Neoplastic Response of Various Tissues to the Administration of Urethan*

ALBERT TANNENBAUM AND CESARE MALTONIT

(Department of Cancer Research,‡ Medical Research Institute, Michael Reese Hospital and Medical Center, Chicago, Illinois)

SUMMARY

Investigations on the multipotential carcinogenicity of urethan (ethyl carbamate) in mice have been extended by a series of three experiments in which the agent was administered in drinking water. Fifty-three-week-old DBA and 5-week-old C57 inbred mice were permitted to drink either 0.1 per cent or 0.3 per cent solutions and were kept on the respective treatments for periods ranging from 6½ to 31 weeks.

The effects produced further confirmed and extended the documentation that urethan is a multipotential carcinogen, regardless of the route by which it is administered. The formation of the following tumors was augmented through the action of urethan: three neoplastic lesions of the lung—adenoma, adenomatosis, and squamous-cell tumor—leukemia, mammary carcinoma, and malignant mesenchymal tumors of the interscapular fat pad. Although it is now known that urethan augments the formation of tumors in various tissues of the body, it is of special interest that in a single organ—namely, the lung—the genesis of three distinct neoplastic epithelial lesions was potentiated by this carcinogen.

Present results are correlated with previous findings from our laboratory and others, and the relationship of experimental factors to carcinogenesis is discussed.

The history of urethan carcinogenesis and the findings which led to the designation of urethan as a multipotential carcinogen were given in our initial publication concerned with the results obtained after chronic topical application of an acetone solution of this relatively simple organic compound to the skin of a number of strains of mice (20). The results of additional studies, with intraperitoneally administered urethan, and a more extended analysis were presented in 1959 at a symposium on Functional Components of Carcinogenesis, held in Rehovoth, Israel (19).

Repetition of the discussion and references in these two publications would serve no necessary purpose. It is important, however, to bring attention to more recent work which extends the documentation that urethan is a multipotential carcinogen. The high incidence of spontaneous benign hepatoma, in three substrains of C57 male mice, was further increased by intraperitoneally injected urethan (5); this effect has been confirmed (10). Oral administration of urethan to Syrian golden hamsters resulted in development of melanotic tumors of the skin and papillomas of the forestomach (14) and a number of other types of neoplasms (21). Many investigators have reported an influence of urethan upon the appearance of leukemia in mice (1, 3, 7–10, 13, 21); this will be discussed later.

The experiments reported here relate to the effects produced in mice administered urethan (ethyl carbamate) in drinking water. They were undertaken with the expectation that through this route of administration and possibly more continuous exposure to urethan, a greater number of tissues might respond. The experimental results confirmed our previous findings regarding the influence on the pulmonary adenoma, mammary carcinoma, and malignant mesenchymal tumors of the interscapular fat pad; but in addition it was found that urethan potentiated the genesis of two other types of lung tumors and of leukemia.

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† Research Associate during 1959 and 1960. Present address: Istituto di Patologia Generale, Università di Bologna, Italy.

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MATERIALS AND METHODS

The mice utilized were from inbred strains DBA and C3H, raised in this laboratory by brother-to-sister mating for many years. The animals were weaned at about 30 days of age, at which time they were divided into groups by litter-mate distribution, insofar as possible, and numbered. From weaning they were fed ad libitum an adequate commercial diet, Purina Laboratory Chow Checkers. The animals were housed in sets of five in metal wire cages with solid bottoms; bedding consi-}

sisted of a shallow layer of a mixture of sterilized wood shavings and peat moss. The mice were kept in a temperature-controlled laboratory at 80°F. Weighed amounts of white, crystalline urethan (ethyl carbamate), reagent-grade, were made up to measured volumes in distilled water and placed in the drinking bottles of the treated groups. The solutions were either 0.1 or 0.3 per cent in concentration. The construction of the bottles prevented loss through spillage, and the graduations permitted the determination of urethan solution consumed by the animals. Distilled water in similar drinking bottles was available to the mice of the control groups.

Consumption of water by the controls and of urethan solutions by the treated mice was measured several times during the course of treatment, and averages were estimated for the whole period. The mice were weighed and examined for general condition and external tumors at 2-week intervals. (When the average weights of the groups appeared to have stabilized, weighing was performed at 4-week intervals.)

