The Significance of Perinatal Age Periods and the Dose of Urethan on the Tumor Profile in the MRC Rat

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SUMMARY

The role of the prenatal, neonatal, and postweaning age periods in the carcinogenesis of various tissues of MRC rats was investigated by exposure to urethan. The animals were given either one injection (2 or 4 days prenatally) or six injections (postnatally) of specified solutions of urethan at 3-day intervals. The first urethan injections were given on the 1st, 28th, or 46th day of life.

The newborn rats developed tumors at specific sites in significantly higher incidence than the animals treated at other age periods. Rats which were exposed to urethan in utero developed liver tumors and Anitschkow cell sarcomas of the heart. Neonatally treated animals had gliomas of the brain, neurilemomas, and embryonal kidney tumors in addition to the latter two types of tumors. The urethan-treated weanlings showed none or brain neoplasms but did develop the other types of tumors with low frequency.

It was concluded that the neoplastic competence of the tissue at a given age governs the outcome of its carcinogenesis independently from its carcinogenic potential at other times and from the carcinogenesis in the other organs.

INTRODUCTION

Our recent studies on the neoplastic potentialities of various tissues of newborn and infant rats demonstrated a broad spectrum of tumor response following the administration of urethan. In these studies, all survivors were sacrificed at 110 weeks of age, at which time non-treate controls showed only a threshold level of neoplastic expression, while urethan-treated animals bore 1 tumor or more which originated in 11 different organs or tissues. Termination of the experiment at the above age made possible the demonstration of the effect of urethan on the development of several types of tumors such as pituitary adenomas and mammary gland fibroadenomas, which usually develop late in the life of nontreated animals.

These studies showed that the liver of rats, as well as that of mice, possesses a high neoplastic competence during the early postnatal age periods. The lymphopoietic system, however, appeared to be significantly less prone to neoplastic change in rats than in mice. Our original studies showed that 1-day-old mice were significantly more responsive to urethan leukemogenesis than the 1-week-old animals. In addition, a slight increase in the dose of urethan delivered (from 2.1 to 4.2 mg/g of body weight) raised the incidence of leukemogenesis significantly. Similarly, hepatocarcinogenesis by urethan has been shown to be most efficient at birth (1, 2, 4, 5, 13). The mice of the prenatal age also responded by carcinogenesis in various tissues.

In order to learn more about the general significance of some of these biological factors in carcinogenesis, an additional series of experiments with rats as the biological system and urethan as the multicarcinogen has been initiated with several objectives in mind: (a) to explore prenatal carcinogenesis in this species, (b) to reinvestigate the susceptibility of newborn rats to leukemogenesis (by increasing the carcinogenic dose), and (c) to assess the neoplastic potential of the liver of weanlings and young adults.

The specified age periods imposed certain limitations not allowing experimental execution as it was originally conceived. Pregnant rats did not tolerate repeated administration of urethan when given during the second half of gestation. The exposure to more than 1 injection of urethan resulted in the death of the mother and/or fetus. Only animals exposed once in utero survived the treatment and they were left to live their life-span. Approximately one-half of the litters was exposed to urethan 2 days before birth and the other one-half was exposed 4 days before birth. Furthermore, the maximal total dose tolerated by newborn rats was 6 mg of urethan/g of body weight. At this dose level there was a 30% mortality rate; consequently, we did not administer the anticipated dose of 7.2 mg/g of body weight.

In spite of these shortcomings, the results clearly indicated that tumor response is dependent more upon the age of the animals at the time of the carcinogenic treatment than upon the administered dose of the carcinogen. Not only the liver but also the brain, heart, and kidneys of newborn animals were significantly more susceptible to carcinogenesis by urethan than those of weanling and young adult animals. The increase in the carcinogenic dose, however, failed to reveal an enhanced susceptibility to the development of malignant lymphoma. On the other hand, the single low-dose...
exposure of rats to urethan in utero resulted in marginal liver and heart carcinogenesis.

