Hormonal Influences on Urethan Carcinogenesis in C3H/f Mice*  

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SUMMARY  
A single dose of urethan was administered to newborn and young adult C3H/f mice of both sexes. The incidences of liver tumors and reticular tissue neoplasms in the treated young adult mice were not significantly increased over those in control mice. However, a striking increase was observed in the incidence of hepatomas in the males which received urethan at newborn age. Females treated by injection with urethan at birth showed a significant increase in the incidence of liver tumors and reticular tissue neoplasms. These two types of neoplasms occurred either alone or together. The incidence of lung tumors was increased in animals of both sexes given injections of urethan at birth. All males that developed hepatomas had stimulated seminal vesicles; the microscopic appearance of the submaxillary glands and kidneys indicated androgen stimulation. The female mice with liver tumors also showed morphologic evidence of androgen stimulation, whereas females with reticular tissue neoplasms showed morphologic evidence of estrogen stimulation or of a castrate state. No apparent relationship was noted between the type of sex hormone stimulation observed at autopsy and the incidence of lung tumors. Adrenal cortical adenomas and ovarian atrophy were found in the females that received urethan at newborn age. These data suggest that the changes produced by urethan in cells of different tissues were variably expressed as recognizable neoplasms. The presence or absence of tumors in various tissues appeared to be dependent to some extent upon the hormonal environment of the host.

Urethan was once considered to be a carcinogen specific for lung tissue (29). Subsequently, it has been shown to produce tumors in a variety of tissues of the mouse (37). Hemorrhagic lesions (6, 34, 37) and hemangiomas (20, 22, 32) in the livers of mice have been observed following the administration of urethan. Heston et al. (18) provided data obtained from urethan-treated C3H mice that permitted the addition of hepatomas to the list of neoplasms induced or potentiated by urethan. Klein (23) reported a striking increase in the incidence of hepatomas in B6AF1/J hybrids following the oral administration of urethan to newborn mice. Of interest was the higher incidence of hepatomas in males than in females.

Urethan was reported to augment leukemogenesis in mice treated with x-irradiation, methylcholanthrene, and estrogens, but alone was found to have no leukemogenic effects (7, 19). Also, when urethan was administered alone to young adult mice of the high leukemia strains, AKR and C58, no enhancement of leukemia was observed (20). It has been suggested that urethan acts as a “promoting factor” in leukemogenesis in x-irradiated C57BL mice (7).

A recent report described the development of malignant lymphomas in Swiss mice receiving urethan in the drinking water as adults (38). The age of the animal at the time of administration of urethan has been shown to be of significance in the manifestation of its leukemogenic potential. Several investigators have described the development of neoplasms of lymphatic tissue following the administration of urethan by the parenteral route to newborn Swiss mice (13, 30) or after oral administration to B6AF1/J hybrids (23). The greater responsiveness of newborn mice to chemical carcinogens has been appreciated and emphasized by several investigators (9, 11, 21, 23, 33), in keeping with previous studies of the susceptibility of newborn mice to virus-induced tumors.

In a preliminary report (27), we described the increased incidence of hepatomas in C3H/f mice treated by injection with urethan at birth. It was the purpose of the present investigation to extend these studies to determine the effects of urethan injected into newborn and young adult C3H/f mice of both sexes with respect to the development of neoplasms as influenced by hormonal factors.

MATERIALS AND METHODS  
The C3H/f mice used in these studies were originally obtained from the late Dr. J. J. Bittner who designated...
them, and have been inbred by brother-sister matings in the Kirschbaum Memorial Research Laboratory for 22 generations. All animals were housed in wooden boxes, 6 X 12 X 6 in., with four to six mice per cage. Purina lab chow was fed ad libitum. A 3.5 per cent solution of urethan (ethyl carbamate) was prepared periodically or estrogenic stimulation at the time of autopsy (14).

A histochemical technic specific for the localization of phenolic groups of protein was employed to emphasize the secretory granules of the submaxillary gland (35). The alcian blue-periodic acid-Schiff technic was also utilized to localize glycogen in the normal and neoplastic liver cells (28). In several instances, animals were autopsied after post-mortem changes had taken place. The method of the $\chi^2$ was used for statistical analysis.

