exerted by the quid is a desirable effect, because this mimicked the oral condition of human in practice.

We demonstrated that the Taiwan betel quid was capable of enhancing tumor formation, whereas no tumor was yielded in animals using betel quid as an initiator. The results inferred that the betel quid chewed in this region was not a significant carcinogen, if it was at all. Instead, it acted to promote carcinogenesis. Few studies have been done with respect to the tumor promoting effect of the betel quid or its constituents. Stich *et al.*[27] introduced areca nut extracts to the