During the course of the studies, superficial tu-

mors were recognized, described, and recorded. Mice dying during the experiment and those sacrificed at its termination were examined for neoplasms and other pathology. Specimens were taken from all tumors, and often from other tissues, for histologic study. The tissues were fixed in 10 per cent formalin, processed and sectioned, and stained with hematoxylin and eosin.

Each of the three studies described consisted of two or three groups. To lessen repetition (and crowding of data in the tables) a summary of the experimental design and factors other than tumor formation is given in Table 1.

TABLE 1

<table>
<thead>
<tr>
<th>EXPERIMENT AND GROUP NO.</th>
<th>MICE</th>
<th>TREATMENT</th>
<th>AV. WEIGHT OF MICE</th>
<th>DURATION OF EXPERIMENT, START TO END (WK.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>URETHAN IN DRINKING WATER (PER CENT)</td>
<td>Av. intake per mouse (mg.)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DURATION (WK.)</td>
<td>WEEKLY</td>
</tr>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td>None</td>
<td>31</td>
</tr>
<tr>
<td>Group 1</td>
<td>DBA♂</td>
<td>61</td>
<td>53</td>
<td>31</td>
</tr>
<tr>
<td>Group 2</td>
<td>DBA♀</td>
<td>62</td>
<td>53</td>
<td>0.1</td>
</tr>
<tr>
<td>Group 2</td>
<td>DBA♂</td>
<td>57</td>
<td>53</td>
<td>0.3</td>
</tr>
<tr>
<td>Group 3</td>
<td>DBA♀</td>
<td>54</td>
<td>53</td>
<td>22</td>
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<td>53</td>
<td>53</td>
<td>22</td>
</tr>
<tr>
<td>Group 4</td>
<td>CSH♀</td>
<td>56</td>
<td>5</td>
<td>None</td>
</tr>
<tr>
<td>Group 4</td>
<td>CSH♂</td>
<td>56</td>
<td>5</td>
<td>None</td>
</tr>
</tbody>
</table>

RESULTS

The experiments proceeded smoothly and satisfactorily. The only untoward occurrence was the general toxic effect, particularly in Group 3, Experiment 1, receiving 0.3 per cent urethan in drinking water. This is mirrored in (a) the disparity between the number of mice in a group at the beginning of an experiment and at the time the first tumor appeared, indicating the deaths that had occurred (compare "corrected number" in Tables 2, 3, and 4 with "number at start" in Table 1); and (b) the average body weights of the animals at specified periods during the study (Table 1).

NEOPLASMS OF THE LUNG

The carcinogenicity of urethan was first demonstrated in 1943 when Nettleship, Henshaw, and Meyer (12) observed that this agent readily enhanced the formation of the pulmonary adenoma in the mouse. This finding has been amply documented by many investigators. Of interest in the
present study is the appearance of two other pulmonary neoplasms: adenomatosis (alveolar-cell tumor) and squamous-cell tumor.

Papillary adenoma.—In all three experiments significant numbers of pulmonary adenomas arose in the urethan-treated mice. Only two control C3H mice, in Group 1, Experiment 3, developed this tumor, whereas none were found in the control DBA mice of Experiments 1 and 2. However, we have observed these pulmonary adenomas in low incidence in DBA mice over 20 months of age, and others have also reported a very low incidence of this tumor in their sublines of strains C3H and DBA. These facts support the view that urethan nodules generally found on the surface of the lung—a significant number of urethan-treated DBA mice exhibited subpleural, whitish-gray plaques, flush with or slightly depressed from the surface of the lung. These lesions were not sharply demarcated from the surrounding lung tissue and at times were in close proximity to or confluent with adenomas. They measured up to a few millimeters in size.

Microscopically, the most prominent characteristic of these lesions is a replacement of the normal alveolar cells by a single-layered cuboidal or columnar nonciliated epithelium (Figs. 6 and 7). The nuclei of the lining cells are well outlined and stain relatively deeply with hematoxylin, and the cyto-

