Limited Response to Propylthiouracil in the Tumor-bearing Rat*

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The accidental observation that the thyroid gland of the tumor-bearing rat was not enlarged after the oral administration of thiouracil was made while investigating the possible role of the thyrotrophic hormone (TSH) as a fat-mobilizing hormone (14). Further experiments were carried out to ensure that the effect was not limited to a particular strain of rat or type of tumor, or due to the action of inanition on the pituitary (10, 15). The results of these experiments are recorded in this paper, though the underlying mechanism is not understood.

MATERIALS AND METHODS

The majority of rats in these experiments were male Sprague-Dawley1 rats, maintained on fox chow2 and tap water. In some experiments Wistar3 or hooded4 rats were used. The Walker 256 carcinoma grafted intramuscularly in the thighs was the standard tumor. A transplantable fibrosarcoma5 was grafted subcutaneously in one experiment. The rats were force-fed a high-fat diet (20) in some experiments. The same diet was used, without the added water, in the caloric restriction experiments. The high-protein diet was that of previous experiments (20), minus the water, and with the substitution of 52.5 gm. casein and 10.3 gm. brewers' yeast for the egg albumen and lactalbumin.

The term “caloric restriction” refers to feeding the non-tumor-bearing rats an amount of diet less than that fed to the tumor-bearing rats, the carcass weight of the nontumor-bearing rats being maintained at the same level as that of the tumor-bearing rats (8). The carcass weight of the tumor-bearing rats (body weight less tumor weight) was estimated during the course of the experiment by subtracting the weight of the tumor, determined by the method of Schrek (17). At the end of the experiment the rats were killed, and carcass and tumors weighed, the latter being 20–25 per cent of the total body weight.

Thiouracil (TU) and propylthiouracil (PTU) were given as a 0.05 per cent solution in the drinking water, or by the subcutaneous injection of 5 mg. daily, dissolved in 5 per cent gum acacia; 5 mg. ACTH (Connaught)6 in saline was injected intraperitoneally in the morning, and 5 mg. in gelatin subcutaneously in the afternoon. Cortisone acetate6 was injected at a level of 5 mg. daily. Rats were adrenalectomized by the lumbar approach and maintained on saline, deoxycorticosterone acetate (0.8 mg.), and 0.1 mg. cortisone acetate daily. The phenylthiourea was given as a single dose of 30 mg/100 gm, and deaths in 48 hours were recorded.

RESULTS

The original observation from which these experiments arose was the lack of response of the tumor-bearing rat to thiouracil in the drinking water (Table 1). It was established that the more potent goitrogen, propylthiouracil, in drinking water, gave some response in the tumor-bearing rat, but not at the level of the control. To exclude differences in water intake, or absorption from the gastro-intestinal tract, propylthiouracil was given by injection. The results show that the response in the tumor-bearing rat is only a fraction of that in the control. The same result is found in the tumor-bearing rat if the goitrogen is started at the time of tumor implantation (20 days), or at the time the tumor becomes palpable (18 days).

Experiments were carried out to test the effect of variation in sex and strain of rat and type of tumor. The difference in response is present in the Wistar rat with the Walker tumor, and in the female hooded rat with a transplantable fibrosarcoma, though this is not so marked as in the Sprague-Dawley strain.

Experiments were performed on caloric-restricted control and force-fed tumor-bearing rats, to note the effect of the nutritional state of the rat on response to the goitrogen. Tumor-bearing rats have a lesser response than the nontumor-bearing rat subjected to a severe dietary restriction (Table 2). Force-feeding of both groups gave a difference at the 0.02 level of significance. This difference is

* Aided by a grant from the National Cancer Institute of Canada. Preliminary reports of this work have been published (2, 3, 4).
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1 Obtained from Sprague-Dawley, Inc., Madison, Wis.
2 Master Fox Cubes, Toronto Elevators Ltd.
3 Bred in the laboratory from stock obtained at the Carworth Farms.
4 From a strain originated by Dr. J. B. Collip at McGill University.
5 Tumor D2S chemically induced in this laboratory by Dr. R. L. Noble.
6 Provided by the Advisory Committee on Medical Research, National Research Council of Canada.
the result of a poor response of the control rats, rather than an increased response of the tumor-bearing rats.

