DEVELOPMENT OF GOITRE
AFTER METHYLTHIOURACIL TREATMENT OF MICE WITH HYPOTHALAMIC LESIONS

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SUMMARY
Lesions aimed at the area of the paraventricular hypothalamic nuclei were made by means of stereotaxic electrocoagulation in intact and gonadectomized mice, which were subsequently treated with methylthiouracil. Lesions could be placed with an acceptable degree of precision and with moderate postoperative mortality. Correctly placed lesions made in intact females and castrated males caused a marked inhibition of goitre development, indicating a depressed secretion of thyrotrophin. However, in intact males and spayed females inhibition of goitre formation could not be demonstrated convincingly.

INTRODUCTION
The mouse is the animal of choice in studies on the relation between endocrine function and carcinogenesis. On the other hand, hypothalamic control of anterior pituitary function is now regarded as an essential element in endocrine regulation. It is therefore of interest to examine the effect of electrocoagulation of specific hypothalamic areas on hypothalamo-pituitary relationships in the mouse. We are aware of only two reports on stereotaxic brain surgery in the mouse (Slotnick & Essman, 1964; Montemurro & Toh, 1968). In this study we report the effects in mice of lesions in the area of the paraventricular nuclei (PVN) on goitre development induced by methylthiouracil. The experiments were prompted by the finding that in the rat such lesions inhibit thyrotrophin (TSH) secretion (Averill, Purves & Sirett, 1961; Van Rees & Moll, 1968) and experimental goitrogenesis (De Jong & Moll, 1965). This study was the first step in exploring hypothalamic influence on the induction and growth of thyrotrophic pituitary tumours (Furth & Clifton, 1966).

MATERIAL AND METHODS
Male and female C57 black mice with an initial body weight of 21–27 g. were used. Hypothalamic lesions were made with a stereotaxic instrument developed for the rat. The incisor bar was placed at a height of 2·8 mm. above the plane of the ear pins. This position brings bregma and lambda in one horizontal plane and brings the bregma, the anterior commissure and the posterior part of the optic chiasma in one vertical plane, whereas the paraventricular nuclei are situated in a plane 1 mm. more

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All lesions were aimed at the paraventricular nuclei and were made with radio-frequency current under ether anaesthesia. The electrode had a diameter of 0.3 mm. and an uninsulated tip of 0.5 mm. The lesions were ellipsoidal with a mean transverse diameter of 1 mm. and a mean long axis of 1.6 mm.

Three series of experiments were performed. In series I, lesions were made in otherwise intact male and female mice. They were fed normal pellets ad libitum during the first postoperative week and pellets containing 0.3% methylthiouracil (MTU) during the second postoperative week. At the end of this week the mice were killed. Thyroid and body weights were determined and the brains were processed for histological control of the localization of the lesions, which were classified as follows: correct lesions, i.e. complete destruction of PVN; incorrect lesions, i.e. PVN partly

or completely intact. The localization of the correct lesions is shown in Fig. 1. Unoperated animals served as controls. Separate experiments, in which goitre development in sham-operated and intact animals was compared, showed that the use of unoperated controls was justified. In series II, gonadectomized and sham-operated male and female mice without brain lesions received for a period of 2 weeks, starting on the day of operation, pellets containing 0.5% MTU ad libitum. At the end of this period the mice were killed and thyroid and body weights were determined. In series III,
lesions aimed at the PVN area were made in male and female mice, gonadectomized 4 days earlier. Sham-operated animals, in which the coagulation needle was brought down into the hypothalamus without passing a current, served as controls. The experimental schedule was in all other respects the same as in the first series.

RESULTS

Mortality of the lesioned animals decreased from approximately 50% in series I to 25% in series III. The percentage of incorrectly placed lesions also decreased: it was about 40% in series I and less than 10% in series II. Therefore, in series I the number of animals with incorrectly placed lesions was large enough to include observations on such animals, but this was not the case in series III. Some lesioned animals lost weight postoperatively and some seemed to develop hyperphagia. Results from animals with a postoperative change in body weight of more than 3 g. were discarded.

Table 1. Effects of hypothalamic lesions on goitre development in intact mice receiving methylthiouracil

(Means ± s.e. Number of animals in parentheses.)

<table>
<thead>
<tr>
<th>Sex</th>
<th>Controls</th>
<th>'PVN' lesions*</th>
<th>'Incorrect' lesions*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>5·3 ± 0·13 (38)</td>
<td>4·9 ± 0·23 (22)</td>
<td>5·2 ± 0·19 (11)</td>
</tr>
<tr>
<td>Female</td>
<td>5·9 ± 0·21 (22)</td>
<td>4·0 ± 0·26 (10)</td>
<td>5·2 ± 0·28 (8)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sex</th>
<th>Controls</th>
<th>'PVN' lesions</th>
<th>'Incorrect' lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>22·4 ± 0·60 (38)</td>
<td>19·2 ± 0·86 (22)</td>
<td>22·8 ± 0·79 (11)</td>
</tr>
<tr>
<td>Female</td>
<td>25·7 ± 0·79 (22)</td>
<td>17·5 ± 1·21 (10)</td>
<td>24·7 ± 1·11 (8)</td>
</tr>
</tbody>
</table>

* See text; PVN = paraventricular nucleus.
† P > 0·05, compared with controls and compared with incorrect lesions.
‡ % of thyroid weight in controls.
§ P < 0·05, compared with controls and compared with incorrect lesions.

