Multipotential Carcinogenesis with Urethan in the Syrian Golden Hamster*

BELA TOTH, LORENZO TOMATIS, AND PHILIPPE SHUBIK

(Division of Oncology, The Chicago Medical School, Chicago, Illinois)

SUMMARY

Urethan in the drinking water (0.2-0.4 per cent) was administered to 5- to 7-week-old Syrian golden hamsters and continued for 42 weeks with an 8-week interval between the 40th and 48th week. This treatment has been found to give rise to melanotic tumors of the skin, papillomas and carcinomas of the forestomach, adenomatous polyps of the cecum, pulmonary adenomatosis, mammary tumors, hepatomas, hemangiosarcomas. Certain other tumors occurring in the controls appear to have been enhanced. After the 40th week, all the surviving animals in the urethan-treated groups developed one or more tumors.

Urethan has been found to be a multipotential carcinogen in several strains of mice (17, 18). In these long-term experiments, five separate lesions were induced or potentiated by urethan; pulmonary adenomas, mammary carcinomas, malignant mesenchymal tumors in the interscapular fat, cystadenomas of the lacrimal gland, and blood cysts in the liver.

Urethan has been reported to have various roles in relation to the occurrence of leukemias and lymphomas in mice. It has been reported to potentiate the leukemogenic action of x-rays, estrogens, and methylcholanthrene in mice (2, 10); it has been found to accelerate the onset of leukemia in AKR and C38 strains of mice (11) and to give rise to an early onset of lymphoma when injected into Swiss mice at birth (6, 13). More recently, we have observed that the incidence of lymphoma was increased and accelerated when large doses of urethan were administered to adult Swiss mice (19). In rats, the urethan treatment resulted in pulmonary adenomas, hepatomas, and a few other tumors (9). However, in guinea pigs, rabbits, and chicks, urethan seems to be ineffective (3, 16).

Pietra and Shubik (14) reported that the administration of urethan in the drinking water of Syrian golden hamsters resulted in a high incidence of melanotic tumors of the skin and papillomas of the forestomach.

In the present experiments the effects of urethan in the Syrian golden hamster were further investigated with a slightly modified experimental approach, with the use of younger animals and higher concentrations of urethan.

MATERIALS AND METHODS

Syrian golden hamsters from a colony originally obtained from Abrams Small Stock Breeders, Chicago, Illinois, and bred randomly in our laboratory since 1959, were used. They were housed in plastic cages with wood shavings in groups of five, according to sex, and fed Rockland mouse diet in pellets and tap water ad libitum.

In the experimental group, 30 females and 31 males, 31-41 days of age, the females weighing 61.5 gm. (average), the males weighing 59.4 gm. (average), were used and given 0.2 per cent urethan (Fisher Scientific Co.) in the drinking water. At the beginning of the 20th week of treatment, the dose of urethan was increased to 0.4 per cent, and it was given until the 40th week, at which time the treatment was discontinued because of diarrhea. The treatment was resumed 8 weeks later and maintained for 2 weeks, which again resulted in an outbreak of diarrhea. Therefore, it was decided to stop the treatment.

The total dose of urethan administered during the whole course of treatment to each hamster could only be estimated with a certain degree of
approximation to be 10 gm. for each female and 7 gm. for each male.

The average daily water consumption per animal was 12 ml. in females and 8 ml. in males, as calculated from the water consumption for each cage, recorded throughout the treatment.

As a control, 47 females and 54 males were kept untreated.

Both experimental and control animals were carefully checked and weighed at weekly intervals, and skin changes were recorded on graph paper. Those pigmented lesions having a nodular appearance and extending for at least 2 mm. in their greatest diameter were classified as melanotic tumors. Other cutaneous lesions were also carefully recorded.

All the animals were allowed to die spontaneously or were killed with ether when in poor condition. A complete necropsy was performed on every animal, with the exception of five females and four males in the treated groups, which were lost through cannibalism. All organs were examined macroscopically, and all pathological lesions were submitted to histological diagnosis. Sections were routinely stained with hematoxylin and eosin and with special technics when needed.

RESULTS

The survival rate and the number of different types of tumors of both experimental and control groups are recorded in Tables 1 and 2.

Tumors of the forestomach.—Eighteen females

TABLE 1  
SURVIVAL RATE OF HAMSTERS TREATED WITH URETHAN AND OF UNTREATED HAMSTERS

<table>
<thead>
<tr>
<th>Hamsters</th>
<th>INITIAL NO. ANIMALS</th>
<th>No. SURVIVORS AT WEEKS OF AGE INDICATED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Urethan-treated*</td>
<td>30♀</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>31♂</td>
<td>30</td>
</tr>
<tr>
<td>Untreated</td>
<td>47♀</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>54♂</td>
<td>42</td>
</tr>
</tbody>
</table>

* 0.2–0.4 per cent in drinking water.

