EXPERIMENTAL PARENTERAL AND AEROSOL TRANSMISSION
OF ADENOVIRUS-12 IN HAMSTERS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By


The Ohio State University
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CHAPTER I

MINIMAL INTRAPULMONIC ONCOGENIC DOSE OF
ADENOVIRUS-12 IN NEWBORN HAMSTERS

Introduction

Oncogenesis in hamsters induced by adenovirus-12 is influenced by many factors including dose of virus (1,2), route of inoculation (3-5), and immunological competence (6-8) and sex (9) of the host. Injection of large doses of adenovirus-12 into immunologically incompetent newborn hamsters results in a high incidence of undifferentiated sarcomas containing intranuclear T-antigen but no progeny virus (2,10-13). The 50% tumor dose following subcutaneous injection of adenovirus-12 is $10^{5.90} \text{TCD}_{50}$ for females and $10^{6.27} \text{TCD}_{50}$ for males (1). The sex differences in incidence and rate of appearance of tumors are more marked at $10^{5.0} \text{TCD}_{50}$ than at the 50% tumor dose. Variations in tumor incidence related to route of injection are also more apparent at the lower virus doses (3).

The minimal oncogenic dose of adenovirus-12 in newborn hamsters is not known. Hamsters inoculated by the intrapulmonic route of injection are more sensitive than those inoculated by the subcutaneous route to oncogenesis from low doses of adenovirus-12 (3). The lowest intrapulmonic dose previously reported to have induced tumors was approximately $10^{3.7} \text{TCD}_{50}$ (2). Recently, one of ninety-eight newborn hamsters
exposed to an aerosol dose of $10^{2.1}$ TCD$_{50}$ developed a tumor (4).

Injection of adenovirus-12 into newborn hamsters also induces specific adenovirus-12 T-antibody (13,14). Initially T-antibody was detected only by complement-fixation in tumor bearing animals and was quantitatively related to the size (antigenic mass) and duration of the viral induced neoplasm. Subsequently adenovirus-12 T-antibody was demonstrated in hamsters after regression of their tumors as well as in nontumor-bearing hamsters (1). The T-antibody response of adult hamsters after inoculation as newborns and its relationship to viral oncogenesis and tumor incidence following injection of low doses of adenovirus-12 has not been reported.

The objectives of this study were (1) to determine the minimal oncogenic dose of adenovirus-12 in newborn female and male hamsters following intrapulmonic inoculation and (2) to correlate the appearance of adenovirus-12 specific T-antibody with tumor incidence following injection of low doses of virus.

**Materials and Methods**

*Virus inoculum.* - The passage history and production methods used to propagate the adenovirus-12 have been reported previously (4). All the virus used here was produced in one suspension of KB cells. After harvesting, the infected cell suspension was frozen and thawed three times and sonicated. Duplicate samples titered twice averaged $10^{7.90}$ TCD$_{50}$/ml. The virus pool was free of mycoplasma (15). Appropriate dilutions of the original virus pool were prepared in maintenance medium (4) to provide $10^6, 10^5, 10^4.7, 10^4.0, 10^3.7, 10^3.0,$ and $10^2$
TCD50/0.05 ml of inoculum. Aliquots of each dilution were reassayed.

**Cell cultures.** - KB cell suspension cultures\(^1\) were used for virus production (16). Secondary monolayer cultures of human embryonic kidney (HEK) cells grown in Leighton tubes were used to assay adenovirus-12. The cultures were originally seeded in 100 ml bottles from frozen primary cultures of HEK cells\(^2\) and subsequently transferred to Leighton tubes. Methods have previously been reported for the growth and maintenance of the cultures (4,17).

**Infectivity assays.** - Duplicate tube dilutions (5 tubes/dilution) in secondary monolayer cultures of HEK cells were employed for the infectivity assays. The methods used to inoculate and maintain the cultures have been reported (4). The 50% tissue-culture infectious dose (TCD\(_{50}\)) was calculated according to the method of Reed and Muench twenty-one days after inoculation (18).

**Hamsters.** - Litters of random-bred, newborn Syrian hamsters were caged individually. After one week of age all hamsters were observed daily and palpated twice weekly until death or termination of the experiment at 180 days. At three weeks of age animals were weaned and segregated according to sex. Serum was collected monthly from ten per cent of the hamsters in each virus dilution group and at six months from all nontumor-bearing hamsters for detection of adenovirus-12 T-antibody. All animals

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\(^1\)The KB cells were supplied by Dr. Maurice Green, University of St. Louis, Missouri.

\(^2\)The adenovirus-12 and HEK cells were obtained from the Special Virus Cancer Program, National Cancer Institute, Bethesda, Maryland.
were necropsied and tissues collected in 10% formalin for histopathology or frozen in liquid nitrogen for immunofluorescence.

**Intrapulmonic injections.** - Eight groups of newborn hamsters (470 hamsters) were inoculated intrapulmonically with 0.05 ml of the appropriate dilution of virus or control suspension within 24 hours after birth. The virus was inoculated into the center of the right diaphragmatic lobe which was easily visualized through the thin thoracic wall. The accuracy of the inoculation technique had been previously evaluated by injecting a water soluble dye (Wright's stain) intrapulmonically and observing the distribution of the dye.

**Lung assay.** - Immediately after inoculation, five hamsters from each group were necropsied. The five lungs were removed and washed in 5 ml of Hank's balanced salt solution (HBS) to remove surface virus. The lungs were weighed, homogenized in cell culture medium to a 10% suspension, and frozen at -90°C. Lung suspensions and pleural washings from each virus dilution group were titrated to compare the inoculated dose with the amount of recoverable infectious virus. Control hamsters received 0.05 ml of non-infected tissue culture suspension from the same subpassage of KB cells. Control lung samples were collected and assayed as described above.

**Gross and microscopic examination.** - Complete necropsies were performed on all dead hamsters. Representative sections of all the organs were fixed in 10% phosphate-buffered formalin. The tissues were embedded in paraffin, sectioned at 6 μ, and stained with hematoxy-
lin and eosin. Macroscopic lesions, multiple sections of lungs from nontumor-bearing hamsters, and representative sections of the other organs were examined microscopically.

**Immunofluorescence.** - Tumor tissue was frozen in liquid nitrogen and stored at -90°C. Cryostat sections of the tumors were fixed for 10 minutes in cold acetone. They were stained for 1 hour at 37°C with fluorescein isothiocyanate-conjugated adenovirus-12 T-antibody^3^, diluted 1:5 in PBS, pH 7.4. The cells were then washed three times in PBS, mounted in buffered glycerol, and examined for T-antigen by ultraviolet microscopy using UG-12 excitor and 530-mu barrier filters.

Adenovirus-12 T-antibody was demonstrated by indirect immunofluorescence. Hamster sera diluted 1:3 in phosphate-buffered saline containing 5% bovine albumin plus 1 mg/ml of rhodamine-labeled bovine albumin was the primary reagent. The secondary reagent was fluorescein conjugated anti-hamster gamma-globulin rabbit antiserum^4^ adsorbed with hamster adenovirus-12 tumor cells. The indicator cells were KB monolayer cultures infected with adenovirus-12 at a multiplicity of twenty-five and harvested 18 hours later. Fixation and staining have been previously reported (1,4). The same filter system was used as for examination of the direct stains.

**Results**

**Comparison of virus dose, sex, and tumor incidence.** - The incidence of tumors in both female and male random-bred hamsters was greatly in-

^3^Flow Laboratories, Rockville, Maryland.

fluenced by the amount of virus injected into the lung (Table 1). Intrapulmonic injection of $10^{6.2} \text{TCD}_{50}$ induced tumors in a significantly higher per cent (76 per cent) of the female hamsters than males (45 per cent) ($P = < 0.05$). Similar significant differences resulted following injection of $10^{5.0} \text{TCD}_{50}$ when 53 per cent of the females and 16 per cent of the males developed tumors ($P = < 0.05$). The differences following injection of $10^{4.7} \text{TCD}_{50}$ were less significant with 22 per cent and 14 per cent tumor incidence in females and males, respectively ($P = < 0.05$). Injection of $10^{4.0} \text{TCD}_{50}$ resulted in a higher incidence of tumors in males (15 per cent) than females (9 per cent). The difference, however, was not statistically significant. The one tumor detected in hamsters inoculated intrapulmonically with $10^{3.7} \text{TCD}_{50}$ of adenovirus-12 occurred in a female. Figure 1 graphically illustrates the statistically significant and non-significant relationships between the log-10 \text{TCD}_{50} of adenovirus-12 injected intrapulmonically into newborn hamsters and the per cent tumor incidence in females and males.

**Tumor latent period in females and males.** - Tumor formation and resultant death of the hamsters were detected earlier in females than males at each virus dilution. Tumors developed between 28 and 119 days post-inoculation (p.i.d.) in female hamsters injected with $10^{6.2} \text{TCD}_{50}$ and retaining $10^{5.2} \text{TCD}_{50}$ (Fig. 2). The mean latent period for females in this group was 57 days. Males retaining $10^{5.2} \text{TCD}_{50}$ of virus developed tumors between 49 and 167 p.i.d. with a mean latent period of 81 days. The latent period increased in both males and females as the virus dose injected and recovered from the lung decreased. Tumors killed female
Table 1. - Relationship between low intrapulmonic doses of adenovirus-12 and tumor incidence in female and male Syrian hamsters.

