Induction of Tumors in the Syrian Hamster with Diethylnitrosamine (N-Nitrosodiethylamine)

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SUMMARY

In the Syrian hamster (Mesocricetus auratus) both intragastric feeding and intratracheal instillation of diethylnitrosamine (DENA) induced multiple squamous-cell papillomas of the trachea and bronchi and carcinomas of the ethmoid region of the nasal cavity. Feeding DENA also induced carcinomas of the liver and proliferative lesions of the kidney. The possibility is discussed that carcinogenic substances may reach the human lung by pathways other than the respiratory tract.

Tumors of the liver, kidneys, and lungs have been induced in rats by the feeding of dimethyl nitrosamine (DMN) and diethylnitrosamine (DENA) (1, 11, 12, 18). Dontenwill et al. (3, 4) reported that squamous-cell carcinoma of the trachea and lung of Syrian hamsters followed administration of DENA by each of three approaches—tube feeding, inhalation, and subcutaneous injection. They observed cirrhosis of the liver but no hepatic cancer.

The tumors of the trachea described by Dontenwill and Mohr histologically resemble the squamous-cell papillomas of the trachea and bronchi induced by us following the intratracheal instillation of benzo[a]pyrene suspended in Tween 60 (9). The experiments reported in the present study were designed to compare tumors of the respiratory system that were induced by DENA with similar tumors induced by benzo[a]pyrene in our earlier experiment.

MATERIALS AND METHODS

Animals.—Male and female Syrian hamsters (Mesocricetus auratus) were obtained from the Animal Production Section of the National Institutes of Health. They were separated by sex and housed in plastic cages in groups of four or five. The animals were fed Purina Laboratory Chow daily, supplemented with kale, carrots, and apples 3 times a week. Drinking water was always available. The animals were 2-34 months old when the experiment was started.

Test substance.—Diethylnitrosamine (Eastman Organic Chemical Company) was made up with distilled water in a 1:250 solution for feeding by intragastric tube, and in a 1:14 solution for intratracheal instillation.

Experimental groups.—The hamsters were divided into two experimental groups separated by sex. There were fifteen female and fifteen male animals in each group. In addition, there were five females and five males in a control group that received distilled water by the intratracheal route.

Procedures.—The technic for intratracheal instillation was the same as that described previously (9): .05 ml. of the solution of DENA was administered once a week for periods up to 6 months. Prior to feeding by intragastric tube the hamsters were fasted from 4:00 P.M. of the preceding day.

An Argyle infant feeding tube (size 8, French, 15" long), with attached tuberculin syringe, was filled with the solution, and before insertion the outside of the tube was wiped clean to reduce the likelihood of the solution's entering the tracheobronchial tree. The tip of the tube was guided into the forestomach, and 0.4 ml. was administered. Hamsters were fed in this manner twice a week for periods up to 7 months. The animals in both experimental groups received approximately the same weekly dosage of DENA.

Post-mortem studies.—Complete autopsies were performed on all animals killed or found dead. Animals were killed either because of respiratory difficulty due to obstruction of the trachea by tumor or because of large abdominal masses. After intratracheal instillation of fixative the entire
respiratory tract was removed en bloc and hardened in buffered 10 per cent formalin solution. Transverse sections were made of the complete trachea and main-stem bronchi, and longitudinal sections of all lobes of the lung. The tissues were embedded in paraffin and sectioned at 6 μ. All sections were stained with hematoxylin and eosin.

RESULTS

A summary of the results is shown in Table 1. The table includes the 53 hamsters that survived the treatment for more than 1 month. It is of interest that, prior to death, and occasionally as early as 2 months after starting the treatment, hamsters with tracheal tumors breathed with difficulty and showed a characteristic tracheal tug. The total life span ranged from 6 to 124 months.

Respiratory system.—Papillary tumors of the trachea were observed in all the hamsters treated with DENA. More than a third of these hamsters also had similar tumors in the bronchi. Nine of the animals in Group 1 that had tracheal or both tracheal and bronchial tumors also had primary tumors of the liver and ethmoturbinals. The same distribution of primary sites was found in four animals of Group 2, except that there were no primary tumors of the liver.

Gross examination of the tracheal and bronchial tumors revealed soft, elevated, papillary nodular masses that were 2—3 mm. in diameter and obstructed the lumen of the trachea or bronchus. The tracheal tumors were multiple and were located in the upper, middle, and lower portions, with the result that distally there was marked dilatation of the entire tracheobronchial tree. On histologic examination most of the tumors were well differentiated squamous-cell papillomas (Fig. 1) but there were occasional less well differentiated tumors (Fig. 2). None of the tumors showed invasion, even though some of them were serially sectioned. Squamous-cell papillomas from four of the animals were transplanted subcutaneously to weanling hamsters. No evidence of growth has been observed after 6 months.