A high-protein diet was given to both groups to exclude the possibility that the supply of amino acids was limiting the response of the tumor-bearing rat. This accentuated, rather than reduced, the difference in the two groups. The diet resulted in very large adrenals in the tumor-bearing rats, one rat having adrenals of 113 mg.

An attempt was made to see if ethionine would interfere with the synthesis of anterior pituitary hormones. The different response to the goitrogen was not altered at the dose level used, but the adrenal increased in size in both groups.

The inhibition of ACTH production in the pituitary of the tumor rat by the administration of cortisone was attempted, with a view to improved response from the goitrogen. Some measure of success was attained, but there is still a difference, significant at the 0.05 level (Table 3). That ACTH production was inhibited is seen from the reduced adrenal weights.

Stimulation of ACTH production in the nontumor-bearing rat by adrenalectomy might limit the response of the rat to a goitrogen. This did lead to a smaller mean thyroid weight, but not at a significant level. ACTH or cortisone administration did not interfere with the response to the goitrogen.

Goitrogens can protect against toxic thioureas (7). If the goitrogens were being metabolized in an

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### TABLE 1

Goitrogenic Response of Tumor-Bearing Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Strain</th>
<th>Tumor</th>
<th>Thyroid wt. (mg.)</th>
<th>Thyroid wt. (mg.)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>S-D, M</td>
<td>W-256</td>
<td>10 ± 0.8§</td>
<td>5 ± 2.5§</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PTU, 5 mg. daily for 13 days</td>
<td>S-D, M</td>
<td>W-256</td>
<td>27 ± 1.1</td>
<td>5 ± 2.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PTU, 5 mg. daily for 20 days</td>
<td>S-D, M</td>
<td>W-256</td>
<td>50 ± 4.2</td>
<td>6 ± 2.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PTU, 0.05 per cent in drinking water for 17 days</td>
<td>W, M</td>
<td>W-256</td>
<td>30 ± 5.2</td>
<td>6 ± 2.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PTU, 5 mg. daily for 10 days</td>
<td>H, F</td>
<td>D72S</td>
<td>26 ± 1.2</td>
<td>5 ± 0.5</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

* TU = thioracil; PTU = propylthiouracil.
† Strains: S-D = Sprague-Dawley; W = Wistar; H = Hooded. M = male; F = female.
§ Tumors' W-256 = Walker 256 carcinoma; D72S = fibrosarcoma.

### TABLE 2

Relation of Goitrogenic Response to Nutrition

Male Sprague-Dawley rats bearing Walker 256 carcinoma as indicated.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Carcass (gm.)</th>
<th>Adrenals (mg.)</th>
<th>Thyroid (mg.)</th>
<th>No.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TU, 15 days; nontumor-bearing rats, calorie-restricted</td>
<td>140</td>
<td>33</td>
<td>40 ± 2.5†</td>
<td>6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PTU, 14 days; all rats force-fed</td>
<td>178</td>
<td>41</td>
<td>29 ± 2.6</td>
<td>9</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>PTU, 12 days; all rats on high-protein diet</td>
<td>276</td>
<td>39</td>
<td>47 ± 3.3</td>
<td>6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PTU, 14 days; all rats given four doses, 20 mg. ethionine on alternate days</td>
<td>198</td>
<td>51</td>
<td>39 ± 1.9</td>
<td>6</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

* TU = thioracil; PTU = propylthiouracil, 5 mg. daily, for times indicated.
† Standard error of the mean.

### TABLE 3

Relation of Goitrogenic Response to the Adrenal

Male Sprague-Dawley rats bearing Walker 256 carcinoma as indicated.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Adrenals (mg.)</th>
<th>Thyroid (mg.)</th>
<th>No.</th>
<th>Thyroid (mg.)</th>
<th>No.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTU, 5 mg. daily for 9 days. Cortisone, 5 mg. daily for 9 days</td>
<td>20</td>
<td>54 ± 3.7</td>
<td>6</td>
<td>51</td>
<td>25 ± 1.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>PTU, 0.05 per cent in drinking water for 13 days</td>
<td>32</td>
<td>33 ± 3.5</td>
<td>6</td>
<td>31</td>
<td>33 ± 3.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>PTU+ACTH, 5 mg. twice daily for 10 days</td>
<td>52</td>
<td>51 ± 3.5</td>
<td>6</td>
<td>50</td>
<td>37 ± 2.8*</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>PTU+cortisone, 5 mg. daily for 10 days</td>
<td>52</td>
<td>51 ± 3.5</td>
<td>6</td>
<td>52</td>
<td>51 ± 3.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PTU+adrenalectomy (10 days)</td>
<td>25 ± 2.8†</td>
<td></td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Standard error of the mean.
abnormal manner, such protection might not be afforded. Pretreatment with propylthiouracil conferred protection on both control and tumor-bearing rats (Table 4).