**RESULTS**

_Tumor incidence._—The incidence of the various types of tumors occurring in C3H/f untreated, castrated, and urethan-treated males and females is shown in Table 1.

Hepatomas developed in 14 per cent (14/97) and 1 per cent (1/77) of the untreated males and females, respectively. Reticular tissue neoplasms were observed in 2 per cent (2/97) of the males and 8 per cent (6/77) of the females. The majority of the control males and females died free of tumors at a mean age of 520 (349-610) days, respectively. Reticular tissue neoplasms were observed. No liver tumors were found in the

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**TABLE 1**

INCIDENCE OF VARIOUS TYPES OF NEOPLASMS IN CONTROL AND URETHAN-TREATED C3H/f MALES AND FEMALES

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Sex</th>
<th>Type of tumor</th>
<th>Average No. of Liver Tumors per Animal</th>
<th>Incidence No. of animals</th>
<th>Per cent</th>
<th>Survival Time of Animals Dying with Tumors (Days)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>♂</td>
<td>Hepatoma</td>
<td>1.1</td>
<td>14/97</td>
<td>14%</td>
<td>495 (361-648)</td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td>Reticular tissue neoplasm</td>
<td>—</td>
<td>2/97</td>
<td>2%</td>
<td>385 and 410</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>Hepatoma</td>
<td>1.0</td>
<td>1/77</td>
<td>1%</td>
<td>540</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>Reticular tissue neoplasm</td>
<td>—</td>
<td>6/77</td>
<td>5%</td>
<td>509 (390-629)</td>
</tr>
<tr>
<td>Castrate</td>
<td>♂</td>
<td>Hepatoma</td>
<td>1.0</td>
<td>1/23</td>
<td>4%</td>
<td>545</td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td>Reticular tissue neoplasm</td>
<td>—</td>
<td>0/23</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>Hepatoma</td>
<td>—</td>
<td>0/22</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>Reticular tissue neoplasm</td>
<td>—</td>
<td>4/22</td>
<td>18%</td>
<td>376 (312-432)</td>
</tr>
<tr>
<td>Urethan at 8-10 weeks</td>
<td>♂</td>
<td>Hepatoma</td>
<td>1.7</td>
<td>6/25</td>
<td>24%</td>
<td>481 (349-610)</td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td>Reticular tissue neoplasm</td>
<td>—</td>
<td>0/25</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>Lung tumor</td>
<td>—</td>
<td>2/25</td>
<td>5%</td>
<td>506 (402-610)</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>Hepatoma</td>
<td>—</td>
<td>0/32</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>Reticular tissue neoplasm</td>
<td>—</td>
<td>4/32</td>
<td>13%</td>
<td>453 (398-510)</td>
</tr>
<tr>
<td>Urethan at newborn age</td>
<td>♂</td>
<td>Hepatoma</td>
<td>6.8</td>
<td>27/30</td>
<td>90%</td>
<td>321 (148-531)</td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td>Reticular tissue neoplasm</td>
<td>—</td>
<td>4/30</td>
<td>13%</td>
<td>285 (145-383)</td>
</tr>
<tr>
<td></td>
<td>♂</td>
<td>Lung tumor</td>
<td>—</td>
<td>14/30</td>
<td>46%</td>
<td>401 (341-445)</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>Hemangiomata (liver)</td>
<td>—</td>
<td>1/30</td>
<td>3%</td>
<td>339</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>Hepatoma</td>
<td>4.2</td>
<td>18/39</td>
<td>46%</td>
<td>434 (252-538)</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>Reticular tissue neoplasm</td>
<td>—</td>
<td>22/39</td>
<td>50%</td>
<td>343 (154-538)</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>Lung tumor</td>
<td>—</td>
<td>10/39</td>
<td>48%</td>
<td>408 (252-538)</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>Ovarian tumor</td>
<td>—</td>
<td>1/39</td>
<td>—</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>Stomach tumor</td>
<td>—</td>
<td>1/39</td>
<td>—</td>
<td>520</td>
</tr>
</tbody>
</table>

