Multipotential Carcinogenicity of Urethan in the Sprague-Dawley Rat*

ALBERT TANNENBAUM, S. D. VESSELINOVITCH, CESARE MALTONI,† and D. STRYZAK MITCHELL

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

SUMMARY

These investigations are concerned with the neoplastic response to urethan (ethyl carbamate) of inbred Sprague-Dawley rats permitted to live out their life span. The carcinogen was administered to male and female animals either intraperitoneally or in drinking water.

Urethan significantly potentiated the formation of benign mammary tumors and carcinomas of the sebaceous gland (Zymbal) of the external auditory canal; it apparently enhanced the development of angiomas and sarcomas at various sites; and it induced malignant lymphomas, tumors of the kidney, and neurofibrosarcomas of the ear. In addition, other tumor types were observed in appreciable numbers, but their significance is equivocated by comparison with similar tumors arising in controls that had a longer average life span.

Untreated controls, inbred Sprague-Dawley rats, developed many types of neoplasms, and the findings augment and extend the information in the literature on the incidence and average age at appearance of these spontaneous tumors. Three of 105 untreated control animals developed carcinomas of the sebaceous gland (Zymbal) of the external auditory canal at an average age of 115 weeks. As far as we know, this tumor has never been previously reported as occurring spontaneously.

The multipotential carcinogenicity of urethan in a number of species and the similarity of response of a particular tissue to various carcinogenic agents are discussed.

The multipotential carcinogenicity of urethan was first demonstrated in the mouse (reviewed in [18–20]). Later, its broad spectrum action in the Syrian golden hamster was clearly shown (22). Although it has been claimed that chickens and guinea pigs do not develop tumors of the lung or other organs through prolonged urethan administration (2), the small number of animals utilized and the limited duration of the experiments render these findings inconclusive.

This report is concerned with investigations on the neoplastic response to urethan of inbred Sprague-Dawley rats permitted to live out their life span. It enforces the documentation that urethan has a carcinogenic action upon many tissues of the body and adds some information as to how urethan may produce its effects.

MATERIALS AND METHODS

Rats.—The animals utilized in the investigations were from our colony of Sprague-Dawley rats. They originated from a litter purchased in 1951 from the Sprague-Dawley Company, Madison, Wisconsin, and have been inbred since then by brother-to-sister mating. The animals were weaned and classified as to sex when they were 4–5 weeks old, at which time they were numbered by ear punch and divided into groups by litter-mate distribution, insofar as possible.

From weaning, the animals were fed ad libitum...
an adequate commercial diet, Purina Laboratory Chow Checkers. They were housed in groups of three in metal wire cages with solid bottoms; a shallow layer of a mixture of sterilized wood shavings and peat moss served as bedding. The rats were kept in a temperature-controlled laboratory at 80° F.

**Urethan administration.**—The experimental groups were administered urethan (ethyl carbamate) either intraperitoneally or in drinking water. For the intraperitoneal injections, weighed amounts of white, crystalline urethan, reagent-grade, were made up to measured volumes in distilled water; this was done biweekly, and the solution was kept in a refrigerator. Groups receiving urethan orally were given 0.1 per cent concentration of the agent in drinking water. The construction of the drinking bottles prevented loss through spillage, and the graduations permitted the determination of urethan solution consumed by the animals. Tap water in similar drinking bottles was available to the rats of the control groups and to those given urethan intraperitoneally.

**Procedures during experiments.**—Consumption of water or of urethan solution was measured at various times during the course of an experiment, and averages were estimated for the whole period. The rats were inspected at 2-week intervals for their general condition and for external tumors. They were weighed at 4-week intervals.

During the course of the studies superficial tumors were described and recorded. All the rats were allowed to live their life span—only those that were moribund were killed. At autopsy, gross descriptions of all external and internal tumors, as well as other pathologic conditions, were recorded. Specimens were taken from all neoplasms, and often from other tissues, for histologic study. The tissues were fixed in 10 per cent formalin, processed and sectioned, and routinely stained with hematoxylin and eosin. In some instances special stains were employed.