<table>
<thead>
<tr>
<th>Lung Dose</th>
<th>Inoculated</th>
<th>Recovered</th>
<th>Proportion and (%) of hamsters with tumors</th>
<th>Probability by $\chi^2$ (Females vs. Males)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Females</td>
<td>Males</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>$10^6$.2</td>
<td>19/25 (76)</td>
<td>10/22 (45)</td>
<td>29/47 (61.8)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>$10^5.0$</td>
<td>18/34 (53)</td>
<td>6/37 (16)</td>
<td>24/71 (33.9)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>$10^4.7$</td>
<td>7/32 (22)</td>
<td>4/28 (14)</td>
<td>11/60 (18.4)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>$10^4.0$</td>
<td>2/23 (9)</td>
<td>4/26 (15)</td>
<td>6/49 (12.2)</td>
<td>-</td>
</tr>
<tr>
<td>$10^3.7$</td>
<td>1/37 (3)</td>
<td>0/33 -</td>
<td>1/70 (1.4)</td>
<td>-</td>
</tr>
<tr>
<td>$10^2.8$</td>
<td>0/26 -</td>
<td>0/27 -</td>
<td>0/53 -</td>
<td>-</td>
</tr>
<tr>
<td>$10^1.8$</td>
<td>0/22 -</td>
<td>0/28 -</td>
<td>0/50 -</td>
<td>-</td>
</tr>
<tr>
<td>0</td>
<td>0/37 -</td>
<td>0/33 -</td>
<td>0/70 -</td>
<td>-</td>
</tr>
</tbody>
</table>

$^a$TCD$_{50}$/lung (HEK) immediately after intrapulmonic injection in newborn hamsters.

$^b$Hamsters were observed for six months.
Fig. 1. The relationship between the log₁₀ TCID₅₀ of adenovirus-12 injected intrapulmonically into newborn hamsters and frequency of tumors in female and male hamsters.
Fig. 1

PERCENT HAMSTERS DYING WITH TUMORS

- FEMALES
- MALES

TCD50 OF ADENO VIRUS-12 INJECTED PULMONICALLY

10^35 10^40 10^45 10^50 10^55 10^60
Fig. 2. Tumor incidence and rate of tumor appearance in female and male Syrian hamsters injected intrapulmonically at birth with different doses of adenovirus-12.
hamsters given $10^{5.0}$ TCD$_{50}$ (retained $10^{4.7}$ TCD$_{50}$) between 43 and 154 p.i.d. The mean latent period was 80 days. Males receiving the same dose of virus died between 42 and 173 p.i.d. and the mean latent period was 111 days. At lower virus doses less difference was observed between sexes and mean latent periods. Female and male hamsters injected with $10^{4.7}$ TCD$_{50}$ and retaining $10^{4.1}$ TCD$_{50}$ in the lung developed tumors between 40 and 121 and 70 and 101 days, respectively. The mean latent periods were 83 and 89 days. A lower dose recovered from hamster lungs ($10^{3.7}$ TCD$_{50}$) induced tumors in females between 64 and 130 days and in males between 45 and 146 days. The mean latent periods were 97 and 92 days, respectively. A total of 59, 42, 18, and 17 per cent of the hamsters developing tumors died during the first 60 p.i.d. in the groups retaining $10^{5.2}$, $10^{4.7}$, $10^{4.1}$, and $10^{3.7}$ TCD$_{50}$, respectively. In the same virus groups 38, 42, and 73 per cent of the hamsters died with tumors between 61 and 120 p.i.d. and 4, 17, and 9 per cent between 121 and 180 p.i.d.

**Recovered lung dose.** - There was a linear relationship between virus inoculated and recovered (Table 2). Total virus recovery in the seven groups varied between $10^{0.3}$ - $10^{1.0}$ TCD$_{50}$ less than the virus inoculated. The mean and median recovery rates were 20 per cent. The efficiency of recovery of virus at the lower dilutions compared favorably with those at higher dilutions. No virus was recovered from pleural washings nor from lungs of control hamsters.

**Gross and histologic appearance of the tumors.** - Sixty-three of the seventy-one (85%) adenovirus-12 induced tumors originated from the
Table 2 - Recovery of adenovirus-12 from newborn hamster lungs immediately after intrapulmonic injection.

<table>
<thead>
<tr>
<th>Virus Inoculated</th>
<th>Virus Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^{6.2a}$</td>
<td>$10^{5.2b}$</td>
</tr>
<tr>
<td>$10^{5.0}$</td>
<td>$10^{4.5}$</td>
</tr>
<tr>
<td>$10^{4.7}$</td>
<td>$10^{4.1}$</td>
</tr>
<tr>
<td>$10^{4.0}$</td>
<td>$10^{3.7}$</td>
</tr>
<tr>
<td>$10^{3.7}$</td>
<td>$10^{3.0}$</td>
</tr>
<tr>
<td>$10^{2.8}$</td>
<td>$10^{2.0}$</td>
</tr>
<tr>
<td>$10^{1.8}$</td>
<td>$10^{0.9}$</td>
</tr>
<tr>
<td>Media Control</td>
<td>0</td>
</tr>
</tbody>
</table>

$^a$TCD$_{50}$/0.05 ml titered in tube dilutions of secondary human embryonic kidney monolayer cultures.

$^b$TCD$_{50}$/lung. Five lungs were pooled and assayed and then the mean TCD$_{50}$/lung calculated.
right diaphragmatic lobe of the lung. Only eight of the seventy-one tumor-bearing hamsters had no pulmonary tumors. Six of these tumors were located subcutaneously at the inoculation site, one was confined to the posterior mediastinum and the base of the heart, and the other non-pulmonary tumor filled the abdominal cavity and incorporated most of the visceral tissues. Nine of the sixty-three lung tumors also extended into the left diaphragmatic lobe and eighteen demonstrated varying degrees of attachment to the thoracic wall or diaphragm. Intrathoracic hemorrhage was frequently observed at necropsy and was the most common immediate cause of acute death. No correlations were noted between the size or location of tumors and dose of virus.

Eleven tumors (16%) occurred in the liver, four (6%) subcutaneously and one each attached to the mesentary, spleen, stomach, and kidney. These were similar grossly to the lung tumors which were also present in each of these hamsters.

The lung tumors characteristically replaced the right and compressed the left lung (Fig. 3). Grossly they appeared as large, nodular but usually solitary, white neoplasms. Cut sections of the tumors revealed homogeneous white masses of viable neoplastic cells. Microscopically the tumors appeared similar to the undifferentiated sarcomas reported by others following inoculation of adenovirus-12 by different routes (3,12). The viable areas of the tumors were composed of dense sheets of large cells with oval vesicular nuclei and granular lightly eosinophilic cytoplasm. Interspersed between the foci were areas of hemorrhage and necrosis. Subcutaneous tumors were similar except slightly larger and had larger zones of necrosis, suppurative inflamma-
Fig. 3. Dorsal surface of the lungs from a 107 day old female Syrian hamster injected at birth in the right diaphragmatic lobe with $10^{5.0}$ TCD$_{50}$ of adenovirus-12. The solitary, nodular, undifferentiated sarcoma has replaced the right lung (top) and the margin (arrows) of the neoplasm is compressing the left lung.
T-antibody response. — There was a direct relationship between the adenovirus-12 T-antibody response and tumor incidence in the five hamsters (approximately 10%) randomly selected for monthly bleeding (Table 3). Results from this sample suggested that a nononcogenic dose of adenovirus-12 might result in cellular transformation and induction of T-antigen synthesis without the formation of detectable tumors. Since $10^{3.0}$ TCD$_{50}$ was the lowest dose that induced a tumor, the remaining nontumor-bearing hamsters in the four closely associated virus dilutions were bled six months after inoculation. No T-antibody was detected in the nontumor-bearing animals that had retained $10^{2.0}$ TCD$_{50}$ of adenovirus-12. The one tumor-bearing hamster and forty-three per cent of the nontumor-bearing hamsters retaining $10^{3.0}$ TCD$_{50}$, however, had a detectable T-antibody response. Sixty-three per cent of the nontumor-bearing hamsters injected with $10^{3.7}$ TCD$_{50}$ had T-antibody in their sera. T-antibody could still be detected in previously T-antibody positive animals nine months after exposure.

Discussion

The studies, employing lower doses of adenovirus-12 and a different route of injection, have extended and confirmed the previous observations that oncogenesis by adenovirus-12 is dose dependent (1) and a greater proportion of female than male hamsters develop tumors (6,7,9). There was a significant difference between the incidence of
Table 3 - Incidence of adenovirus-12 T-antibody in hamsters inoculated with different doses of virus.

<table>
<thead>
<tr>
<th>Virus Inoculated</th>
<th>60 p.i.d.</th>
<th>180 p.i.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^6.2$</td>
<td>5/5</td>
<td></td>
</tr>
<tr>
<td>$10^5.0$</td>
<td>5/5</td>
<td></td>
</tr>
<tr>
<td>$10^4.7$</td>
<td>3/5</td>
<td>30/48</td>
</tr>
<tr>
<td>$10^4.0$</td>
<td>2/5</td>
<td>27/42</td>
</tr>
<tr>
<td>$10^3.7$</td>
<td>1/5</td>
<td>28/65</td>
</tr>
<tr>
<td>$10^2.8$</td>
<td>1/5</td>
<td>0/41</td>
</tr>
<tr>
<td>$10^1.8$</td>
<td>0/5</td>
<td></td>
</tr>
</tbody>
</table>

The 60 p.i.d. samples were from five hamsters randomly selected from each virus dilution group. The 180 p.i.d. samples represent sera collected from the nontumor-bearing hamsters at the end of the experiment.

