Induction of Lung Adenomas Following Exposure of Pregnant, Newborn, and Immature Male Mice to Urethan*

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In a recent publication (3) it was reported that, when pregnant mice near term were given injections of urethan, and the young removed 1 or 5 hours thereafter, the offspring had developed numerous lung tumors by the age of 6 months. Inasmuch as the results suggested a rapid passage of carcinogen across the placenta, it was decided to confirm and extend this observation by reducing the time between injection and cesarean delivery to 5 minutes. Since the cesarean-delivered mice had been exposed to a mixture of 95 per cent O₂ and 5 per cent CO₂, the possibility that this might have influenced tumorigenesis was investigated.

It has been suggested that the amount of lung tissue vulnerable to urethan increases with age (2). On the other hand, Rogers (5) reported that young mice were more susceptible than older ones to this carcinogen, while Klein (3) observed about the same degree of tumor susceptibility in urethanized mothers as in their offspring. In view of these conflicting results, the influence of age on lung tumorigenesis was re-examined.

MATERIALS AND METHODS

Albino mice related to strain A (3) and including pregnant females, newborn, and 47-day-old males were given one injection intraperitoneally of a 10 per cent solution of urethan in distilled water. The pregnant females (average weight, 37 gm.) each received 25 mg. of the carcinogen 19 days following discovery of a vaginal plug, while the newborn and 47-day-old male mice received 1 mg of urethan/gm body weight. Fetuses were removed by cesarean section and placed with untreated foster mothers of the same strain. Operative procedures differed from those previously detailed (3) in that the delivered young were not exposed to a mixture of 95 per cent O₂ and 5 per cent CO₂. All mice were sacrificed 6 months following the administration of urethan, and the lungs were excised and fixed in Telyesnieczky's fluid. Lung tumors were counted with the unaided eye or under 12x magnification. The great majority of tumors were greater than 0.5 mm. in diameter. All doubtful nodules were sectioned and stained with hematoxylin-eosin, and these tumors were diagnosed histologically. The weights of all mice were determined periodically, and those animals which were in poor condition were eliminated from the experiment.

The experiment consisted of seven groups. Groups 1, 2 (3), and 3 included mice delivered by cesarean section following injection of pregnant females with urethan. The time between injection and delivery was 5 minutes in Group 1 and 5 hours in Groups 2 and 3. Group 4 contained 58-day-old nonurethanized male mice. These, together with the mice from Group 2, were exposed ½ hour to a mixture of 95 per cent O₂ and 5 per cent CO₂. The mice in Groups 5 and 6 were injected with urethan at birth and at 47 days of age, respectively. Groups 7a and 7b were composed of untreated mice sacrificed at 180 or 240 days of age.

RESULTS AND DISCUSSION

Tumor incidences for the various groups were compared, by the χ² formula corrected for continuity (Yates), while, for tumor multiplicity, the means were compared using "Student's" t test. Statistical significance is recorded in terms of P. The results of the investigation are summarized in Table 1.

It is evident from the data that effective amounts of carcinogen were able to cross the placenta, despite the fact that the time interval between administration of urethan and removal of the fetuses was decreased to 5 minutes (Group 1). A tumor incidence of 100 per cent and an average of 19.1 tumors per lung had been reported previously when fetuses were removed 1 hour after the injection of urethan (3). Statistical analysis of the data in the 1-hour and 5-minute groups demonstrated that, after the longer exposure in utero, both tumor incidence and tumor multiplicity

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were significantly increased—$P < .001$. Similar comparisons, but between the cesarean-delivered 5-minute and 5-hour mice (Group 1 vs. Group 3) also were significant at the 0.001 level. These results may be explained if less urethan reached the fetal lungs during the 5-minute as compared to the 1- or 5-hour exposure. The demonstration that pulmonary adenomas may be induced in cesarean-delivered mice after so short a time interval strongly suggests that the intact urethan molecule rather than a metabolite thereof crossed the placentae.

In an earlier experiment (3), pregnant mice near term were injected with urethan; some were permitted to bear litters, while in other cases the young were removed by cesarean section. To assure a high rate of survival, the latter were exposed to a mixture of 95 per cent $O_2$ and 5 per cent $CO_2$ (Group 2). When it was subsequently observed that tumor multiplicity was significantly higher in the cesarean-delivered mice than in the others, it was suspected that administration of the gas mixture might have contributed to the results obtained. The data in the present experiment, however, do not support this hypothesis, for the differences in tumor incidence or tumor multiplicity in Groups 2 and 3 are of doubtful significance, $P > .05$. Similarly, no significant effect was observed on spontaneous lung tumorigenesis when nonurethanized male mice were exposed to a 95 per cent $O_2$ and 5 per cent $CO_2$ mixture (Group 4 vs. Group 7b). It would appear that the increased number of tumors per lung in the cesarean-delivered mice was the result of operative procedures associated with removal of the fetuses. It is known that contractions of the gravid uterus may force blood from placenta to fetus and produce a noticeable rise in blood volume (4). If such contractions were induced in the present experiment (Group 5 vs. Groups 2, 3). Although significantly more tumors per lung were observed among the newborn as compared to the cesarean-delivered 5-minute mice (Groups 1, 5) $P < .01$, this may be accounted for if a comparatively small amount of urethan crossed the placenta during the 5-minute interval.