**Plan of the studies.**—Altogether seven experiments are reported, four in which virgin females were utilized and three with males. There were one to four groups to an experiment, and each group contained fifteen rats. The experiments were begun at different times over a 1-year period. The factors of the experiments and of urethan administration are given in Table 1.

**RESULTS**

The longevity of the control animals in the several experiments—an average life span of 106 weeks for females, 110 weeks for males—is a measure of their satisfactory course. In the main, rats in both control and experimental groups died of neoplasms and/or the chronic toxic effect of the carcinogen. These events are mirrored in the relative survival rates shown in Table 1, columns 10-12. The studies were singularly undisturbed by intercurrent infections or laboratory accidents.

Although the dosage of urethan, duration of treatment, and route of administration were not the same for each experiment, the qualitative findings were in general of the same order. It was learned that urethan potentiates the formation of many types of tumors that occur spontaneously in the Sprague-Dawley rat and also induces other types not observed by us in untreated controls.

**Mammary Tumors**

In this report, the designation of mammary tumors encompasses all neoplasms that arose in mammary tissue. The great majority of these were benign, and individual tumors ranged from those that had predominantly fibrous elements (Fig. 1) to those that were mainly glandular (Fig. 2) (adenofibroma, fibroadenoma, adenoma). In many histologic sections combinations of the various types were found, and therefore they were classified as a group—i.e., fibroadenomas. Some of these neoplasms showed the additional feature of secretory activity and cysts (cystadenoma and papillary cystadenoma), and, indeed, this was expected from the gross recognition of milk cysts. Malignant mammary tumors were observed in a small per cent of rats.

**Untreated control female rats.**—It is obviously necessary to have a baseline for evaluation of the influence of urethan administration on the formation of mammary tumors. The spontaneous occurrence of such neoplasms in Sprague-Dawley rats has been reported by a number of investigators (3, 10, 21). In general, their rats had been purchased from dealers, and often the animals were randomly bred females that had raised litters. Our subline has been inbred, and the females utilized were virgin.

Four groups of fifteen animals each were designated as untreated controls (Groups 1 of Experiments 1, 2, 3, and 4). These came from three different breeding schedules over a period of 1 year, and the rats were litter-mates of those given urethan. Chart 1 presents the cumulative curve of the appearance of mammary tumors in these untreated control female Sprague-Dawley rats. Only the age at which animals developed their first mammary tumors was taken into account, regardless of whether they bore more than one such neoplasm. The curve, fitted by eye, is S-shaped and reveals: (a) The tumors did not appear until the
rats were about 50 weeks of age, (b) 50 per cent of the animals bore this neoplasm when approximately 90 weeks old, and (c) late in life, the over-all incidence was as high as 85 per cent (18/15, 11/15, 13/15, and 14/15), even though calculated on the basis of the number of animals at the beginning of the experiments (not taking into account deaths from other neoplasms and non-neoplastic pathology). The actual incidences and other data are given in more detail in Table 1.

It is of some interest that the results of other workers, utilizing commercial, randomly bred and often parous, untreated Sprague-Dawley rats, fall close to the curve in Chart 1 for our inbred rats (3, 10, 21).

**Urethan-treated female rats.**—The influence of an experimental agent on mammary carcinogenesis can be measured by three criteria: incidence, average age at appearance, and multiplicity of tumors. In the present studies mammary neoplasms were observed in high frequency in the female rats administered urethan, but this was not surprising since the tumors occur spontaneously in high frequency. What cannot be demonstrated by comparison of incidence, however, is clearly shown by the decided acceleration of the genesis of these mammary tumors in the urethan-treated female rats (shorter latent period). The average age at which the first mammary tumors arose in the rats that received urethan was 58 weeks, in comparison with 87 weeks for the controls. In addition, the treated rats generally developed a larger average number of tumors per animal than did the corresponding controls, even though the latter should have been favored because of a longer life span and the absence of toxicity. This was not invariable, as can be seen in Experiment 3 in which urethan administration was begun when the rats were 32 weeks of age. These data and factors of the experiments are given in Table 1 (Experiments 1, 2, and 3).

