Induction of Tracheobronchial Carcinomas in the Syrian Golden Hamster*

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A number of clinical statistical studies conducted in recent years have pointed to an association between several environmental factors and an increase in the incidence of bronchogenic carcinoma in man (7). Of the numerous factors involved, atmospheric pollution and cigarette smoking are being considered with the most seriousness (4, 6, 8, 9, 15). To supplement the clinical data, experimental studies with incriminated materials are being carried out in animals. These efforts to reproduce the human disease in the laboratory animal have been difficult, and, although some of these substances have been found to induce skin tumors in mice and rabbits (3, 5, 10, 17) and subcutaneous sarcomas in mice (12), the production of carcinoma of the bronchus has not been accomplished with any regularity. The failure to produce this lesion is not owing to any inherent refractoriness of the animals to develop the disease, since Vorwald has induced bronchogenic carcinoma in rats and rabbits following inhalation of beryllium sulfate (16) and Lisco has reported the induction of this lesion in rats following endotracheal administration of radioactive material (13).

In experiments with hydrocarbon carcinogens, Niskanen (14) induced squamous-cell carcinomas in the lung and epithelial changes in the tracheobronchial tree of the rat by injecting 1,2,5,6-dibenzanthracene in olive oil into the bronchi through the tracheal wall. Andervont (1) produced squamous-cell carcinomas by implanting a thread impregnated with dibenzanthracene directly into the lung of the mouse. Kuschner et al. (11) also induced lung carcinomas in the mouse by a similar technic and in the rat with threads and mesh pellets impregnated with 3-methylcholanthrene. They also reported a single squamous-cell carcinoma and lung adenomas in mice after repeated installations of methylcholanthrene through the tracheal wall. Recently, Blacklock (2) described sarcomas and carcinomas of the lung of the rat induced by direct introduction, into the exposed lungs, of 3,4-benzpyrene, methylcholanthrene, and a condensate of cigarette smoke. All these methods are complex and introduce a large element of trauma; in addition, they depart from the normal route of contamination of the human rather drastically. The problem seems to resolve into one of a correct choice of species for study and into the design of a suitable method of administration.

In arriving at the technics to be reported in the present study, prolonged pilot experiments were necessary in which the rat and the mouse were used, with several methods. Eventually, it was found that the Syrian golden hamster was the best animal for our purposes, since direct intubation was easily possible. The hamster also appears to be particularly suitable for lung experiments, in view of the facts that most mice strains develop spontaneous lung adenomas and that rats are frequently affected by lung infections. In our laboratory, among several hundred hamsters, we have never observed lung adenomas or any other type of lung tumors.1 In addition, pulmonary infections seem to be very uncommon among untreated hamsters.

The materials used in our studies were 9,10-dimethyl-1,2-benzanthracene and a cigarette tobacco tar condensate. The selection of a proper solvent for these materials was complicated by the fact that the organic solvents usually employed exhibited marked toxic properties. It was found that colloidal suspensions provided the most suitable form for administration of these test substances into the lungs.

MATERIALS AND METHODS

Syrian golden hamsters, male and female (Abrams Small Stock Breeders, Chicago, Ill.), were used. They were housed

1 Dr. J. G. Fortner observed two lung adenocarcinomas in untreated hamsters (personal communication).
in plastic cages, divided into groups of five or six according to the sex, and fed Rockland mouse diet and water ad libitum.

Two test substances were used: 9,10-dimethyl-1,2-benzanthracene (DMBA) (Eastman Organic Chemicals) purified by chromatography, and a cigarette tobacco tar condensate. Both substances were suspended in a 1 per cent gelatin colloid, alone or in combination in different amounts to be described with the experiments. The colloid suspension was prepared by the rapid addition of DMBA or tobacco tar, dissolved in 5–8 ml. of acetone, to 1 ml. of a 1 per cent solution of gelatine in water. This operation was done in a water bath at approximately 56° C. The acetone was then removed by slowly bubbling nitrogen gas through the suspension. The materials were instilled directly into the tracheobronchial tree. An 18G metal canula, 5 cm. in length, was introduced via the oral route into the trachea; a PE 10 polyethylene tube, attached to a 30 G needle, was then passed through the canula so that 1.5 cm. of the tubing extended beyond the tip. The polyethylene tube was beveled at the end, so that it would easily enter one of the stem bronchi, since the average distance from the upper incisors to the bifurcation, in the hamster, is 5.5 cm. The suspension was injected with a 0.05-ml. tuberculin syringe. The operation was done under light ether anesthesia, once or twice a week, according to the experiment.