| TABLE 2 |
| URETHAN AND PULMONARY NEOPLASMS |

<table>
<thead>
<tr>
<th>EXPERIMENT AND GROUP NO.</th>
<th>MICE</th>
<th>TREATMENT</th>
<th>MICE BEARING INDICATED PULMONARY TUMORS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Strain and sex</td>
<td>Corrected no.*</td>
<td>Urethan in drinking water (per cent)</td>
</tr>
<tr>
<td>-------------------------</td>
<td>----------------</td>
<td>----------------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>Experiment 1</td>
<td>DBA♂</td>
<td>59</td>
<td>None</td>
</tr>
<tr>
<td>Group 1</td>
<td>54</td>
<td>0.1</td>
<td>10</td>
</tr>
<tr>
<td>Group 2</td>
<td>58</td>
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<td>0</td>
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<td>Group 3</td>
<td>22</td>
<td>0.1</td>
<td>11</td>
</tr>
<tr>
<td>Group 4</td>
<td>14</td>
<td>0.3</td>
<td>2</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>CSH♀</td>
<td>38</td>
<td>None</td>
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<tr>
<td>Group 1</td>
<td>32</td>
<td>0.1</td>
<td>15</td>
</tr>
<tr>
<td>Group 2</td>
<td>36</td>
<td>None</td>
<td>0</td>
</tr>
</tbody>
</table>

* Number of mice in each group of an experiment at the time the first adenoma was detected in the particular experiment. Percentages of tumor-bearing animals are based on these corrected numbers of mice.

potentiates the formation of this neoplasm. The data in Table 2 refer only to mice with grossly visible pulmonary adenomas.

The pulmonary adenoma is generally well circumscribed and papillary in structure. It appears to grow by expansion, often compressing the surrounding alveoli and bronchi. A small proportion of the lesions invade bronchi, and a few have histologic characteristics suggestive of papillary adenocarcinoma. Some of these features are illustrated in Figures 1–4. The benignancy or malignancy of these pulmonary lesions has been the subject of discussion for many years; our studies throw no new light on the subject. We take the position that most of these lesions are benign but that a number might be considered malignant.

Adenomatosis (alveolar-cell tumor).—In addition to the readily recognized adenomas of the lung—elevated, pearly white, usually well circumscribed plasm is markedly eosinophilic. The cytoplasm may contain fine granules, generally located between the basally situated nucleus and the free border of the cell. The lesion, which we interpret as a form of pulmonary adenomatosis, does not distort, compress, or invade the bronchi or blood vessels (Fig. 8).

This interesting lesion of the lung, focal or diffuse in distribution, has been designated by various names; pulmonary adenomatosis, alveolar-cell tumor, and alveolar-cell carcinoma are but a few of the terms used in the extensive literature dealing with the subject. These exemplify the diversity of opinion that exists as to the origin and nature of this lesion.

Table 2 lists the number and incidence of these alveolar-cell tumors in the DBA urethan-treated mice of Experiments 1 and 2 (not found in the C3H mice, Experiment 3). None of the control
mice exhibited similar gross findings, but routine sections of the lungs of three of these animals revealed the characteristic lesion (Fig. 5). Thus it appears that urethan augments the formation of the alveolar-cell tumor (adenomatosis) in DBA mice.

**Squamous-cell tumor.**—A third type of neoplastic lesion of the lung was found in the DBA mice, Experiments 1 and 2, but not in their control groups or in the control or urethan-treated CSH mice, Experiment 3. It was generally seen, on microscopic study, in the lungs of animals that also had papillary adenomas and alveolar-cell tumors. The lesions were found in various forms or stages. In the lungs of some mice, clusters of alveoli were partially or completely filled with squamous epithelial cells forming nests which obliterated the lumina (Figs. 11 and 12). These nests were distributed either focally or in a diffuse manner. In some sections, mitoses were seen; in others, signs of invasiveness. In the lungs of other animals, large, nodular lesions were observed grossly. On microscopic examination these lesions did not have the nest structure described above, particularly in the central portion of the tumor where large epithelial pearls were present (Fig. 10). At this time, we regard these lesions as neoplastic but benign and have therefore designated them squamous-cell tumors; however, some of these might be classified as carcinomas. The numbers and frequencies of squamous-cell tumors are summarized in Table 2.

Although this type of lung tumor was not found in any of the untreated DBA mice of Experiments 1 and 2, a pulmonary squamous-cell carcinoma with the characteristics described has been observed in this laboratory in 1 of 24 untreated DBA mice, 22 months of age (Fig. 9).

**Leukemia**

The terminology of neoplastic diseases of the lymphoreticular system, in the mouse as well as in man, abounds in ambiguity and confusion. Recognition and classification of these neoplastic diseases have been based on either histopathology, gross anatomic distribution within the body, or clinical findings (physical signs and hematologic manifestations)—or on combinations of these three criteria.

