Chin Dent J (中華牙誌) 8(4): 162-169 December 1989

Growth of hamster cheek pouch tumors induced by DMBA inhibited under cold environmental stress

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This study was designed to evaluate the effects of a chemical carcinogen on tumor induction in the hamster cheek pouch under hypothermic condition. Twenty hamsters were divided into three groups. The 1st group (8 hamsters) and 2nd group (8 hamsters) were placed in a cold room (2°C) and a room at room temperature, respectively. The 3rd group (4 hamsters) was the control group and animals were placed in the hypothermic condition. 9,10-dimethyl-1,2-benzanthracene was applied to the right cheek pouch of the hamsters in the 1st and 2nd groups three times a week. Five hamsters, 3 in the cold room group and 2 in the room temperature group, were sacrificed during the 4th, 7th, 12th and 15th week. The pouches were observed and the gross changes on them were recorded. In addition, biopsies were done. During the 4th and 7th week, no apparent change was noted on the mucosa grossly and microscopically among these 3 groups. In the 12th week, numerous granular lesions were noted on the mucosal surface of the 1st animal group; epithelial dysplasia was found by microscopic examination. Numerous papilomatous lesions were noted on the mucosal surface of the 2nd animal group and early invasive squamous cell carcinoma was diagnosed microscopically. In the 15th week, numerous papillomas were noted on the mucosal surface of the 1st animal group and large ulcerative lesions were noted on the right cheek pouches of the 2nd animal group. By light microscopy, lesions of both groups were shown to be squamous cell carcinoma. In this study, the average temperature of the cheek pouches of hamsters which were placed in the hypothermic condition was 28.5°C. This temperature was 6°C lower than that of the animals placed at the room temperature. We conclude that in the hypothermic condition the growth rate of the tumor may be temporarily inhibited for a certain period of time.

Key words: hypothermic, carcinogenesis.

低溫的、癌化過程.

The relationship between the development of a chemically induced turmor and systemic stress has been studied[1,2]. Rashkis reported that a moderate degree of stress to rats, produced by forced swimming, would inhibit the tumor growth rate compared with the nonstressed rats[1]. Andervont found the stress effect of crowding on tumor development would delay the onset of spontaneous tumor growth in mice[2].

Epidemiological studies have demonstrated that the growth rate of tumors was faster in summer than in winter and have also shown a seasonal fluctuation in mortality rates[3]. The reason for such a reduced tumor incidence might be due to the inhibition of neoplastic transformation as the rate of cellular metabolism is high in cold conditions[4], which can cause the metabolic degradation of carcinogens[5]. Cold exposure can act as a stressor and elicit nonspecific responses in the host[6]. It is similar to other forms of chronic stressors which also have been reported to inhibit tumor growth[7-10]. Turbiner et al[11] studied the hamster salivary gland tumor under cold environmental conditions (3°C). The results showed that the tumor growth rate was accelerated with larger lesions compared to the non-stressed control group. In review of different results from various physiologic and anatomic neoplastic tissue changes resulting from extended cold stress, a further investigation should be considered.

MATERIALS AND METHODS

The chemical carcinogen, 9,10-dimethyl-1,2,-benzanthracene (DMBA) was used to

induce squamous cell carcinoma in hamster cheek pouches. This cheek pouch cancer model has been established for many years by different investigators[13-15]. Twenty male Syrian hamsters, 6-8 weeks of age and 70-90 grams in weight were used. The experimental animals were divided into three groups. Twelve animals were placed in a cold room and maintained at a temperature of 2°C ± 2°C for the different experimental peroids of time (Table 1). Among the 12 hamsters, 8 served as an experimental group and the other 4 as a control group. The remaining 8 animals were maintained at a room temperature of 24° ± 2°C and served as another experiment group. In the experimental animal groups (8 in cold condition and 8 at room temperature), the right cheek pouch was painted with 0.5% DMBA in heavy mineral oil three times a week with a No. 4 camel's hair brush. The left pouch, however, served as a negative control site. The 4 control animals placed in the cold room had only mineral oil applied to their right pouches.