* Values are means, with range in parentheses.
TABLE 2
OCCURRENCE OF HEPATOMAS, RETICULAR TISSUE NEOPLASMS, AND LUNG TUMORS IN C3H/f MALE MICE TREATED WITH URETHAN AT BIRTH AND RELATIONSHIP TO OCCURRENCE OF ADRENAL CORTICAL ADENOMAS AND TO SEX HORMONE ENVIRONMENT

<table>
<thead>
<tr>
<th>TYPES OF TUMOR</th>
<th>INCIDENCE</th>
<th>SURVIVAL TIME (DAYS)</th>
<th>ADRENAL CORTICAL ADENOMAS, GROSS OR MICROSCOPIC</th>
<th>SEX HORMONE ENVIRONMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatoma only</td>
<td>30% (9/30)</td>
<td>343 (230-408)</td>
<td>2/7</td>
<td>7</td>
</tr>
<tr>
<td>Hepatoma plus lung tumors</td>
<td>43% (13/30)</td>
<td>401 (341-531)</td>
<td>3/11</td>
<td>11</td>
</tr>
<tr>
<td>Hepatoma plus reticular tissue</td>
<td>13% (4/30)</td>
<td>286 (145-383)</td>
<td>2/4</td>
<td>2</td>
</tr>
<tr>
<td>neoplasm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatoma plus hemangioma</td>
<td>3% (1/30)</td>
<td>339</td>
<td>0/1</td>
<td>1</td>
</tr>
<tr>
<td>Lung tumor only</td>
<td>3% (1/30)</td>
<td>351</td>
<td>0/1</td>
<td>1</td>
</tr>
<tr>
<td>Hepatomas, total</td>
<td>90% (27/30)</td>
<td>321 (148-531)</td>
<td>7/24</td>
<td>22</td>
</tr>
</tbody>
</table>

* Values given are means, with range in parentheses.

gonadectomized females. The incidence of reticular tissue neoplasms was increased to 18 per cent (4/22) as compared to 8 per cent in controls, but the difference was not statistically significant (P > 0.05).

Males treated with urethan at 8–10 weeks of age had an incidence of hepatomas of 24 per cent (6/25) and of lung tumors of 8 per cent (2/25). However, this increase in the incidence of liver tumors was not statistically significant when compared to the incidence of hepatomas in controls (P > 0.05). The average number of liver tumors per mouse was 1.7 compared with 1.0 in controls. No reticular tissue neoplasms were noted. Four of 32 (13 per cent) females receiving urethan at 6–8 weeks of age developed reticular tissue neoplasms. This also was a slight increase in the incidence of this type of neoplasm over that seen in untreated females, but this was not statistically significant (P > 0.05). No hepatomas or lung tumors were observed in any of the 32 females.

Urethan injected at newborn age caused a striking increase in the incidence of hepatomas (90 per cent) in males (P < 0.02). The liver tumors occurred either alone or in association with another type of neoplasm (lung tumor or reticular tissue neoplasm) (Table 2). The average number of hepatomas (greater than 5 × 5 mm.) was increased to 6.8 per liver (Fig. 1). The time of appearance of hepatomas was accelerated. Of the males that developed hepatomas following urethan injection as newborns, 50 per cent showed evidence of tumor earlier than any male of either the control, castrated, or urethan-treated young adults that died with a hepatoma (Chart 1). The hepatomas closely reproduced the structure of normal liver (Fig. 2) and were morphologically similar to hepatomas described by other investigators (3). Five representative hepatomas from different mice have been successfully transplanted. Appropriate cytochemical stains revealed a decrease and abnormal distribution of glycogen in the majority of tumor cells as compared to normal liver. Eosinophilic inclusions as large as several micra in diameter were found in the cytoplasms of parenchymal cells (Fig. 3a) of approximately 90 per cent of the hepatomas from urethan-treated males and females. This inclusions were weakly periodic acid-Schiff-positive after diastase digestion and strongly positive for phenolic groups of protein (Fig. 3b). Similar inclusions have been described in spontaneous, chemically induced, and transplanted hepatomas in mice (4, 33). Only four spontaneous hepatomas of the fourteen studied from control animals had similar inclusions.