In Chart 2, the probits of the percentages of rats bearing mammary tumors are plotted against the logarithm of the age of the rats in weeks, for the combined controls and three experimental groups, and the straight lines were fitted by inspection. Very nearly linear relationships are obtained in each case, and the shift from the control curve to the left reveals that urethan accelerates the formation of this neoplasm. Shown in the chart are only the experimental groups in which toxicity played a minor role. It is recognized that the experiments differ in dosage utilized, and in the duration and route of treatment. Although this series does not represent an age-response study, the impression is gained that, at least in animals up to 7 months of age, the latent period of mammary tumor formation is relatively the same (about 45 weeks) if calculated from the time at which urethan was first administered.

**Urethan-treated male rats.**—Mammary tumors were observed in three to five rats in each of five groups composed of fifteen males administered
urothelial. In comparison, such tumors were found in the three corresponding groups of untreated controls in five, seven, and eight animals. It is probable that the treatment with the carcinogen did not augment the incidence of mammary tumors in male rats because of toxicity and greater mortality. However, potentiation was suggested by a shortening of the latent period. These points are found in the data of Experiments 5, 6, and 7 in Table 1.

Mammary adenocarcinoma.—In addition to the neoplasm most commonly encountered, benign mammary tumors, a small number of malignant mammary neoplasms (adenocarcinoma, cystadenocarcinoma, and papillary cystadenocarcinoma) were observed in both male and female rats (Figs. 3, 4). During inspection, they were recognized as differing from the benign tumors in that the former appeared to be attached to the dermis and generally ulcerated while relatively small. At autopsy, it was difficult to separate them from surrounding tissue. These characteristics are in contrast to those of benign mammary tumors, which when small (1–2 cm.) were relatively movable, not being firmly attached to the skin. Ulceration of the skin was generally found, however, when these benign tumors became much larger.

Urethane administration appeared to have had no demonstrated effect upon the genesis of mammary adenocarcinomas: When male and female controls and corresponding urethane-treated animals were combined, both groups had an over-all incidence of about 4 per cent.

Carcinomas of Sebaceous Gland (Zymbal) of the External Auditory Canal

The sebaceous gland of the external auditory canal has been described by Zymbal (27) and Zawisch-Ossenitz (26). It is a specialized gland found in rodents and insectivores (Fig. 5) and is located in the loose connective tissue adjacent to the external auditory canal, anterior and inferior to the tympanum. The normal gland, in the rat, is 2–4 mm. in size and remains attached to the external auditory canal when the ear is dissected from the animal.

Tumors of these sebaceous glands have many designations, a few being: carcinomas arising adjacent to the external auditory canal (25), tumors of the ductus acusticus externus (1), squamous carcinomas of the periauricular sebaceous gland (18), squamous-cell carcinomas of the ear duct (19), and Zymbal gland tumors.

In the present studies, urethane influenced the formation of carcinomas of the sebaceous gland of the external auditory canal. They arose as subcutaneous masses below and slightly anterior to the ear in the region of the mandibulo-maxillary junction, and grew rapidly. Often the overlying skin ulcerated. Necrosis was a common feature. Although there was some variation in their histologic appearance, from tumor to tumor and often within the same section, the majority could be classified as carcinomas of the sebaceous gland; cysts containing sebaceous material were not uncommon (Figs. 6, 7). A few neoplasms exhibited nests of squamous cells with epithelial pearls (Fig. 8), whereas others were frank squamous-cell carcinomas. In one rat metastases were found in the lung, in another in a regional lymph node.