The hamsters, two in each plastic cage, were kept for different experimental peroids of time. The body weight of the animals, gross changes of the pouches, dimension and number of tumor growth in the pouches were recorded once per week. The animals were given a lethal dose of diethyl-ether. The cheek pouches were removed from each animal by inverting and extending them with forceps and excising them with scissors. The specimens were fixed in 10% formalin for 24 hours, dehydrated in ascending alcohols, cleared in xylene, and embedded in paraffin. Sections were cut at 5 microns and stained with hematoxylin and eosin. All the sections were examined by a light microscope. The tissue changes including the degree of dysplasia and malignant transformation were studied and recorded.

Table 1. Number of hamsters sacrificed at different time periods

	Cold room		Room temperature
	DMBA	Control	DMBA
4th week	2	1	2
7th week	2	1	2
12th week	2	1	2
15th week	2	1	2

RESULTS

At the time of sacrifice the cold-stressed animals on the average weighed significantly less than those in the room temperature group (Figure 1). The cold-stressed hamsters displayed cutaneous ulcerations, especially on the tails or in the penis area.

Gross examination of the pouches revealed that the progress of the induced tumors began with erythema, ulceration, papilloma and a tumor mass with uniformity of response. Tumors developed in all the animals painted with DMBA. Papillomatous lesions were first found in the animals of the room temperature group in the 11th week. By the end of the 12th week in DMBA-painted animals of the room temperature group, there were apparent papillomatous masses on all the right pouches (Figure 2A). In the cold room group with DMBA painting for 12 weeks, however, only tiny whitish granules were found on the irregular mucosal surface (Figure 2B). These papillomatous lesions with marked changes in color, texture and consistency were indicative of premalignancy.

By the end of the 15th week, the animals in the room temperature group showed several

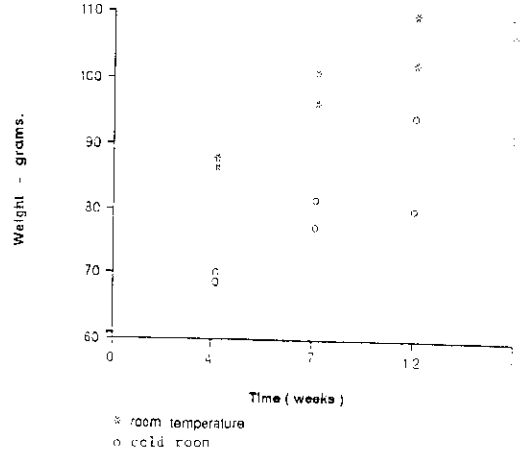

Figure 1. Body weight of animals at time of sacrifice.

Figure 2. (A) Numerous papillomatous lesions are noted on the check pouch treated with DMBA for 12 weeks at room temperature.
(B) Tiny whitish granules are noted on irregular mucosal surface of pouch treated with DMBA for 12 weeks in hypothermic condition.
(C) ulcerative exophytic tumor masses are noted on the pouch treated with DMBA for 15 weeks at room temperature.
(D) Papillomatous tumor masses are noted on pouch treated with DMBA for 15 weeks in hypotheric condition.

