The Development of Neurogenic Neoplasms, Embryonal Kidney Tumors, Harderian Gland Adenomas, Anitschkow Cell Sarcomas of the Heart, and Other Neoplasms in Urethan-treated Newborn Rats

S. D. Vesselinovitch and N. Mihailovich

Division of Oncology, Institute for Medical Research, The Chicago Medical School, Chicago, Illinois 60612

SUMMARY

The neoplastic potentialities of various tissues of newborn and infant rats were investigated by postnatal administration of urethan. Randomly bred MRC rats were exposed to repeated i.p. injections of 10% urethan; the first injection was given within 24 hours of birth and subsequent ones at 3-day intervals. The animals received a total of either 6 or 10 urethan injections, each consisting of 0.5 mg/gm body weight. Nontreated breeding mates served as controls. The experiment was terminated when the animals were 110 weeks of age.

Seven percent of the nontreated controls developed tumors in four different organs (pituitary, uterus, peripheral nerve, and mammary gland). However, 82.5% of the urethan-treated animals showed one or more primary tumors at the time of death. Urethan influenced the development of gliomas of the brain, epithelial and embryonal renal tumors, pituitary adenomas, Harderian gland adenomas, Anitschkow cell sarcomas of the heart and hemangiopericytomas, in addition to previously reported hepatomas, hepatocarcinomas, cholangiomas, and cholangiocarcinomas (10), and neurofibrosarcomas, mammary gland fibroadenomas, uterine sarcomas, Zymbal gland adenomas, and malignant lymphomas (6).

These findings demonstrated that newborn and infant rats possess broad neoplastic potentialities, as revealed by exposure to urethan.

INTRODUCTION

As part of an integrated study on the neoplastic potentialities of the various tissues of newborn and infant animals (7–9), MRC rats were exposed to the multicarcinogenic action of urethan immediately after birth. The effect of this urethan treatment on the survival rate, and the weights of the animals and the development of benign and malignant liver tumors has been reported in the preceding paper (10).

The present paper reports on the neoplastic response of the nervous system (central and peripheral), kidneys, pituitaries, Harderian glands, heart, vascular tissues, mammary glands, uteri, Zymbal glands, and the lymphoreticular system.

MATERIALS AND METHODS

The essential facts for the understanding of the present work are given in Table 1. The additional information has been presented in the preceding paper (10).

Autopsies were performed on all control and urethan-treated animals. The brains were removed in toto, and any abnormalities grossly observed were recorded at that time. The pituitaries were similarly inspected for their size, color, and shape. Both Zymbal glands were exposed in each case and all glands larger than 2–3 mm in diameter were excised. Enlarged Harderian glands were also removed. The hearts of all animals were opened and inspected for the presence of any gross changes. All sections were routinely stained with hematoxylin and eosin and studied microscopically.

RESULTS

Table 2 lists the overall incidence of tumors resulting from the postnatal administration of urethan. On the average, 82.5% of the treated animals had either single or multiple primary tumors. The increase in the dose of urethan delivered resulted in higher frequency and a broader spectrum of primary tumors.

Neurogenic Tumors. Tumors of the neurogenic stromal tissue developed not only at the site of the peripheral nerves (most frequently involving branches of the N. auricularis) but also in the brain substance proper (Table 3). While the peripheral variety of neoplasms were identified as neurofibrosarcomas, the intracranial neoplastic lesions of the brain which originated from the glial cells were classified as either astrocytoma (Fig. 1) or oligodendroma (Fig. 2). The male rats developed significantly more brain tumors than the females (12/80 in Groups 2 and 3 combined vs. 3/80 in Groups 4 and 5; P < 0.05).

Kidney Tumors. Seven percent of the animals (11/160) showed neoplastic lesions in the kidneys (Table 3). In most cases (7/11) tumors were large (up to 175 gm), and they...
Urethan Carcinogenesis in Newborn Rats

Table 1

<table>
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<th>Group</th>
<th>Sex</th>
<th>Number</th>
<th>Age at start (days)</th>
<th>Effective number*</th>
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<th>Age at last injection (days)</th>
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Experimental factors.

* Animals alive at 20 weeks of age.

b 10% (w/v) water solution made shortly before treatment. Injections were delivered intraperitoneally at 3-day intervals.

c Each animal received 0.5 mg (0.005 ml of solution) / gm body weight / injection.