The data with regard to the incidence and average age at appearance of these carcinomas in the urethane-treated groups are brought together in Table 1. The over-all incidence in the males was 17 per cent, in the females 14 per cent; these appeared at an average age of 92 weeks (Table 2).

An unexpected and rewarding observation was the occurrence of three Zymbal gland carcinomas in untreated control rats—two in males, at 105 and 123 weeks of age, and one in a female, at 117 weeks at age. The significance of this finding will be discussed later.

Malignant Lymphomas

Five male and five female treated rats developed malignant lymphomas, in contrast to none in the controls. At autopsy malignant lymphoma was suspected from enlargement of the spleen and often of the liver (in two animals the liver contained scattered gray nodules, partly hemorrhagic). Microscopic study resulted in the following classification of the ten neoplasms: six lymphoid lymphomas, two lymphatic leukemias, and two reticulum-cell sarcomas.

Neurofibrosarcomas of the Ear

Only the rats initially administered urethane when they were 1–2 weeks of age developed neoplasms of the ear (Experiments 1, 5). The total number of such tumor-bearing animals was six (7 per cent), one of them having bilateral involvement. The neoplastic nodules were 3–6 mm. in diameter, situated close to the free border of the ear lobe and in no way related to the punched hole used for identification. On cutting they were grayish-white, glistening, and firm in consistency.

A striking histologic feature of these tumors was their dumbbell shape, being present on both sides of the cartilaginous plate (Fig. 9). The neoplasms were similar, in general, although some features varied: cells were spindle- or "sausage"-shaped; one tumor showed typical Verocay bodies (Fig. 10); and there
### Table 1

**Neoplastic Response of Sprague-Dawley Rats to Urethan**

<table>
<thead>
<tr>
<th>Exp. and Group</th>
<th>Exp. 1 F:</th>
<th>Exp. 1 G:</th>
<th>Exp. 2 F:</th>
<th>Exp. 2 G:</th>
<th>Exp. 3 F:</th>
<th>Exp. 3 G:</th>
<th>Exp. 4 F:</th>
<th>Exp. 5 M:</th>
<th>Exp. 6 M:</th>
<th>Exp. 7 M:</th>
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<tr>
<td>AV. AGE AT START (WEEKS)</td>
<td>1</td>
<td>2</td>
<td>7</td>
<td>32</td>
<td>1</td>
<td>2</td>
<td>58</td>
<td>1</td>
<td>7</td>
<td>32</td>
</tr>
<tr>
<td>AV. WEIGHT AT START (GM.)</td>
<td>8</td>
<td>22</td>
<td>138</td>
<td>265</td>
<td>8</td>
<td>22</td>
<td>311</td>
<td>8</td>
<td>181</td>
<td>418</td>
</tr>
<tr>
<td>Administration of Urethan</td>
<td>None</td>
<td>I.P. 0.5/gm B.W. 3X week</td>
<td>None</td>
<td>P.O. 38/day</td>
<td>None</td>
<td>I.P. 100/rat 2X week</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td></td>
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<tr>
<td>Total amount (mg.)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
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<td>Frequency</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
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<tr>
<td>Duration (weeks)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
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</tr>
<tr>
<td>No. rats alive at age (in weeks):</td>
<td>15 15 13</td>
<td>15 12 10</td>
<td>15 11 7</td>
<td>15 11 15</td>
<td>15 15 10</td>
<td>15 15 10</td>
<td>15 15 15</td>
<td>15 15 15</td>
<td>15 15 15</td>
<td>15 15 15</td>
</tr>
<tr>
<td>MAMMARY TUMORS</td>
<td>13 87 85 9</td>
<td>12 80 58 9</td>
<td>7 47 47 3</td>
<td>10 67 75 2</td>
<td>8 30 68 1.6</td>
<td>7 47 111 1.0</td>
<td>8 53 100 1.1</td>
<td>8 53 99 1.6</td>
<td>5 53 88 1</td>
<td>5 53 88 1</td>
</tr>
<tr>
<td>Average age at appearance of tumors per rat (weeks):</td>
<td>1.3</td>
<td>0.7</td>
<td>0.7</td>
<td>2.3</td>
<td>1.8</td>
<td>1.0</td>
<td>1.1</td>
<td>1.6</td>
<td>1.0</td>
<td>1.1</td>
</tr>
<tr>
<td>Average no. of tumors per T-B rat</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SEROUS GLAND (ETRIMAL) CARCINOMAS</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