The animals were left to die spontaneously or were sacrificed when moribund or at the end of the period of observation. Several were lost by cannibalism or because of advanced post mortem deterioration. At autopsy the entire respiratory tract, including the hypopharynx and the upper portion of the esophagus, was removed en bloc. These tissues were embedded in three separate paraffin blocks; the first block included the upper portion down to mid-trachea; the second, the lower segment of the trachea and the proximal portion of the bronchial tree; the third, the peripheral area of each of the five lobes.

**RESULTS**

**Group 1.**—Twenty-one male hamsters, 15 weeks old, received weekly instillations of 50 μg. of DMBA in 0.05 ml. of colloid. At the 20th week, seventeen animals survived. The treatment was stopped after 45 weeks; at that time eight animals survived. At the 50th week only one animal was still alive, and it was sacrificed moribund at the 70th week of the experiment. Most of the animals died spontaneously with evident respiratory distress; some were killed when moribund.

Pathological study was performed in all the animals dying before the 20th week and in ten out of the seventeen surviving after the 20th week; the other seven were lost because of advanced post-mortem decomposition or because of cannibalism. Gross examination constantly showed bronchopneumonia involving one or more lobes. Occasionally, small papillomatous growths were seen in the forestomach. Histological examination revealed acute suppurative tracheobronchitis and bronchopneumonia with areas of carnification. The bronchial mucosa frequently showed areas of necrosis. In the animals that received twenty or more instillations, the epithelium of the lower third of the trachea and of the stem bronchi consistently showed basal-cell hyperplasia and well differentiated squamous metaplasia. In addition, in focal areas of the lower third of the tracheas, the epithelium presented histological and cytological atypicality (Fig. 1). An invasive carcinoma in this lower segment of the trachea was found in one animal that died 8 weeks after the treatment was stopped (Fig. 2). Another animal, dying after 35 weeks of treatment, had an invasive squamous-cell carcinoma in the arytenoid region. The one animal sacrificed 25 weeks after the end of the treatment presented marked suppurative tracheobronchitis and a moderate degree of acanthosis and parakeratosis of the metaplastic squamous epithelium of the larynx. In the trachea and main bronchi, the epithelium showed no squamous metaplasia, but only a slight piling of basal cells.

**Group 2.**—Twenty-one female hamsters, 7–9 months old, received instillations of 200 μg. of tobacco tar in 0.05 ml. of colloid suspension twice a week. Seventeen hamsters had survived by the 20th week of treatment. The treatment lasted for 32 weeks, at which time there were twelve survivors. These animals were kept under observation until spontaneous death, the last animal dying at the 55th week of the experiment.

A complete pathological study was possible in eleven out of the seventeen animals surviving 30 weeks of treatment. Sections invariably showed acute and chronic tracheobronchitis and occasional foci of bronchopneumonia. The epithelium of the tracheobronchial tree showed no remarkable changes, nor was squamous metaplasia seen in the animals dying during the treatment or surviving afterwards. No papillomas were noted in the stomach.

**Group 3.**—Ten male hamsters 10 weeks old received twelve instillations of 50 μg. of DMBA in 0.05 ml. of colloid suspension once weekly. Two animals died accidentally during the treatment after two and four instillations, respectively. Histological examinations revealed intra-alveolar hemorrhages and no remarkable changes in the bronchial mucosa. Eight animals survived at the end of the treatment; two of them died 10 and 11 months later and were lost for pathological examination; the last six were alive and in good condition 1 year after the treatment was stopped and were sacrificed. In five of them, a few small papillomas were present in the forestomach. Histological examination of the lung showed chronic tracheobronchitis and no remarkable changes of the tracheobronchial epithelium.