Table 2

<table>
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<th>Treatment with urethan</th>
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Table 3

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<th>Treatment with urethan</th>
<th>Survivors at the end (118 weeks)</th>
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The overall incidence of tumors in MRC rats following postnatal administration of urethan.

a 0.5 mg/gm body weight of urethan was delivered i.p. at each injection; treatment began on the 1st day of life (<24 hours) and continued at 3-day intervals for 6 (3 mg) or 10 (5 mg) times.
b Animals with any type(s) of primary tumors.
c Animals with liver tumors (benign or malignant).
d Animals with any type of primary tumor(s) regardless of whether they bear liver neoplasms.
e Number of animals bearing either single or multiple primary tumors.

practically always originated in the right kidney. Usually only a very small portion of the involved kidney could be recognized at the autopsy. Morphologically, these kidney tumors were of embryonal or mixed variety (Figs. 3-6). In addition to this type of kidney tumor, 3 animals had cortical adenocarcinomas and one animal showed a transitional-cell carcinoma of the renal pelvis. On the average, the animals with embryonal kidney tumors had a shorter life span (62 weeks) than did the rats with epithelial type of neoplasms (78 weeks), apparently due to the faster growth of the former type of tumors.

Pituitary Gland Adenomas. The pituitary glands which were abnormal in size and/or appearance contained either hyperplastic and hyperemic nodules or frank chromophobe ade-
The induction of pituitary adenomas, mammary fibroadenomas, and uterine sarcomas by urethan.

- 0.5 mg/gm body weight of urethan was delivered i.p. at each injection; treatment began on the 1st day of life (<24 hours) and continued at 3-day intervals for 6 (3 mg) or 10 (5 mg) times.

- Average age of animals at which internal tumor was found at autopsy or external tumor was recognized at inspection.

The neoplastic pituitaries were more frequently seen in the urethan-treated female animals (in 22.5% at an average age of 87 weeks) than in the males (in 2.5% at an average age of 104 weeks; P < 0.001). Only 2 nontreated female mice developed hemorrhagic adenomas (5%).

**Harderian Gland Adenomas.** Table 5 shows that the animals that received a total of 5 mg of urethan per gm body weight (10 injections) developed adenomas of the Harderian gland in an incidence of 7.3% and 9.5% for males and females, respectively (Fig. 8).

**Anitschkow Cell Sarcomas of the Heart.** Careful inspection of the heart muscles revealed in several instances the presence of grayish, 2-3 mm nodules. Microscopic study of those lesions showed their sarcomatous character (Figs. 9, 10) and peculiar morphology worthy of comment (Figs. 11, 12). The chromatin was dense and occupied the central portion of the nucleus. From this main core of chromatin, fine "threads" were extending to the nuclear membrane. Because the nuclei were elongated and the cells had a tendency to grow in interlacing sheets, the microscopic section revealed either their longitudinal or perpendicular aspect. Therefore, depending upon the direction of the cut, the nuclei had either a "caterpillar" or "owl-eye" appearance. This morphology was so reminiscent of "Anitschkow myocytes" that we classified these lesions of the heart as Anitschkow cell sarcomas.

**Hemangiomases.** Vascular neoplasias were found with low frequency at various sites such as the area posterior to the kidney, the intestinal wall, and the thoracic cavity (Table 6).

**Other Tumors.** The developments of mammary gland fibroadenomas (23/80 females; Table 4), Zymbal gland adenomas (7/160 rats; Table 5), and malignant lymphomas (7/160 animals; Table 5) were positively affected by the administration of urethan. These data confirm our previous findings (6), so that present observations are given only in tabular form.

**Miscellaneous Tumors.** In addition to the above-mentioned neoplasias, a few isolated types of tumors were found clinically and/or at the autopsy. The two less frequently encountered tumors were an adamantinoma (Figs. 13-15) and an embryonal liver tumor. The other tumors were carcinoma of the adrenal cortex (two), papillary adenocarcinoma of the thyroid gland (one; Fig. 16), and adenocarcinoma of the small intestines (one).

The adamantinoma, presented as a submandibular mass, was 2.5 cm in diameter, weighted 9.3 gm, and had a hard bone-like consistency and a spongy appearance on the cut surface. Microscopic study of this mass revealed a classical case of adamantinoma. The tumor consisted of a meshwork of interlacing strands of epithelial tumor cells with the connective tissue stroma. The tumor cells had a columnar appearance and resembled ameloblasts, while the stromal cells were of a stellate shape, similar to the stellate reticulum of the enamel organ.