Changes in the lining epithelium of the tracheobronchial tree were observed in both experimental groups, but they were more marked in Group 2 than in Group 1. The lesions were basal-cell hyperplasia, papillary excrescences, squamous metaplasia, and epithelial atypism. Although there were some very large cells with bizarre-shaped hyperchromatic nuclei (Fig. 3), the nuclear-cytoplasmic ratio was not altered. In five animals of Group 2 small nests of well differentiated squamous epithelium were observed in the peripheral areas of the lung (Fig. 4). An origin from the bronchiolar epithelium (Fig. 5) could be demonstrated in only one of four instances in which serial sections were examined. The characteristic peri-bronchiolar proliferation previously described after intratracheal instillation of benzo[a]pyrene suspended in Tween 60 (9) or noncarcinogenic substances (16, 17) was not observed.

Liver.—Twenty-two of the animals that received DENA by tube feeding (Group 1) developed hepatocellular carcinomas. These were often multiple and varied in size from pinpoint lesions to those 3.5 cm. in diameter. The histologic patterns of the liver tumors were similar to those described in the rat by Stewart and Snell (15). Although the trabecular carcinomatous pattern (Fig. 6) was the type most frequently observed, some of the tumors revealed a combination of patterns. The sex of the animal did not appear to influence the frequency of tumor development. Tumors were successfully transplanted.

Extrahepatic metastases were observed in six animals, and sites involved included the lungs,

### Table 1

**SUMMARY OF EXPERIMENTS IN WHICH HAMSTERS WERE TREATED WITH DIETHYL-NITROSOAMINE (N-NITROSOACETAMIDE)**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>SEX</th>
<th>NUMBER</th>
<th>Trachea</th>
<th>Bronchi</th>
<th>Liver</th>
<th>Ethmoturbinals*</th>
<th>Kidney†</th>
</tr>
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<tr>
<td>Intragastric DENA</td>
<td>F</td>
<td>15</td>
<td>15/15</td>
<td>6/15</td>
<td>10/15</td>
<td>6/11</td>
<td>7/15</td>
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<td></td>
<td>M</td>
<td>13</td>
<td>18/13</td>
<td>4/13</td>
<td>12/13</td>
<td>4/9</td>
<td>5/13</td>
</tr>
<tr>
<td>Intratracheal DENA</td>
<td>F</td>
<td>11</td>
<td>11/11</td>
<td>4/11</td>
<td>0/11</td>
<td>3/9</td>
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<td>10/14</td>
<td>0/14</td>
<td>1/13</td>
<td>0/14</td>
</tr>
</tbody>
</table>

* Eleven of the 53 hamsters are not included in the totals because of cannibalism of head.
† Proliferative lesions (see text on p. 775).
undersurface of the diaphragm, peripancreatic lymph nodes, peritoneum, adventitial tissue of the kidney, adrenal gland, and uterine horns (Figs. 7, 8).

Changes observed in the liver in addition to carcinoma were focal necrosis, inflammatory cell infiltrates, interstitial hemorrhage, fatty metamorphosis, regenerating nodules, large atypical hepatic cells, multilocular cysts, and areas of erythropoiesis and myelopoiesis. Cirrhosis was observed in only one animal, and this animal did not have a hepatoma. Dilatation of the common bile duct and pancreatic ducts and marked hyperplasia of the lining epithelium were occasionally seen. These changes may have resulted from obstruction of the biliary tree by the large tumor masses. Tumors of the liver were not found in the animals that received DENA by intratracheal instillation (Group 2). Histologic examination revealed small, discrete, often round areas of fatty metamorphosis and necrosis. Foci composed of small numbers of atypical hepatic cells were observed in the peripheral or in the mid-zonal regions of the lobules. These cells were larger than normal hepatic cells and often had bizarre-shaped vesicular nuclei and a single large prominent nucleolus.

Kidney.—Microscopic lesions of the kidney located in the cortex were observed in twelve of 28 animals in Group 1 and in none of Group 2 (Table 1). The lesions were sharply demarcated from the renal parenchyma, and the architectural pattern mimicked that of tubules (Figs. 9, 10). The cells were small, with basophilic cytoplasm, and showed variation in shape, nuclear size, and chromatin content. Some of the cells had small deeply basophilic nuclei, and infrequent mitotic figures were noted. Focal alteration of the epithelium of the cortical tubules was observed in ten of the Group 1 animals and in seven of the Group 2 animals. The enlarged cortical epithelial cells contained large, atypical nuclei and resembled the cells in the kidneys of rats fed DMN as described by Zak et al. (18) and later by Magee and Barnes (12). This change may represent an early stage in tumorigenesis, whereas the tubular and intracystic papillary lesions illustrated in Figures 9 and 10 may represent a later stage.