When 47-day-old male mice were injected with urethan (Group 6), significantly more tumors per lung were induced in them than in the newborn mice which had been exposed to a comparable dose of the carcinogen—$P < .001$. Similarly, more tumors per lung were observed in the mice in Group 6 as compared to the cesarean-delivered mice in Groups 2 and 3—$P < .001$. These data support those of Henshaw and Meyer (2), inasmuch as an increased susceptibility to lung tumors was evident in the older mice. In a previous investigation in which pregnant mice were injected with urethan and permitted to litter, the mothers bore an average of 8.9 tumors per lung at 6 months (3). This is significantly less than

| TABLE 1 |
| LUNG TUMORS IN URETHANIZED NEWBORN, 47-DAY-OLD, AND CESAREAN-DELIVERED MICE |

<table>
<thead>
<tr>
<th>Group*</th>
<th>Sex</th>
<th>Total survivors (no.)</th>
<th>Tumor incidence (per cent)</th>
<th>Av. tumors/lung tumor-bearers (no.)</th>
<th>Av. age at death (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Cesarean, 5 min.</td>
<td>$\sigma / \sigma$</td>
<td>49</td>
<td>62.0</td>
<td>5.6</td>
<td>180</td>
</tr>
<tr>
<td>2 Cesarean, 5 hr. 95 $O_2$: 5 $CO_2$</td>
<td>$\sigma / \varnothing$</td>
<td>60</td>
<td>97.1</td>
<td>16.4</td>
<td>180</td>
</tr>
<tr>
<td>3 Cesarean, 5 hr.</td>
<td>$\sigma / \sigma$</td>
<td>66</td>
<td>100.0</td>
<td>13.7</td>
<td>180</td>
</tr>
<tr>
<td>4 No urethan, 95 $O_2$: 5 $CO_2$</td>
<td>$\sigma$</td>
<td>36</td>
<td>25.0</td>
<td>1.3</td>
<td>233</td>
</tr>
<tr>
<td>5 Newborn</td>
<td>$\sigma / \varnothing$</td>
<td>31</td>
<td>100.0</td>
<td>9.2</td>
<td>180</td>
</tr>
<tr>
<td>6 47-Day-old</td>
<td>$\sigma$</td>
<td>38</td>
<td>100.0</td>
<td>22.4</td>
<td>227</td>
</tr>
<tr>
<td>7a Intact controls, no urethan</td>
<td>$\sigma / \sigma$</td>
<td>177</td>
<td>7.9</td>
<td>1.3</td>
<td>180</td>
</tr>
<tr>
<td>7b Intact controls, no urethan</td>
<td>$\sigma / \varnothing$</td>
<td>139</td>
<td>24.5</td>
<td>1.1</td>
<td>240</td>
</tr>
</tbody>
</table>

* Fetuses delivered from pregnant mice which received 85 mg. urethan intraperitoneally (I.P.); 47-day-old and newborn mice received 1 mg urethan/gm body weight, I.P.

† Data for Group 2 from previous experiment (3).

‡ 1-Hr. exposure following cesarean delivery (Group 4) or at start of experiment (Group 4).

Research.
was observed in the 47-day-old males in Group 6—
P < .001. Although the pregnant mice were in-
jected with approximately 0.7 mg. and the 47-day
males with 1.0 mg urethan/gm body weight, this
variation in dosage is not considered sufficient to
account for the observed difference in tumor mul-
tiplicity. Assuming the pregnant state did not alter
the response of the mice to urethan, the results
here obtained tend to support the conclusions of
Rogers (5), since now younger mice were found to
be more susceptible than older ones. The contra-
dictory results obtained from these and other ex-
periments (2, 3, 5) emphasize the need for further
study before a valid conclusion may be drawn on
the influence of age on urethan lung tumorigenesis.

SUMMARY

Numerous lung tumors were observed at 6
months in fetuses exposed for 5 minutes to urethan
transplacentally. This suggests that the intact
urethan molecule rather than a metabolite crossed
the placenta. Significantly more lung tumors arose
when the time between injection and cesarean
delivery was extended to 5 hours. Administration
of a gas mixture containing 95 per cent O₂ and 5
per cent CO₂ appeared to have no effect on spon-
taneous or on urethan-induced lung tumorigenesis.
Significantly more tumors per lung were observed
among cesarean-sectioned mice delivered 5 hours
following urethan injection than among urethan-
ized newborn mice. However, cesarean-delivered
mice exposed in utero 5 minutes bore significantly
fewer tumors per lung, probably because a smaller
quantity of carcinogen crossed the placenta during
this brief time interval. Significantly more tumors
per lung were observed in mice injected with ure-
than at 47 days than at birth, suggesting an in-
creased susceptibility with age. On the other hand,
more lung tumors were observed among the 47-
day-old mice than among 178-day-old urethanized
pregnant females.

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