Sera not tested.
tumors in female and male hamsters following injection of $10^{6.2}$ or $10^{5.0}$ TCD$_{50}$ of adenovirus-12. Tumors were present in 31 and 37 per cent more females than males, respectively. Injection of lower doses, $10^{4.7}$ or $10^{4.0}$ TCD$_{50}$ of virus, produced less significant differences in incidence of tumors between females and males.

The incidence of tumors following intrapulmonic injection of $10^{6.2}$ TCD$_{50}$ was similar to that observed following subcutaneous injection of $10^{6.3}$ TCD$_{50}$ of adenovirus-12. Intrapulmonic injection of $10^{6.2}$ TCD$_{50}$ induced tumors in 76 per cent of the females and 45% of the males. Subcutaneous injection of $10^{6.3}$ TCD$_{50}$ of virus induced tumors in 70 per cent of the females and 51 per cent of the males (1). Therefore, at these doses, the route of injection did not appear to markedly influence the incidence of tumors. Contrary to previous reports (2,3), at lower doses the intrapulmonic route appears to be only slightly more sensitive than the subcutaneous route of injection. After intrapulmonic injections of $10^{5.0}$ TCD$_{50}$ of adenovirus-12, 44 and 13.5 per cent of the females and males developed tumors within 120 days, respectively, while 31 and 12.5 per cent developed tumors within 120 days after subcutaneous inoculation. Comparison of these two studies was restricted because the intrapulmonic route doesn't permit evaluation of tumor regression and because secondary rather than primary human embryonic kidney cultures were used to assay for viral infectivity. Assays on secondary HEK cells are reproducible (19) but slightly less sensitive than primary cultures.

Tumor formation and resultant deaths were detected earlier in females than males injected intrapulmonically with $10^{3.0}$ to $10^{6.2}$ TCD$_{50}$
of adenovirus-12. The death rates were highest between 40 to 80 days after inoculation. Sex differences regarding rate of appearance of tumors were most apparent in hamsters inoculated with $10^6.2$ and $10^5.0 \text{TCD}_{50}$ of adenovirus-12. Previous studies using doses between $10^5.0$ and $10^7.0 \text{TCD}_{50}$ (primary HEK cultures) showed a more marked difference with virus doses of $10^6.0 \text{TCD}_{50}$ or less, than with higher doses (1). Assuming the response follows the $S$-shaped normal dose response curves, it is not surprising that the differences between tumor incidence in males and females was most apparent at medium virus dosages.

The uniformity and efficiency of adenovirus-12 recovery from lungs immediately after intrapulmonic inoculation was examined. Assay of lung suspensions collected from animals immediately after inoculation showed a mean loss of $10^{0.7} \text{TCD}_{50}$ of inoculated virus. The accuracy of the inoculation technique was supported by preliminary dye retention studies, absence of detectable virus in pleural washings, and high incidence of tumors involving the right diaphragmatic lobe of the lung. There was no detectable direct inhibition of viral infectivity by suspensions of normal lung parenchyma. The observation that a small amount of inoculum often leaks from the nasal and oral cavities, via the bronchi and trachea, following intrapulmonic inoculation of hamsters (3) suggests that loss of virus may be higher after intrapulmonic inoculation than following other routes such as subcutaneous or intraperitoneal. Neither the fate of the remaining virus nor all the factors that might influence recovery were determined.
Comparison of the inoculated and recovered lung doses strengthen the observation that only a small amount of virus is actually required to induce cellular alterations in vivo and viral oncogenesis or formation of T-antibody in the hamsters (1). These studies also permit a comparison of the direct intrapulmonic and aerosol routes of exposure (4). In studies characterizing the aerosol route of exposure the virus recovery from lungs could be measured but not the exact aerosolized dose.

The neoplasms induced by intrapulmonic injection of adenovirus-12 were undifferentiated sarcomas similar to those described by others (3,12,20,21). The target cell(s) for adenovirus-12 oncogenesis is not known but the route of injection does not appear to influence the histologic appearance of the tumors. In this study animals were observed until moribund or dead and therefore the pathogenesis of the lesions was not followed. The resulting tumors had extensively infiltrated the lung and other involved tissues. Neither the histologic appearance of the neoplasms nor relationships with normal tissues indicated any origin from neuroectoderm (22) or lymphoid tissues (23). The liver and subcutaneous neoplasms also appeared as solid undifferentiated sarcomas with sheets of densely packed oval cells but no adenocarcinomatous pattern (24).

The appearance of 89 per cent of the tumors in the right diaphragmatic lobe supports the previous observation that neoplasms induced by adenovirus-12 usually appear at the site of inoculation. Other sites of involvement originated as local extensions or infiltrations of the pulmonary tumors. These sites included thoracic wall, diaphragm,
peritoneal cavity (especially the liver), and the subcutaneous site of injection.

Not all the non-pulmonary tumors, however, originated by local extension from primary tumors in the lungs. Eight animals had only non-pulmonary tumors. Six were located subcutaneously at the inoculation site. The mediastinal and peritoneal tumors probably represent errors in inoculation. A high incidence of adenovirus-12 induced tumors occurs in the liver (11,23). Following intravenously injection of virus the highest incidence of tumors occurs in the liver (2,5). The highest incidence (15%) of non-pulmonary tumors in our study also occurred in the liver. Five of the eleven hepatic tumors did not originate by local extension from the lungs.

Adenovirus-12 T-antibody appeared in a high percentage of non-tumor-bearing hamsters. It was detected by indirect immunofluorescence in 62, 63, and 43 per cent of the nontumor-bearing hamsters 180 days after they had been injected intrapulmonically with $10^{4.7}$, $10^{4.0}$, or $10^{3.7}$ TCD$_{50}$ of virus, respectively. In previous studies, T-antibody had been detected in 50% of the sera tested from nontumor-bearing hamsters 120 days following injection of high doses ($10^{6.0} - 10^{6.3}$ TCD$_{50}$) of virus at birth (1). Detectable levels of T-antibody were not present in animals injected intrapulmonically with $10^{2.8}$ TCD$_{50}$ and retaining $10^{2.0}$ TCD$_{50}$. This was unexpected since, following aerosol exposure, pooled sera from hamsters retaining $10^{2.1}$ TCD$_{50}$ of adenovirus-12 contained T-antibody up to 18 months after exposure (4).
The only known source of T-antigen in the hamster is transformed neoplastic cells. Therefore, these results suggest that T-antigen is synthesized in most hamsters following injection of a minimal dose of adenovirus-12. Furthermore, the minimal detectable immunogenic dose appears to be similar to the minimal oncogenic dose. Neither tumors nor T-antibody could be detected in hamsters six months after injection with $10^{2.8} \text{TCD}_{50}$ of virus. T-antibody, however, was present in many nontumor-bearing hamsters following injection of $10^{3.7} \text{TCD}_{50}$ or more of virus. The numerous host factors that influence the eventual oncogenic and immunogenic response could easily account for this observation (1,4). Some of the nontumor-bearing hamsters may have had undetectable regressing intrapulmonary tumors. The initial presence of undetected slowly proliferating tumor cells which were rejected immunologically could have provided the initial immunogenic stimulus. Subsequent studies showed that tumor cells were probably rejected early and not continually proliferating in the hamsters. Nontumor-bearing hamsters, injected intrapulmonically at birth with between $10^{6.2}$ - $10^{3.7} \text{TCD}_{50}$ of adenovirus-12 and containing T-antibody in their sera, were suppressed immunologically with 2.5 mg cortisone per week (7). No tumors developed in these 273 hamsters during a 90 day observation period.

**Summary**

Oncogenesis by adenovirus-12 is significantly influenced by dose of virus and sex but not route of inoculation. Intrapulmonic injection of $10^{6.2}$ or $10^{5.0} \text{TCD}_{50}$ of adenovirus-12 resulted in significant
differences (*p* = 0.05) in the incidence of tumors in female (76%) and male (45%) Syrian hamsters. Similar significant differences resulted following injection of $10^{5.0} \text{TCD}_{50}$ when 53% of the females and 16% of the males developed tumors. Injection of $10^{4.0} \text{TCD}_{50}$ induced tumors in 15% of the males and 9% of the females. Intrapulmonic injection of $10^{3.7} \text{TCD}_{50}$ killed only one of 70 inoculated hamsters. Tumors were detected earlier in female than male hamsters at each dose level. The mean latent periods were significantly different in female and male hamsters injected with $10^{6.2}$ or $10^{5.0} \text{TCD}_{50}$, but not $10^{4.7}$ or $10^{4.0} \text{TCD}_{50}$.

Assays of lung suspensions from hamsters injected intrapulmonically with adenovirus-12 were reproducible but only 20% of the virus originally inoculated was recovered. Eighty-nine per cent of the tumors originated at the inoculation site in the right diaphragmatic lobe.

The adenovirus-12 T-antibody response was dose dependent. T-antibody was present in 43% of the sera tested from nontumor-bearing hamsters injected with $10^{3.0} \text{TCD}_{50}$ of adenovirus-12, while hamsters injected with $10^{2.0} \text{TCD}_{50}$ of virus developed no detectable T-antibody.
CHAPTER II

EFFECT OF RELATIVE HUMIDITY ON DYNAMIC AEROSOLS OF ADENOVIRUS-12

Introduction

Previous studies of the factors affecting survival of airborne bacteria (1,2) and viruses (3-7) have established that the effect of relative humidity is an important and unpredictable factor. Aerosols of influenza, vaccinia, Venezuelan equine encephalomyelitis viruses (3), parainfluenza (4), and Semliki Forest viruses (5), are more stable at a low relative humidity. Yaba (6), Rous sarcoma (7) and polioviruses (3) are more stable at a high relative humidity.