MATERIALS AND METHODS

Rats. The randomly bred MRC rats were used in the present experiment (11). The animals were bred at 3 months of age, and the fetuses, newborns, weanlings, and young adults were treated as indicated below. The rats were weaned and separated by sex when 30 days old, following which they were weighed and inspected at 2-week intervals. The animals were kept in a temperature-controlled laboratory at 78°F and were fed Rockland diet and given water ad libitum.

The experiment ended when the animals were 146 weeks old. At autopsy, specimens were taken from all the internal organs, tumors, endocrine glands, and the brain. All sections were routinely stained with hematoxylin and eosin and studied microscopically.

Treatment. The solutions of urethan of specific concentration (percentage of w/v in redistilled water) were injected i.p., 0.005 ml/g of body weight, at each treatment. Pregnant mothers were exposed once to a 10% solution either 2 or 4 days before parturition (Groups 2 and 3). All other groups (except one-half of Group 6) received 6 injections at 3-day intervals, the first delivered on Day 1 (Groups 4 to 6), Day 28 (Groups 7 and 8), or Day 46 (Groups 9 and 10). The percentage of urethan solution varied from 10% (Groups 2, 3, 4, 7, and 9) to 16% (Groups 5, 8, and 10). Group 6 received a total of 6 mg of urethan/g body weight; one-half of the group was given 6 injections of 20% solution and the other one-half was given 5 injections of 24% solution of urethan. Because of early mortality, the treatment was stopped after the fifth injection in the latter case. Because in both instances the animals received the same total amount of urethan, which resulted in similar tumor response, the subgroups and the results were combined and presented as such. Control group (Group 1) received no treatment.

RESULTS

Animals surviving the treatment showed good longevity, living on the average 100 weeks. A variety of tumors developed throughout their life-span, but the incidence of a certain type of tumors was dependent upon the age of the rats at the time of the treatment. The type of tumors which showed this phenomenon are listed in Table 1.

The animals which were exposed in utero 4 days before birth (Group 3) developed liver tumors and heart sarcomas (Anitschkow cell type) with an extremely low incidence (4.5%). The absence of these lesions in the controls, in conjunction with the extremely low dose of urethan delivered (0.5 mg/g of body weight), is indicative of the high susceptibility of these 2 tissues to urethan carcinogenesis during the fetal development. Thirty-nine fetuses which were treated with urethan in the same fashion, but 2 days before birth (Group 2), did not develop either of these tumors. The low incidence of tumors in Group 3 and the small size of both groups does not allow any conclusion as to the possible effect of intrauterine age (4 versus 2 days antepartum) on carcinogenesis.

The animals which were repeatedly exposed to urethan as newborns (Groups 4 to 6) developed tumors in several other organs or tissues in addition to hepatocarcinomas and heart

<table>
<thead>
<tr>
<th>Group No.</th>
<th>MRC rats</th>
<th>Urethan treatment, mg/g of body weight</th>
<th>Liver tumors</th>
<th>Heart tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>118</td>
<td>None</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>39</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>44</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2 and 3</td>
<td>83</td>
<td>3.0</td>
<td>15</td>
<td>2.2</td>
</tr>
<tr>
<td>4</td>
<td>77</td>
<td>4.8</td>
<td>11</td>
<td>1.4</td>
</tr>
<tr>
<td>5</td>
<td>73</td>
<td>27.0</td>
<td>11.7</td>
<td>3.2</td>
</tr>
<tr>
<td>6</td>
<td>76</td>
<td>3.0</td>
<td>13.1</td>
<td>3.9</td>
</tr>
<tr>
<td>7</td>
<td>49</td>
<td>1.8</td>
<td>1.2</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>54</td>
<td>1.8</td>
<td>1.2</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>48</td>
<td>1.8</td>
<td>1.2</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>44</td>
<td>4.8</td>
<td>2.4</td>
<td>1.4</td>
</tr>
<tr>
<td>7 to 10</td>
<td>195</td>
<td>3</td>
<td>1.5</td>
<td>0</td>
</tr>
</tbody>
</table>

*Animals alive at 20 weeks of age.