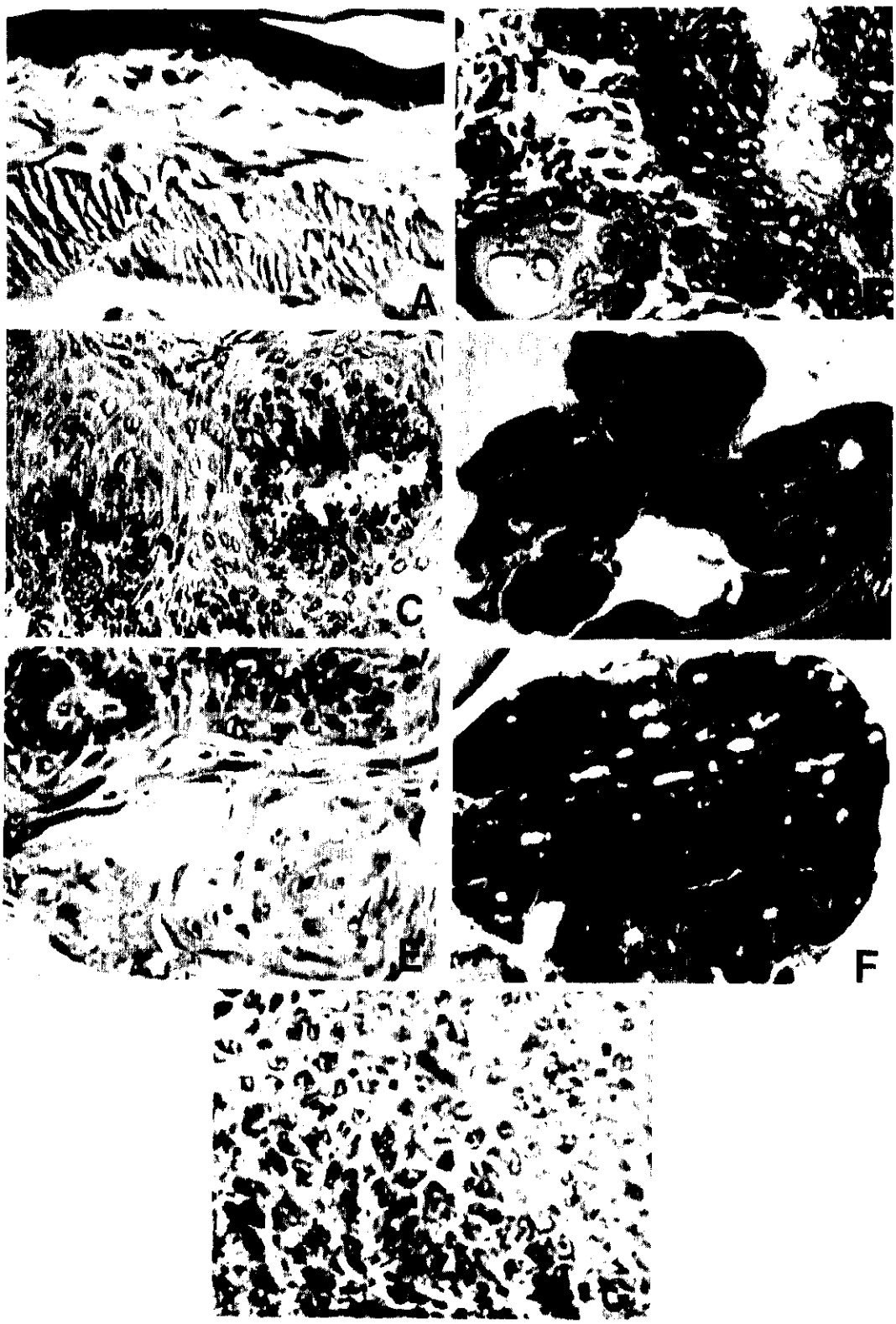

Figure 3. (A) Histologic section from a control pouch showing stratified squamous epithelium with flattened rete ridge and distinct basal cell layer, covered by a thin layer of keratin. Subepithelial connective tissue is not remarkable.

(B) Cheek pouches painted with DMBA for 12 weeks at room temperature showed dysplastic epithelium with invasive tumor nests of squamous cell carcinoma in the lamina propria.
(C) 12-week DMBA-treated cheek pouches of the cold room hamster revealed mild or moderate dysplasia of epithelium.
(D) An exophytic mass profliferating from surface epithelium of cheek pouch treated with DMBA for 15 weeks in hypothermic condition.
(E) High power view of the tumor was shown in (D). Note invading epithelial nests of squamous cell carcinoma characterized by tumor cells with plemorphism, hyperchromatism and increased number of mitotic figures.
(F) A large exophytic mass proliferating from surface epithelium of cheek pouch treated with DMBA for 15 weeks at room temperature.
(G) High power view of tumor was shown in (F). Obviously, a moderately differentiated squamous cell carcinoma.

(H&E, (A) 100X; (B), (C), (E) and (G) 400X; (D) (and (F) 40X)

large tumor masses with multiple purulent ulcerative lesions on the cheek pouch (Figure 2C), which were characteristic of a malignancy. The cold room group animals with 15-week DMBA painting demonstrated numerous small exophytic whitish papillomatous nodules on the affected pouch mucosa (Figure 2D).