The mixed or embryonal liver tumor was composed of epithelial, mesenchymal, and osteoid elements.
The development of Anitschkow cell sarcomas of the heart, hemangiomas, and miscellaneous tumors following the administration of urethan.

- 0.5 mg/gm body weight of urethan was delivered i.p. at each injection; treatment began on the 1st day of life (<24 hours) and continued at 3-day intervals for 6 (3 mg) or 10 (5 mg) times.
- Average age of animals at which internal tumor was found at autopsy or external tumor was recognized at inspection.
- Thyroid carcinoma.
- Liver embryoma; intestinal adenocarcinoma.
- Adamantinoma; cortical adenoma of the adrenal gland.
- Cortical adenoma of the adrenal gland.

**DISCUSSION**

The main objective of the present study was to explore the neoplastic potentialities of various tissues of newborn and infant rats by administering urethan during these early postnatal age periods. The broad spectrum potentialities were demonstrated, as it was shown that 82.5% of the animals which received urethan bore one or more primary tumors which originated in 11 different organs or tissues. The present studies also extended our knowledge regarding urethan multicarcinogenicity, as several types of tumors were related to the carcinogenic action of this agent for the first time.

Neurofibrosarcomas of the N. auricularis have already been observed in rats treated with urethan (6), ergot (2), and dimethylnitrosourea (1). However, the finding of neoplastic lesions in the brain (gliomas) is of interest because they developed following the neonatal administration of urethan. Similar tumors were seen by Druckrey et al. (1) in BD rats which were exposed diaplacentally to ethylnitrosourea. This showed that the supporting tissue of the central nervous system possesses neoplastic capacities, as revealed following the remote administration of carcinogens to the newborn animals or fetuses. Apparently the absence of the blood-brain barrier and the stage of the animals' development at the time of carcinogenic action were essential factors in the genesis of tumors in the central nervous system.

The development of the embryonal kidney tumors following treatment with urethan also represents a new finding. Their embryonal character may be due to the administration of urethan immediately after birth, so that the carcinogen was able to act upon immature, actively dividing, bipotential cells which lent themselves readily to carcinogenesis. This speculation may be supported by the observation of an embryonal liver tumor and an adamantinoma of the salivary gland in the present series. Riopelle and Jasmin (4) are of the opinion that both the well-differentiated epithelial tumors and the less well-differentiated neoplastic mixed tumors of rats originate from the same, undifferentiated, bipotential kidney cells, which only occasionally achieve differentiation during carcinogenesis.

Pituitary adenomas develop spontaneously in most rat strains late in life. In the present case, the experiment was terminated before these spontaneous tendencies became manifested. This enabled us to observe a positive effect of the urethan on the development of the pituitary gland adenomas when it was administered to rats immediately after birth. It is likely that similar conditions to those suggested in connection with the nervous system carcinogenesis were also operative in this instance.

Although a high frequency of Harderian gland adenomas have been reported in urethan-treated mice (9), the development of this tumor has not previously been observed in rats administered urethan. Thus, the present finding indicates that the difference between these two species in the neoplastic potentialities of their Harderian glands was only of a quantitative nature.

The finding of Anitschkow cell sarcomas of the heart in several urethan-treated rats is of interest because of the rarity of this neoplastic lesion. To our knowledge, thus far only one such case has been reported by Morris et al. (3) in a rat fed N-2-fluorenylacetamide for more than 7 months.

The development of this great variety of primary tumors in various tissues of the animals exposed to urethan as newborns contrasts with the extremely low incidence of tumor-bearing animals found in the nontreated controls or urethan-treated adults (6). This indicates that the majority of tissues of newborn rats are prone to undergo neoplastic change, which can be realized only if the animals are exposed during this particular age period to a carcinogen acting systemically.

**Tumors in Nontreated Rats.** While 82.5% of the urethan-treated rats developed primary tumors of various tissues, only 6.2% of the nontreated controls showed single primary neoplasms. In addition, tumors of the control groups were seen practically only at the termination of the experiment, while tumors in the urethan-treated groups developed within a shorter latent period.