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Table 1

The effect of age of rats and the dose of urethan on the tumor profile

Groups 2, 3, 4, 7, and 9 were given injections of 10% solution of urethan (w/v), Groups 5, 8, and 10 received 16% solution, and Group 6 was treated with 20 and 24% solutions (see text). Injections were delivered i.p. at 3-day intervals and began at specified age.
sarcomas. Variation in the total dose of urethan from 3 to 6 mg/g of body weight (Groups 4 to 6) had no effect on the incidence of any of these tumors.

Statistical analysis of the data from the comparison of similarly treated newborns (Groups 4 and 5 combined) with weanling and young adults (Groups 7 to 10) revealed a highly significant difference in the incidence of tumors. The degree of significance for each tumor type was as follows: liver, \( p < 0.001 \) (18.0 versus 1.5%); gliomas of the brain, \( p < 0.01 \) (7.3 versus 0.0%); kidney tumors, \( p < 0.01 \) (6.0 versus 0.5%); and thyroid tumors, \( p < 0.05 \) (0.4 versus 4.1%). In the case of thyroid carcinomas, young adults developed more of these tumors than newborns. Peripheral neurogenic tumors (neurilemomas) developed with the same frequency in postweanling animals as in the newborns (3.5 versus 8.0%, respectively, \( p < 0.05 \)). These tumors were exclusively seen on animal ears originating in the nauricularis.

Table 2 presents the incidence of mammary gland fibroadenomas, pituitary adenomas, and fibrosarcomas at various sites (mainly the uterus) observed in the female controls and the urethan-treated rats at various ages. All surviving controls were sacrificed at 110 weeks of age; thus all controls lived on the average 95 weeks and showed a low spontaneous neoplastic expression (11, 12). However, control animals sacrificed at 146 weeks of age (controls, Group 1, Table 2) developed significantly more tumors. The neonatally treated rats had a much shorter life-span due to variety of tumors which developed as listed in Table 1. For this reason, the pituitary and mammary gland tumors developed in significantly lower incidence than in the controls (controls, Group 1, Table 2). The rats treated with urethan postweanling survived just as well as controls (146 weeks), so that they developed tumors listed in Table 2 with similar frequencies to untreated animals, which did not permit assessment of positive urethan effect as shown earlier (12). No significant difference in weight has been observed between various groups.

Rats treated with urethan as newborns, therefore, showed the highest incidence and the broadest spectrum of neoplasms (Table 1), so that the range of the neoplastic expression narrowed with the age of the animals.

**DISCUSSION**

In spite of experimental limitations, the main questions posed at the inception of this series of experiments were answered. Thus it has been shown that a single exposure of fetuses to urethan resulted in development of liver tumors and heart sarcomas. A similar incidence of liver tumors was observed in urethan-treated weanlings and young adults, even though they received 6 to 8 times the dose given prenatally. It may, therefore, be concluded that the fetal liver possesses a higher neoplastic competence than does the liver of weanlings. On the other hand, the newborn rats were significantly more prone to hepatocarcinogenesis than the older animals. Thus it was shown that the neoplastic potentiality of rat liver varied with age, as does mouse liver (1, 2, 4, 5, 13). Heart sarcomas were also observed in the animals treated at newborn age but not in the urethan-treated adults. The histological appearance of this lesion has been described in detail in our recent publication (12).

Neurogenic brain tumors were previously observed in MRC rats treated as newborns (12). Similar tumors were seen by Druckrey et al. (3) in BD rats exposed transplacentally to ethylnitrosourea. These findings showed that the supporting tissue of the central nervous system possesses neoplastic competence revealed only following the administration of systemically acting carcinogens to the newborn animals or fetuses. Apparently, the absence of the blood-brain barrier and the stage of animal brain development at the time of carcinogenic action are essential factors in the genesis of these tumors. The dose of urethan given prenatally to Groups 2 and 3 was apparently below the threshold amount necessary to induce brain tumors.