During studies on the transmission of oncogenic adenovirus-12 to newborn hamsters (8) it was necessary to evaluate the effects of relative humidity on dynamic aerosolization of the virus. The only reported relationship between adenovirus aerosols and relative humidity were obtained using a static aerosol chamber. Adenoviruses 4 and 7 are more stable in static chamber aerosols at 80% relative humidity than at 50 or 20% (4).

The objectives of this study were (1) to determine the effects of relative humidity on the recovery of infectious adenovirus-12 after dynamic aerosolization in a Henderson apparatus and (2) to determine the relationship between relative humidity of the aerosol and recovery
of infectious virus from newborn Syrian hamster lungs.

Materials and Methods

Hamsters. - Sixty-five newborn, random-bred, Syrian hamsters (Mesocricetus auratus) were obtained from our closed hamster colony. Twelve to eighteen hours after birth the hamsters were placed in nylon mesh exposure cups suspended in the center of a 12.5 x 15.0 x 47.5 cm stainless steel exposure box of the Henderson apparatus (9). At 0, 10, 30, and 60 minutes following exposure for 20 minutes to an aerosol of adenovirus-12, groups of five animals were euthanatized and lungs collected for assay of virus infectivity.

Virus inoculum. - The same stock of adenovirus-12 served for all aerosol exposures. The methods for production, concentration, and assay of this stock have been previously reported (8). It was produced and assayed on monolayer cultures of human embryonic kidney cells. Before being aerosolized the virus suspension was frozen and thawed 3 times at -90°C and 37°C respectively and sonicated for 3 minutes at 60 cps in a Raytheon sonicator. The total protein concentration of the virus suspension was 900 mg/100 ml (10). Immediately prior to aerosolization 1% antifoam was added to the virus suspension. Preliminary experiments showed that the addition of 1% antifoam to spray or impinger bottles had no measurable effect on viral infectivity.

1Antifoam A, Dow Corning Co., Midland, Michigan.
Aerosol exposure. - The three aerosols of adenovirus-12 were generated from the same Collison atomizer of a Henderson apparatus (9). The virus was aerosolized into the animal exposure chamber for 20 minutes at the rate of 0.305 or 0.310 ml/minute and air dilutions of 12.5 or 28.4 liters/minute (Table 4). At 0-1, 10-15, and 19-20 minutes during each exposure, 12.5 liters/minute of air leaving the exposure chamber were diverted through low point all glass impingers (AGI) containing 20 ml of Hank's balanced salt solution (HBSS), and 1% antifoam. The temperature during each exposure remained constant at 28 ± 0° C or 29.5 ± 0.5° C (Table 4). Adenovirus-12 is highly stable at these temperatures. All work was conducted within a freon-tight biological safety cabinet system.

Control of relative humidity. - The medium relative humidity was produced in the aerosol chamber by aerosolizing 0.31 ml virus suspension in 28.4 liters of air per minute (Table 4). Relative humidity, measured by a psychrometer interposed in the exhaust air stream, varied between 49-53% during the twenty-minute exposure period. The temperature of the air leaving the exposure chamber varied between 29-30° C.

For the high relative humidity experiment, the relative humidity of the make-up air was increased by reducing the air flow to 12.5 liters per minute and sparging the air through a water-soaked natural sponge placed in the bottom of a 3 liter flask. The relative humidity varied between 85-93% during the last 17 minutes of the exposure after an initial 3 minute adjustment period of 72 ± 13% required because of the decreased air flow. The temperature of the exhaust air remained constant at 28° C.
Table 4. - Exposure data on aerosolization of adenovirus-12 in a Henderson apparatus at different relative humidities

<table>
<thead>
<tr>
<th>% Relative humidity</th>
<th>Aerosol temperature °C</th>
<th>Aerosolization rate ml/min</th>
<th>Air dilution factor 1./min.</th>
<th>Collison bottle titer&lt;sup&gt;a&lt;/sup&gt; 0 min.</th>
<th>Collison bottle titer&lt;sup&gt;a&lt;/sup&gt; 20 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>89 ± 4</td>
<td>28 ± 0</td>
<td>0.305</td>
<td>12.5</td>
<td>10&lt;sup&gt;7.2&lt;/sup&gt; ± 0.2</td>
<td>-</td>
</tr>
<tr>
<td>51 ± 2</td>
<td>29.5 ± 0.5</td>
<td>0.310</td>
<td>28.4</td>
<td>10&lt;sup&gt;7.2&lt;/sup&gt; ± 0.2</td>
<td>10&lt;sup&gt;7.78&lt;/sup&gt; ± 0.08</td>
</tr>
<tr>
<td>32 ± 1</td>
<td>29.5 ± 0.5</td>
<td>0.305</td>
<td>28.4</td>
<td>10&lt;sup&gt;7.2&lt;/sup&gt; ± 0.2</td>
<td>10&lt;sup&gt;7.2&lt;/sup&gt; ± 1.6</td>
</tr>
</tbody>
</table>

<sup>a</sup>TCD<sub>50</sub>/ml as titered in human embryonic kidney cells.
Low relative humidity was produced by initially cooling then warming 20.5 liters per minute of make-up air. Moisture was removed from the make-up air by passing the air through cold copper tubing immersed in an acetone-dry ice bath and allowing water to freeze on the inner surface of the tubing. The air then was warmed by passing it through a similar copper coil and a 70° water bath. The relative humidity of warmed make-up air was as low as 10%. After mixing with the 8 liters/minute of air flowing through the spray bottle, the total flow of 28.5 liters/minute varied in relative humidity between 31-35% and in temperature between 29-30° C.

Assay procedures. - Spray and impinger bottle samples were assayed in secondary monolayer cultures of human embryonic kidney cells grown in Leighton tubes (8,11). Ten-fold dilutions of the virus samples were made in Hank's balanced salt solution (HBSS). Confluent monolayer cultures grown in Leighton tubes were washed once with phosphate-buffered saline (PBS) at pH 7.2, inoculated, and incubated 1 hour at 37° C. Maintenance medium was then added and changed every fourth day. Cultures were examined for the characteristic adenovirus cytopathic effect (CPE). The 50% tissue culture dose (TCD₅₀) was calculated according to the method of Reed and Muench twenty-one days after inoculation (12).

The TCD₅₀ of virus retained per hamster lung represents the mean amount of infectious virus retained in five hamster lungs collected and pooled 10 minutes after exposure. This time was used because preliminary exposures at the medium relative humidity resulted in similar titers at 10, 30, and 60 minutes post-exposure of 10².16, 10¹.97 and
10^2.10 TCD50, respectively. The hearts and large pulmonary and aortic vessels were removed and the surfaces of the lungs washed with HBSS. Each group of five lungs was then pooled, weighed, made up to a 20% suspension in cell culture medium without serum, and frozen at -90°C until titrated. Lungs from hamsters aerosolized with uninfected cell culture suspensions at medium relative humidity served as controls.

Table 5 illustrates the calculations used for determining the total virus aerosolized, total and per cent virus recovery from aerosols and lungs, theoretical maximum inhaled dose, and TCD50/ml of aerosol.

**Results**

**Virus recovery from aerosols.** - There was marked variation in recovery of infectious adenovirus-12 when it was aerosolized in a Henderson apparatus at different levels of relative humidity (Fig. 4). Maximum virus survival occurred at a high relative humidity. At 89 ± 4% relative humidity 10^5.4 TCD50/minute or a total of 10^6.7 TCD50 of adenovirus-12 was recovered from the 20-minute dynamic aerosol exposure (Fig. 4). Only 20% (10^6.0 TCD50) as much infectious virus was present in aerosols at 51 ± 2% relative humidity and 0.4% (10^4.3 TCD50) at 32 ± 1% relative humidity (Fig. 4). The total virus recovery however, was relatively low at each relative humidity (Table 6).

**Virus recovery from lungs.** - The total adenovirus-12 retained in the lung also varied after 20-minute aerosol exposures at the three different levels of relative humidity (Fig. 4). The highest pulmonary reten-
Table 5. - Calculation of virus aerosolized and recovered from aerosols and lungs.

Total virus aerosolized = 

\[ \text{spray bottle titer} \times \text{volume of virus aerosolized} \]

Total virus recovery from aerosols\(^2\) = 

\[ \frac{\text{AGI titer} \times \text{AGI diluent volume}}{\frac{\text{length of exposure}}{\text{length of AGI collection}}} \]

Per cent virus recovery from aerosols = 

\[ \frac{\text{total virus recovery from aerosol}}{\text{total virus aerosolized}} \times 100 \]

Per cent virus recovery from lungs = 

\[ \frac{\text{total virus recovery from lungs}}{\text{theoretical maximum inhaled dose}} \times 100 \]

Theoretical maximum inhaled dose = 

\[ \frac{\text{TCD}_{50}/\text{ml of aerosol} \times \text{minute respiratory volume of hamster (13)}}{\text{length of exposure}} \]

\[ \text{TCD}_{50}/\text{ml aerosol} = \frac{\text{total virus aerosolized}}{\text{total air dilution}} \]

\(^2\)Impinger titers were multiplied by 2.28 in the medium and low relative humidity exposures because only 12.5 liters/minute of the total 28.5 liters/minute were diverted through the impinger.
Figure 4. - Effect of relative humidity on the recovery of adenovirus-12 from dynamic aerosols and newborn hamster lungs.
Fig. 4

RELATIVE HUMIDITY
89±4 %
51±2 %
32±1 %
tion of adenovirus-12 occurred in newborn hamsters exposed to the high relative humidity aerosols. At 89 ± 4% relative humidity there was a total recovered lung dose of $10^{3.9}$ TCD$_{50}$. Only 25% as much virus was recovered from lungs exposed to medium relative humidity ($10^{2.4}$ TCD$_{50}$) and 1% after the low relative humidity exposure ($10^{1.0}$ TCD$_{50}$). Since there was blood in the pulmonary vessels, blood was assayed separately but virus could not be re-isolated. No virus was detected in the washings from the pleural surface. The parallel assay of lungs from control littermate hamsters produced no cytopathic effects in the human embryonic kidney cultures.