All the left pouches and right control pouches painted with mineral oil only were smooth and pinkish in color and had no evidence of gross pathologic lesions. Microscopically, the control pouch mucosa was covered by the stratified squamous epithelium with a thin layer of keratin on the surface, flattened rete ridge, and distinct basal cell layer, The subepithelial connective tissue was not remarkable (Figure 3A). All the 4 and 7-week DMBA-treated cheek pouches showed the histologic findings similar to those found in the control animals except that the epithelium was slightly acanthotic in the DMBA-treated animals. The cheek pouches painted with DMBA for 12 weeks at room temperature showed dysplastic epithelium with invasive tumor nests of squamous cell carcinoma in the lamina propria (Figure 3B). The 12-week DMBA-treated cheek pouches in animals of the cold room group, however, revealed only mild or moderate dysplasia of the epithelium (Figure 3C).

After a 15-week exposure of DMBA, animals in both the cold room and room temperature groups showed squamous cell carcinoma characterized by tumor cells with pleomorphism, hyperchromatism and increased number of mitotic figures (Figure 3D-G). No obvious microscopic difference was found between these two groups except the size of the tumor mass.

DISCUSSION

Exposure to low temperature caused an immediate and sustained change in the metabolic rate as indicated by a doubling of oxygen consumption and food intake[18]. In the present study, it may be related to increased metabolic rate to resist the cold-stressed environment. We found the cold-stressed animals weighed less than those in the room temperature group and the former consumed larger quantities of food and water. The initial response to cold was shivering with increased muscular activity which supplied sufficient heat to maintain body temperature. However adaptation to extended cold is achieved by the ability to produce heat without shivering and by increasing the metabolic rate[16,17].

Goldfeder reported temporary retardation in tumor growth when subjects were exposed to acute cold stress[19]. But Bischoff et al stated that induced hibernation did not cause the permanent retardation of the growth of mouse sarcoma 180[20]. The average temperature of the experimental hamster pouches at the room temperature was 34.5°C and that in the cold room was 28.5°C. This difference of the temperature (6°C) might directly cause the tumor retardation in early stages of the carcinogenesis either by gross or

microscopic examinations. However, no obvious microscopic difference between the cold room and room temperature groups could be found in the late stages of the tumor growth except the size of the tumor. Smith and Fay[21] stated that maintenance of body temperature is as important to the fate of the tumor as it is to the general survival of the animal. Tumor cells are many times more susceptible to a reduction in temperature than are normal cells. All phase of the mitosis are shorter in the normal temperature range[22]. It will increase its mitotic phase when the temperature is above or below its normal range. The varied experimental condition, host species and tumor etiology used by those investigators may account for the variability of the results. During the same low temperature with continued chronic and intermittent exposure could produce the opposite effect[23]. Therefore it is possible that physiological alterations of the host in response to the nature, duration and severity of the stress exposure could influence the course of the neoplastic development.

In our study, papilloma was first noted in animals treated with DMBA for 11 weeks at room temperature. It shows a delayed sign of tumor development compared with the other study[15]. This delay may be related to either the amount of DMBA painted on the pouch of the hamster or difference in individual brushing techniques.

We found that there is a critical threshold for tumor growth and inhibition in this study. If the stimulation is within the limit, tumor growth is inhibited; if stimulation is over the limit, tumor growth is promoted. However, it remains to be studied whether the temperature effects are related to the productivity of the tumors as well as the nature and duration of the exposure regime. In addition to increased metabolism, cold stress may act to inhibit tumor growth by increasing the cell-mediated or humoral immunity. Further study is required to substantiate this theory.

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低温抑制DMBA誘發倉鼠口腔癌之生長

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本論文研究在低溫環境下，化學致癌劑對癌細胞所產生的作用。20隻倉鼠共分成三組，第一及第二組（各別8隻）爲實驗組，倉鼠分別被置於2°C的低溫環境及室溫中，第三組（共4隻）爲對照組，倉鼠置於2°C的低溫中。第一、二組每週定時塗抹三次DMBA於右側頰囊袋黏膜，觀察記錄表皮變化，並做切片檢查。在第四、七週時，三組黏膜表面及切片並沒有明顯的差別。在十二週時，第一組黏膜表面出現很多白色突起的顆粒，切片下呈現表皮細胞的發育異常。第二組的黏膜已出現多個乳頭狀瘤，切片下被診斷爲早期的鱗狀上皮癌。在十五週時，第一組表面出現多個乳頭狀瘤，第二組表面出現大且潰瘍的病竈；但在切片下，兩組同時出現鱗狀上皮癌的特徵。本研究發現置於低溫下倉鼠的頰囊袋溫度爲28.5°C，比置於室溫下低6°C。結論爲低溫於某程度內，可暫時性的抑制癌細胞的快速成長。