*Also includes non-neoplastic epidermal cysts.
† Each group was composed of fifteen rats.
‡ I.P., intraperitoneal; P.O., per os (in drinking water, ad libitum).
§ For individual I.P. injection, mg/gm body weight or per rat; for administration in drinking water, average intake per day.
‖ Average age of rats at which their first mammary tumors arose.
|| Average number of mammary tumors per tumor-bearing rat.
** Uterine sarcomas not included (they are discussed in the text).
†† Numbers in parentheses represent single values.

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TABLE 2
NEOPLASMS IN CONTROL AND URETHAN-TREATED SPRAGUE-DAWLEY RATS*

<table>
<thead>
<tr>
<th>TYPE OF NEOPLASM</th>
<th>FEMALES</th>
<th></th>
<th>Males</th>
<th></th>
<th>FEMALES AND MALES COMBINED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (60 rats)</td>
<td>Urethan (90 rats)</td>
<td>Control (45 rats)</td>
<td>Urethan (75 rats)</td>
<td>Control (105 rats)</td>
</tr>
<tr>
<td></td>
<td>Rats with tumors</td>
<td>Av. time of appear. (weeks)</td>
<td>Rats with tumors</td>
<td>Av. time of appear. (weeks)</td>
<td>Rats with tumors</td>
</tr>
<tr>
<td>Mammary tumors</td>
<td>51</td>
<td>85</td>
<td>51</td>
<td>87</td>
<td>20</td>
</tr>
<tr>
<td>Sebaceous gland (Zymbal) carcinomas</td>
<td>1</td>
<td>2 (117)**</td>
<td>13</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>Malignant lymphomas</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>0</td>
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<tr>
<td>Neurofibrosarcomas of the ear</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Tumors of the kidney</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Angiomas</td>
<td>1</td>
<td>2 (112)</td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Uterine sarcomas</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Sarcomas (other than uterine)</td>
<td>1</td>
<td>2 (89)</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Epidermal cysts</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin tumors</td>
<td>1</td>
<td>2 (117)</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Fibroma</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>0</td>
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<td>11</td>
<td>0</td>
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<tr>
<td>Pituitary adenomas</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Pheochromocytomas of adrenal</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

* Also includes non-neoplastic epidermal cysts.

† Exp. 1, Gp. 1; Exp. 2, Gp. 1; Exp. 3, Gp. 1; and Exp. 4, Gp. 1.
‡ Exp. 1, Gps. 2, 3, and 4; Exp. 2, Gp. 2; and Exp. 3, Gps. 2 and 3.
§ Exp. 5, Gp. 1; Exp. 6, Gp. 1; and Exp. 7, Gp. 1.

Average time at which first external tumor of the type was recognized at inspection, or internal tumor was found at autopsy.

** Numbers in parentheses represent single values.
was a range in the degree of anaplasia (Fig. 11). It was necessary to differentiate between neurofibrosarcoma and leiomyosarcoma, since the tumors had some morphologic and staining qualities (van Gieson and Masson stains) suggesting each. It is our impression that designation of neurofibrosarcoma (malignant neurilemmoma) is applicable to this neoplasm.

