Tumor of the Lung in Rats Following Injections of Urethane (Ethyl Carbamate)*

M. F. Guyer, Ph.D., and P. E. Claus, Ph.D.

(From the Department of Zoology, University of Wisconsin, Madison 6, Wisconsin)

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Following our 1939 paper (1) on treatment of transplanted rat carcinoma, in which it was shown that x-rays were notably more lethal to growing cancers in colchicine-treated rats than to similar cancers in untreated controls, we planned to extend the experiments but, because of war conditions, were unable to secure colchicine. A supply of urethane (ethyl carbamate, N₂H₂COOC₂H₅) was available, however, and since this drug was supposed to have effects on mitosis similar to those of colchicine we proceeded with it. It not only had a delaying effect on mitosis for some 12 to 16 hours, but what is more important, we found from later experiments, of which this is a report, that it induced tumors of the lung in a majority of our experimental animals. Since urethane is sometimes used as a human anesthetic, particularly for children, it seemed important to explore this lead further.

Nettlcship and Henshaw (6) have reported induction of pulmonary tumors in mice with urethane. An inspection of this paper and the later ones of Henshaw and Meyer (3, 4) convinced us that we were obtaining in rats the same effects they got in mice; namely, the induction of multiple pulmonary tumors of adenomatous type. After completion of our present manuscript a study of Jaffé (5) has appeared in which he reports carcinogenic action of ethyl urethane on rats. He secured pulmonary adenomas both by injection and by feeding. Some of his animals also developed tumors of the liver. In our rats, a highly inbred stock, we found no involvement of the liver.

MATERIALS AND METHODS

Because the rat is far less likely than the mouse to develop cancers spontaneously, and particularly because we had a strain of rats selected for its 25 years of immunity to cancer implants (Flexner-Jobling carcinoma), it seemed worthwhile to continue our experiments with urethane. After some experimentation with intraperitoneal dosage, our treatments were standardized to injections of 1 cc. of a 10 per cent aqueous solution of urethane per 100 gm. of body-weight of the rat being treated, all of which were young adults. This was an anesthetizing dose which left the animal inert for several hours. The rats were kept in a room temperature of about 72° F.

Most of the animals received from 3 to 5 injections in all, administered at weekly intervals, although in certain cases only 1 or 2 injections were given. The practice was in each lot of animals, after the first injection, to leave certain ones un.injected each time so that at the end of 5 weeks the group would include individuals having had 1, 2, 3, 4, and 5 injections respectively. After the fifth, no further injections were given and about 11 months later all survivors were killed and autopsies made. Animals that died in the meantime were also dissected and carefully inspected.

The animals received the same diet as that fed regularly to our large rat colony; namely, Wayne Dog Food Blox, which analyzes into:

<table>
<thead>
<tr>
<th>Component</th>
<th>Min. (%)</th>
<th>Max. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>22.0%</td>
<td>24.0%</td>
</tr>
<tr>
<td>Fat</td>
<td>4.0%</td>
<td>6.0%</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>40.0%</td>
<td>42.0%</td>
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<tr>
<td>(nitrogen free extract)</td>
<td></td>
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</tr>
<tr>
<td>Fiber</td>
<td>5.0%</td>
<td>7.0%</td>
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In addition, a bit of fresh lettuce was given each day.

RESULTS

In all, 3 series of experiments were made on rats. In the first set of observations 8 of 11 animals developed lung tumors. In our second experiment...
28 animals (14 males and 14 females) were used—all from a strain selectively bred for many years for immunity to carcinoma transplants. Twenty-three animals developed tumors in the lung. In the third series of unselected though highly inbred rats, 35 of 52 animals revealed pulmonary tumors upon autopsy.

Some 8 to 10 months after the first injection of urethane the treated animals commonly showed evidence of illness, lost weight and died within a month or two, if not killed for study. Inspection usually showed scattered tumor nodules beneath the pleura of the lungs, ranging in size from barely perceptible dots up to 4 or 5 mm. in diameter (Fig. 1), with sections showing occasional nodules more deeply embedded in the lung parenchyma. Males and females were equally affected. Although careful inspection was made of the other internal organs, notably the liver, no tumors were ever found apart from the lungs.

Fig. 1.—Rats’ lung showing adenomatous nodules (whitish areas.)

Microscopic inspection showed many of the tumor cells making their first appearance as gland-like cells associated with the bronchioles (Fig. 2). As early as 8 to 10 weeks after the first treatment, small cellular foci which seemed to precede the tumors were detectable. As each such focal region increased in size the air sacs with which it was in contact became obliterated and replaced by adenomatous tissue. The simultaneous appearance of the nodules indicates that the neoplastic growth does not originate in a single cell but that a number of cells must have transformed into a similar form of neoplasm about the same time.