Females treated with urethan at newborn age had an incidence of hepatomas of 46 per cent (18/39) as contrasted to 1 per cent in untreated controls (P < 0.001). Five animals had only hepatomas, whereas the remaining thirteen animals had either a reticular tissue neoplasm or lung tumor in addition to liver tumors (Table 3). One mouse developed an ovarian tumor, and another developed both stomach and lung tumors. The average number of hepatomas was 4.2 per mouse, fewer than found in males treated in the same manner, but greater than in control animals. The size of the tumors tended to be smaller in the urethan-treated female than in the male (Fig. 4). Reticular tissue neoplasms were present in 56 per cent of the injected females of this group as compared to an incidence of 8 per cent in controls (P < 0.01).
TABLE 3
OCCURRENCE OF HEPATOMAS, RETICULAR TISSUE NEOPLASMS, AND LUNG TUMORS IN C3H/f FEMALE MICE TREATED WITH URETHAN AT BIRTH AND RELATIONSHIP TO OCCURRENCE OF ADRENAL CORTICAL ADENOMAS AND TO SEX HORMONE ENVIRONMENT

<table>
<thead>
<tr>
<th>TYPE OF TUMOR</th>
<th>INCIDENCE</th>
<th>SURVIVAL TIME (DAYS)*</th>
<th>ADRENAL CORTICAL ADENOMAS, GROSS OR MICROSCOPIC</th>
<th>SEX HORMONE ENVIRONMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>♀</td>
<td>♂</td>
</tr>
<tr>
<td>Hepatoma only</td>
<td>13% (5/39)</td>
<td>452 (387–520)</td>
<td>4/5</td>
<td>4</td>
</tr>
<tr>
<td>Hepatoma plus reticular tissue neoplasm</td>
<td>5% (2/39)</td>
<td>372 (343–401)</td>
<td>2/2</td>
<td>—</td>
</tr>
<tr>
<td>Hepatoma plus lung tumors</td>
<td>20% (8/39)</td>
<td>466 (401–502)</td>
<td>7/8</td>
<td>7</td>
</tr>
<tr>
<td>Hepatoma plus lung tumors plus reticular tissue neoplasms</td>
<td>8% (3/39)</td>
<td>361 (252–338)</td>
<td>3/3</td>
<td>2</td>
</tr>
<tr>
<td>Hepatomas, total</td>
<td>46% (18/39)</td>
<td>434 (252–538)</td>
<td>16/18</td>
<td>13</td>
</tr>
<tr>
<td>Reticular tissue neoplasm only</td>
<td>26% (10/39)</td>
<td>339 (165–497)</td>
<td>10/10</td>
<td>—</td>
</tr>
<tr>
<td>Reticular tissue neoplasm plus lung tumors</td>
<td>18% (7/39)</td>
<td>347 (274–465)</td>
<td>7/7</td>
<td>—</td>
</tr>
<tr>
<td>Reticular tissue neoplasms, total</td>
<td>56% (22/39)</td>
<td>343 (165–538)</td>
<td>22/22</td>
<td>2</td>
</tr>
<tr>
<td>Lung tumors, total</td>
<td>48% (19/39)†</td>
<td>408 (252–538)</td>
<td>16/19</td>
<td>10</td>
</tr>
</tbody>
</table>

† One animal developed only lung tumor.
* Values given are means, with range in parentheses.