Received: August 13, 1989
Accepted: December 16, 1989
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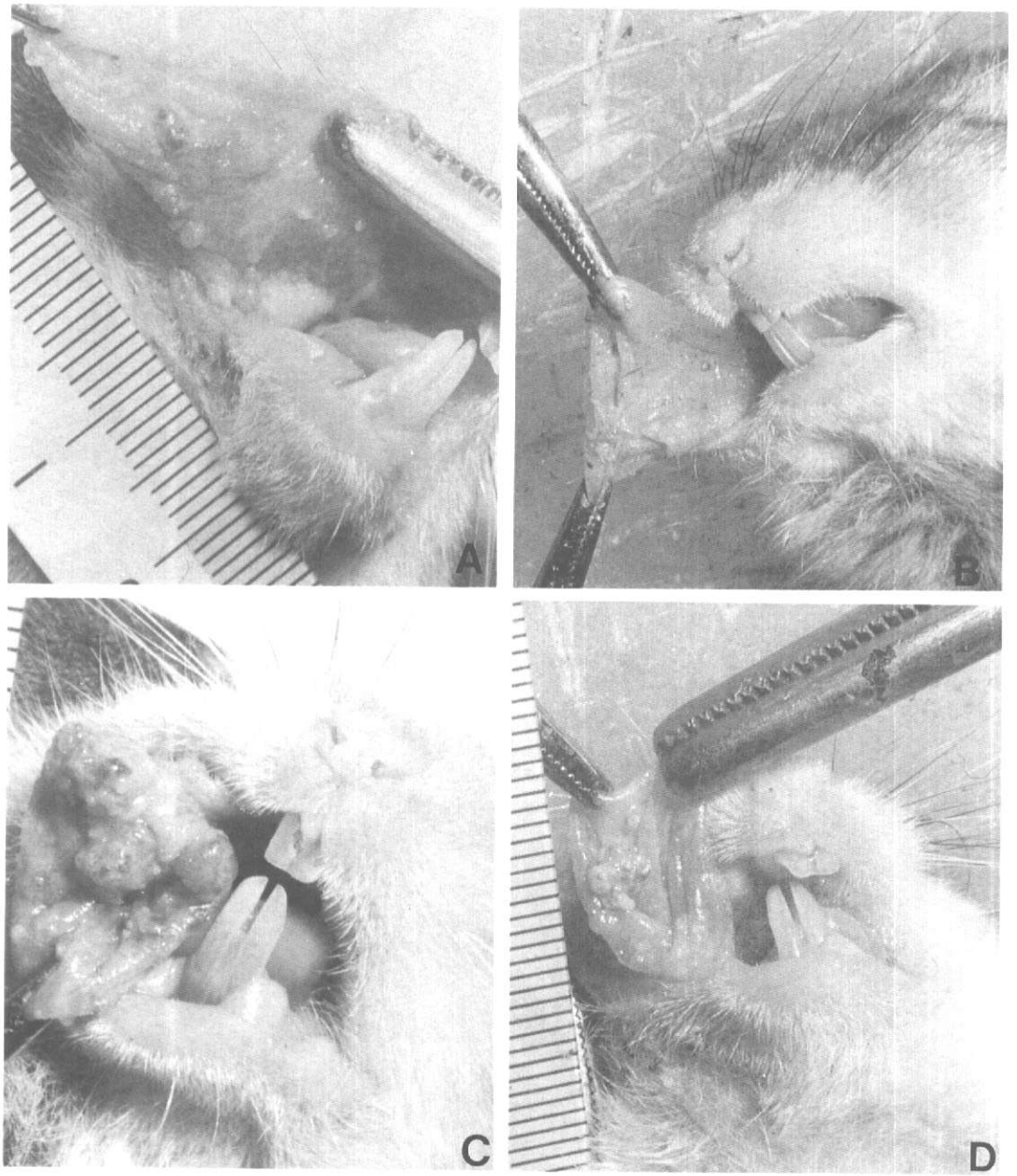