The general structure of the tumors, stained with hematoxylin and eosin and seen at different magnifications, is shown in Figs. 2 to 5. In Fig. 2, under low magnification (×48), the tumor tissue is plainly visible at the left, the much looser normal lung tissue, at the right. In Fig. 3, at a magnification of ×192, and in Fig. 4 at a magnification of ×516, the structure of the tumor itself is shown in greater detail. In Fig. 5, the characteristic appearance of the nuclei of the irregularly arranged tumor cells is seen at a magnification of ×1164. All of the tumors studied were of the same histological type.

That a single dose is sufficient to cause tumor formation is shown by the fact that 3 animals receiving but 1 injection each, developed tumors although not as plentifully as rats receiving multiple injections. Our records show, however, that following only 2 injections, as many as 37 tumors could be counted on the lung surfaces after the death of the animal.

In not a single one of our 50 control rats did tumor of the lung appear, although 1 had cancer of the liver. The several suspicious-looking nodules observed in 5 of the controls proved under microscopic examination to be infections. In several of the treated animals similar non-tumorous centers of bacterial infection were also observed.

Unlike colchicine, which delays mitosis in metaphase for from 15 to 25 hours, although permitting continuance of chromosome divisions, urethane seems to do away with practically all mitotic activity. Inspection of the graphs reproduced in Fig. 6 shows how nearly mitotic activities were brought to a standstill in corneal cells. They summarize the total counts of mitotic activity, made from separate studies of each microscopic field for prophase, metaphase, anaphase and telophase figures.

These graphs are based on sections in which mitotic figures of all phases were counted in every fifth section, with 36 sections counted for each cornea. Forty-two rats (21 males, 21 females) were injected at 9:30 A.M., after which 6 injected and 6 control animals were killed at the following hours: 10 A.M., 2 P.M., 6 P.M., 10 P.M., 2 A.M., 6 A.M., and 10 A.M. The lower graph shown in Fig. 6 is for the 21 injected female rats and their controls. That for the males was not essentially different. The continuous line in the graphs represents the urethane-treated animals, the dotted line, the controls. The horizontal readings indicate the time of day the animals were killed, the upright column shows the average number of division figures per section in 108 sections (3 × 36) of the 3 female rats killed at any one time. For purposes of comparison a set of counts was made on 28 mice, although the counts for each phase were made on 2 instead of 3 individuals.

It will be noted that the controls show the fairly regular diurnal rhythm of mitotic activity commonly seen in mice and rats. Urethane, while not obliterating it, depresses it materially. For from a short time after 9:30 A.M. until about 2 A.M., a
Fig. 2.—Tumor tissue at left, resembling glandular structures associated with a bronchiole. Mag. × 48.

Fig. 3.—Tumor structure is shown in detail. Mag. × 192.

Fig. 4.—Same as Fig. 3. Mag. × 516.

Fig. 5.—Characteristic appearance of nuclei of the irregularly arranged tumor cells. Mag. × 1164.
It is evident from this study that, as affecting mitosis, urethane does not operate in the manner of colchicine by arresting mitosis in metaphase and holding it there, but does away almost entirely with all stages of it for a time. Its effects resemble more closely those of such anesthetics as ether or cocaine, or such mydriatics as ephedrin, which apparently prevent cells from entering upon mitosis. After injections of urethane the several phases of mitosis seem to disappear with equal rapidity, hence more than mere prevention of its inception seems involved. What part, if any, this suppression of mitosis plays in the imitation of pulmonary tumor formation, remains unanswered. It may be merely an accompanying response to the causative agent. It is obvious, however, that the drug has cytological effects on tissues other than those of the lungs. Further studies on the cytological aspects of the problem are being continued.

SUMMARY

Intraperitoneal injections of urethane (1 cc. of a 10 per cent aqueous solution, per 100 gm. of body-weight) induced the formation of multiple pulmonary adenomatous tumors in the majority of 91 rats so treated, including one lot of 28 which had been selectively bred for many years for immunity to carcinoma transplants. Cytological studies of other cells of the body, such as corneal cells in which division figures are easy to observe, showed that the drug noticeably reduced all stages of mitosis for some 12 to 16 hours, thus demonstrating that it has direct or indirect cytological effects not only on lung tissue but on other tissues as well, even though no tumors were found elsewhere. The study indicates also that a number of pulmonary cells underwent similar abnormal change at about the same time—that the neoplastic growth did not originate in a single cell.

REFERENCES

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