Effect of Partial Hepatectomy on Tumor Incidence in BALB/c Mice Treated with Urethan

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had only the median lobe removed to produce an approximate deficit of 30% of the liver.

All animals were permitted to live out their life-span. They were examined daily, but not weighed, and were killed when they became moribund. In general, the diagnosis of tumors was made on gross specimens. The criteria for the diagnosis of hepatomas included size greater than 0.5 cm, solid consistency when cut, and well-developed vascular channels on the surface. Approximately 50% of randomly selected tumors were examined microscopically and, by the criteria of Andervont and Dunn (1), all were diagnosed as hepatomas and resembled urethan-induced hepatomas previously described (14). The method of $\chi^2$ was used for statistical evaluation.

Mitotic Activity Study. Several preliminary studies were carried out which demonstrated that, when hepatectomy was performed as described above, the liver was restored to preoperative weight within 14 days. This is in keeping with results in the literature which also emphasize that there is considerable variation in the response of mice to partial hepatectomy, even among individuals of the same strain (23). However, it was deemed necessary to establish the mitotic activity of the regenerating liver in mice not treated with urethan under the conditions of the present study.

Eighty BALB/c/Ki mice ranging in age from 6 to 8 weeks were assigned at random to 4 groups with at least 9 male and 9 female mice in each group. In the 1st group, 70% of the liver was removed; in the 2nd group, 30% was removed; in the 3rd group no liver was removed but the abdomen was opened and closed according to the routine partial hepatectomy procedure. The anesthetic and surgical procedures have been described above. No surgery was performed on the 4th group prior to autopsy.

All surgery and subsequent autopsy of the animals was done between 9:00 a.m. and 12:00 noon. At least 3 male and 3 female mice in each group were autopsied at 48, 96, and 144 hr after surgery. The remaining liver was removed and placed in Bouin’s fixative for 4 days. The right lateral lobe was then bisected, and the inferior half was submitted for paraffin embedding, sectioning (5 μm), and staining with hematoxylin and eosin. Sections were taken from 3 different tissue levels. The number of hepatocytes containing mitotic figures were counted as well as the total number of hepatocytes in that field. This was done by examining 25 fields (×40) at 3 different tissue levels for each mouse. In this manner, approximately 2500 cells were counted for each liver specimen. The number of mitoses per 1000 cells was determined from this data. To avoid bias, slides were assigned random numbers and coded prior to counting the mitotic figures. The data from the regenerating liver mitotic index study was subjected to a multivariate analysis of variance with 1 variable (mitotic index). A program (IBM package MANOVA) developed in the Biometric Laboratory at the University of Miami was used and performed on an IBM 7094 computer.²

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RESULTS

The incidences of various types of neoplasms in control animals (Group I) and those of experimental groups are shown in Table 1. The occurrence of spontaneous tumor appeared to be limited primarily to the lungs and reticular tissue, with hepatomas occurring in a very low incidence and only in males. A low incidence of mammary tumors in the females was also found.

The incidence of lung tumors in the mice treated with urethan but not subjected to prior partial hepatectomy (Group II) was high in both sexes as anticipated (Table 1). Similarly, a high incidence of lung tumors occurred in the animals that underwent partial hepatectomy followed by urethan treatment (Groups III and IV). All urethan-treated animals had multiple lung tumors visible grossly, but no attempt was made to quantify these. However, the significant finding in the mice subjected to 70% hepatectomy followed by urethan treatment (Group III) was the occurrence of hepatomas in 41% of the males, in addition to lung tumors. Hepatomas also occurred in 17% of the females in Group III, a statistically significant increase when compared to the incidence of hepatomas in female mice of Groups I and V, but not in comparison to the incidence in females of Group II. The high incidence of hepatomas in males of Group III is in contrast to the low incidence of hepatomas in all other groups. The hepatomas were distributed at random throughout the livers without a predominance in a single gross anatomical location. Unlike the results of other authors (21), blood cysts in the liver were not found following urethan treatment.

The mean survival times of urethan-treated groups were shorter than those of groups not treated with urethan, presumably because of the high incidence of pulmonary adenomas. Urethan did not appear to have a life-shortening effect on another basis since the mean survival time of urethan-treated mice that did not develop tumors was as long as that of untreated controls in Group I (Table 1). The incidence of leukemia in all of the groups that received urethan was decreased to 0, with 1 exception.