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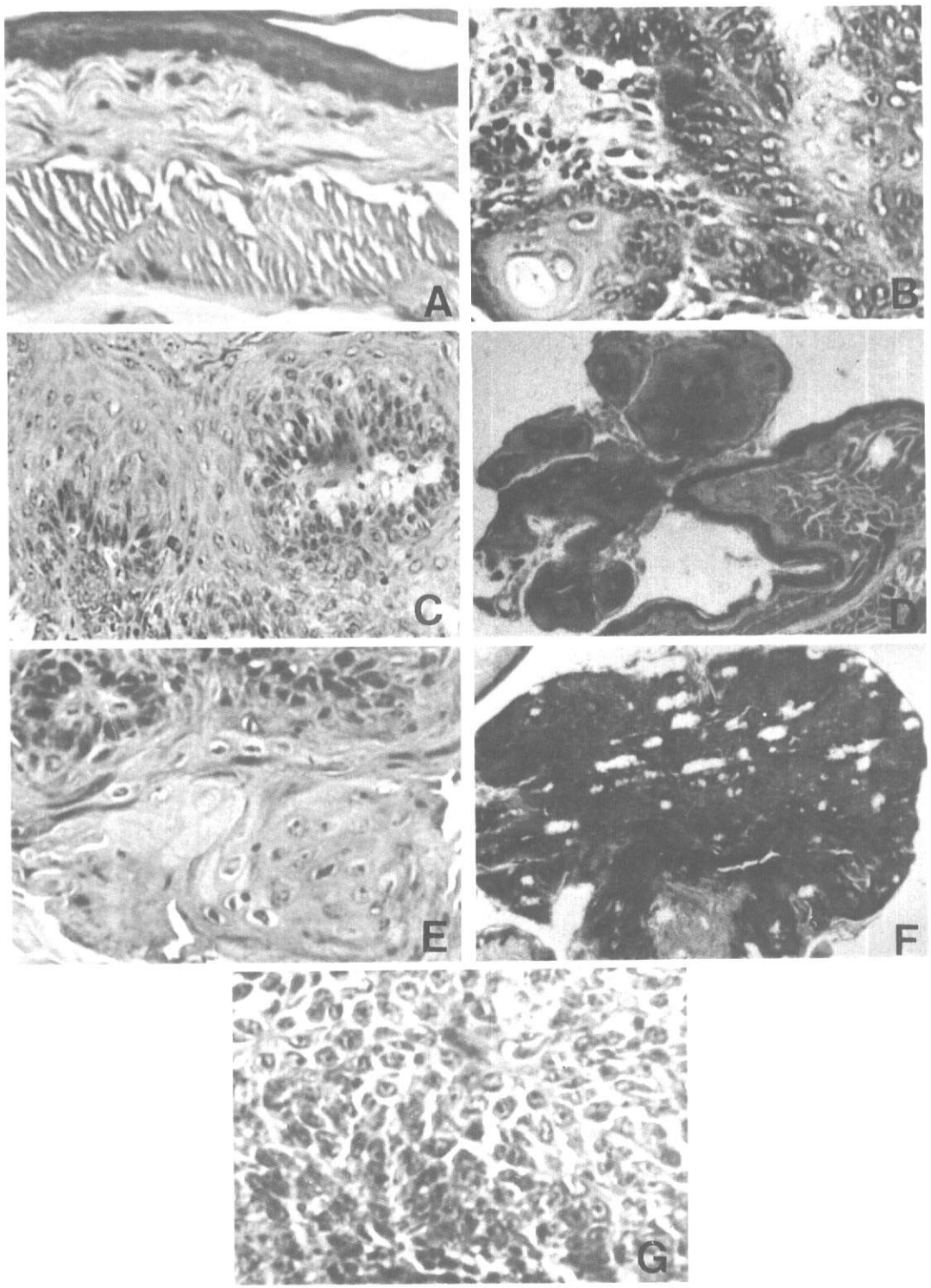

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