TABLE 2  
TUMOR INCIDENCE IN HAMSTERS TREATED WITH URETHAN AND IN UNTREATED HAMSTERS

<table>
<thead>
<tr>
<th>Hamsters</th>
<th>INITIAL NO. ANIMALS</th>
<th>NO. ANIMALS WITH COMPLETE NECROPSY</th>
<th>NO. ANIMALS WITH MULTIPLE TUMORS</th>
<th>NO. ANIMALS/NO. ADENOMATOUS POLYPS OF FORESTOMACH</th>
<th>NO. ANIMALS/NO. MELANOTIC TUMORS OF SKIN</th>
<th>TUMORS OF THE FORESTOMACH</th>
<th>MALIG- NANT LYMPHOMAS</th>
<th>MAMMARY TUMORS</th>
<th>HEPATOMAS</th>
<th>HEMANGIOMAS</th>
<th>HEMANGIOSARCOMAS</th>
<th>PULMONARY ADENOMATOSIS</th>
<th>OTHER TUMORS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urethan-treated*</td>
<td>30♀</td>
<td>25</td>
<td>21</td>
<td>5/12</td>
<td>11/11</td>
<td>18♀</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>1♀</td>
<td>1§</td>
<td>5</td>
<td>4♀</td>
</tr>
<tr>
<td></td>
<td>31♂</td>
<td>27</td>
<td>22</td>
<td>3/18</td>
<td>12/21</td>
<td>22♀</td>
<td>4</td>
<td>0</td>
<td>3</td>
<td>5♀</td>
<td>1♀</td>
<td>3</td>
<td>4♀</td>
</tr>
<tr>
<td>Untreated</td>
<td>47♀</td>
<td>47</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1♀</td>
<td>0</td>
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<td>0</td>
<td>0</td>
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<td>0</td>
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<tr>
<td></td>
<td>54♂</td>
<td>54</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2♀</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

* 0.2–0.4 per cent in drinking water.
† Eighteen animals with multiple papillomas; two animals also with squamous-cell carcinomas.
‡ Hemangiomata of spleen.
§ Hemangiosarcoma of liver.
# One squamous-cell carcinoma of vagina; one mastocytoma; one thecoma; one malignant neurilemmoma.
|| Twenty-two animals with multiple papillomas; three animals also with squamous-cell carcinomas.
** One animal with hemangiomata of spleen; two animals with hemangiomata of liver; one animal with hemangioendothelioma of spleen; one animal with hemangiomata of spleen, liver, and cecum.
†† Hemangiosarcoma of liver.
‡‡ One malignant melanoma; one cholangioma; one clear-cell adenoma of kidney; one adrenal cortical carcinoma.
§§ One papilloma.
## One adenoma of thyroid; one sarcoma of s.c. tissue.
||| One animal with hemangiomata of liver; one animal with hemangiomata of spleen and liver.
*** One cholangioma; four adrenal cortical adenomas.
broad bases were not included in the total number of lesions that appeared grossly as sessile masses with a cystic appearance, and they were often observed, three or four per animal. Only one female had two adenomatous polyps of the mucosa of the cecum. All these tumors were pedunculated. Their size ranged between 2 × 2 × 1 and 5 × 3 × 2 mm. They were supported by a central connective stalk in which an incomplete muscularis mucosae could always be distinguished. Sometimes the glanular formations were dilated up to a cystic appearance, and they were often observed in the thickness of the stalks simulating an early invasion of the stroma. Many mucus-producing cells were noted, several of them exhibiting a high degree of activity. No mitoses and no nuclear abnormalities were seen. The lumen of the dilated glands generally contained mucous material. Two lesions that appeared grossly as sessile masses with broad bases were not included in the total number of tumors: they showed an extensive infiltration with inflammatory cells that masked the histological appearance of the original structure.

Melanotic tumors.—Increased pigmentation of the skin, with the appearance of minute black spots spread over the back and flanks, was seen in many animals. Eleven melanotic tumors were found in eleven females (38 per cent) and twenty-one melanotic tumors in twelve males (39 per cent). The histological characteristics were those described by Della Porta et al. (5) resembling the blue nevi of man.