The mitotic indices following 70%, 30%, and sham hepatectomy at 48, 96, or 144 hr postsurgery are shown in Table 2. Subjecting these data to statistical analysis of variance revealed interaction between certain factors. For determination of the location and strength of these interactions, the group t test (95% confidence limits) for unequal variance was used (13, 20). The following significant differences were noted.

At 48 and 96 hr posthepatectomy, the mitotic index of the male 70% hepatectomy group was significantly higher than the sham-operated males. This pattern was noted only at 48 hr posthepatectomy in the male 30% hepatectomy groups. Forty-eight hr posthepatectomy, the mitotic index of the male 70% hepatectomy group was significantly higher than the male 30% hepatectomy group.

In comparing the mitotic indices of the female mice, the 48 hr, 30% hepatectomy group must be discarded because of its large standard deviation. There were no significant statistical differences between the remaining female groups.
### Table 1

Tumor incidences in BALB/c males and females after partial hepatectomy plus urethan treatment

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Sex</th>
<th>No. of mice</th>
<th>Lung</th>
<th>Hepatoma</th>
<th>Leukemia</th>
<th>Breast</th>
<th>Ovary</th>
<th>With lung tumors and/or hepatomas</th>
<th>With leukemia, breast or ovary tumors</th>
<th>Without tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Colony controls</td>
<td>M</td>
<td>102</td>
<td>18</td>
<td>(17%)</td>
<td>2</td>
<td>(2%)</td>
<td>7</td>
<td>(7%)</td>
<td>0</td>
<td>722  (466–960)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>79</td>
<td>15</td>
<td>(19%)</td>
<td>0</td>
<td>(0%)</td>
<td>22</td>
<td>(28%)</td>
<td>6 (8%)</td>
<td>674  (526–882)</td>
</tr>
<tr>
<td>II. Urethan</td>
<td>M</td>
<td>24</td>
<td>23</td>
<td>(96%)</td>
<td>0</td>
<td>(0%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>559  (520–658)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>18</td>
<td>18</td>
<td>(100%)</td>
<td>1</td>
<td>(6%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>599  (536–703)</td>
</tr>
<tr>
<td>III. 70% hepatectomy + urethan</td>
<td>M</td>
<td>22</td>
<td>20</td>
<td>(90%)</td>
<td>9</td>
<td>(41%)</td>
<td>1</td>
<td>(5%)</td>
<td>0</td>
<td>526  (226–708)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>24</td>
<td>21</td>
<td>(87%)</td>
<td>4</td>
<td>(17%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>532  (322–664)</td>
</tr>
<tr>
<td>IV. 30% hepatectomy + urethan</td>
<td>M</td>
<td>19</td>
<td>17</td>
<td>(89%)</td>
<td>1</td>
<td>(5%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>524  (428–598)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>18</td>
<td>15</td>
<td>(84%)</td>
<td>0</td>
<td>(0%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>561  (398–630)</td>
</tr>
<tr>
<td>V. 70% hepatectomy</td>
<td>M</td>
<td>28</td>
<td>3</td>
<td>(10%)</td>
<td>1</td>
<td>(4%)</td>
<td>2</td>
<td>(7%)</td>
<td>0</td>
<td>774  (565–786)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>32</td>
<td>7</td>
<td>(21%)</td>
<td>0</td>
<td>(0%)</td>
<td>5</td>
<td>(16%)</td>
<td>0</td>
<td>749  (662–786)</td>
</tr>
</tbody>
</table>

*Numbers in parentheses represent range.*

Comparison of the mitotic indices of the paired male and female groups demonstrates that at 48 hr posthepatectomy the 70% hepatectomy males had a significantly higher mitotic index than the correspondingly treated females. No other valid comparisons demonstrated any significant differences between male and female groups. There was no significant statistical difference between the sham hepatectomy controls and the nonoperated controls.