Clinicians, pathologists, hematologists, and those engaged primarily in animal experimentation have adopted various generic nomenclatures such as leukemia; malignant tumors of the lymphoreticular system; and malignant lymphoma. Some authors use malignant lymphoma as the generic term for many of these diseases, even including the myeloid types, whereas others prefer leukemia as the inclusive term, irrespective of whether or not malignant cells are found in the peripheral blood.

It is important for those individuals unfamiliar with the lack of uniformity in terminology and standards of diagnosis in this area of research and clinical oncology to realize that they may be reading about the same disease under a number of different names. There are excellent, extensive articles that present the various arguments and viewpoints (2, 4, 15, 18). Because of its common usage, this section has been arbitrarily entitled “Leukemia” (encompassing a number of more or less related conditions), even though “Malignant Diseases of the Lymphoreticular System” may be more appropriate.

In our studies the clinical condition of the animals and the presence of enlarged superficial lymph nodes were observed and recorded at the regular inspection periods, but examination of the blood and bone marrow was not a part of the experimental technic, either before or after death. However, the results presented here are based on gross autopsy findings and microscopic study of sections.

In a pilot experiment, not recorded in the tables, 50 CSH females, 14 weeks of age, were given 0.3 per cent urethan in drinking water for 6½ weeks. The toxic effects were sufficient to cause an appreciable number of deaths, resulting in a decision to terminate the study when the mice were 52 weeks old. Two of these mice developed lymphatic leukemia, whereas none was found in the 50 control mice. Statistical significance is not attached to these data; they are mentioned only to give one of the indications for Experiment 3, described below.

In Experiment 3, eight of the 36 CSH mice in the group which was given 0.1 per cent urethan developed leukemia (Table 3). The average age at death of the animals bearing these neoplasms was 46 weeks. The involvement and gross and microscopic characteristics of the tissues differed. Five were of the lymphocytic type (lymphatic leukemia) revealing enlargement of superficial and internal lymph nodes, thymus and spleen, and involvement of liver, kidneys, and lung—all or in different combinations. Characteristic involvement and infiltrations were observed histologically. Three mice had myelogenous leukemia.

Leukemia was not found in any of the 36 control CSH mice of this experiment, but has been observed in an unrelated study in four of 129 male, untreated control mice of our CSH subline—one at 13 months and three at 19 months of age.

In Experiments 1 and 2, male and female DBA...
mice first began drinking the urethan solutions when they were 53 weeks of age. They developed lymphatic leukemia in an incidence and at an average age comparable to the controls.

The augmentation of leukemia in CSH female mice, placed on urethan at 5 weeks of age, appears to be greater than in the pilot group that received larger amounts but which began when they were 14 weeks old. Moreover, the effect is in sharp contrast to the lack of response in both male and female DBA mice administered urethan in even greater amounts but at 55 weeks of age. This is readily apparent from Table 3.

MAMMARY CARCINOMA

Administration of urethan in drinking water enhanced the formation of mammary carcinomas, confirming and extending our previous observations (19, 20). The results are shown in Table 4. With this neoplasm it is possible and also helpful to utilize three criteria: incidence, average age at appearance of the first tumor in each mouse, and average number of tumors per tumor-bearing animal.

In Experiment 2 with DBA mice, potentiation of mammary carcinoma formation by urethan is demonstrated by all three indicators: increased in-

TABLE 3
URETHAN AND LEUKEMIA

<table>
<thead>
<tr>
<th>Experiment and group no.</th>
<th>Strain and sex</th>
<th>Corrected no.*</th>
<th>Treatment</th>
<th>Mice with leukemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Urethan in drinking water (per cent)</td>
<td>No.</td>
</tr>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td>None</td>
<td>9</td>
</tr>
<tr>
<td>Group 1</td>
<td>DBA Q</td>
<td>61</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>Group 2</td>
<td>DBA Q</td>
<td>61</td>
<td>0.1</td>
<td>11</td>
</tr>
<tr>
<td>Group 3</td>
<td>DBA Q</td>
<td>57</td>
<td>None</td>
<td>7</td>
</tr>
<tr>
<td>Group 4</td>
<td>DBA Q</td>
<td>54</td>
<td>0.1</td>
<td>8</td>
</tr>
<tr>
<td>Group 5</td>
<td>DBA Q</td>
<td>14</td>
<td>0.3</td>
<td>2</td>
</tr>
<tr>
<td>Group 6</td>
<td>DBA Q</td>
<td>56</td>
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<td>0</td>
</tr>
<tr>
<td>Group 7</td>
<td>DBA Q</td>
<td>56</td>
<td>0.1</td>
<td>8</td>
</tr>
</tbody>
</table>

* Number of mice in each group of an experiment at the time the first leukemia was detected in the particular experiment. Percentages of mice with leukemia are based on these corrected numbers.