One malignant melanoma was recorded in a male hamster killed at the 84th week. The tumor appeared as a brownish nodular mass, 25 × 15 × 5 mm. in size, growing on the skin of the low back, between the two pigmented costovertebral spots, but having no relationship to them. Microscopically, the tumor was composed of polyhedral, round, or spindle-shaped cells, frequently arranged in alveolar patterns. The cytoplasm of most of the cells was loaded with melanotic pigment. Several cells showed the presence of dendritic processes in which minute granules of the pigment were also recognizable and were classified as melanocytes. In other instances, the absence of dendritic processes and the presence of large coarse amounts of pigment helped us to identify some of the cells as macrophages, mainly in the peripheral areas of the tumor. The neoplastic growth appeared well encapsulated and had no relationship to the overlying epithelium. No junctional changes were seen (Fig. 9). A metastasis was found in the lungs.

Hemangiomas and hemangiosarcomas.—In several animals, both females and males, a marked dilation of liver sinusoids was noted. Two females showed large blood lakes in the liver and one a hemorrhagic cyst in the salpinx. One male also had blood lakes in the liver. Only those lesions in which a real proliferation of new vessels and of endothelial tissue was observed were classified as hemangiomas. In five males vessel tumors were seen in different organs, in two animals in the liver, in two other animals in the spleen, and in one animal in the liver, in the spleen, and in the intestine (Fig. 5). Only one hemangioma of the spleen was observed in female hamsters. Grossly, the tumors appeared as slightly elevated, reddish-black areas on the liver surface, ranging from 4 to 9 mm. in diameter. Microscopically, most had the appearance of hemangioma cavernousum, and one had the histological pattern of benign hemangioendothelioma. Thrombosis was a common finding. The surrounding parenchyma was compressed by the tumorous growth, and some areas of necrosis were seen.

Furthermore, a hemangiosarcoma of the liver was found in one female and in one male, killed at the 40th and the 73d week from the beginning of the experiment, respectively (Fig. 6). A metastasis in the spleen was seen in the male.

It is noteworthy that all the above-mentioned findings of dilated vessels or blood lakes were seen in animals which died or were killed between the 40th and the 60th week, whereas the hemangiomas were seen between the 60th and the 80th week, with the exception of one female that died at the 50th week.

Pulmonary adenomatosis.—Areas of hyperplasia of the bronchiolar epithelium with some adenomatous structures were observed in the lungs of six females and nine males. In addition, three males and five females exhibited pulmonary lesions with the following characteristics. Groups of alveoli constituting a focal area without any demarcation from the surrounding parenchyma were lined by one or two layers of cylindrical or columnar cells, several of which secreted mucus. Only in very limited areas did the alveolar structure appear to be destroyed; otherwise, the neoplastic cells formed...
a regular lining coat. The supporting stroma was formed by the alveolar septa, often thickened through an inflammatory reaction. The presence of abundant mucous material in the alveoli lined by the neoplastic cells simulated the presence of glandular structures. Mitoses were extremely rare. These lesions were always in close proximity to a bronchiole, but continuity between the bronchiolar epithelium and the cells lining the alveoli could not be established histologically. We have called these lesions pulmonary adenomatosis, from their similarity to the lesions described in human pathology.

Other tumors and lesions.—Besides those already mentioned, nine tumors were observed in the females. One malignant lymphoma of the lymphocytic type (lymphosarcoma) was seen in one female that died at the 28th week from the beginning of the experiment. One malignant neurilemoma (Schwannoma) was found in a female that was killed at the 29th week. The tumor, 30 × 20 × 20 mm. in size, was solid, well encapsulated, whitish-pink in color. Fragments of the tumor were successfully transplanted to four male young hamsters, and presently the transplant is in its seventh generation. Microscopically the tumor was composed of intermingled bands of elongated cells. Palisading of the nuclei was evident in many fields, and characteristic Verocay bodies were also found (Fig. 10).

In addition, one squamous-cell carcinoma of the vagina, one thecoma, one malignant mastocytoma, two mammary adenocarcinomas, and one mammary fibroadenoma were recorded in the females.

Four malignant lymphomas, all of them of the histiocytic type (reticulum-cell sarcoma) (Fig. 11), two hepatomas, one cortical carcinoma of the adrenal gland, one clear-cell adenoma of the kidney (Fig. 12), and one cholangioma were found in the males.

Lesions in the control group.—Microcysts, bile duct formation, and proliferation of oval cells were observed frequently in the liver. In the females one papilloma of the forestomach, one adenoma of the thyroid, and one sarcoma of the subcutaneous tissue were seen. Four cortical adenomas, two malignant lymphomas of the histiocytic type, one papilloma of the forestomach, one cholangioma, and three hemangiomas were observed in the males. In a few animals focal areas of bronchiolar hyperplasia were found, and areas of pulmonary adenomatosis were noted in one female.