TUMORS OF KIDNEY

At autopsy, six of the rats administered the carcinogen were found to have tumors of the kidney, whereas none were present in control animals. In four rats the tumors were white to pale yellow, circumscribed, slightly elevated nodules which measured 2-5 mm. in diameter. Microscopic examination revealed that they were located in the cortex and were typical renal adenomas composed of cells with pale cytoplasm (Fig. 12).

The other two rats exhibited poorly delimited, larger neoplasms which were grayish in color and extensively hemorrhagic. One, a clear-cell carcinoma (hypernephroma), was 10 mm. in size. The other, an undifferentiated carcinoma, apparently arising from the renal pelvis, measured approximately 4 cm.

ANGIOMAS

Angiomas were observed in ten urethan-treated animals: three hemangiomas of the spleen, two of the lung, and one in the heart, mesocolon, thymus, and inguinal lymph node; two lymphangiomas were present in the same rat, in the thymus and mesenteric lymph node. Lymphangioma of the thymus and hemangioma of the spleen, respectively, were found in two control animals.

SARCOMAS

Nine rats that had been given urethan developed sarcomas (other than uterine) located as follows: three in the neck, two in limbs, and one each in the lung, spleen, and subcutaneous tissue of the flank and tail. These were classified as neurogenic-, neurofibro-, lipo-, angio-, or myxo-sarcomas. One control animal had a neurogenic sarcoma, another a fibrosarcoma—both at the base of the ear.

It is of interest that five sarcomas—three in the neck region of rats given urethan and two at the base of the ear in control animals—were readily differentiated from the sebaceous gland (Zymbal) carcinomas found in the same general region. This distinction was made through the combined criteria of anatomic location, characteristics of the tumor at autopsy, and histologic features.

EPIDERMAL CYSTS

Epidermal cysts were found in sixteen male rats administered urethan, whereas none were observed in the control males or the treated and control females. They were recognized during periodic inspection of the animals as palpable intradermal nodules. These lesions were found in various regions but more generally on the back or flank. Microscopically, they were recognized as epidermal cysts with or without sebaceous gland elements (Fig. 13).

OTHER NEOPLASMS

In addition to the neoplasms and epidermal cysts already described, an appreciable number of tumors of the uterus, pituitary, adrenal, and skin were observed, as well as one or more examples of a variety of lesions.

Sarcomas of the uterus (either leiomyo- or round-cell sarcomas) were found in seven treated and two control rats. Pituitary adenomas occurred in small numbers in both control and treated male rats and in control females, whereas none were found in treated female animals. In both males and females, more pheochromocytomas of the adrenal developed in the controls than in those given urethan. It is our opinion that the lower incidence of pituitary and adrenal tumors in the treated animals is related to the earlier formation of other tumors, and therefore earlier death of the rats. Obviously, neoplasms forming late in life did not have the opportunity to emerge (Table 2).

Three rats which received urethan had malignant tumors of the brain stem (meningioma, round-cell sarcoma, and an invasive mesenchyma neoplasm). Hepatocellular carcinoma was found in two treated animals. Three other rats given urethan developed a neurofibrosarcoma of the stomach, a papillary adenocarcinoma of the ovary, and a pancreatic adenoma, respectively. One control animal had a transitional-cell carcinoma of the urinary bladder.

In Table 1 the experiments and groups are individualized, thus showing the exact, broad-spectrum, significant neoplastic response for each group of fifteen rats. In Table 2, the control groups and urethan-treated groups are combined, respectively, even though various experimental factors differed. This permits an over-all comparison of the significant findings in a much larger number of rats. Other neoplasms, where the action of urethan is more difficult to evaluate, are also listed.

NON-NEOPLASTIC EFFECTS OF URETHAN

Toxic effects of urethan were observed early in animals administered the agent in large amounts (pilot studies): damage to the capillaries and sinusoids, which led subsequently to extravasation of blood and hemorrhages, dilation of blood
vessels, and the formation of blood cysts and blood lakes. These often were present in the affected organs even in the later stages. Some of the organs showing these features were: thymus, liver, spleen, lungs, adrenals, and uterus. Gross manifestation of these tendencies was the presence of hemorrhagic fluid and hemorrhages in the thoracic and abdominal cavities, gastrointestinal tract, and uterine cavity. Hemorrhages also occurred in the nose and conjunctiva.