The per cent recovery of infectious virus from the lungs after aerosol exposure was greater in the high than medium or low relative humidity experiments (Table 6). The theoretical maximum inhaled doses were $10^{3.9}$ TCD$_{50}$ at high relative humidity and $10^{3.5}$ TCD$_{50}$ at medium and low relative humidities. At high relative humidity 12.6 per cent of the theoretical maximum inhaled dose was recovered. Only 8.0 and 0.32 per cent were recovered after the medium and low relative humidity exposures, respectively.

The maximum inhaled dose was also calculated from the total virus recovery in the aerosols rather than total virus aerosolized. This comparison of the virus recovery from aerosols and lungs revealed that the measured lung dose at each relative humidity was higher than the calculated inhaled dose. The latter were $10^{2.6}$ TCD$_{50}$, $10^{1.6}$ TCD$_{50}$, and $10^{-1.4}$ TCD$_{50}$, respectively, at the high, medium, and low relative humidities.
Table 6. - Relationships between relative humidity and recovery of adenovirus-12 from dynamic aerosols and newborn hamster lungs.

<table>
<thead>
<tr>
<th>Relative humidity</th>
<th>Total virus aerosolized TCD&lt;sub&gt;50&lt;/sub&gt;</th>
<th>Virus Recovery from aerosols TCD&lt;sub&gt;50&lt;/sub&gt; %</th>
<th>Virus Recovery from lungs TCD&lt;sub&gt;50&lt;/sub&gt; %</th>
</tr>
</thead>
<tbody>
<tr>
<td>89 ± 4</td>
<td>10&lt;sup&gt;8.0&lt;/sup&gt;</td>
<td>10&lt;sup&gt;6.7&lt;/sup&gt; 4.6</td>
<td>10&lt;sup&gt;3.0&lt;/sup&gt; 12.6</td>
</tr>
<tr>
<td>51 ± 2</td>
<td>10&lt;sup&gt;8.0&lt;/sup&gt;</td>
<td>10&lt;sup&gt;6.0&lt;/sup&gt; 1.05</td>
<td>10&lt;sup&gt;2.4&lt;/sup&gt; 8.0</td>
</tr>
<tr>
<td>32 ± 1</td>
<td>10&lt;sup&gt;8.0&lt;/sup&gt;</td>
<td>10&lt;sup&gt;4.3&lt;/sup&gt; 0.02</td>
<td>10&lt;sup&gt;1.0&lt;/sup&gt; 0.32</td>
</tr>
</tbody>
</table>
Discussion

The relative humidity of dynamic aerosols is an important factor in the recovery of adenovirus-12 from dynamic aerosols. The most infectious virus was recovered from high humidity aerosols while only 20.0% and 0.4% as much virus was present in the medium and low relative humidity aerosols. Similar results have been reported for static aerosols of adenovirus 4 and 7. Miller et al. (4) observed a much lower rate of infectivity loss at 80% than at 50 or 20% relative humidity. Only 3.3% as much adenovirus-7 was present in aerosols at 20% or 50% relative humidity as at 80% relative humidity. Furthermore, a major effect of relative humidity on adenovirus 7 was observed at the first sampling period (5 minutes). Therefore, the effect of relative humidity on adenoviruses in both dynamic and static aerosols appears to be rapid.

Although relative humidity influenced the recovery of infectious virus from dynamic aerosols, all three exposures still resulted in a relatively low recovery of infectious virus. Similar losses across the Henderson exposure chamber occurred following aerosolization of Yaba (6) and Rauscher murine leukemia virus (14). Many factors such as sonification, electrostatic precipitation, physical decay, and impingement could have influenced the survival of the virus. The limited studies on survival of airborne viruses have shown factors such as extraneous protein concentration, salt concentration (5), and the suspension medium (7) markedly affect survival of airborne viruses. These factors remained constant during our experiments and were not evaluated individually.
The primary effect of relative humidity on recovery of virus from the lungs was an indirect one. There was less available viable virus recovered from aerosols at the lower relative humidities. Therefore, it was not surprising to observe a decreased total detectable lung dose of virus in the animals exposed at the low relative humidities. Comparison of virus recovery from aerosols and lungs suggests a good correlation between the two observations although a slightly higher percentage of the available virus was retained at the higher relative humidities.

Interpretation of differences between calculated and measured lung doses was restricted because of the inability to measure the hamster’s small respiratory volume. Based on Guyton’s formula (13) a two gram newborn hamster has a minute respiratory volume of 1 ml/minute. All the measured lung doses (Table 6) were between the calculated doses based on aerosolized and impinged virus. Based on total aerosolized virus the calculated inhaled doses were $10^{3.9}$, $10^{3.5}$, and $10^{3.5}$ TCD$_{50}$ at high, medium, and low relative humidities. When the total virus titers recovered from aerosols (Table 6) were used the inhaled doses were $10^{2.6}$, $10^{1.6}$, and $10^{-1.4}$ TCD$_{50}$. Correlation between these doses is further restricted since neither the percent retention of the inhaled dose nor the relation between particle size and infectivity were determined.

**Summary**

Dynamic aerosols of adenovirus-12 were generated in a Henderson apparatus under conditions of high, medium, and low relative humidity.
Relative humidity influences the recovery of adenovirus-12 from aerosols and lungs of newborn Syrian hamsters. At 89, 51, and 32% relative humidity the total infectious virus recovered from a 20-minute aerosol exposure was $10^6.7$, $10^6.0$, and $10^4.3$ TCID$_{50}$, respectively. Hamsters exposed to these aerosols retained a measured lung dose of $10^3.0$, $10^2.4$, and $10^1.0$ TCID$_{50}$, respectively. The measured retained lung doses are compared to calculated inhaled lung doses based on both total virus aerosolized and total virus recovery from the aerosols.
CHAPTER III

EXPERIMENTAL AEROSOL TRANSMISSION OF
ADENOVIRUS-12 TO HAMSTERS

Introduction

The airborne route is a common mode of transmission for many viral diseases. Recently representatives of both DNA and RNA oncogenic viruses have been transmitted by the aerosol route. Yaba virus (1), Rauscher murine leukemia (2), and avian leukemia (3,4) have been transmitted by aerosols to monkeys, mice, and chickens, respectively. Each of these viruses replicates intracytoplasmically and produces progeny virus in the cells of the exposed host. Many other oncogenic viruses, when injected parenterally, induce tumors without viral replication. The risk associated with exposure of man and animals to aerosols of these non-replicating oncogenic viruses has not been evaluated.

Adenovirus-12 is an intranuclear DNA oncogenic virus that induces undifferentiated sarcomas but no progeny virus when parenterally inoculated into a susceptible host (5-7). The presence of viral specific T-antigens in the transformed cells and T-antibody provide a positive relationship between the tumors and virus (8-11). Although the specific target cell(s) for in vivo viral transformation is not known (12), pulmonary tumors do develop in hamsters after intravenous (12) or intrapulmonic (7) inoculation of adenovirus-12. Adequate in vitro methods
are available for production, concentration, purification, and assay of infectious adenovirus-12 (13-15).

Adenoviruses can be aerosolized experimentally as demonstrated by the infection of human volunteers with adenovirus-4 (16). More recently Couch et al. (17) have reported the natural aerosol transmission of an adenovirus to man.

The aim of this study was to determine the susceptibility of newborn hamsters to infection and tumor formation from inhalation of dynamic aerosols of adenovirus-12. The objectives were (1) to compare the measured and calculated doses recovered from aerosols and lungs; (2) to determine the fate of an aerosolized non-replicating DNA oncogenic virus by measuring recoverable infectious virus in the pulmonary, integumentary, gastrointestinal, and urinary systems; (3) to determine whether an immunologic response occurs in hamsters exposed to infectious viral aerosols as measured by formation of antibodies to adenovirus-12 T-antigen, and (4) to determine the incidence of tumors following aerosol exposure to adenovirus-12.

Materials and Methods

Host. - The random-bred Syrian hamster (Mesocricetus auratus) was selected because it is the species most susceptible to adenovirus-12 oncogenesis and has been used most frequently in previous parenteral studies. Within twenty-four hours after birth, 188 newborn hamsters were removed from their dams and exposed to an aerosol of suspensions of adenovirus-12. Following exposure 43 hamsters were euthanatized at various times for tissue sample collection and the remaining
140 hamsters were maintained in flexible plastic isolators. The latter hamsters were observed daily and palpated weekly until death or termination of the experiment at 18 months. Complete necropsies were performed on all moribund or dead animals.