Ethmoid region, nasal cavity.—All fourteen tumors of the ethmoturbinals were classified as undifferentiated carcinomas. The neoplastic cells were densely packed together with small, round, oval, or fusiform hyperchromatic nuclei, scanty cytoplasm, and indistinct cell boundaries (Fig. 11). Sometimes the cells were arranged in rosettes and tubules, and their cytoplasm was relatively abundant. In one hamster the tumor invaded the maxillary sinus and extended into the olfactory space, olfactory bulb, and bone marrow (Fig. 12). Atypical epithelial changes were observed in the mucous membrane of the ethmoturbinals despite the presence or absence of carcinoma in this region. The atypism was most prominent in the basal cells of the lining epithelium.

We did not find significant abnormalities in the control group of animals that received distilled water intratracheally.

DISCUSSION

The results of these experiments agree with the observations of Dontenwill et al. (3, 4) that DENA induces tumors of the trachea and bronchi in Syrian hamsters. Although they stated that not all their tracheal tumors showed infiltrative growth, they designated the tumors as carcinomas. They believed that the absence of invasion might have been due to early death of the animal from asphyxiation as a result of obstruction of the trachea by tumor.

In the present experiment none of the tracheal or bronchial tumors showed microscopic evidence of invasion even when serial sections were examined. Transplantability is one criterion of malignancy. Although histologically similar tumors induced by benzo[a]pyrene suspended in Tween 60 have been successfully transplanted, the tissue from squamous-cell papillomas induced by DENA has not shown evidence of growth 6 months after transplantation.

The dosage of DENA and the technic for intratracheal instillation differed from that of Dontenwill et al. (4) They administered DENA without use of a solvent, by means of a hand atomizer introduced into the oral cavity. The dosage and method for the intragastric tube feeding in the present experiment were the same as those used by Dontenwill et al., but feeding was continued in our experiment for a period of 51–7 months instead of 1–3 months. Druckrey et al. (5, 7, 8) have shown that the localization of tumors produced with dialkylnitrosamines depends not only on the nature of the carcinogen but also on the dosage.

In our experiment, administration of DENA produced hepatocellular carcinomas, carcinomas of the ethmoturbinals, and lesions in the kidney, as well as tumors of the trachea and bronchus. We believe that the kidney lesions may represent an early stage in tumor development. In support of this viewpoint is the induction by DMN of kidney tumors in rats as reported by Zak et al. and Magee and Barnes (12, 18). Another possibility is that the lesions are metastases from hepatic carcinoma. However, their histologic appearance is not char-
acteristic of the primary tumors that arose in the liver or of their extrahepatic metastases.

We observed carcinoma in the ethmoturbinals of fourteen treated hamsters. Similar tumors have not been seen in the ethmoidal region of the nasal cavity of 200 hamsters previously examined. We have not found reports that carcinoma develops spontaneously at this site in the Syrian hamster. We have observed frequently, in untreated hamsters, pathologic changes in the nasal cavity that vary from mild inflammation to acute suppuration with necrosis and hemorrhage. Kelemen and Sargent (10) have described similar nasal findings in laboratory rats.

There is an overflow of instilled substances from the esophagus into the trachea of laboratory animals as has been demonstrated with India ink and radioactive isotopes (4). The possibility that this occurred must be kept in mind in attempting to explain the development of tumors in the trachea and bronchi of hamsters treated by the intragastric route. However, Don tenwill et al. (4) produced squamous-cell papillomas of the trachea in hamsters by repeated subcutaneous injections of DENA, and we have made a similar observation.1

The work of Druckrey and his colleagues (5, 6) has been considered as supporting a theory that the dialkyl nitrosamines are carcinogenic by virtue of their metabolic conversion to active alkylating agents, diazoalkanes. Recent biochemical studies suggest that the dialkyl nitrosamines may cause acute damage to the liver by methylation of proteins and nucleic acids (18, 14). Magee and Farber (13) demonstrated in rats the methylation by DMN of liver and kidney ribonucleic acid (RNA). Their observations suggest the possibility that an alteration in RNA may play a part in the induction of cancer by DMN. It is not known what are the capacities of the trachea, bronchi, lungs, and nasal cavity to metabolize the dialkyl nitrosamines. The theory has been proposed that a specific "dealkylizing" enzyme is not required for each tissue and that enzymatic oxidation in the alpha carbon atom is sufficient, with the result that the alkyl residue is quickly split off because the oxida-

tion products are chemically unstable, and the diazoalkanes are formed (5).