**Group 4.**—Ten female hamsters, 10 weeks old,
received twelve instillations of DMBA as in Group 3, followed in nine survivors by twice-weekly instillations of 200 µg. of tobacco tar in 0.05 ml. of colloid suspension. This second treatment lasted for 30 weeks with no deaths. The nine survivors were kept under observation without any further treatment. Six of them died between the 9th and the 15th week after the treatment was stopped. Three animals were alive in good condition 64 weeks from the beginning of the experiment and were sacrificed.

Pathological study was not possible in the one animal that died during the DMBA treatment. Histological examination of the group of six dying spontaneously showed acute and chronic inflammation of the trachea and bronchi; their epithelium showed focal areas of hyperplasia and no squamous metaplasia. In the lower trachea and main bronchi there were several pedunculated or sessile polyps, always covered by cylindrical epithelium (Fig. 3). In one instance a focal area of bronchiolar proliferation with an adenomatous pattern was seen. Also, in the group of three animals killed 22 weeks after the end of the treatment, squamous metaplasia was absent in the tracheobronchial mucosa. One of these three animals had a chondroma in the tracheal wall. Papillomas of the forestomach were noted in almost all the animals of this experimental group.

Group 5.—Nine male and eleven female hamsters, 15 weeks old, received weekly instillations of 100 µg. of DMBA in 0.05 ml. of colloid suspension. After twelve instillations, seven males and seven females survived, and five males and three females were alive after seventeen instillations; therefore, the treatment was stopped. At the 25th week from the beginning of the experiment, one male and one female survived; the male died at the 31st week and the female at the 35th week.

Pathological study was possible in seven out of the eight animals surviving at the end of the treatment. The first two deaths, one female and one male, occurred by accident during the treatment at the end of the 5th and 8th week, respectively. Histological study, in both animals, revealed well differentiated squamous metaplasia in the larynx and upper trachea, while in the lower trachea the epithelium was formed by irregularly arranged cells, with large nuclei and very abundant granular cytoplasm (Fig. 4). Numerous inflammatory cells infiltrated both the epithelium and the submucosa. No areas of necrosis were seen. The epithelium of the main bronchi presented piling of oblong cells with absence of the ciliated cylindrical layer (Fig. 5). The small bronchi and bronchioles showed no changes in the epithelium.

**TABLE 1**

<table>
<thead>
<tr>
<th>No. of animals</th>
<th>Treatment</th>
<th>Effective no. of animals</th>
<th>No. of animals with carcinomas</th>
<th>Location and no. of carcinomas</th>
</tr>
</thead>
<tbody>
<tr>
<td>21♂</td>
<td>DMBA, 50 µg. weekly for 45 weeks</td>
<td>10*</td>
<td>2</td>
<td>Trachea: 1</td>
</tr>
<tr>
<td>21♂</td>
<td>Tobacco tar, 200 µg. twice weekly for 32 weeks</td>
<td>11*</td>
<td>2</td>
<td>Larynx: 1</td>
</tr>
<tr>
<td>10♂</td>
<td>DMBA, 50 µg. weekly for 12 weeks</td>
<td>6†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10♀</td>
<td>DMBA, 50 µg. weekly for 12 weeks followed by tobacco tar, 500 µg. twice weekly for 30 weeks</td>
<td>9†</td>
<td>4</td>
<td>Trachea: 3</td>
</tr>
<tr>
<td>9♂ 11♀</td>
<td>DMBA, 100 µg. weekly for 17 weeks</td>
<td>7†</td>
<td>4</td>
<td>Larynx: 1, Esophagus: 1, Stem bronchus: 1</td>
</tr>
<tr>
<td>10♂ 10♀</td>
<td>DMBA, 100 µg.+tobacco tar, 500 µg. weekly for 20 weeks</td>
<td>9†</td>
<td>3</td>
<td>Larynx: 2</td>
</tr>
</tbody>
</table>

* Animals surviving 20 wk. and available for pathological examination.
† Animals surviving at the end of the treatment and available for pathological examination.