In the experiments reported, where smaller dosages were utilized, the above toxic manifestations were of a lesser degree. As the animals became older, anemia was often present.

An interesting lesion was found in the lungs of a number of treated rats, possibly related to urethan. It was observed at autopsy as a 2- to 3-mm. gray nodule, usually located immediately below the pleura. Grossly, it appeared that these nodules might be pulmonary tumors, but microscopic study dispelled this consideration. The lesions were not sharply demarcated from surrounding lung tissue, as seen in pulmonary adenoma of the mouse. Acini were lined with bronchiolar cells, and clumps of these cells were also found in the lumina, as well as histocytic cells, some containing blood pigment. It is our opinion that the lesions represent the end stage of an organizing pneumonia, rather than neoplasms. This experience has impressed us with the difficulty of assessing the real nature of pulmonary lesions found in rats given urethan.

DISCUSSION

There have been a limited number of investigations concerned with the neoplastic response of rats to urethan (7, 11, 14). These claim that the agent had an influence on the formation of pulmonary, and perhaps other tumors. We have found no evidence of pulmonary adenoma induction in our subline of inbred Sprague-Dawley rat.

The present investigations demonstrate that urethan exhibits multipotential carcinogenicity in the rat. As expected, incorporating the agent had an influence on the formation of pulmonay, and perhaps other tumors. We have found no evidence of pulmonary adenoma induction in our subline of inbred Sprague-Dawley rat.

The recognition of these periauricular sebaceous

and carcinomas of the sebaceous gland (Zymal) of the external auditory canal; it apparently enhanced the development of angiomas and sarcomas at various sites; and it induced malignant lymphomas, tumors of the kidney, and neurofibrosarcomas of the ear. A considerable number of male rats given urethan developed epidermal cysts, whereas none were observed in the male controls or in females.

In addition, neoplasms of the adrenal and pituitary were observed late in life in both untreated and treated rats; but a meaningful comparison could not be made, inasmuch as the treated animals died relatively early from other tumors or the late effects of urethan toxicity. Other neoplasms, one or more of a number of different types, were found in either or both the urethan-treated and control animals. It would not be wise to assume that these incidental findings have biological significance.

As in other biologic investigations of this nature it was not possible to evaluate and interpret the influence of urethan without a precise knowledge of the incidence of neoplasms in the untreated control rats. The findings with regard to the panorama of spontaneous tumors in our inbred subline of Sprague-Dawley rat do not differ greatly from those of other investigators who utilized commercial Sprague-Dawley rats (3, 10, 21). It was, however, most gratifying to unearth sebaceous gland (Zymal) carcinomas in three of our control rats. They were found, on inspection, in animals having an average age of 115 weeks. Our method of numbering, housing, and transferring these rats from soiled to clean cages precludes the possibility of contamination or mistaken identity. Moreover, these three rats showed no pathologic evidence of having been exposed to urethan.

As far as we know, sebaceous gland (Zymal) carcinomas have never been previously reported to occur spontaneously. The finding is probably not related to the strain of animal, because other workers have also utilized Sprague-Dawley rats. More likely, the fact that our rats were in good health and were allowed to live out their full life span permitted these tumors to emerge. It is recognized that there are advantages to short-term experiments (more can be completed in a given time), but also disadvantages (possibly missing neoplasms that occur in low incidence at a late age). Perhaps others have observed tumors of the face and neck in their untreated groups, some possible Zymal gland carcinomas that were not recognized in the variety of tumors that occur spontaneously in this region.