**Cell culture.** - Initially human embryonic kidney (HEK) monolayer cell cultures were used for both virus production and assay (16). Frozen HEK cells were propagated in basal medium Eagle's in Hank's balanced salt solution (HBME) supplemented with 10% bovine serum, 1% glutamine (200 mM), 0.05% NaHCO₃, and 1.0% antibiotic stock (1 gm. streptomycin, 250,000 units penicillin and 115,000 units mycostatin per 100 ml). After virus inoculation the cultures were maintained in HBME supplemented with 2% gamma globulin-free calf serum, 1% glutamine, 0.1% NaHCO₃, and 1% antibiotics. Later, larger pools of virus were produced in suspension cultures of KB cells. The growth medium was minimum essential medium (MEM) suspension medium supplemented with 10% horse serum, 1% glutamine, 0.05% NaHCO₃, and 1% antibiotic stock. The growth medium for KB monolayer cultures used for indirect immunofluorescence was MEM supplemented with 10% bovine serum, 1% glutamine, 0.05% NaHCO₃, and 1% antibiotic stock. Following virus inoculation, both suspension

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1 HEK cells were obtained from The Special Virus Cancer Program, National Cancer Institute, Bethesda, Maryland.

2 The KB cells were supplied by Dr. Maurice Green, University of St. Louis, St. Louis, Missouri.
and monolayer cultures were supplemented with 3% gamma globulin-free bovine serum.

**Infectivity assay procedures.** - All viral infectivity assays were duplicate tube dilutions (5 tubes/dilution) in secondary monolayer cell cultures of HEK. The average standard deviation between duplicate samples was \(10^{0.2} + \text{TCD}_{50}\). Ten-fold dilutions of virus were made in HBSS. Confluent monolayer cultures grown in Leighton tubes were washed once with phosphate-buffered saline (PBS) at pH 7.2, inoculated, and incubated one hour at 37°C. Maintenance medium was then added and changed every fourth day. Cultures were examined for the characteristic adenovirus cytopathic effect (CPE). The 50% tissue-culture infectious dose (TCD_{50}) was calculated according to the method of Reed and Huench twenty-one days after inoculation (18).

**Virus production and concentration.** - Adenovirus-12 (Huie) stock was initially passaged in our laboratory in secondary HEK monolayer cultures (16). Later virus pools were prepared in suspension cultures of KB cells. Titers of \(10^{7.0} - 10^{7.5} \text{TCD}_{50}/\text{ml}\) were obtained in HEK monolayer cultures. Suspension cultures of KB cells yielded \(10^{7.5} - 10^{8.0} \text{TCD}_{50}/\text{ml}\). Virus pools were centrifuged at 600 X g for 10 minutes, resuspended in 10% of the original volume of maintenance medium, and stored at -90°C. The inoculum was further concentrated to \(10^{8.5}\)

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3 The adenovirus-12 stock was supplied by The National Cancer Institute, Bethesda, Maryland.
TCID_{50}/ml by ultracentrifugation at 20,000 rpm for 8 hours at 40 C
over a cushion of RbCl, 1.43 gm/ml. The RbCl was removed by dialysis
at 40 C against 0.01 M Tris buffer, pH 8.1 (15). Before being aerosol-
ized the virus pools were frozen and thawed three times and sonicated.
Virus samples were negative for mycoplasma following tests in both our
laboratory and Dr. Leonard Hayflick's (19).

Aerosol experiments. - The first two experiments were designed to
measure the relationships between theoretical and measured, aerosolized
and recovered virus doses. Newborn hamsters between 12-24 hours old
were exposed for 20 or 200-minutes to dynamic aerosols of adenovirus-
12 generated from the Collison atomizer of a Henderson apparatus (20,21).
The atomizer delivered 0.3 ml of virus inoculum per minute in experi-
ments one, two and three and 0.12 ml per minute in experiment four.
In the first three experiments the total air flow through the exposure
chamber was 28.5 liters/minute and the relative humidity of the aerosol
varied between 46-48%. Subsequent experiments established that a
high relative humidity favored the survival of adenovirus-12 in a dy-
namic aerosol (22). Therefore, the fourth group of hamsters was ex-
posed to an aerosol at a relative humidity varying between 85-96%. The
relative humidity was increased by reducing the total air flow to 12.5
liters/minute and sparging the air through a water-soaked sponge
placed in a flask. The exposure period was increased to 200 minutes.

The aerosols were sampled for 1-minute or 5-minute periods in
low point, all glass impingers (AGI) (20) containing 20 ml of HBSS
plus 1% antifoam and subsequently titered to determine the number of TCD$_{50}$/minute of aerosol. An average of 0.5 ml/minute of medium evaporated from the impinger during the sampling period. The AGI is over 90% efficient in entrapping particles less than 5 μm (23). Virus samples were also collected from the spray bottle at the beginning and end of each aerosol exposure. After exposure the animals were air-washed for approximately 30 seconds and then returned to their mothers.

For determining the particle size distribution of the polydisperse aerosols, adenovirus-12 was collected for seven minutes in an Anderson sampler (24) modified to a flow rate of 12.5 liters/minute. The collection plates were prepared with 21 ml hard agar base and an overlay of 6 ml of 5% gelatin and stored at 4°C until immediately before use. After sampling, the gelatin in the plates was liquefied at room temperature and harvested for virus assay. The relative humidity of the aerosol was 71-78 per cent during the 7 minute sampling period.

Control animals were exposed to a 200-minute aerosol exposure of uninoculated KB cell suspension (600,000 cells/ml.) prepared in the same manner as was the virus inoculum. Impinger, spray bottle, and tissue samples were collected and assayed as described above.

Measured virus dose. - At 0, 12, 24, and 48 hours after aerosol exposure the lungs, gastrointestinal contents, and urine were collected from five hamsters. The lungs from the five animals were individually

pooled, weighed, made up to a 10% suspension in cell culture medium, and frozen at -90° C until titration. The gastrointestinal contents from the five hamsters were collected the same way as the lungs. The urine was collected directly from the bladder. Residual virus on the epidermis was detected by repeatedly immersing the newborn hamster to the dorsal cervical region, in 10 ml of cell culture medium. Before titration each sample was frozen and thawed three times. Each TCD$_{50}$ is the mean of the tissues pooled from five hamsters. Tissues from aerosolized control hamsters were collected and assayed as described above.

**Data analysis.** - Table 7 lists the formulas used to calculate the total virus aerosolized, total and per cent virus recovery from aerosols and lungs, the theoretical maximum inhaled dose, and the TCD$_{50}$/ml in the aerosol.

**Histopathologic examination.** - Complete necropsies were performed on all dead or moribund hamsters. Representative sections of all organs were fixed in 10% phosphate-buffered formalin. The tissues were embedded in paraffin, sectioned at 6 u, and stained with hematoxylin and eosin. Macroscopic lesions, multiple sections from each lung, and representative sections of other organs were studied microscopically.

**Immunofluorescence.** - Tissue samples and serum for immunofluorescence were collected from two hamsters at 0, 1/2, 1, 2, 4, 8, 12, and 24 hours
Table 7. - Calculations of the virus aerosolized and recovered from aerosols and lungs

Total virus aerosolized =

spray bottle titer X volume of virus aerosolized

Total virus recovery from aerosols

AGI titer X AGI diluent volume
X length of exposure
length of AGI collection

Per cent virus recovery from aerosols =

\[
\frac{\text{total virus recovery from aerosol}}{\text{total virus aerosolized}} \times 100
\]

Per cent virus recovery from lungs =

\[
\frac{\text{total virus recovery from lungs}}{\text{theoretical maximum inhaled dose}} \times 100
\]

Theoretical maximum inhaled dose =

\[
\text{TCD}_{50}/\text{ml of aerosol} \times \text{minute respiratory volume of hamster (25)} \times \text{length of exposure.}
\]

\[
\text{TCD}_{50}/\text{ml aerosol} = \frac{\text{total virus aerosolized}}{\text{total air dilution}}
\]

Impinger titers were multiplied by 2.28 in the medium and low relative humidity exposures because only 12.5 liters/minute of the total 28.5 liters/minute were diverted through the Impinger.
and 2, 4, 7, 14, 21, and 28 days after aerosol exposure and frozen in liquid nitrogen. Cryostat sections of the tissues were fixed for 10 minutes in acetone. They were stained for 1 hour at 37° C with fluorescein isothiocyanate-conjugated adenovirus-12 T-antibody\(^5\) diluted 1:5 in PBS, pH 7.4. Following three washings in PBS they were examined with ultraviolet microscopy using an UG-12 excitor and a 530 μm barrier filter.

Adenovirus-12 T-antibody was demonstrated by indirect immunofluorescence. Hamster sera diluted 1:3 in PBS containing 5% bovine serum albumin plus 1 mg/ml of rhodamine-labeled bovine albumin was the primary reagent. The secondary reagent was fluorescein-conjugated, anti-hamster gamma-globulin, rabbit antiserum\(^6\) absorbed with hamster adenovirus-12 tumor cells in our laboratory. The indicator cells were KB monolayer cultures grown on 11 X 35 mm Leighton slips and harvested at 12-24 hours or KB cultures inhibited with 15 μg/ml of cytosine arabinocide 12 hours after infection and harvested at 36 hours post-inoculation. Fixation and staining techniques have previously been reported (11). The cells were examined by ultraviolet microscopy as described above.

**Results**

**Virus recovery from aerosols.** - Infectious adenovirus-12 was recovered from dynamic aerosols generated in a Henderson apparatus (Table 8). In

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\(^5\) Flow Laboratories, Rockville, Maryland.