These neoplasms tended to occur earlier than the hepatomas (Chart 2) and were found either alone or in association with other neoplasms. On the basis of available tissue specimens, fourteen of these 22 animals with reticular tissue neoplasms had reticulum-cell sarcomas of type A as classified by Dunn (12), and three animals had lymphoendothelial leukemia. Histologic material was not obtained from five animals, but all had greatly enlarged mesenteric lymph nodes and/or spleens (Fig. 5b). No thymic involvement was noted. Several animals had a uniform enlargement of the liver, which on microscopic study revealed the presence of reticulum-type cells (Fig. 6). Grossly this particular type of lesion was found to be quite distinct from hepatomas. Interestingly, six animals had almost complete replacement of the myometrium of both horns of the uterus with the reticulum-type cells (Fig. 7). In all instances these six animals had enlarged mesenteric lymph nodes due to reticulum-cell proliferation. Lung tumors were found in 48 per cent of the urethan-treated females, whereas none were found in control mice.

Incidence of adrenal cortical adenomas and/or hyperplasia.
—No gross adrenal cortical adenomas were observed in either untreated males or females. Microscopic studies were carried out on random sections of adrenals from a total of eighteen untreated males and females; approximately 13 per cent of the animals had evidence of α-cell proliferation, and none demonstrated β-cell proliferation (14). Of the eighteen castrated males from which tissue specimens were obtained, twelve had adrenal cortical adenomas and/or hyperplasia of the β-cell type. Tissue specimens were obtained from sixteen of 22 gonadecto-
teen of the 22 animals were diagnosed as having adrenal cortical adenomas and/or hyperplasia.

No gross adrenal cortical adenomas were noted in the intact males and females treated by urethan injection as young adults that developed liver or reticular tissue tumors; no detailed microscopic study was carried out.

In the males that received injections of urethan at birth, seven of the 24 (28 per cent) that were examined had adrenal cortical adenomas as determined by either gross or microscopic study of three to four random sections of each adrenal gland (Table 2). The adenomas and/or hyperplasia were primarily of the β-cell type (Fig. 8), although occasional α-cell types were noted. The testes of fifteen animals with or without adrenal cortical adenomas were studied microscopically and were considered to be similar to those of untreated controls of comparable age.

Of the eighteen females that had received injections of urethan at birth and that developed hepatomas either alone or with another type of neoplasm, sixteen had adrenal cortical adenomas (Table 3). All 22 animals that developed reticular tissue neoplasms were found to have adrenal cortical adenomas and/or hyperplasia. The ovaries of twelve of the animals with adrenal cortical adenomas were studied microscopically, and all were found to show an extreme atrophy, including hyaline degeneration and absence of follicles (Fig. 9).

Relationship between androgenic and estrogenic stimulation at autopsy and type of tumors.—No attempt was made to correlate the type of sex hormone stimulation found at autopsy and the type of tumor that developed in untreated control males and females. The one castrated male that developed a hepatoma had adrenal cortical adenomas, and the seminal vesicles were not stimulated. Tissue specimens were obtained from eighteen out of 23 castrated males, and only two of these animals had stimulated seminal vesicles with evidence of androgen stimulation of the kidney and submaxillary glands; the remaining sixteen animals had castrate-type seminal vesicles.

All four of the 22 gonadectomized females that developed reticular tissue neoplasms had adrenal cortical adenomas. Evidence for estrogenic stimulation was present in two of these four mice; the remaining two demonstrated a castrate-type reproductive tract. Animals that had neither liver nor reticular tissue neoplasms were not studied in detail to assess hormonal environment.

All six males which developed liver tumors after treatment with urethan as young adults had seminal vesicles compatible with androgen stimulation. The four females that developed reticular tissue neoplasms showed evidence of estrogen stimulation. Animals that did not exhibit tumor development were not studied for type of sex hormone stimulation.

The seminal vesicles were grossly stimulated in 21 of the 27 males treated with urethan at birth that subsequently developed hepatomas (Table 2). Of the four males that developed reticulum-cell sarcomas in addition to liver tumors, two had stimulated seminal vesicles whereas the other two were considered to be castrate (or possibly estrogenic activity).