**DISCUSSION**

The finding of a high incidence of melanotic tumors of the skin and of squamous-cell papillomas of the forestomach in urethan-treated hamsters was expected from previous results obtained in this laboratory by Pietra and Shubik (14). In addition, we observed the occurrence of squamous-cell carcinomas of the forestomach in five animals, which was not previously reported.

The large number of adenomatous polyps of the cecum is noteworthy, since we did not find any in our present control groups and since these tumors have not been reported in other species following urethan administration. However, in our laboratory, small inflammatory polyps of the cecum were observed in another control group (4), and Fortner (7) reported that he found a number of intestinal polyps and adenocarcinomas in untreated golden hamsters.

The hemorrhagic lesions in the liver of urethan-treated mice have been found by several investigators and differently interpreted (8, 11, 12, 15, 17, 18). Recently, Kawamoto et al. (11), using AKR and C58 strains of mice, reported that urethan induced the formation of hemangiomata of liver, intestine, and pancreas. In our present study we observed dilated vessels and blood lakes in the liver of several urethan-treated hamsters. Furthermore, we have found hemangiomas and hemangiosarcomas in different organs of the urethan-treated animals. However, in our control group we have seen two animals with hemangiomas in the liver and spleen.

With regard to pulmonary lesions, eight animals of the urethan-treated groups developed pulmonary adenomatosis, as compared with one.
Fig. 5.—Hemangioenoma of the intestine, in a male hamster dying 78 weeks after beginning of the treatment. H. & E., ×115.

Fig. 6.—Hemangiosarcoma of the liver in a male hamster killed 73 weeks after beginning of the treatment. H. & E., ×300.

Fig. 7.—Papilloma and carcinomata of the forestomach in a male hamster killed 85 weeks after beginning of the treatment. H. & E., ×50.

Fig. 8.—Enlargement of Figure 7. Carcinomatous portion of the tumor: undifferentiated carcinoma. H. & E., ×180.
Fig. 9.—Malignant melanoma, poorly pigmented, in a male hamster killed 84 weeks after beginning of the treatment. H. & E., ×300.

Fig. 10.—Malignant neurilemmoma (Schwannoma) with so-called “Verocay bodies” in a female hamster killed 29 weeks after beginning of the treatment. H. & E., ×115.

Fig. 11.—Malignant lymphoma, histiocytic type (reticulum-cell sarcoma), lymph node in a male hamster killed 80 weeks after beginning of the treatment. H. & E., ×450.

Fig. 12.—Clear-cell adenoma of the kidney in a male hamster killed 88 weeks after beginning of the treatment. H. & E., ×110.
animal in the untreated groups. These findings suggest that the growth of these tumors is poten-
tiated by urethan.

The finding of mammary tumors and hepato-
mas appears significant, because of their extreme
rarity in the untreated animals.

One of the questions to be asked is why the
results of the present study with urethan differ
from the previous report from this laboratory in
which fewer tumors of different types were report-
ed in hamsters (14). The first factor that may be
implicated is the age of the animals. It has been
shown recently that newborn mice are particularly
sensitive to small doses of different chemical
carcinogens (13). Not only are tumors induced in
newborn mice with very small doses of carcinogen,
as compared with those required by the adult, but
the type of tumor occurring is different. In the
present study 5- to 7-week-old hamsters have been
found to develop many different tumors following
administration of urethan, as compared with 8-
to 10-week-old animals. Unfortunately, it is im-
possible to attribute this difference entirely to the
age of the animals, since the dosage of carcinogen
differed from the previous study. In the original
study, 0.2 per cent urethan was administered to
hamsters continuously; in the present study, ure-
than was given at a dosage of 0.3 per cent in the
drinking water for the first 20 weeks, and then at
a level of 0.4 per cent for 22 weeks with an inter-
ruption between the 40th and 48th weeks. It is
thus impossible to say which of these variants is
the more important. It is, however, of some inter-
rest to note once again that the full range of ac-
tivity of a carcinogen may be determined only
after many complex studies. This has been par-
ticularly notable in the instance of urethan, a
compound originally thought only to give rise to
lung adenomas and now known to have perhaps
the most widespread activity of any chemical
carcinogen.

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Multipotential Carcinogenesis with Urethan in the Syrian Golden Hamster

Bela Toth, Lorenzo Tomatis and Philippe Shubik


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