Papilloma Formation in the Forestomach of the Mouse Following Oral Administration of Urethan (Ethyl Carbamate)

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The experiments presented here form part of a larger project, intended to account for the reason why the glandular mucosa of the mouse's stomach fails to respond to the action of carcinogenic hydrocarbons when administered from within the lumen (see reviews 1, 6, 7, 12). Such carcinogens are known to induce tumors of glandular mucosa when injected into the submucosa (4, 5, 11) but induce only tumors of the squamous epithelium of the forestomach when administered by mouth (2, 8, 10). On the assumption that the "mucus barrier" (6) might prevent water-insoluble carcinogens from penetrating the glandular mucosa, the use of water-soluble substances, known to be carcinogenic to other organs, seemed a promising approach.

Urethan (ethyl carbamate) was chosen for the purpose, administered continuously (for complete carcinogenesis) and for short periods (as possible initiator), followed by other water-soluble substances (to serve as promoters). The use of iodoacetic acid as a possible promoter was based on the knowledge that this substance acts as such on the skin (3).

While the primary objective was not realized, the finding that urethan was mildly carcinogenic for the squamous epithelium of the forestomach was sufficiently interesting to warrant this publication.

MATERIALS AND METHODS

The animals were male mice of the Swiss strain, 3–3½ months old at the start of the experiment, from a colony bred in this laboratory by brother-sister matings for 10–12 generations. The mice were kept in transparent plastic cages and housed in an air-conditioned room at 21°–23° C. The diet consisted of Purina Laboratory Chow and water ad libitum, except for the periods of treatment, as described below.

The administration of urethan, iodoacetic acid, and control solvents was carried out with the use of a polyethylene stomach tube of 1-mm. bore, with a closed end and lateral opening, as previously described (2). Before the first of the three primary treatments, the mice were kept on a milk diet for 3 days and then on water alone for a further 18 hours, so that the stomachs would be empty at the time of treatment (2). Before each of the subsequent treatments, the mice were submitted to 18 hours of starvation (i.e., receiving water alone), the Purina being returned to the cages 2 hours after the treatment. (The 3 days' milk diet, if repeated weekly throughout the experiment, would have seriously interfered with the nutrition of the animals.)

Both the urethan (5 and 10 per cent) and iodoacetic acid (0.4 per cent) solutions were made up in distilled water. The amounts given per feeding were 0.4 ml. for each primary treatment and 0.2 ml. for each secondary treatment. The total amounts administered amounted, therefore, to 190 mg. and 60 mg. in the case of 10 and 5 per cent urethan, respectively, for primary treatment, and 900 and 450 mg., respectively, for the secondary treatment. The total dose of iodoacetic acid for secondary treatment (0.2 ml. of a 0.4 per cent solution, X45) amounted to 36 mg. Distilled water alone was used as control for the urethan, and dilute acetic acid-acetate buffer, at pH 2.4, as control for the iodoacetic acid. Both primary and secondary treatments were given once weekly, with an interval of 3 weeks between the end of the primary and the commencement of the secondary treatment.

At the end of each experiment, or in the case of death of an animal during the course of the experiment, the stomach of each mouse was distended with fixative solution (acid Zenker's fluid) before being opened. Paraffin sections of whole stomachs, and in many cases of particular segments which showed gross evidence of tumors, were cut and stained with hematoxylin and eosin and, in some cases, with Schiff's periodic acid stain.
RESULTS

In the glandular mucosa, no pathological changes were observed, except in one animal of the group receiving three feedings of 5 per cent urethan followed by 45 feedings of iodoacetic acid. The lesion, 7 X 2 mm. in size, in the region of the pylorus, had the histological features of an adenoma with atypical growth. A second group of 32 mice was set up, receiving the same treatment, except for a prolongation of the iodoacetic acid feeding to 52 weeks. None of the survivors of this group developed such a lesion. The isolated observation cannot, therefore, be considered related to the treatment received (though no such lesion has been encountered by us in over 1,500 mice of the Swiss strain in which the stomachs have been examined histologically).

DISCUSSION

The evaluation of the results of this experiment involves some difficulties, in common with certain other forms of carcinogenesis (e.g., mammary carcinogenesis or leukemia induction), in which a low but appreciable incidence of the particular form of tumor exists spontaneously. One of the criteria used in the other examples quoted is the earlier average age at which the tumors develop; but this could not be used in the case of gastric carcinogenesis without sacrificing large numbers of animals during the course of the experiment. One is restricted, therefore, to an analysis of the differences in incidences between the experimental and control groups.