More complex findings were observed in the females treated by injection with urethan at birth (Table 3). Of the eighteen females that developed hepatomas either alone or with another type of neoplasm, thirteen showed evidence of androgenic activity, one had estrogenic activity, and four had a morphologic picture compatible with a castrate state (Fig. 5a). The appearance of the secretory cells of the submaxillary glands as revealed with the specific cytochemical stain appeared to be the most sensitive indicator of androgen activity (Fig. 10, a–d). Thirteen of the 22 urethan-treated females that developed reticular tissue neoplasms had estrogenic activity; seven were considered to be of the castrate type, and two had androgenic activity. The latter two animals had also developed liver and lung tumors in addition to the reticular tissue tumors. All 22 of these animals had adrenal cortical adenomas.

The sex hormone environment in the nineteen treated females that developed liver tumors in addition to either liver tumors or reticular tissue neoplasms was as follows: ten, male; six, female; and three, castrate. Sixteen of these nineteen females had adrenal cortical adenomas and/or hyperplasia.

DISCUSSION

The results of this study confirm two well-documented characteristics of urethan as a carcinogenic agent: (a) urethan is capable of inducing neoplasms in a variety of tissues, and (b) the carcinogenic properties of urethan are more readily manifested when the drug is administered to newborn mice. In the present study, a significant enhancement of hepatomas, reticular tissue neoplasms, and lung tumors occurred in C3H/f male and female mice given a single injection of urethan within 24 hr after birth. Young adult mice of the same strain, when treated by injection with a comparable dose of urethan, failed to show this striking increase in incidence of neoplasms.

A relationship was noted between the type of sex hormone stimulation observed at autopsy in either sex and the type of neoplasm that developed in mice treated with urethan at birth. It was found that, in general, the occurrence of hepatomas was associated with evidence of androgenic stimulation, whereas reticular tissue neoplasms were found to occur in association with estrogenic stimulation or a castrate state. The type of sex hormone stimulation was assessed on the basis of gross and microscopic examination of the gonads, reproductive tract, kidneys, and submaxillary glands. Several authors have described the sexual dimorphism of the mouse kidney and submaxillary gland (14, 24). A cytochemical stain for phenolic end groups of protein was found to be very useful for demonstrating the secretory granules in the submaxillary gland. The morphology of this gland was regarded to be the most sensitive indicator for assessing the presence of androgenic activity in the female. Morphologic criteria for determining the presence of androgenic or estrogenic stimulation are biologic indices of predominance of one sex steroid over the others, and methods for quantitation of sex steroids in small biologic samples will be required to overcome the shortcomings of the qualitative data presented in this study.
Several investigations have demonstrated the capacity of adrenal cortical adenomas to secrete sex steroids; these neoplasms usually arise following surgical or “physiologic” castration (14, 31, 39). The incidence of adrenal cortical adenomas in females treated with urethan at birth was comparable to that seen in gonadectomized females. On the other hand, males that received urethan injections at birth had significantly fewer adrenal cortical adenomas when compared with castrated males. Administration of urethan to young adults did not influence the incidence of adrenal tumors. Ovaries of mice treated with urethan at birth showed extreme atrophy with absence of follicles and corpora lutea at autopsy; the testes of similarly treated males showed essentially normal histology. Urethan administered to adult male mice had no damaging effects on the testes (10), but urethan has been shown to inhibit mitotic activity in ovaries of young female mice (15). In unpublished studies we have found that ovarian atrophy was present as early as 10 days of age following the injection of urethan in newborn C3H/f females, but the testes appeared normal in similarly treated newborn males. This difference in response between testes and ovaries could account for the higher incidence of adrenal neoplasms in the female. However, the possible direct action of urethan on the adrenal cortical cells or hypophysis must also be considered.

Estrogenic hormones have been considered to be leukemogenic (16), whereas androgenic steroids have been found to inhibit either spontaneous or methylcholanthrene-induced leukemia in mice (26). The susceptibility of the male to the induction of hepatomas following the administration of urethan has been described (18, 23). However, the susceptibility of males and females to the development of lymphocytic neoplasms and liver tumors after urethan treatment has been found to differ with different strains of mice (9, 23, 38). The absence of data in these reports concerned with the hormonal status of the animals at autopsy prevents any attempt to compare the findings with those of the present study. It is of interest that the administration of urethan to newborn mice of a high leukemic subline C3Hf*/Lw, in which Moloney leukemogenic virus is transmitted through the mother's milk to successive generations, resulted in a decrease in the incidence of leukemia (25). Hormonal changes may have played a role in these results, but were not described.