† Age at recognition of the disease—in some instances clinically, in others at post-mortem examination.

TABLE 4
URETHAN AND MAMMARY CARCINOMAS

<table>
<thead>
<tr>
<th>Experiment and group no.</th>
<th>Strain and sex</th>
<th>Corrected no.*</th>
<th>Treatment</th>
<th>Mice with mammary carcinomas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td>Urethan in drinking water (per cent)</td>
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</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td>None</td>
<td>13</td>
</tr>
<tr>
<td>Group 1</td>
<td>DBA Q</td>
<td>57</td>
<td></td>
<td>54</td>
</tr>
<tr>
<td>Group 2</td>
<td>DBA Q</td>
<td>54</td>
<td>0.1</td>
<td>54</td>
</tr>
<tr>
<td>Group 3</td>
<td>DBA Q</td>
<td>14</td>
<td>0.3</td>
<td>11</td>
</tr>
<tr>
<td>Group 4</td>
<td>DBA Q</td>
<td>56</td>
<td>None</td>
<td>29</td>
</tr>
<tr>
<td>Group 5</td>
<td>DBA Q</td>
<td>56</td>
<td>0.1</td>
<td>30</td>
</tr>
</tbody>
</table>

* Number of mice in each group of an experiment at the time the first mammary carcinoma was detected in the particular experiment. Percentages of tumor-bearing animals are based on these corrected numbers of mice.

† Average of the ages of mice at the time their first mammary tumors appeared.
cidence, earlier average time of appearance, and greater multiplicity. The latter two criteria also clearly indicate the augmenting effect on mammary carcinoma in the C3H mice of Experiment 3. However, the potentiating action is not shown by the incidence data, but this is expected in a situation where the normal or spontaneous incidence approaches 100 per cent. Generally all our female CSH mice develop this neoplasm spontaneously if they are permitted to live out their life-span. However, this experiment was terminated when the mice were 81 weeks of age, at which time there were five survivors without mammary tumors, all in the control group. The lower than expected incidence of mammary tumors in the urethan-treated group was due to early deaths of several mice having leukemia.

TUMORS OF INTERSCAPULAR FAT PAD
Malignant mesenchymal neoplasms of the interscapular fat pad were found in six mice given urethan orally: three DBA mice of Group 2, Experiment 1, and three C3H mice of Group 2, Experiment 3. They were recognized clinically as soft, subcutaneous interscapular swellings which on post-mortem examination revealed well circumscribed, spongy, blood-filled tumors. Tumors of this type were not found in any of the control mice.

VASCULAR-HEMORRHAGIC LESIONS
Vascular-hemorrhagic lesions were observed in the liver, interscapular fat pad, mammary tumors, lungs, and other tissues of the urethan-treated mice. They were of two types: blood cysts and frank hemorrhages. In our opinion these lesions are mainly the end result of vascular injury and are not neoplasia.

In the liver, passive hyperemia and dilatation of the sinusoids were noted; this process often progressed to the formation of blood cysts or lakes that could be recognized grossly. The incidences in the groups ranged from 11 to 48 per cent. The lesions were, on the average, less extensive than those previously reported.

Although a few urethan-treated mice developed neoplasms of the interscapular fat pad, many more had pinpoint hemorrhages or blood-filled blebs in this tissue—found at autopsy on retraction of overlying skin.

The histologic sections of the mammary tumors of the urethan-administered mice revealed interesting findings: (a) dilatation of blood vessels; (b) dilated mammary ducts filled with secretion; (c) hemorrhage into some of the mammary ducts; and (d) diffuse hemorrhage into the stromal tissue.

The mammary tumors of the untreated mice also revealed ducts filled with secretion and some contained hemorrhage, but in these the findings were of far lesser degree than those observed in the urethan-treated mice.

It is of note that the mammary carcinomas, along with the dilatation of blood vessels and increased amount of secretion and hemorrhage in the ducts, occur long after the termination of urethan treatment. Are these phenomena related to injury of blood vessels by urethan or indirect hormonal modifications, or both?