\(^6\) Microbiological Associates, Inc., Bethesda, Maryland.
Table 8. - Recovery of infectious adenovirus-12 from dynamic aerosols generated in a Henderson apparatus

<table>
<thead>
<tr>
<th>Aerosol experiment</th>
<th>Total virus (^a) aerosolized (TCD(_{50}))</th>
<th>Impinger samples (^b) (TCD(_{50})/minute)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-1 minute 5-10 minutes 19-20 minutes</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>(10^8.9) (10^4.7) (10^4.2) (10^4.7)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>(10^8.7) (10^4.8) (10^4.2) (10^4.9)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Twenty-minute exposure period

\(^b\) TCD\(_{50}\)/minute of aerosol collected in low point all glass liquid impingers and assayed on secondary HEK cells
two parallel preliminary experiments a total of $10^{8.9}$ TCD$_{50}$ or $10^{8.7}$ TCD$_{50}$ were generated during a 20-minute exposure period by aerosolizing 0.3 ml/minute of virus titering $10^{8.1}$ or $10^{7.9}$ TCD$_{50}$/ml and 20.5 liters/minute of make-up air. Virus was recovered from AG1 samples taken at the beginning, middle, and end of each exposure. Recovery in the impingers from both experiments was uniform. A slight variation appeared between early or late one-minute AG1 samples and the five-minute AG1 samples collected midway through the exposure periods. From three to five times more virus was recovered per minute from the one-minute impingers. The mean virus recovery in experiments one and two was $10^{4.5}$ and $10^{4.6}$ TCD$_{50}$/minute, respectively. The total virus recovery during the 20-minute exposure was $10^{5.8}$ and $10^{5.9}$ TCD$_{50}$.

**Virus infectivity vs. aerosol particle size.** - The total virus aerosolized during the seven-minute collection period in the Anderson sampler was $10^{7.4}$ TCD$_{50}$. The total virus recovery from the seven-minute aerosol, as calculated from a one-minute AG1 sample, was $10^{6.9}$ TCD$_{50}$. Slightly more total virus ($10^{7.0}$ TCD$_{50}$) was collected in the six collection plates of the Anderson sampler (24) during the same period. Fifty per cent ($10^{6.7}$ TCD$_{50}$) of the total $10^{7.0}$ TCD$_{50}$ of adenovirus-12 collected was related to 1.0-1.5 u particles. Forty per cent ($10^{6.6}$ TCD$_{50}$) of the virus was associated with 0.5-1.0 u particles. All but 0.3% of the remaining virus was associated with particles larger than 1.5 u.
Virus recovery from lungs. — Adenovirus-12 was transmitted to newborn Syrian hamsters by the aerosol route. Newborn hamsters exposed in the Henderson apparatus to a total aerosol virus dose of $10^{8.8} \text{TCD}_{50}$ at 46-48 per cent relative humidity retained a measurable lung dose of $10^{2.1} \text{TCD}_{50}$ (Table 9). By (a) increasing the relative humidity of the aerosol (22) to 85-96 per cent, (b) increasing the total virus aerosolized 12.6 times by increasing the virus titer to $10^{8.5} \text{TCD}_{50}/\text{ml}$ and (c) extending the exposure period to 200 minutes, a 32-fold increase in the adenovirus-12 recovery from lungs resulted (Table 9). This occurred even though the calculated lung dose increased only 3.2 times because of a greater total air dilution in the 200-minute aerosol exposure.

Distribution of virus. — Infectious adenovirus-12 was reisolated from the lungs, epidermis, intestinal contents, and urine of newborn hamsters after aerosol exposure for 200 minutes to a total of $10^{8.9} \text{TCD}_{50}$ of virus (Table 10). A total of $10^{5.2} \text{TCD}_{50}$ or 2.5 per cent of the virus recovered from the aerosol was reisolated from the four tissues immediately after exposure. More virus was ingested than inhaled and retained in the lungs. $10^{5.1} \text{TCD}_{50}$ of the recovered virus was in the intestine, $10^{4.5} \text{TCD}_{50}$ in the epidermis, $10^{3.0} \text{TCD}_{50}$ in the lungs, and $10^{2.2} \text{TCD}_{50}$ in the urine. The virus could be recovered from urine only immediately after aerosol exposure. It persisted in the intestine for 12 hours and lung and epidermis for 24 hours post-exposure. Titers dropped rapidly in each tissue. The titer in the lungs at 24 hours
Table 9. - Aerosol transmission of adenovirus-12 to newborn hamsters

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Relative humidity (%)</th>
<th>No. hamsters</th>
<th>Total virus&lt;sup&gt;a&lt;/sup&gt; ( \text{TCD}_{50} )</th>
<th>Total air&lt;sup&gt;a&lt;/sup&gt; [ml]</th>
<th>Lung dose&lt;sup&gt;b&lt;/sup&gt; [TCID&lt;sub&gt;50&lt;/sub&gt;]</th>
<th>Calculated</th>
<th>Measured</th>
<th>No. tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>46-48</td>
<td>98</td>
<td>( 10^{8.8} )</td>
<td>( 5.7 \times 10^{5} )</td>
<td>( 10^{3.0} )</td>
<td>( 10^{2.1} )</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>85-96</td>
<td>42</td>
<td>( 10^{9.9} )</td>
<td>( 2.5 \times 10^{5} )</td>
<td>( 10^{3.5} )</td>
<td>( 10^{3.6} )</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> The length of exposure was 20 minutes

<sup>b</sup> Average of lungs pooled from five hamsters immediately after aerosolization, weighed, homogenized into a 10% suspension, further disrupted by freezing and thawing, and titered on HEK monolayer cultures.
Table 10. - Distribution of infectious adenovirus-12 in selected tissues of the newborn hamster following aerosol exposure$^a$

<table>
<thead>
<tr>
<th>Hours post-exposure</th>
<th>Lungs</th>
<th>Epidermis</th>
<th>Intestinal contents</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>$10^{3.0}$</td>
<td>$10^{4.5}$</td>
<td>$10^{5.1}$</td>
<td>$10^{2.2}$</td>
</tr>
<tr>
<td>12</td>
<td>$10^{2.1}$</td>
<td>$10^{3.1}$</td>
<td>$10^{4.1}$</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>$10^{1.5}$</td>
<td>$10^{2.0}$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>48</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

$^a$ TCD$_{50}$/hamster
was $10^{1.5}$ TCD$_{50}$ or 1.4% of the initial amount of recoverable virus ($10^{3.0}$ TCD$_{50}$). No detectable virus was present in the lungs, epidermis, intestinal contents, or urine from 48 hours to 3 weeks after exposure.

**T-antigen and T-antibody.** - None of the tissues examined from hamsters retaining $10^{3.0}$ TCD$_{50}$ of infectious virus contained detectable levels of T-antigen. Numerous transverse sections of each of the two hamsters collected at the 0 through 24 hour post-exposure sampling periods were directly stained and examined. Specific tissues examined in each animal included lung, liver, spleen, heart, intestine, and kidney. In addition, the lungs, liver, spleen, and kidneys from hamsters necropsied 7, 14, 21, and 28 days post-exposure were also examined. The sections or impression smears of adenovirus-12-induced tumor used as positive controls showed the characteristic intranuclear bundles of T-antigen.

Pooled serum samples collected monthly from five animals in the fourth aerosol transmission experiment (Table 9) were positive by indirect immunofluorescence for adenovirus-12 T-antibody between 3 and 12 months after exposure. Individual serum samples collected terminally from two hamsters at 1, 2, and 3 weeks post-exposure were negative. Ten pooled samples containing serum from 7-10 hamsters were harvested 18 months post-exposure and examined by indirect immunofluorescence. Four of the 10 pooled samples contained T-antibody.

**Tumor Incidence.** - Only one of the 140 hamsters developed an adenovirus-12-induced neoplasm (Table 9). The female hamster was moribund thirteen months after receiving a retained lung dose of $10^{2.1}$ TCD$_{50}$
of adenovirus-12. The primary tumor completely incorporated both apical and cardiac lobes and the anterior mediastinum to the thoracic inlet (Fig. 5). The mass was firmly attached to the ventral thoracic vertebrae (T1-T4) involving an area of 1.0 cm. The diaphragmatic lobes of the lung were unaffected. Sectioning the tumor revealed a white, homogeneous, moderately firm neoplasm with central focal areas of necrosis and hemorrhage involving sixty per cent of the mass. The neoplastic cells, secondary inflammatory reaction, necrosis, and hemorrhage had completely replaced the affected lobes of the lung.

Microscopically there was a dense sheet of neoplastic cells with scattered bundles of collagenous connective tissue (Fig. 6). The neoplastic cells had little cytoplasm and a prominent oval to round vesicular nucleus with clumped chromatin. Aggregates of inflammatory cells including lymphocytes, plasma cells, and neutrophils infiltrated the margin between necrotic and viable tumor tissue. The tumor cells contained the intranuclear adenovirus-12 specific T-antigen. Neoplastic cells were also present in the liver and right auxiliary lymph node. On the surface and cut sections of both tissues were numerous 1-2mm white foci of neoplastic cells. The entire liver was enlarged and swollen. The anterior and posterior poles of the right lateral lobe also contained large solitary tumor nodules measuring 1.5 cm and 1.2 cm, respectively (Fig. 7). On sectioning they were well delineated nodules composed of homogeneous white lobules of neoplastic cells. In contrast with pulmonary lesions the hepatic lesions did not contain prominent
Fig. 5. The lung from a 13 month old Syrian hamster that was exposed, as a newborn, to a dynamic aerosol of adenovirus-12. The tumor (T) has replaced the apical and middle lobes of the lung. On cut section (arrow) it appears as a dense, white, homogeneous neoplasm. The diaphragmatic lobes (D) of the lung were not involved.
Fig. 6. Photomicrograph of the lung tumor. The neoplastic cells have filled the respiratory spaces and replaced all but scattered strands of the interstitial stroma. The cells have a moderate amount of cytoplasm and discrete oval to round nuclei with clumped chromatin. Tumor cells with pyknotic nuclei and mononuclear inflammatory cells are also present.
Fig. 7. The liver from the 13-month-old Syrian hamster that was exposed, as a newborn, to a dynamic aerosol of adenovirus-12. The entire liver is enlarged. In addition, the anterior and posterior poles of the right lateral lobe contain large, solitary, lobulated tumor nodules measuring 1.5 cm and 1.2 cm, respectively (arrows).
areas of hemorrhage. The sinusoids were filled and hepatocytes replaced by a population of neoplastic cells similar to those in the lung (Fig. 8).