In all the other animals dying during the period of treatment, bronchopneumonia, squamous metaplasia of the trachea, and basal-cell hyperplasia of the bronchial epithelium (Fig. 6) were always observed. In one female that died immediately after the thirteenth instillation, the squamous metaplasia was well differentiated and particularly extensive, involving the main bronchi (Figs. 7 and 8). Purulent exudation in the bronchi was very marked, with necrosis of the epithelium of the small bronchi and necrotizing pneumonia. In the same animal, in the lower third of the trachea, the squamous metaplasia was abruptly replaced by an irregularly composed epithelium, in which atypical histological and cytological findings were very prominent with the characteristics of the intraepithelial carcinoma of human pathology (Fig. 9). In another female that died 4 weeks after the treatment was stopped, a large papillomatous growth was found in the lower third of the trachea,
just above the bifurcation, almost completely occluding the tracheal lumen (Fig. 10). This papilloma was partially necrotic and composed of bands of squamous epithelium with plugs of keratin. At its base there was evident invasion of the submucosa and of the external layers of the trachea, by cords of small undifferentiated cells. In the same animal there was acanthosis of the squamous epithelium in the larynx, while in a male dying 1 week later there was an invasive squamous-cell carcinoma of the larynx (Fig. 11).

In a male dying at the 25th week of the experiment, an invasive squamous-cell carcinoma arose at the upper end of the esophagus, and a second invasive squamous-cell carcinoma was present in the middle third of the trachea. The last male died at the 31st week, 15 weeks after the treatment had stopped; sections showed a papilloma just below the larynx and no squamous metaplasia either in the trachea or in the bronchi, while extensive bronchopneumonia was present. The last female was moribund at 35th week and was sacrificed. In the lower third of the trachea there was a carcinoma invading the mediastinal soft tissue, either with a glandular pattern (Fig. 12) or with epidermoid characteristics (Fig. 13). The remainder of the tracheobronchial epithelium did not show squamous metaplasia.

Group 6.—Ten male and ten female hamsters, 15 weeks old, received weekly instillations of a mixture of 100 ¡¿g. of DMBA and 500 ¡¿g. of tobacco tar in 0.05 ml. of colloid suspension. This suspension was unsatisfactory, since some of the DMBA tended to precipitate out. Seven males and seven females survived after the fifteenth instillation, and seven males and five females after the twentieth and last instillation. Ten weeks later, four males and two females were still alive. The last animal, a female, was killed moribund at the 35th week of the experiment, presenting intestinal infection.

Pathological study was done in sixteen out of the twenty animals used and in nine out of the twelve surviving at the end of the treatment. In all the animals that died during the period of treatment, no remarkable changes were noted in the tracheobronchial epithelium besides a moderate degree of hyperplasia and focal areas of squamous metaplasia. Tracheobronchitis and focal bronchopneumonia were constantly present. In one male dying 6 weeks after the treatment was stopped, there was an invasive squamous-cell carcinoma in the larynx and small polyoid elevations in the trachea, covered by cylindrical epithelium (Fig. 14). No squamous metaplasia was seen elsewhere; there was marked purulent bronchitis with pneumonia.

One female dying at the same time had a large carcinomatous growth almost completely occluding one of the main bronchi (Fig. 15); this tumor was composed mainly of an irregular squamous epithelium, but also showed an area with a glandular pattern. In another male that died at the 30th week, sections revealed an invasive squamous-cell carcinoma of the larynx, squamous metaplasia in the trachea, and a squamous papilloma in the lower third with atypical histological and cytological characteristics. In the other animals that died at the same time or later on, different degrees of inflammatory lesions were seen, without remarkable changes of the epithelium.

DISCUSSION

A relatively simple technic for studying the effect of carcinogens in the mucosa of the respiratory tract has been described. It has been shown that invasive squamous-cell carcinomas may be induced in the larynx, trachea, and major bronchi by repeated instillations of the carcinogen 9,10-dimethyl-1,2-benzanthracene in the hamster.

The dosage of carcinogen in the individual treatments appears to be an important factor in the effectiveness of this method. Whereas 45 repeated doses of 50 ¡¿g. of DMBA yielded only borderline carcinogenic action on the tracheobronchial epithelium, seventeen doses of 100 ¡¿g. induced a significant number of tumors in this location.

In Table 1 the number and location of the carcinomas observed are recorded. It is impossible to establish incidence rates from these results because of the small number of animals surviving at the end of the treatment or reaching a hypothetical threshold of tumor latency. Moreover, not all these animals were available for pathological examination.

