The Inhibiting Effect of Ethyl Urethane on the Development of Lymphatic Leukemia in Rats*

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Hawkins and Murphy (2) reported in 1925 that animals anesthetized with ethyl urethane (ethylcarbamate) showed changes in the lymphoid system strikingly similar to those observed following a general exposure of the animal to x-ray.1 The present investigation is a test of the effect of urethane on malignant lymphoid cells, as exemplified by a transplantable lymphatic leukemia and a lymphosarcoma of the rat. Previous investigations by Haddow and Sexton (1), in which they tested the effect of urethane on experimental animal tumors, showed a definite effect on the Walker Rat Carcinoma 256, but the action was more striking on leukemic cells. Paterson, Thomas, Haddow and Watkinson (9) reported that in human leukemia, urethane produces a fall in the white blood cell count, diminution in size of the spleen and involved lymph nodes, changes which "are remarkably similar to those obtained by standard methods of deep x-ray therapy."

MATERIAL AND METHODS

The transplantable lymphatic leukemia used in the tests has been carried in this laboratory since 1940 (5). The leukemic cells inoculated intraperitoneally cause the development of typical leukemia with marked increase in the circulating lymphocytes, and extreme involvement of the thymus and lymph nodes. Subcutaneous inoculation of the leukemic cells in the groin results in a rapidly-growing lymphosarcoma which may occasionally show metastases to the regional lymph nodes but rarely progresses into leukemia. At the time of the present tests about 84 per cent of the rats inoculated intraperitoneally developed leukemia which caused death in 8 to 10 days. Lymphosarcoma grew in approximately 86 per cent of the animals inoculated subcutaneously, with a fatal outcome between 12 and 18 days.

A 10 per cent concentration of ethyl urethane in 0.85 per cent salt solution was injected subcutaneously 5 times weekly and the doses varied from 25 to 100 mgm. per 100 gm. of body weight.

Effect of ethyl urethane on leukemia. In 8 tests, 79 rats inoculated intraperitoneally with leukemic cells were treated with subcutaneous injections of ethyl urethane. In the first experiment, treatment was started an hour after inoculation and the rats were given 100 mgm. of the drug per 100 gm. of body weight and this was repeated 5 times weekly. This gave complete protection against the development of leukemia but there was a question of how great a role the general toxic effect of the drug had on the result. In a second experiment, the same dose of urethane was given 3 times weekly and this also resulted in complete protection. A third experiment in which the dose was reduced to 75 mgm. given 5 times weekly gave equally good protection. Five groups of inoculated rats were treated with 50 mgm. 5 times a week. There was slight, if any, manifest toxic effect from these doses of urethane, and the animals remained in good condition throughout the treatment period. As controls for these tests, 71 rats from the same strain were inoculated at the same time and with the same material but were given no treatment. The details and results of the individual experiments are given in Table I.

It will be noted that the degree of inhibition on the development of leukemia was almost as high with 50 mgm. of urethane as with the larger dose. In 6 of the experiments, treatment was initiated 1 hour after inoculation but the results in two experiments in which treatment was started 24 hours later were just as definite.

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Effect of urethane on lymphosarcoma.—In three experiments 60 rats were inoculated subcutaneously with leukemia cells. Of these, 30 rats were given injections of 50 to 75 mgm. of ethyl urethane per 100 gm. of body weight, starting 24 hours after inoculation, and the dose was repeated 5 times a week. In the treated rats 53.3 per cent failed to develop tumors; 16.6 per cent of the tumors that developed later retrogressed. Thus, only 30 per cent showed progressive tumors. This may be contrasted with the untreated controls where 86.6 per cent of the 30 rats had progressive tumors (Table II). The treated animals in which the tumors progressed showed a definite effect of the treatment in that they survived 12 to 14 days longer than the untreated controls (Table III).

Effect of ethyl urethane on white blood cells, lymphoid organs and adrenals.—Blood counts on groups of animals from the foregoing experiments confirm our earlier observations (2). The urethane-treated rats showed a rapid fall in the circulating lymphocytes, reducing the number of both normal lymphocytes and leukemic cells to approximately a third of the previous level.