**Discussion**

It was demonstrated that oncogenic adenovirus-12 can be reproducibly aerosolized in a Henderson apparatus. The stability of aerosols of other adenoviruses had been investigated previously only statically in a rotating drum (26). Biological and physical decay rates were measured but not the effects of continuous dynamic aerosolization on virus recovery in aerosols or lungs.

Ninety per cent of the infectious virus in aerosols generated in the Henderson apparatus at 71-78% relative humidity was associated with 0.5 - 1.5 μ particles. This result is similar to previous dynamic aerosol studies using the Henderson apparatus (1). Since alveolar retention is maximal with particles measuring 0.5 - 2.0 μ, the viral aerosols generated should have been retained in lower respiratory spaces. Increasing the relative humidity increases the average particle size (22). Therefore, the relationship between particle size and infectivity can not be extrapolated directly to the other experiments because they were conducted at slightly different relative humidities.

The measured and calculated lung doses were different in each aerosol experiment. The dose variation was greater in the low relative humidity experiment where the measured lung dose was 10^0.9 TCD_{50} less than the calculated dose. When the relative humidity and exposure period were increased the measured dose was actually 10^0.1 TCD_{50}
Fig. 8. Photomicrograph of one of the large tumor nodules in the liver showing the margin (arrows) between hepatocytes (top) and the neoplastic cells which have filled the sinusoids and replaced the normal parenchymal cells. Pyknosis and karyorrhexis of the neoplastic cells were observed in the larger tumor nodules.
higher than the calculated dose. The calculated lung dose while serving as an approximate dose fails to account for such influences as the aerosol environment, per cent pulmonary retention, or direct influences from the pulmonary tissue on actual lung dose. Measured lung doses, although affected by factors such as the reproducibility of the assay system or substances in the lung, do provide a more accurate appraisal of the actual lung dose. Although in our experiments there was a uniform virus recovery from aerosols, a total of approximately $10^{3.0}$ TCD$_{50}$ was lost during aerosolization. Similar early but less marked losses ($10^{1.0}$ TCD$_{50}$) have been reported for adenovirus-4 with a cloud age of one hour (26). Subsequent studies showed that increasing the relative humidity decreased the loss (22). It was also shown that temperature or antifoam was not responsible for the loss.

The susceptibility of hamsters to dynamic adenovirus-12 aerosols was demonstrated by the induction of adenovirus-12 specific T-antibody and by the appearance of one undifferentiated sarcoma. Immediately after aerosol exposure infectious virus was reisolated from the lung, intestine, epidermis, and urine. The epidermal retention was expected because the newborn hamsters were placed directly within the exposure box. The virus present in the intestinal tract represented both the direct oral dose during the exposure period and that subsequently cleared from the lung and swallowed. Over 90% of the materials initially retained in the lung are subsequently cleared (27). Most clearance is via the respiratory tree rather than the lymphatic system and the majority of this cleared material is swallowed.
The marked decrease in titer of virus recovered at 12 and 24 hours and the lack of infectious virus after 48 hours post-exposure indicates that no replication occurred after aerosol exposure of hamsters. The virus persisted longer in the lung and intestine than has been reported following subcutaneous inoculation. Chino et al. (28) found that the inoculated virus penetrated into mesenchymal cells within 2 hours after inoculation. Since adenovirus virions go into an eclipse phase 60-90 minutes after penetration of the cell the persistent virus probably represents unadsorbed virions. Substances such as surfactant or mucus in the lung or intestinal contents might have inhibited cellular penetration.

Lung, liver, spleen, heart, intestine, and kidney were negative for adenovirus-12 T-antigen between 0 and 28 days post-exposure. Levinthal et al. (29) inoculated adenovirus-12 subcutaneously into newborn hamsters and then demonstrated T-antigen 6 hours later in the mesenchymal cells at the site of inoculation. Essentially 100 per cent of hamster cells growing in vitro produce T-antigen within twenty-four hours after inoculation (30). Chino et al. (28) showed T-antigen in mesenchymal cells at the inoculation site within twenty-four hours after virus inoculation. Lack of demonstrable T-antigen in the tissues may indicate a wide pattern of distribution following aerosol exposure restricting the usefulness of indirect immunofluorescence or a failure to examine the correct tissues. The presence of T-antibody in these same animals, indicates some cells underwent viral transformation and T-antigen production.
The presence of adenovirus-12 specific T-antibody in the serum between three and twelve months post-exposure indicates that the aerosolized virus did penetrate and transform a susceptible cell population following aerosolization into the newborn hamster. Absence of the T-antibody during the first three weeks post-exposure, the hamster's age and immunological response, and initial exposure of the disrupted virus inoculum to ultracentrifugation all suggest that soluble residual T-antigen in the partially purified inoculum was not responsible for the T-antibody response. The detection of adenovirus-12 T-antibody by immunofluorescence in nontumor-bearing hamsters has previously been reported. Yohn et al. (11) interpreted the presence of T-antibody to indicate that T-antigen is synthesized in most inoculated hamsters in amounts sufficient to induce an immunologic response. He detected T-antibodies in 50% of the sera tested from nontumor-bearing hamsters and in 97% of the sera tested from hamsters in which tumors regressed. The exposure of hamsters by the aerosol route restricted evaluation of tumor regression and its relationship to T-antibody response.

The presence of detectable levels of adenovirus-12 T-antibody at 18 months after aerosol exposure to low doses of adenovirus-12, was of interest. Subsequent studies indicated that T-antibody was induced and persisted for nine months in 43% of the hamsters retaining $10^{3.0} \text{TCD}_{50}$. None of the hamsters that retained $10^{2.0} \text{TCD}_{50}$ had a detectable T-antibody response. The reason for the persistence of the T-antibody was not determined. T-antibody is a 7 S immunoglobulin (31) and would be expected to have a half-life of approximately twenty days. This suggests that a single stimulus was not responsible for the persistence
of detectable amounts of antibody. Lack of information on the specific behavior of the T-antibody or the host target cell(s) following aerosolization of adenovirus-12, restricts further interpretation of the observations. The appearance of T-antibody does suggest that aerosolization of adenovirus-12 resulted in a host cell-virus interaction, cellular transformation, and induction of T-antigen. The subsequent fate of the transformed cells in all but one of the exposed hamsters was probably related to host mediated factors that influence cellular replication rates or tumor regression mediated, presumably, immunologically.

Only 1 of the 140 hamsters developed an adenovirus-12 induced neoplasm during the eighteen-month observation period. The one tumor was an undifferentiated sarcoma which appeared grossly and microscopically similar to those previously reported (6,7,32). The measured lung dose in animals exposed at the same time was $10^{2.1}$ TCD$_{50}$ per hamster. Subsequent studies showed that this retained dose following intrapulmonic inoculation is equivalent to injection of $10^{2.9}$ TCD$_{50}$ (33). Adenovirus-12 tumorigenesis is strongly dose dependent and the lowest intrapulmonic dose previously reported to have induced a tumor was $10^{3.7}$ TCD$_{50}$ (7). Recently it has been shown that intrapulmonic injection of $10^{3.7}$ TCD$_{50}$ produces an immediate retained lung dose of $10^{3.0}$ TCD$_{50}$ and 1.4% tumor incidence (33). An incidence of 1.1% tumors in hamsters eighteen months after they received a measured lung dose of $10^{2.1}$ TCD$_{50}$ suggests that the aerosol route is comparable to parenteral routes in sensitivity. A smaller group of 42 hamsters, however, that retained a measurable lung dose of $10^{3.6}$ TCD$_{50}$ failed to develop neoplasms. In hamsters retaining $10^{3.7}$ TCD$_{50}$ after direct intrapulmonic inoculation 6/49 (12.2%) develop
tumors within 6 months (33). Definitive comparison of the sensitivities of aerosol and intrapulmonic routes was inhibited by the inability to produce a higher aerosol dose of adenovirus-12 primarily because of the hamster's small respiratory volume and the loss of infectious virus during aerosolization.

Summary

Aerosol transmission of low doses of adenovirus-12 to 140 newborn Syrian hamsters was demonstrated by isolation of infectious virus, induction of adenovirus-12 T-antibody, and appearance of one undifferentiated sarcoma containing adenovirus-12 T-antigen. Following aerosol exposure a maximum of $10^{3.6} \text{TCD}_{50}$ was recovered from the newborn hamster's lung. Infectious virus was isolated from urine immediately after exposure, intestine at 0 and 12 hours post-exposure, and lung and epidermis at 0, 12, and 24 hours post-exposure. T-antibody was present in sera between 3 and 12 months post-exposure but no T-antigen could be demonstrated in the tissues. It was shown that the measured lung dose varied from the calculated maximum inhaled dose. The small total retained lung dose of virus limited the tumor incidence and therefore any definitive comparison of the sensitivity of aerosol and parenteral routes of exposure. It was concluded that the potential biohazard to other experimental animals or man following aerosol exposure to this DNA virus under non-replicating conditions are minimal and of short duration.
Chapter 1


Chapter II


Chapter III