DISCUSSION
In the present investigation, it is demonstrated that urethan augments the formation of six neoplastic lesions in the mouse: pulmonary adenoma, pulmonary adenomatosis, pulmonary squamous-cell tumor, leukemia, mammary carcinoma, and malignant mesenchymal tumors of the interscapular fat pad. The enhancement of the genesis of three distinct neoplastic epithelial lesions in a single organ, the lung, emphasizes the multipotentiality of this carcinogen.

The augmentation of the pulmonary adenoma, mammary carcinoma, and malignant mesenchymal tumors of the interscapular fat pad occurred in the same qualitative manner as previously reported for other routes of administration. However, the appearance of pulmonary adenomatosis, pulmonary squamous-cell tumor, and leukemia was not observed in our earlier studies. A discussion of these tumors and the relationship of our findings to those of other investigators are therefore warranted.

Pulmonary adenomatosis (alveolar-cell tumor).—As far as we know, the first report of pulmonary adenomatosis in mice appeared in 1947. Various strains of mice were given orally an aqueous olive-oil emulsion containing either methylcholanthrene or dibenzanthracene in place of drinking water. Additional details of these experiments in a later publication reveal that nine of 40 DBA mice, ranging from 5 to 9 months in age, developed pulmonary adenomatosis. Only one lesion of this nature was seen in the lungs of 125 untreated mice examined. The authors also report the finding of pulmonary adenomatosis in an untreated, virgin wild mouse and in an untreated male CSHb mouse, both more than 19 months of age.

In the present studies with DBA mice, 43 of 120 mice which were given urethan in drinking water developed pulmonary adenomatosis. Only three of 115 untreated controls exhibited such lesions. None were observed in either the treated or untreated C3H mice. Of interest and significance is a
recent publication which reports the occurrence of pulmonary adenomatosis in the Syrian golden hamster given urethan in drinking water (22).

Pulmonary adenomatosis is of importance from both health and economic viewpoints in that it is a disease not only affecting laboratory animals but also man, sheep, goat, horse, etc. (16, 17).

Pulmonary squamous-cell tumors.—Nests of squamous cells in the alveoli or frank squamous-cell tumors were observed in eighteen of 120 DBA mice treated with urethan. None were found in the DBA control mice or in C3H mice, treated or untreated. However, a pulmonary squamous-cell carcinoma was found in one of 24 untreated 22-month-old DBA mice of an unrelated experiment. Only a small proportion of primary spontaneous tumors of the lungs of mice are malignant; of these, relatively few have been considered to be squamous-cell carcinomas (23, 24).

What are the reasons for our use of the term tumor for this lesion rather than carcinoma? The lesions were found, almost always, on histologic examination of the sections, so that no opportunity existed for transplantation. They were always observed in old animals, and no metastases were evident. Thus the main criteria are histologic, and here there is room for disagreement. Are the lesions metaplastic or neoplastic, and, if the latter, benign or malignant? After much study and excellent consultation, we have reached the opinion that the conservative course would be to designate these lesions as pulmonary squamous-cell tumors. Obviously, experiments specially designed to clarify this area are indicated.

Leukemia (malignant lymphoma).—That urethan is leukemogenic was first demonstrated by Kirschbaum and associates: it remarkably augmented the leukemogenic activity of x-rays, estrogenic hormones, and methylcholanthrene in strains C57BL, BALB/c, and DBA/2 mice (7, 9). Urethan was not found to be leukemogenic for these low-leukemic strains. Later, these workers claimed that urethan alone accelerated the onset of leukemia in high-leukemia strains, AKR and C58 (8).

Other investigators have reported that urethan administered after x-rays had an augmenting effect in C57BL/6 mice (1) and that urethan alone significantly increased the formation of malignant lymphoma (leukemia) in newborn and young adult Swiss (3, 13, 21) and newborn C3Hf mice (10). Malignant lymphomas have been reported in both untreated and urethan-treated Syrian golden hamsters (22) and, although the frequencies were low, it is likely that urethan enhances the formation of this neoplasm.

Our findings in the C3H mice (Experiment 3), in which urethan administration in drinking water was begun when they were 5 weeks of age, confirm and extend the fact that urethan is leukemogenic. The various studies reported, utilizing numerous strains of mice, suggest the phenomenon has generality and that with adequately chosen experimental factors the leukemogenic-augmenting property of urethan will be demonstrated for many low- and high-leukemic strains of mice, and even other species.

