Failure to Transmit Diethylnitrosamine Tumorigenicity from Transplacentally Exposed F₁ Generation Syrian Hamsters to the Respiratory Tract of F₂ and F₃ Generations

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ABSTRACT

A multigeneration study with four successive generations of Syrian hamsters was conducted to determine whether a single s.c. injection of different doses of diethylnitrosamine (DEN) (1.25, 2.5, 5, 10, and 20 mg/kg body weight) on day 15 of pregnancy induces respiratory tract tumors not only in the treated P generation mothers and their F₁ progeny but also in F₂ and F₃ generations. In this study, the P generation mothers only were given a single injection of DEN during the period of gestation. Fifty-six % of the 36 DEN-treated mothers and 52% of their F₁ generation offspring (total, 233 animals) developed neoplasms in the respiratory tract. A single respiratory tract tumor was found in one DEN-unexposed F₁ generation control hamster as well as in one F₂ generation animal (total, 209 animals) descended from DEN-exposed P generation. Both tumors were considered to have arisen spontaneously. No respiratory tract tumors were observed in the F₂ generation (total, 160 animals) descended from a DEN-exposed P generation. Thus our results indicate that the vertical transmission of the tumorigenic effect of DEN in Syrian hamsters is limited to one generation and does not persist in the F₂ and F₃ generations.

INTRODUCTION

Transplacental induction of tumors by DEN² has been reported in the first generation of Syrian hamsters (1, 2), Djungarian hamsters (3), mice (4–6), and rats (7, 8). In the Syrian hamster, the respiratory tract proved to be the main target organ for DEN tumorigenesis, regardless of the route of administration of the carcinogen (9). However, only animals whose mothers had received DEN on one of the last 4 days (12–15) of pregnancy developed neoplasms in the respiratory tract (10). It is only at this late stage of pregnancy that the respiratory tract mucosa has attained the capacity to enzymatically activate the indirect acting DEN to its ultimate carcinogen (11, 12).

A particular aspect of transplacental carcinogenesis is that in experiments using certain animal species and certain types of carcinogens the carcinogenic risk can be observed not only in the F₁ generation but also in subsequent Fₒ and F₃ generations. Such experiments include those carried out in mice and rats with 3-methylcholanthrene (13), DMBA (14–17), o-aminoazobenzene; ENU, ethyl nitrosourea; TBA, tumor-bearing animals.

RESULTS

Fig. 1 shows the mating schedule and the group-based and total effective number of males and females in both the control and experimental hamsters over 4 generations. While each of the 6 F₁ groups (total, 273 hamsters) was derived from 6–10 P generation mothers (total, 42 females), the 7 F₂ (total, 251 animals) and 7 F₃ groups (total, 178 animals) were delivered by only 5–6 mothers per group of F₁ and F₂ mothers, respectively. Since most deliveries occurred during the night, the preweaning mortality through miscarriage, cannibalism, and perinatal death was especially high in the F₃ generation. After autopsy, all organs were fixed in 10% buffered formalin, the skulls were decalcified with Decal (Scientific Products, Evanston, IL), and all tissues were embedded in Paraplast. Sections (5 μm thick) were stained routinely with hematoxylin and eosin and, if necessary, also with special stains. Step sections were prepared from the nasal cavities, larynx, trachea with stem bronchi, and lungs. To determine statistical significance, Fisher’s one-sided exact probability test was used. Calculations were based on the number of hamsters histologically examined (= effective number of animals), since some animals were lost due to cannibalism.

MATERIALS AND METHODS

Thirty-six female 12-week-old Syrian hamsters (TNO, Zeist, The Netherlands) were mated and divided into 5 treatment groups consisting of 6–10 animals each (Fig. 1). The day of mating was counted as day 0. On day 15 of gestation, all animals (P generation) received a single s.c. injection of 0.2 ml of physiological saline in which 1.25, 2.5, 5.0, 10.0, and 20.0 mg of DEN (provided by Professor Preussmann, German Cancer Research Center, Heidelberg, Federal Republic of Germany; purity, 98%), respectively, had been dissolved. A group of 6 hamsters treated with the solvent alone on day 15 of gestation served as the vehicle control. After delivery, the F₁ generation was raised. Because of the limitations on space and the costs of keeping progeny over 3 successive generations, the sister-brother mating of F₁ generation was carried out only for two representative DEN doses, i.e., the intermediate (5-mg) and the highest (20-mg) dose. In order to check the possibility of any sex-associated transmission of carcinogenic risk to the F₂ generation, F₁ animals exposed in utero to the two representative DEN doses were cross-mated with the control F₂ animals of either sex. The F₃ generation was obtained by brother-sister mating of F₂ litters (Fig. 1) only. The hamsters were mated when they were 12 weeks old.