Liver tumors in mice occur spontaneously and predominantly in the male (2, 8, 36). Administration of estrogen to C3H male mice tended to prevent the occurrence of hepatomas, and, conversely, injection of testosterone to female C3H mice seemed to increase the susceptibility to development of spontaneous hepatomas (1). Recently, the incidence of hepatomas in gold thioglucose-obese CBA male mice was found to be significantly increased as compared to controls, but comparable obese females of this same strain showed no increase in the incidence of liver tumors (17). Andervont (3) has recently shown that C3H females were more susceptible than males to hepatoma induction with azo dyes, while males were more susceptible than females to the development of hepatomas induced with carbon tetrachloride. It becomes apparent that further investigation is required to determine the interrelationship(s) between the induction of tumors with chemical carcinogens and hormonal factors.

The results of the present study suggest that the changes produced by urethan in cells of different tissues of newborn C3H/f mice were variably expressed as recognizable tumors. The presence or absence of liver tumors and reticular tissue tumors appeared to be dependent to some extent upon the hormonal environment of the host. This apparent relationship would be in agreement with the two-stage mechanism of carcinogenesis (5), with urethan acting as the “initiator” and the sex steroids of either gonadal or adrenal origin acting as “promoters.” However, the manifestations of a neoplastic transformation in other tissues, such as those of lung, are not as evidently influenced by sex steroids. Studies utilizing more rigidly controlled hormonal conditions and extension of the present type of study to other strains of mice may provide additional information bearing on this problem.

ACKNOWLEDGMENTS

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REFERENCES

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Fig. 1.—The occurrence of multiple hepatomas in C3H/f male mice that had received a single injection of urethan when newborn. Fig. 2.—Microscopic appearance of a hepatoma (HEP) and adjacent nontumor liver tissue (LIV) stained with hematoxylin and eosin. X 120.

Fig. 3.—Microscopic appearance of a urethan-induced hepatoma stained with hematoxylin and eosin (a) and for phenolic groups of proteins (b). Note the numerous cytoplasmic inclusions, varying markedly in size. X 282.

Fig. 4.—Livers from three urethan-treated C3H/f mice (A and C, males; B, female). Note greater difference in size and number of hepatomas in males as compared to female.

Fig. 5.—Gross appearance of two urethan-treated C3H/f females: A, Multiple hepatomas with a small and thin uterus. B, Enlarged mesenteric lymph node (MLN) and “stimulated” uterus.
FIG. 6.—Microscopic appearance of liver from C3H/f urethan-treated female, demonstrating infiltration of neoplastic histiocytic-type cells with numerous mitotic figures. X 120.

FIG. 7.—Microscopic appearance of uterus of C3H/f urethan-treated female, showing infiltration of neoplastic histiocytic-type cells. Note occurrence of numerous, large, palely staining cells. X 120.

FIG. 8.—Microscopic appearance of a portion of zona fasciculata (Fasc.) of adrenal cortex from a C3H/f urethan-treated female demonstrating nests of beta-cell types. X 282.

FIG. 9.—Microscopic appearance of ovary from a 158-day-old C3H/f urethan-treated female which had received a single injection of urethan at newborn age. Note complete absence of follicles and corpora lutea and marked hyaline degeneration. X 132.

FIG. 10.—Microscopic appearance of portions of submaxillary gland stained for phenolic groups of protein from an untreated, intact C3H/f male (a) and castrated C3H/f male (b) as compared to an untreated, intact C3H/f female (c) and an intact C3H/f urethan-treated female (d) which had atrophied ovaries and adrenal cortical adenomas. Note the presence of secretion granules in section d, which are considered to be the result of androgenic hormonal stimulation. X 282.


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