Following partial hepatectomy, the increasing rate of change of the mitotic index with time reached a peak between the 2nd and 4th days. The rate of increase in mitotic activity and the time that peak activity occurs are dependent on such variables as the sex and age of the animal, the species being studied, and the amount of liver removed. Therefore, the statistical analysis of the mitotic index of male and female mice at identical intervals following the removal of varying amount of liver must be approached with caution (4). Nevertheless, it appears that the male 70% hepatectomy group showed a significantly higher mitotic index than either the sham or the 30% hepatectomy group. There appears to be a significant difference in the mitotic index between males and females in the 70% hepatectomy groups, the mitotic index in the male being higher than in the female.

### DISCUSSION

Pound and Withers (18) found that urethan had an initiating action in the development of skin tumors in mice in which croton oil and other skin irritants served as promoting agents. Of significance was the observation that the tumor yield was significantly augmented when the urethan was injected 24 hr after the treatment of the skin with one of the irritants. The augmenting effect was related...
to the cellular proliferation in the skin evoked by the irritant, suggesting that dividing cells may be more susceptible to the initiating action of urethan. It has also been shown that the period of greatest susceptibility to the Gross passage A virus corresponds to the period when the greatest number of large cells of the outer zone of the thymic cortex in newborn mice are proliferating (2).

Recently, kidney tumors have been described in animals subjected to X-irradiation to the remaining kidney following unilateral nephrectomy (19). Although it is generally held that the remaining kidney after unilateral nephrectomy undergoes primarily hypertrophy, there is evidence indicating a hyperplasia also occurs (7).

Haran-Ghera et al. (8) had as their objective the confirmation of studies indicating that regeneration following partial hepatectomy functions as a "promoting influence" in liver carcinogenesis. X-irradiation was used as the "initiating" agent and multiple subtotal hepatectomies as a "promoting" agent. The results were negative in that few hepatomas occurred.

In a preliminary report of the present study (12), we demonstrated that the administration of urethan to 70% hepatectomized young adult mice 2, 4, and 6 days after partial hepatectomy in males compared to intact mice receiving the same dose and schedule of the carcinogen. It was suggested that rapidly proliferating liver cells may be more susceptible to the action of a carcinogen. Subsequently, Pound (17) reported that one-third hepatectomy followed by urethan injection at intervals up to 6 days increased the incidence of liver tumors over that observed in unoperated urethan-treated mice and suggested a similar explanation for this phenomenon. He also used mice with a low susceptibility to hepatoma formation after treatment with urethan, but did not present data on the sex of the animals. Hollander and Bentvelzen (10) confirmed an increased incidence of hepatomas in young mice administered urethan after partial hepatectomy compared to intact urethan-treated animals of the same age. These workers studied male mice only of a strain highly susceptible to hepatoma formation after urethan treatment.

Some of the possible explanations for these results include these: (a) rapidly proliferating liver cells are more susceptible to the carcinogenic effects of urethan than resting cells; (b) urethan is concentrated by regenerating liver cells to a greater degree than by resting cells; (c) removal of a large portion of the liver results in a slower rate of elimination of urethan so that there is a greater exposure of tissues to the carcinogen. It was found in the present study that the mitotic activity in the liver was influenced by the amount of liver removed and the sex of the animal. These findings are in agreement with those of others (4). The incidence of hepatomas in mice subjected to 30% hepatectomy plus urethan treatment was lower than in those subjected to 70% hepatectomy plus urethan treatment. The highest mitotic index was found in male mice subjected to 70% hepatectomy. These results support the hypothesis that increased cell proliferation enhances susceptibility to a carcinogen.

The slower rate of elimination of urethan from newborn mice as compared to adults has been described, and it has been suggested that the longer time during which urethan remains in the body may explain the greater carcinogenic action of urethan in newborn mice (15). The accompanying paper (6) describes studies on the rate of elimination of urethan and on its concentration in liver cells and subcellular fractions in intact and 70% hepatectomized BALB/c Ki mice. The results support the view that rapidly proliferating liver cells are more susceptible than resting cells to the carcinogenic action of urethan.

The low incidence of leukemia in all urethan-treated groups compared to untreated controls was notable in these studies. While the mean survival times of urethan-treated animals dying with lung tumors and/or hepatomas were 67 to 153 days shorter than those of colony controls dying with leukemia, there is considerable overlap in the ranges of survival for these groups and the median survival times were similar. One would have expected a similar incidence of leukemia based on duration of survival. We are unaware of similar observations in the literature and cannot explain this result.

REFERENCES


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