All animals of the P, F₁, F₂, and F₃ generations were housed individually in Makrolon cages, type III (E. Becker and Co., Castrop-Rauxel, Federal Republic of Germany), under standard laboratory conditions (room temperature, 22 ± 2°C; relative humidity, 55 ± 5%; air change, 8 times/h) and were given a pelleted diet (RMH-TMB; Hope Farms, Woerden, The Netherlands) and tap water ad libitum. They were checked twice daily, weighed at weekly intervals, and either kept until spontaneous death or killed when moribund. After autopsy, all organs were fixed in 10% buffered formalin, the skulls were decalcified with Decal (Scientific Products, Evanston, IL), and all tissues were embedded in Paraplast. Sections (5 μm thick) were stained routinely with hematoxylin and eosin and, if necessary, also with special stains. Step sections were prepared from the nasal cavities, larynx, trachea with stem bronchi, and lungs. To determine statistical significance, Fisher’s one-sided exact probability test was used. Calculations were based on the number of hamsters histologically examined (= effective number of animals), since some animals were lost due to cannibalism.

The present study was undertaken to investigate whether similar vertical transmission of tumorigenicity over multiple successive generations also occurs in Syrian hamsters when treated with DEN.

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1 To whom requests for reprints should be addressed.
2 The abbreviations used are: DEN, diethylnitrosamine; DMBA, 7,12-dimethylnitrosamethylnitrosourea; ENU, ethyl nitrosoureas; TBA, tumor-bearing animals.
offsprings where some entire litters were cannibalized by their mothers. Fertility rates in terms of the total number of animals at weaning showed no significant differences between controls and treatment groups of each generation or between animal groups of different generations.

Data on survival times and tumor incidences are summarized in Table 1. Although the percentage of TBA, the overall tumor incidence, and the tumor multiplicity (number of tumors per hamster) of the DEN-treated P mothers and their F₁ progeny were markedly higher than in the untreated controls, the life span was apparently little affected. The average survival time of DEN-treated P mothers was 77 weeks as compared to 81 weeks in the untreated controls (TBA, 72.2 versus 50%). The number of tumors per hamster was nearly twice as high in the DEN-treated P mothers and their F₁ progeny as in the controls. No significant differences were observed between the percentages of TBA, the overall tumor incidence, and the tumor multiplicity of F₂ and F₃ descendants from DEN-treated mothers and from untreated control animals.

The overall incidences of respiratory tract tumors induced by DEN at 1.25 to 20 mg/kg body weight were 55.5% in the treated P mothers and 47.5% in their male and 56.6% in their female F₁ descendants. A single respiratory tract tumor was found in one F₁ control animal (papilloma of the larynx) and in one F₂ descendant of a DEN-treated mother (adenocarcinoma of the nasal mucosa). No respiratory tract tumors could be detected in any of the F₃ animals.

Table 2 shows the incidence, type, and organ distribution of respiratory tract tumors in the DEN-exposed P and F₁ generations. In both generations, the main target organ for DEN tumorigenesis was the trachea. This was followed in decreasing order of incidence by the larynx, lungs, and nasal cavities in the F₁ generation and by the larynx, nasal cavities, and lungs in the P generation. A positive dose-response relationship was evident except in the highest dose group of the F₁ generation (Fig. 2). At as little as 10 and 20 mg/kg body weight fetal respiratory tissues appeared to be less responsive to DEN tumorigenicity than the adult tissues. At a much higher dose (45 mg/kg body weight) this difference still remained consistent (10).

Histologically, the observed benign tumors were papillomas and adenomas of the nasal cavities, papillomas of the larynx and trachea, and adenomas of the lung. At ages ranging from 3 to 8 months these benign tumors became clinically apparent due to sonorous rales caused by obstruction of the airways. Additionally, 1 adenocarcinoma of the nasal cavities, 2 adenocarcinomas, and 1 primary fibrosarcoma of the lungs were observed in F₂ generation.

The incidence of various tumors of other locations (Table 1) showed large fluctuations between the treatment and control groups of one and of different generations (range, 25–63.2%). However, these differences were statistically insignificant and none of the tumors could be causally related to DEN treatment. Furthermore, there was no obvious correlation between the occurrence of tumors at any sites in the P, F₁, and F₂ generations and their respective progeny either in the control animals or in the treatment groups.