### Table 6

<table>
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<tr>
<th>Group number</th>
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<th>Heart sarcomas</th>
<th>Hemangiomas</th>
<th>Miscellaneous tumors</th>
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<td>%</td>
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The development of Anitschkow cell sarcomas of the heart, hemangiomas, and miscellaneous tumors following the administration of urethan.
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range of the neoplastic potentialities usually narrows with the age of the animals, so that, in general, fewer tissues of adults become neoplastic following the administration of a multicarcinogen. It is likely that several factors, such as the degree of cell differentiation, the mitotic rate of the relevant tissue, the metabolic efficacy of the animal, the immunologic competence of the hormonal constitution of the host, and the state of the blood-brain barrier may complement each other in modifying either the inception and/or the development of tumors. Further knowledge of these factors, and especially of the mechanisms of their action, should be helpful in better comprehending the process of carcinogenesis.

ACKNOWLEDGMENTS

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REFERENCES


Fig. 1. Histologic section through a well-differentiated astrocytoma of the cerebrum. Note the “thread-like” pattern of cell distribution. Cell nuclei are large and relatively uniform with delicate and evenly distributed chromatin. H & E, × 450.

Fig. 2. Highly cellular oligodendroma of the cerebrum. Note the small, closely arranged cells with deeply stained nuclei, most of which are demarcated by clear spaces from the remains of the cytoplasm. Compare the size and staining characteristics of these cell nuclei with those presented in Fig. 1. H & E, × 450.

Fig. 3. Histologic section through a “soft” part of an actively proliferating embryonal kidney tumor, showing a sarcomatoid cell pattern. H & E, × 80.

Fig. 4. Higher magnification of the same tumor as in Fig. 3. Nuclear chromatin is mainly distributed along the nuclear membrane. The spindle and anastomosing stellate cells are loosely arranged. H & E, × 450.

Fig. 5. “Mixed” kidney tumor consisting of a combination of spindle cells of the fibroblastic type and the epithelial elements arranged in tubules. This type of tumor showed a firmer consistency than the type of tumor presented in Fig. 4. H & E, × 120.

Fig. 6. Histologic section of part of a fast-growing kidney tumor which showed a “hard” consistency. This embryonal tumor is characterized by high cellularity and a dense, homogenous intercellular substance lying throughout the tumor mass. H & E, × 80.

Fig. 7. Histologic sections through a pituitary gland chromophobe adenoma. Note diffuse and sinusoidal structure of this tumor. H & E, × 130.

Fig. 8. Histologic section of a Harderian gland adenoma. Note irregularity of gland structure and papillary proliferations. H & E, × 130.

Fig. 9. Histologic section through the heart muscle containing a highly cellular tumor mass. The muscle tissue is seen in the lower left corner. H & E, × 80.

Fig. 10. Higher magnification of the same tumor as in Fig. 9. Note the sarcomatous cells infiltrating the heart muscle. Two mitotic figures are clearly visible, one toward the lower left and the other toward the upper right corner of the photomicrograph. H & E, × 450.

Fig. 11. Neoplastic lesion involving the heart muscle. The cells in this case have a tendency to grow in sheets. This is especially visible in the central section of the photomicrograph, where cell nuclei were cut longitudinally. H & E, × 300.

Fig. 12. Higher magnification of the central portion of the lesion presented in Fig. 11. Note the peculiar distribution of the chromatin. A densely stained and relatively wide chromatin band occupies the central portion of the nucleus, from which fine “threads” extend to the nuclear membrane. A longitudinal section of these nuclei shows a “caterpillar” appearance (central part of the photomicrograph) while a cross section (cell in the lower right corner of the photomicrograph) shows an “owl-eye” look. H & E, × 750.

Fig. 13. Photomicrograph of an adamantinoma. Note meshwork of interlacing strands of the columnar epithelial cells with the connective tissue stroma. H & E, × 180.

Fig. 14. Higher magnification of the same tumor as in Fig. 13. Palisaded arrangement of the columnar epithelial cells and the connective tissue papilla are clearly visible. H & E, × 450.

Fig. 15. Histologic section from another part of the tumor presented in Figs. 13 and 14. Note the bone-like spicule (osteoid metaplasia) within a columnar epithelial cell strand. H & E, × 450.

Fig. 16. Thyroid gland papillary adenocarcinoma. The cytoplasm of the spheroidal cells is inconspicuous, and the cell boundaries are ill-defined, so that relatively clear nuclei are packed closely together forming a bead-like structure. H & E, × 450.
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