Argus et al. (2) have reported that the mechanism of carcinogenesis by the dialkynitrosamines is probably through protein denaturation. Our experiment suggests three possible mechanisms for the carcinogenic effect of DENA on the respiratory system, liver, and ethmoturbinals. These include: (a) a local action of DENA; (b) that the DENA or a metabolite present in the circulating blood is selectively deposited in various tissues where it is metabolized to a carcinogen; or (c) that a carcinogenic metabolite is excreted via the respiratory system.

The functions of the lung that have always been emphasized are those concerned with the physiology of respiration. According to Gilman2 the role of the lung in general metabolism is not generally appreciated, and diphosphopyridine nucleotide, for example, may be present in much greater concentration in the lung than in the liver. Additional evidence of the role of the lungs in general metabolism is that neutral fats pass first to the lacteals and then through the heart and lungs before they reach the general circulation. If DENA is administered to Syrian hamsters locally, orally, or subcutaneously, tumors are induced in the trachea, bronchi, and ethmoturbinals. These experiments suggest that the carcinogenic substance circulates in the blood and is selectively deposited or metabolized in certain tissues. Experiments are now in progress to attempt to determine whether DENA exerts a local carcinogenic effect and to investigate its mode of action at distant sites.

The increased incidence of lung cancer reported for man has been attributed to cigarette smoking and atmospheric pollution, thus stressing that the respiratory route plays a major role in carcinogenesis of the lung. The fact that DENA induces tumors of the respiratory tree and ethmoturbinals when administered to animals by intragastric feeding and induces tumors at the same sites when given subcutaneously poses the question whether or not carcinogens are carried to the human lung through other pathways than the respiratory tract.

1 Herrold and Dunham, unpublished data.

FIG. 1.—Trachea and bronchus of male hamster 8 months of age. Intratracheal instillation of DENA. Squamous-cell papillomas obstructing lumen. Hematoxylin and eosin (H. & E.), X39.

FIG. 2.—Trachea of female hamster 9 months of age. Intratracheal instillation of DENA. Poorly differentiated squamous-cell papilloma. H. & E., X56.

FIG. 3.—Bronchus of female hamster 9 months of age. Intratracheal instillation of DENA. Basal-cell hyperplasia and epithelial atypism. Note large cells with enlarged hyperchromatic nuclei. H. & E., X610.

FIG. 4.—Lung of male hamster 9 months of age. Intragastric feeding of DENA. Basal-cell hyperplasia and epithelial atypism. Note large cells with enlarged hyperchromatic nuclei. H. & E., X138.
FIG. 5.—Bronchiole. Serial section of lung shown in Figure 4. Atypical lining epithelium with extension of cells into adjacent alveoli indicates origin of nests of squamous epithelium from bronchiole. H. & E., X 610.

FIG. 6.—Liver of female hamster 10 months of age. Intragastric feeding of DENA. Trabecular carcinoma composed of sheets of neoplastic cells with large vesicular nuclei and prominent nucleoli. H. & E., X 100.

FIG. 7.—Diaphragm of female hamster 10 months of age. Intragastric feeding of DENA. Implants of hepatocellular carcinoma on surface of diaphragm. H. & E., X 125.

FIG. 8.—Lung. Metastases from hepatocellular carcinoma shown in Figure 6. H. & E., X 380.
FIG. 9.—Kidney of female hamster 9 months of age. Intragastric feeding of DENA. Nests of atypical epithelial cells with distinct basophilic cytoplasm and vesicular nuclei. H. & E., X290.

FIG. 10.—Kidney of female hamster 9½ months of age. Intragastric feeding of DENA. Cystic lesion in cortex lined with atypical epithelial cells and papillary excrescences. H. & E., X380.

FIG. 11.—Frontal section, ethmoid region of male hamster 10 months of age. Intragastric feeding of DENA. Undifferentiated carcinoma involving the ethmoturbinal. The neoplastic cells are small with hyperchromatic nuclei and scanty cytoplasm. H. & E., X84.

FIG. 12.—Frontal section of skull of male hamster 12 months of age. Intratracheal instillation of DENA. Extension into olfactory space of carcinoma that arose in ethmoturbinal. Arrow points to cluster of tumor cells in bone marrow. H. & E., X50.
REFERENCES

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