Factors of the experiments and carcinogenesis.—The appearance of pulmonary adenomatosis and pulmonary squamous-cell tumors in DBA mice—not observed in our previous studies—probably resulted from larger dosages of urethan (mirrored by greater toxicity) and was detected because of more extensive histologic examination of the tissues. In addition to obvious gross lesions, only a very limited number of sections of lung tissue were generally studied. In all probability, a morphologic study in which the tissues were examined serially would reveal a far higher frequency of pulmonary lesions than reported herein.

It is likely that the reason for obtaining a leukemogenic response in Experiment 3 (C3H mice) is the early age at which these animals were placed on urethan. Although the response was not high, it was in sharp contrast to the lack of effect in the C3H and DBA mice of our earlier investigations (19) or in the DBA mice of Experiments 1 and 2 of the present study. These groups, not showing enhancement of leukemogenesis, were placed on experimental treatment at ages ranging from 7 to 53 weeks. Although other factors, such as administering the carcinogen in a shorter period, might have played a role in evoking leukemia in Experiment 3, it appears that the major factor was the greater susceptibility of the young mice. We hazard the guess that young DBA mice would also respond to the agent.

It is not possible at this time to give the exact reasons for diverse results in our experiments and in those of others. However, there are host, environmental, and experimental factors that might dictate differences. In this respect urethan is not unique—as with other carcinogens, tumor response is dependent on the genetic characteristics of the species and strain; route of administration of the agent (distribution and concentration of the carcinogen in various tissues); dosage; periodicity and duration of treatment; toxicity and effect on metabolism and body weight; age at which treatment is instituted; and the duration of the experiment. We have previously emphasized the great importance of excellent laboratory and housing condi-
tions that permit a long, healthy life-span for animals and thereby a complete expression of neoplastic potentials.

Practically all the tumor-types reported by us and others to be influenced by urethan administration occur spontaneously ("normally expected"), sometimes in low frequency at a late age, in the animals that have been utilized. If and when urethan administration increases this frequency, as indicated by both higher incidence and shortened average latent period, the action can be looked upon as augmentation, enhancement, or potentiation rather than induction de novo. This concept has been discussed in our previous urethan publications (19, 20).

REFERENCES

FIG. 1—Urethan and papillary adenoma
OF THE LUNG

FIG. 1.—Low power of a papillary adenoma of the lung in a treated mouse showing its circumscribed nature. Hematoxylon and eosin, X75.

FIG. 2.—Pulmonary papillary adenoma in a treated mouse. Note the large vesicular nuclei. Hematoxylon and eosin, X170.

FIG. 3.—Pulmonary papillary adenoma in a treated mouse. Note the small, darkly stained nuclei. Hematoxylon and eosin, X170.

FIG. 4.—Pulmonary papillary tumor in a treated mouse. Note the tumor tissue projecting into a bronchus. Hematoxylon and eosin, X170.
FIGS. 5–8.—URETHAN AND ADENOMATOSIS (ALVEOLAR-CELL TUMOR) OF THE LUNG

Fig. 5.—Pulmonary adenomatosis in a control DBA mouse. Hematoxylin and eosin, ×300.

Fig. 6.—Pulmonary adenomatosis in a treated DBA mouse. Hematoxylin and eosin, ×300.

Fig. 7.—Pulmonary adenomatosis in a treated DBA mouse. Under high magnification the glandular structure and single-layered cuboidal epithelium are evident. Hematoxylin and eosin, ×450.

Fig. 8.—Pulmonary adenomatosis in a treated DBA mouse. Note the lack of compression of the bronchus. Hematoxylin and eosin, ×300.
Figs. 9–12.—Urethan and squamous-cell tumor of the lung

Fig. 9.—Nodular squamous-cell pulmonary tumor in an old, untreated DBA mouse. Note the epithelial pearls. Hematoxylin and eosin, X170.

Fig. 10.—Nodular squamous-cell pulmonary tumor in a treated DBA mouse, also showing large epithelial pearls. Hematoxylin and eosin, X170.

Fig. 11.—Squamous-cell pulmonary tumor in a treated DBA mouse, showing nests of tumor cells in the alveoli. Hematoxylin and eosin, X285.

Fig. 12.—High magnification of another squamous-cell pulmonary tumor, with nests of cells, in a treated DBA mouse. Hematoxylin and eosin, X540.
Neoplastic Response of Various Tissues to the Administration of Urethan

Albert Tannenbaum and Cesare Maltoni


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