Table IV shows the average weights of the lymphoid organs and adrenals of 80 rats treated with urethane and the same data from 71 untreated controls. It will be noted that, in the first group of treated animals developing leukemia, the shrinkage of the thymus and spleen was less pronounced than in the other groups and there was no significant hypertrophy of the adrenals. In as yet an unpublished study of the effect of the growth of rat lymphosarcoma on the lymphoid organs, a pronounced regression of the thymus was noted. The controls of group 3 give an example of this reaction with 3 of the animals showing complete atrophy of the thymus. Averaging the weights of the several organs from 80 urethane-treated rats and the 71 controls gives the following results. The thymus was present in only 58.7 per cent of the treated rats; when present, the average weight of this organ was a third of that of the average weight of the thymus in the controls. The cervical nodes and spleens in the treated animals were reduced approximately by a half.

It will also be noted in Table IV that the adenals of the controls of group 4 animals, resistant to the lymphosarcoma, are larger than the average for the other untreated rats. This same condition has been observed in a large number of rats in another study, the details of which will be published later. With the exception of group 1, the urethane-treated animals were found to have definitely enlarged adrenals, approximately 40 per cent larger than these organs from the untreated controls.

DISCUSSION

It has previously been shown in this laboratory that adrenalectomized rats have a definitely in-

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>Number of rats</th>
<th>Amount urethane per 100 gm. body weight</th>
<th>Time after inoculation</th>
<th>Frequency per week</th>
<th>Negative</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>100 mgm.</td>
<td>1 hour</td>
<td>5</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>100 mgm.</td>
<td>1 hour</td>
<td>3</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>75 mgm.</td>
<td>1 hour</td>
<td>5</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>50 mgm.</td>
<td>1 hour</td>
<td>3</td>
<td>100</td>
<td>*</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>50 mgm.</td>
<td>1 hour</td>
<td>3</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>50 mgm.</td>
<td>1 hour</td>
<td>5</td>
<td>88.8</td>
<td>10</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>50 mgm.</td>
<td>24 hours</td>
<td>5</td>
<td>80</td>
<td>11</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>50 mgm.</td>
<td>24 hours</td>
<td>3</td>
<td>100</td>
<td>10</td>
</tr>
<tr>
<td>Total 8</td>
<td>79</td>
<td></td>
<td></td>
<td></td>
<td>91.1</td>
<td>71</td>
</tr>
</tbody>
</table>

* Controls for this group were the same as those in Experiment 2.
creased susceptibility to transplanted lymphatic leukemia (6, 7) and that adrenal cortical and pituitary adrenotropic hormones have a definite inhibiting effect on the development of this disease (7). In an as yet unpublished study it was noted that rats with growing lymphosarcoma have hypertrophy of the adrenals (25 per cent) and that rats which develop resistance to this growth show even more pronounced enlargement of these glands (34.4 per cent). In the present investigation, definite enlargement of the adrenals (40 per cent) is noted in the rats treated with ethyl urethane. Attempts have been made to determine if the effect of this treatment on leukemia was due to the stimulation of the adrenals rather than to direct action on the leukemic cells. Unfortunately, adrenalectomized rats do not withstand even a small amount of urethane, so the answer to the question must await a different approach.

It was demonstrated in this laboratory that alkalosis induced by injections of sodium bicarbonate has a destructive effect on the lymphoid system quite comparable to that resulting from general x-ray exposure (3, 4). As noted above, ethyl urethane causes a similar destruction of the lymphoid system, and this chemical also induced uncompensated alkalosis (2). These observations suggest the possibility, perhaps remote, that the urethane effect may depend on the induced alkalosis. This question is now under investigation.

Among 79 rats inoculated with leukemic cells and treated with ethyl urethane in doses ranging from 50 to 100 mgm. per 100 gm. of body weight, repeated 3 to 5 times weekly, 91.1 per cent failed to develop the disease. In 71 inoculated but untreated controls only 16.9 per cent were resistant. A similar inhibiting effect was found in the treatment of rats with lymphosarcoma. Among 30 rats given ethyl urethane only 30 per cent developed progressive tumors while 86 per cent of the 30 controls died with large tumors. The thymus, lymph nodes and spleen in rats receiving urethane treatment were reduced to a fraction of their normal weights. The adrenals of these animals increased 40 per cent in weight over that of the normal glands.

### REFERENCES

5. MURPHY, J. B., and Sturm, E. The Transmission of an Induced Lymphatic Leukemia and Lympho-
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