In several experiments the thyroid glands were examined histologically, and in general the degree of hyperplasia has been in keeping with the thyroid weights.

**TABLE 4**

PROTECTION AGAINST PHENYLTHIOUREA* 
BY PROPYLTHIOURACIL†

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>No. deaths in 48 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nontumor</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Nontumor, PTU</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Tumor-bearer</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Tumor-bearer, PTU</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

* Phenylthiourea, 50 mg/100 g after PTU.
† Sprague-Dawley rats bearing Walker 256 carcinoma as indicated.

PTU, 5 mg. daily for 3 days.

Attempts at isolation and chromatography of nucleic acids from tumors and tissues of tumor rats, to ascertain if the uracil derivative had become incorporated, were negative (21, 22).

**DISCUSSION**

The goitrogenic response of a tumor-bearing rat is limited, with variation in the strain and sex of the rat, the type of tumor, and the route of administration.

It is apparent from the experiments on caloric restriction and forced feeding that inanition is not responsible for the effect. The experiments with cortisone are associated with a loss of body weight but do not interfere with response to the goitrogen. Feeding a high-protein diet, and the presumed additional supply of amino acids, has no effect on the goitrogenic response of the tumor-bearer, but appears to provide additional ACTH to both the nontumor- and tumor-bearing animals (19). The presence of an iodine-trapping substance in tumor-bearing rats has been reported (18).

It is possible that an abnormal metabolism of the goitrogen prevents an adequate concentration from reaching the thyroid. Thiouracil may be incorporated into liver nucleic acids (6), and uracil into the nucleic acid of tumors (16). Effects on nucleic metabolism of the host have been reported in tumor-bearing rats (11). The methods used to detect possible incorporation into nucleic acids in this study were too crude to yield valid results. This approach might be exploited with C14-labeled thiouracil.

That the goitrogen gives protection against phenylthiourea (7) in the tumor-bearing rat suggests that it is metabolized in a manner similar to that of the nontumor-bearing rat. Protection against the toxic thioureas may be afforded in the thyroidectomized animal (7), and thus the present experiments do not prove that all the goitrogen reaches the thyroid.

If a goitrogen is functioning in a normal manner, the synthesis of thyroxine should be blocked (1). No attempt has been made to date to study thyroid hormone production in nontumor- and tumor-bearing rats under the influence of a goitrogen, nor to assay the TSH content of the pituitary.

Tumor-bearing rats have large adrenals, presumed to be the result of increased release of ACTH from the anterior pituitary (3). The total synthetic ability of the pituitary for proteins may be limited, and the formation of large amounts of one tropic hormone might lead to inability to increase the production of another (9).

As a guide to experimentation it has been assumed that the ability of the tumor-bearing rat to produce adequate amounts of TSH is limited, in the face of the large amounts of ACTH being formed by the pituitary. Both experimental approaches to the problem, i.e., increasing the production of ACTH in the nontumorous rat by adrenalectomy and depressing the production of ACTH in the tumor-bearing rat by the administration of cortisone, have led to suggestive but inconclusive results. Ethionine did not affect the

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production of TSH in either the nontumor- or tumor-bearing rat, but appears to increase the release of ACTH in both groups, possibly by a toxic reaction.

SUMMARY

The increase in thyroid weight after the administration of a goitrogen to a tumor-bearing rat is less than that observed in comparable control rats. The limited response to the goitrogen is not sex-, strain-, or tumor-limited, nor is it the result of inanition.

ACKNOWLEDGMENTS

The advice of Dean J. B. Collip and Dr. R. L. Noble has been of great value, and our indebtedness to Miss Luise Schmidt and Mr. George Carpenter for technical assistance is acknowledged gratefully.

REFERENCES

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