In the animals dying in the early phases of the experiments, a sequence of pathological changes in the tracheobronchial epithelium was observed. Basal-cell hyperplasia was the earliest and most common lesion (Figs. 5 and 6): the columnar ciliated layer was still present at the surface or was largely desquamated with beginning of superficial stratification. Squamous metaplasia was also a frequent and extensive change (Figs. 7 and 8), with different degrees of differentiation and acanthosis; acanthosis was particularly common in the larynx and upper trachea. A third category of epithelial changes comprised the lesions in which there were atypical histological and cytological characteristics (Figs. 1 and 4). This sort of dysplastic lesion seemed to arise directly from the columnar epithelium or during the process of
formation of the squamous metaplasia, or as a subsequent stage of transformation of the well differentiated squamous metaplasia. These lesions were located mostly in the lower third of the trachea and in the stem bronchi; in one case they were associated with an area of a more atypical epithelium (Fig. 9) closely resembling the intraepithelial carcinoma of human pathology. Eventually, frank invasive carcinomas arose at the same location. The animals, free of tumors, that survived for a certain length of time after treatment was stopped showed a reversion to a normal columnar epithelium; several had polypoid or papillomatous growths.

Although it is permissible to draw conclusions from the groups in which carcinogen was administered, the negative results using tobacco tar alone are of less significance. Only female hamsters were in this group, and low doses of tar were used, although for a long period. However, it is interesting that the tobacco tar alone was unable to bring about even the early epithelial changes.

Two different combinations of DMBA and tobacco tar were used. The tar was instilled in one group after an amount of DMBA was given that by itself did not induce remarkable changes in the tracheobronchial epithelium. The second treatment did not produce any further pathological findings. A second group of animals received a mixture of DMBA and tobacco tar, but this treatment did not add any valuable information in spite of the increased amount of tar. However, any action of the tar would have been difficult to detect, in view of the large dose of the carcinogen.

SUMMARY
1. A technic has been described for the direct endotracheal introduction of carcinogens in the Syrian golden hamster.
2. In experiments with repeated endotracheal instillations of 9,10-dimethyl-1,2-benzanthracene in a colloidal suspension, hyperplasia, squamous metaplasia, atypical changes of the tracheobronchial epithelium, squamous-cell and adenocarcinomas of the trachea and bronchi have been produced.
3. Administration of tobacco tar, also as a colloidal suspension, produced no histologically demonstrable lesions.

ACKNOWLEDGMENTS
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REFERENCES
Fig. 10.—Four weeks after seventeen instillations DMBA, 100 μg. Squamous-cell carcinoma of lower third of trachea. ×90.

Fig. 11.—Same as Figure 10. Invasive squamous-cell carcinoma of the larynx. ×230.
Fig. 4.—Seven instillations DMBA, 100 μg. Atypical epithelium of the lower trachea. X320.

Fig. 5.—Seven instillations DMBA, 100 μg. Hyperplasia of the bronchial epithelium. X550.

Fig. 6.—Fifteen instillations DMBA, 100 μg. Hyperplasia of the bronchial epithelium. X950.
Fig. 7.—Thirteen instillations DMBA, 100 µg. Squamous metaplasia of main bronchi. ×70.

Fig. 8.—Same as Figure 7. Well differentiated squamous metaplasia of main bronchus. ×400.

Fig. 9.—Same as Figure 7. Intraepithelial carcinoma of lower trachea. ×560.
Fig. 10.—Four weeks after seventeen instillations DMBPA, 100 μg. Squamous-cell carcinoma of lower third of trachea. ×90.

Fig. 11.—Same as Figure 10. Invasive squamous-cell carcinoma of the larynx. ×490.
Fig. 12.—Nineteen weeks after seventeen instillations of DMBA, 100 µg. Carcinoma of lower trachea invading the mediastinal soft tissue with a glandular pattern. X170.

Fig. 13.—Same as Figure 12. Squamous pattern of the same carcinoma. X170.

Fig. 14.—Six weeks after twenty instillations of DMBA, 100 µg., and tobacco tar, 500 µg. Polypoid formation in trachea, covered by normal epithelium. X430.

Fig. 15.—Seven weeks after twenty instillations of DMBA, 100 µg., and tobacco tar, 500 µg. Carcinoma of main bronchus. X50.


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