Neurilemomas (12) in newborns developed with similar incidence as in weanlings and young adults (\( p > 0.05 \)). This indicates that age variation of the host had no modifying effect on carcinogenesis of this particular tissue. On the other hand, the embryonal kidney tumors (12) developed with significantly higher frequency in animals treated as newborns than as adults.

It is obvious that the liver, heart, brain, and kidney showed a higher neoplastic competence shortly after birth than later in life. It may be assumed that partly the higher dose of the carcinogen at the level of these tissues, but mainly the

**Table 2**

The incidence of mammary gland and pituitary tumors and sarcomas that developed in female rats

<table>
<thead>
<tr>
<th>Experimental series (group)</th>
<th>Mammary gland fibroadenoma</th>
<th>Pituitary adenoma</th>
<th>Fibrosarcoma^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nontreated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls^b</td>
<td>40</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>Controls (1)</td>
<td>60</td>
<td>15</td>
<td>25.0</td>
</tr>
<tr>
<td>Urethan-treated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prenatally (2, 3)</td>
<td>41</td>
<td>10</td>
<td>24.3</td>
</tr>
<tr>
<td>Neonatally (4 to 6)^c</td>
<td>115</td>
<td>12</td>
<td>10.4</td>
</tr>
<tr>
<td>Postweaning (7 to 10)</td>
<td>96</td>
<td>27</td>
<td>28.1</td>
</tr>
</tbody>
</table>

^a At various sites, but mainly in the uterus.

^b From experiments reported previously (Refs. 11 and 12). All surviving animals of this group were killed at 110 weeks of age.

^c This series of animals had significantly shorter life-span (\( p < 0.01 \)) than those of the other nontreated or urethan-treated groups.
presence of cohorts of relatively immature and actively replicating cells during that period of life, made these tissues readily prone to carcinogenesis.

In contrast, urethan does not enhance carcinogenesis in lymphoid tissue of newborn rats, which is at variance with its high leukemogenicity for newborn mice. The reason for this discrepancy is not known at present. However, one should consider that the genetic background (6, 7), the viral participation, and the interaction between the bone marrow and the thymus might have been factor(s) differentially modulating the leukemogenesis in these 2 species.

In a previous publication, we showed a positive effect of urethan on the development of pituitary adenomas, Harderian and Zymbal gland adenomas, and mammary fibroadenomas (12). Those experiments were terminated at 110 weeks, which was before spontaneous neoplastic tendencies in these organs were manifested. The longer duration of the current experiment allowed neoplastic expression in the current experiment to be observed. Therefore, it is apparent that urethan, at least descriptively, "accelerated" the development of the latter group of neoplasms, while it "induced" liver, heart, brain, and kidney tumorogenesis. It is likely that the basic mode of action of urethan on the molecular level is the same in both instances. If this is the case, the observed phenomena (acceleration versus induction) may be more apparent than real.

From the viewpoint of comparative oncogenesis, it is of interest that similar treatment with urethan of newborn hamsters (Syrian white), rats (MRC), and mice [(C57BL × C3H) F1] resulted in different tumor spectra. The main tumor types that developed as a result of urethan treatment were malignant melanomas in hamsters (15), hepatocarcinomas in rats (11), and hepatomas and thymic lymphomas in mice (8–10). In contrast, none of these tumors were readily induced in the adult animals. Thus, apparently, the genetic background governs which tumor types might develop following the exposure to a chemical carcinogen, while the age of the animal and the stage of tissue development at the time of carcinogenic action are decisive factors in the occurrence of carcinogenesis. It is easily conceived that the degree of specific carcinogen-tissue interaction varies with age, due to the biochemical kinetics at the tissue level and the general metabolic competence of the whole animal. Apparently, the intrinsic predisposition of the tissue or neoplastic competence at the time of carcinogenic action governs the outcome of its carcinogenesis independently from the carcinogenic potential at other times and the carcinogenesis in the other tissues.

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