**DISCUSSION**

Our results confirm previous studies (1, 4) that the administration of DEN to pregnant Syrian hamsters at the late stage

**Table 1** Animal numbers, survival times, tumor multiplicity, and tumor incidences in DEN-exposed and control Syrian hamsters of 4 generations

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex</th>
<th>Total no./ effective no. of animals</th>
<th>Av. survival time (wk ± SD)</th>
<th>TBA</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEN</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>F</td>
<td>36/36</td>
<td>77 ± 1</td>
<td>26</td>
<td>72.2</td>
<td>44 (3) 1.22</td>
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<tr>
<td>F₁</td>
<td>M</td>
<td>121/120</td>
<td>90 ± 15</td>
<td>77</td>
<td>64.2</td>
<td>121 (13) 1.01</td>
</tr>
<tr>
<td>F₁</td>
<td>F</td>
<td>115/113</td>
<td>76 ± 15</td>
<td>86</td>
<td>76.1</td>
<td>132 (22) 1.18</td>
</tr>
<tr>
<td>F₂</td>
<td>M</td>
<td>104/104</td>
<td>97 ± 21</td>
<td>51</td>
<td>49.0</td>
<td>68 (27) 0.65</td>
</tr>
<tr>
<td>F₂</td>
<td>F</td>
<td>105/105</td>
<td>84 ± 15</td>
<td>43</td>
<td>41.0</td>
<td>55 (22) 0.52</td>
</tr>
<tr>
<td>F₃</td>
<td>M</td>
<td>80/79</td>
<td>99 ± 18</td>
<td>40</td>
<td>50.6</td>
<td>45 (11) 0.57</td>
</tr>
<tr>
<td>F₃</td>
<td>F</td>
<td>86/81</td>
<td>84 ± 18</td>
<td>26</td>
<td>32.1</td>
<td>30 (7) 0.37</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>F</td>
<td>6/6</td>
<td>81 ± 14</td>
<td>3</td>
<td>50.0</td>
<td>4 (1) 0.67</td>
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<tr>
<td>F₁</td>
<td>M</td>
<td>21/21</td>
<td>83 ± 27</td>
<td>7</td>
<td>33.3</td>
<td>10 (0) 0.48</td>
</tr>
<tr>
<td>F₁</td>
<td>F</td>
<td>19/19</td>
<td>83 ± 14</td>
<td>11</td>
<td>57.9</td>
<td>15 (6) 0.79</td>
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<tr>
<td>F₂</td>
<td>M</td>
<td>25/25</td>
<td>97 ± 11</td>
<td>12</td>
<td>48.0</td>
<td>17 (11) 0.68</td>
</tr>
<tr>
<td>F₂</td>
<td>F</td>
<td>17/17</td>
<td>93 ± 12</td>
<td>7</td>
<td>41.2</td>
<td>8 (4) 0.47</td>
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<tr>
<td>F₃</td>
<td>M</td>
<td>5/5</td>
<td>99 ± 19</td>
<td>1</td>
<td>25.0</td>
<td>1 (1) 0.25</td>
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<tr>
<td>F₃</td>
<td>F</td>
<td>14/14</td>
<td>90 ± 13</td>
<td>5</td>
<td>35.7</td>
<td>5 (3) 0.63</td>
</tr>
</tbody>
</table>

* At time of weaning.

* Malignant tumors in parentheses.

* Animals of which both parents were descendants of DEN-exposed mothers and animals of which only one parent (male or female) was descendant of a DEN-exposed mother were grouped together.
MULTIGENERATION TUMORIGINICITY OF DEN

Table 2 Incidence, type, and organ distribution of respiratory tract tumors in the DEN-exposed P and F1 generations

<table>
<thead>
<tr>
<th>Generation (sex)</th>
<th>Organ</th>
<th>Tumor</th>
<th>Treatment group</th>
<th>No.</th>
<th>%</th>
<th>No.</th>
<th>%</th>
<th>No.</th>
<th>%</th>
<th>No.</th>
<th>%</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>P (F) Nasal</td>
<td>Papilloma</td>
<td>Den 1.25</td>
<td>1 (10)</td>
<td>10.0</td>
<td>1 (8)</td>
<td>12.5</td>
<td></td>
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<tr>
<td>cavities</td>
<td>Adenoma</td>
<td>Den 2.5</td>
<td></td>
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<tr>
<td>Larynx</td>
<td>Papilloma (ta)</td>
<td>Den 5</td>
<td>1 (6)</td>
<td>16.7</td>
<td>4 (6)</td>
<td>66.7</td>
<td></td>
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</tr>
<tr>
<td>Trachea</td>
<td>Papilloma (ta)</td>
<td>Den 10</td>
<td>6 (6)</td>
<td>100.0</td>
<td>6 (6)</td>
<td>100.0</td>
<td></td>
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<tr>
<td>Lungs</td>
<td>Adenoma</td>
<td>Den 20</td>
<td>1 (6)</td>
<td>16.7</td>
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<tr>
<td>F1 (M, F) Nasal</td>
<td>Adenoma</td>
<td>Control</td>
<td>1 (41)</td>
<td>2.4</td>
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<tr>
<td>cavities</td>
<td>Adenocarcinoma</td>
<td>Den 1.25</td>
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<tr>
<td>Larynx</td>
<td>Papilloma (ta)</td>
<td>Den 2.5</td>
<td>1 (35)</td>
<td>2.9</td>
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<td>Trachea</td>
<td>Papilloma (ta)</td>
<td>Den 5</td>
<td>17 (35)</td>
<td>43.1</td>
<td>22 (51)</td>
<td>43.1</td>
<td></td>
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<tr>
<td>Lungs</td>
<td>Adenoma (ta)</td>
<td>Den 10</td>
<td>3 (35)</td>
<td>8.6</td>
<td>5 (34)</td>
<td>14.7</td>
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<td>Adenocarcinoma</td>
<td>Fibrosarcoma</td>
<td>Den 20</td>
<td>1 (34)</td>
<td>2.9</td>
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</table>