The recognition of these periauricular sebaceous
gland carcinomas in untreated rats and their significant acceleration by administered urethan adds evidence to the concept that many types of neoplasms are not induced de novo but are enhanced by particular nutritional or pharmacological means (18–20). A considerable number of tumor types found in untreated mice, Syrian golden hamsters, and rats are potentiated by urethan. There are other types found in urethan-treated animals, however, which as yet have not been observed in controls.

Potentiation or induction of mammary tumors in the rat has been achieved through administration of estrogen (5), 2-acetylaminofluorene (1), 4-aminostilbene (8), carcinogenic polycyclic hydrocarbons (6, 9, 15), and following irradiation (4, 16). This report documents the effectiveness of urethan.

Urethan can also now be added to the agents known to influence the formation of Zymbal gland carcinomas, others being 2-acetylaminofluorene (25), benzidine (17), 3,2-dimethyl-4-aminobiphenyl (24), 4-aminostilbene (8, 22), 9,10-dimethyl-1,2-benzanthracene (6), and 3-methoxy-4-aminoazobenzene (13).

From investigations on mice, it has been learned that estrogens, carcinogenic polycyclic hydrocarbons, and irradiation enhance leukemogenesis. In the past few years a large body of evidence has been accumulated showing that urethan is leukemogenic in the mouse (reviewed in [19])—and, as presented here, in the rat.

Therefore, it is becoming more and more obvious that a particular tissue or organ may respond to a large variety of agents, resulting in neoplasms of the same type. It is an intriguing speculation that the metabolism and function of a tissue or organ may be of greater significance than the specific agent in determining whether or not carcinogenesis occurs.

The carcinogen utilized in this study, urethan, influences the formation of mammary tumors, leukemia, and many other neoplasms in the mouse and rat. Accumulating data and inference suggest that such general action may occur in many tissues of a number of species. Whereas in the early days of experimental carcinogenesis the tendency was to think in terms of carcinogenic action in a particular tissue, it is now clear that most carcinogens are multipotential. The conditions must be favorable, however, for them to express this property. Urethan does so remarkably.

REFERENCES


![Fig. 1. Fibroadenoma of breast in urethan-treated rat showing predominance of fibrous element. Hematoxylin and eosin stain (H. & E.), X170.](Image)

![Fig. 2. Fibroadenoma of breast in treated rat showing predominance of adenomatous component. H. & E., X170.](Image)

![Fig. 3. Adenocarcinoma of breast in rat given urethan. H. & E., X170.](Image)

![Fig. 4. Same as Figure 3, under higher magnification. H. & E., X450.](Image)

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Fig. 5.—Normal sebaceous gland (Zymbal) of the external auditory canal in control rat. Note the ear canal with the cartilagenous plate on left and part of the gland on right. H. & E., ×70.

Fig. 6.—Carcinoma of Zymbal gland in treated rat showing central cystic area containing sebaceous material, a characteristic feature of this tumor. H. & E., ×170.

Fig. 7.—Same as Figure 6, under high magnification. Note the numerous malignant sebaceous cells and mitoses. H. & E., ×450.

Fig. 8.—A Zymbal gland carcinoma in a rat given urethan showing squamous-cell features and epithelial pearls. H. & E., ×60.
FIG. 9.—Low power of neurofibrosarcoma of the ear in a urethan-treated rat. Note the dumbbell shape of the tumor. H. & E., X7.

FIG. 10.—Another neurofibrosarcoma of the ear in a rat administered urethan, showing typical Verocay body. H. & E., X60.

FIG. 11.—Neurofibrosarcoma of the ear in a treated rat exhibiting anaplasia and a mitotic figure at middle of right field. H. & E., X450.

FIG. 12.—Adenoma of the kidney in a rat given urethan. Note the clear cells of this tumor. H. & E., X75.

FIG. 13.—Epidermal cyst in a male rat treated with urethan. H. & E., X50.


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Albert Tannenbaum, S. D. Vesseliovitch, Cesare Maltoni, et al.


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