The results, summarized in Table 1, strongly suggest that the differences are significant: The incidence of tumor-bearing animals to total survivors is highest in the group receiving the largest doses of urethan; it is moderately high in all the other groups receiving urethan with or without other forms of treatment; and it is very low in the water or untreated controls. Similarly, the average numbers of tumors per animal show a similar trend. Furthermore, it was observed (though not actually evident from the table) that, while the papillomas that occurred in the control mice were invariably solitary and very small in size, those

<table>
<thead>
<tr>
<th>PRIMARY TREATMENT</th>
<th>SECONDARY TREATMENT</th>
<th>No. survivors</th>
<th>No. tumors/cent</th>
<th>Av. no. tumors/survivor</th>
</tr>
</thead>
<tbody>
<tr>
<td>(0.4 ml. X 5)</td>
<td>(0.5 ml. X 45)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 per cent urethan</td>
<td>Dist. water</td>
<td>40</td>
<td>18/30</td>
<td>0.1 ± 0.3</td>
</tr>
<tr>
<td>5 per cent urethan</td>
<td>Dist. water</td>
<td>57</td>
<td>7/37</td>
<td>0.3 ± 0.09</td>
</tr>
<tr>
<td>—</td>
<td>Dist. water</td>
<td>29</td>
<td>4/25</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>—</td>
<td>Untreated controls</td>
<td>48</td>
<td>3/42</td>
<td>0.07 ± 0.03</td>
</tr>
<tr>
<td>10 per cent urethan</td>
<td>0.4 per cent iodoacetic acid</td>
<td>27</td>
<td>9/17</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>5 per cent urethan</td>
<td>—</td>
<td>54</td>
<td>7/45†</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>5 per cent urethan</td>
<td>—</td>
<td>48</td>
<td>12/45</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>—</td>
<td>Dil. buffer (pH 4.4)</td>
<td>38</td>
<td>5/38</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>0.4 per cent iodoacetic acid</td>
<td>—</td>
<td>58</td>
<td>2/40</td>
<td>0.07 ± 0.05</td>
</tr>
</tbody>
</table>

* Survivors: excluding animals that died before 10th week of secondary treatment.
† Iodoacetic acid treatment continued, in this group, for 52 instead of 45 weeks.

In the squamous mucosa of the forestomach, papillomas were observed in all the groups, including the controls (Table 1), though the percentage of animals bearing such tumors and, more especially, the average number per animal were much higher in those receiving urethan (the average number per animal ranging, in the various groups, from 0.3 to 1.2) than in the water and untreated controls (averaging 0.03 and 0.07 per animal) or the group receiving iodoacetic acid alone (0.07 per animal). An illustration of multiple papillomas of the forestomach following treatment with urethan is shown in Figure 1. In contrast to the other controls, the small group receiving the dilute acetic acid-acetate buffer after the primary treatment with urethan yielded a moderately high incidence (0.5 per animal).

The two highest yields in the whole series were in the group receiving 10 per cent urethan continuously and in the one receiving three feedings of 10 per cent urethan followed by continuous iodoacetic acid feeding.

(Attention should be drawn to the care needed in distinguishing true early papillomas from pseudopapillomatous foci of hyperplasia, the latter sometimes including fragments of hair embedded in the mucosa.)
Fig. 1.—Multiple papillomas of the epithelium of the forestomach in a mouse receiving 23 weekly feedings of 0.4 ml. of 10 per cent urethan in distilled water. ×16.
in the urethan-treated mice were often multiple (see Fig. 1), in some cases numbering as many as eight, and often up to 5 mm. in diameter.

The unusually high incidence of gastric papillomas in the group receiving three doses of 10 per cent urethan, followed by 45 doses of iodoacetic acid, suggests the possibility that the latter agent acted as a promoting agent for this tissue. The fact that no such increase in incidence was observed in the two groups receiving 5 per cent urethan followed by iodoacetic acid makes one hesitant in accepting such a conclusion, however.

The one apparently anomalous result was the moderately high incidence of gastric papillomas in the group receiving acetic acid-acetate buffer at pH 2.4 after three doses of urethan. In this connection, the results of Mori (9) must be recalled, who claimed that acetic acid was carcinogenic for the squamous epithelium of the forestomach of the rat.

SUMMARY

The administration of large doses of urethan (ethyl carbamate) to Swiss strain mice by stomach tube led to the development of multiple papillomas of the squamous epithelium of the forestomach.

Spontaneous solitary papillomas of the forestomach were observed in a small proportion of control mice of this strain.

REFERENCES


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