* Dose of DEN given in mg/kg body weight.
* Effective number of animals in parentheses.
* \( p < 0.05. 
* \( p < 0.001. 
* \( p < 0.01. 

Fig. 2. Relationship between DEN doses and percentage ratios of the number of animals with at least one respiratory tract tumor to the total effective number of animals. The difference between P and F1 is not statistically significant. b.w., body weight.

of pregnancy results in a dose-related high incidence of tumors of the respiratory tract, the latter being the main target for DEN tumorigenesis in both mothers and their F1 offspring. This organotropism of DEN in Syrian hamsters has been long established (1, 4, 9). It is evident from the present study that DEN treatment of pregnant P hamsters did not produce respiratory tract tumors in their F2 and F3 descendants. The adenocarcinoma of the nasal cavities, which was found in one F2 hamster, and the laryngeal papilloma in one F1 control animal are most likely to be spontaneous tumors, although, as has been reported by other authors (27), the spontaneous incidence of these tumors should usually be very low (<2%). The incidence and spectrum of tumors of other locations, including liver and kidney tumors which might have been initiated by DEN treatments (28), showed neither statistically significant differences between control and treatment groups, each including P, F1, F2, and F3 generations, nor evidence for a causal relationship to DEN treatment. Thus the respiratory tract tumorigenesis in the Syrian hamsters is the direct result of DEN carcinogenicity and not merely the enhancement of spontaneous tumor incidence.

Our results do not conform to those of the majority of multigeneration carcinogenicity studies which have shown elevated incidences of neoplasms not only in the F1 but also in the F2 or even F3 generations (13-15, 17, 19-22, 24-26, 29). There are contradictory results for the carcinogens ENU and DMBA. Whereas Tomatis et al. (21) reported an increased incidence of neurogenic tumors in the F1 and F2 offspring of ENU-treated pregnant rats, Ogawa et al. (23) observed no difference in the incidence of preneoplastic hepatic lesions in the F2 and F3 progeny derived from F1 rats exposed to ENU in utero. In contrast to the reports of Tomatis (14), Tomatis and Goodall (15), and Rao (17) who observed an increased tumor incidence in F1 and F2 descendants of mice treated with DMBA during pregnancy, Schneider (16) could confirm these data for the F1 but not for the F2 generation. Although the exact mechanisms of vertical tumor induction in F2 and F3 generations having no direct contact with carcinogens are still unclear, various hypotheses have been put forward to explain the phenomenon of multigeneration carcinogenicity (Refs. 15, 17, 19, 26 and 30; see also Ref. 31).

In the present study, no DEN-induced respiratory tract tumors of F2 and F3 Syrian hamsters were found, suggesting that there are additional factors (or mechanisms) to consider when interpreting the multigeneration transfer of carcinogenicity.

One of the above-mentioned hypotheses, the enhancement of an inherited susceptibility (15), could explain the induction of lung tumors in F2 and F3 Swiss or ICR mice or of lymphomas in F2 and F3 MA mice; these strains of mice are all genetically predisposed to develop the respective tumors (15, 24). This genetic disposition to specific tumor types implies a ready response to exogenously induced light perturbation of heritable materials. By contrast, as regards the induction of hyperplastic (preneoplastic) hepatic nodules by ENU in F1 generation F-344 rats (23) and, in this study, papillary respiratory tract tumors caused by the organotropism of the administered carcinogens and was not due to the acceleration of processes responsible for spontaneous tumorigenesis. Therefore, neither tumors nor preneoplastic changes could be induced in the target organs of F2 and F3 animals.

In order to verify these assumptions, additional experiments seem necessary with the use of various species and strains of animals with low and high spontaneous tumor incidences and diverse carcinogens.

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REFERENCES


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