Series I

Table 1 shows that goitre development was subnormal in otherwise intact females with lesions in the PVN area, but not in females with lesions with incorrect localization. This observation holds for both absolute thyroid weights and for thyroid weights/100 g. body weight. In contrast, in the males there was no significant difference in thyroid weights between the lesioned animals and the controls.

Series II

Table 2 shows that gonadectomy caused a small but significant decrease in goitre development in the males, whereas in the females goitre development did not differ significantly between intact and gonadectomized animals.
Table 3 shows that in castrated males the lesions caused an inhibition of goitre development, which was of the same degree on the basis of both absolute and relative thyroid weight. In the spayed females only relative thyroid weight was lower in the lesioned animals.

Table 2. Effects of gonadectomy on goitre development in mice receiving methylthiouracil

(Means ± s.e. Number of animals in parentheses.)

<table>
<thead>
<tr>
<th></th>
<th>Absolute thyroid weights (mg.)</th>
<th>Thyroid weight/100 g. body weight (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>Gonadectomized</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>6.9 ± 0.16 (13)</td>
<td>6.0 ± 0.18 (17)* 87 % †</td>
</tr>
<tr>
<td>Female</td>
<td>6.5 ± 0.20 (15)</td>
<td>7.0 ± 0.14 (18)‡ 108 %</td>
</tr>
</tbody>
</table>

* P < 0.05, compared with controls. † % of thyroid weight in controls.
‡ P > 0.05, compared with controls.

Table 3. Effects of hypothalamic lesions on goitre development in gonadectomized mice receiving methylthiouracil

(Means ± s.e. Number of animals in parentheses.)

<table>
<thead>
<tr>
<th></th>
<th>Absolute thyroid weights (mg.)</th>
<th>Thyroid weight/100 g. body weight (mg.)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td>'PVN' lesions</td>
</tr>
<tr>
<td>Male</td>
<td>4.3 ± 0.17 (23)</td>
<td>3.1 ± 0.14 (23)* 72 % †</td>
</tr>
<tr>
<td>Female</td>
<td>5.1 ± 0.24 (19)</td>
<td>4.9 ± 0.19 (29)‡ 96 %</td>
</tr>
</tbody>
</table>

* P < 0.05, compared with controls. † % of thyroid weight in controls.
‡ P > 0.05, compared with controls.

DISCUSSION

These observations show that in mice it is possible to destroy, with an acceptable degree of precision, specific areas of the brain by stereotaxic electro-coagulation. Montemurro & Toh (1968 and personal communication) used a stereotaxic apparatus similar to ours; Slotnick & Essman, however (1964 and personal communication) used a nasal-palatine bone clamp instead of incisor bar and ear pins. The accuracy achieved in the experiments of these authors seems similar to that of the present study. Therefore, the method of fixation of the skull is probably not of great influence on the accuracy of the operative procedure. Both Slotnick & Essman (1964) and
Montemurro & Toh (1968) made d.c. lesions. In our experience and that of Averill et al. (1961), radio-frequency lesions are to be preferred, since they are more clearly demarcated and show more regular contours. It seems likely that the increase in survival rate and in accuracy of placement of the lesions, which we encountered (compare series I and III), was a matter of experience.

Our results are in agreement with previous observations in the rat (see Brown-Grant, 1966; De Jong & Moll, 1965) which also show an inhibition of goitre development after the placement of lesions in the PVN area, and it seems justified to assume that the observed inhibition of goitre formation is due to diminished TSH secretion. In this respect it is of interest that the 'incorrect' lesions of the female mice of series I (Table 1) differed in localization only slightly from the correct lesions, but did not cause a significant goitre inhibition. This supports the interpretation that the effective lesions inhibited TSH secretion, since in the rat (De Jong & Moll, 1965) the effect of hypothalamic lesions on pituitary-thyroid activity is markedly dependent on their localization. It is not justified to speak of 'goitre blocking' lesions, since the lesioned mice had thyroid weights higher than those of animals on normal food. Mean thyroid weight in such animals has not been studied systematically, but was below 3.0 mg. in intact males and females.

Finally, the influence of gonadal hormones on development of goitre should be discussed. Each separate experiment was performed with males or females only. Therefore, no valid comparison of thyroid weights of male and female mice is possible. However, series II (Table 2) revealed that gonadectomy inhibits goitre development in male mice but is without effect in the female. This observation may be explained on the basis of data in the literature demonstrating an influence of gonadal hormones on pituitary-thyroid activity (Van Rees, Noach & Van Dielen, 1965; Bithel & Brown-Grant, 1968; Deutinger-Skrube, Hagmüller & Hellauer, 1968; D'Angelo & Fisher, 1969). However, of greater interest is a possible sex difference in the results of the lesion experiments. A positive result, i.e. marked inhibition of goitre development, was observed in intact females and castrated males, but not in intact males and spayed females. We are inclined to attach more importance to the positive results for the following reason. Experiments with antithyroid compounds given with the food allow valid conclusions only when intake of food is the same in the experimental and control groups, and this can be easily ascertained by comparing absolute and relative thyroid weights. If these do not show similar changes, intake of food and of the antithyroid compound may have been different and may have influenced the results. Such an effect might be involved in the groups without definite goitre inhibition, i.e. the non-castrated males and spayed females, since in these groups the relative thyroid weights indicated a greater effect of the lesions than the absolute thyroid weights. However, such a discrepancy did not occur in the groups with marked inhibition of goitre development, i.e. the intact females and the castrated males, indicating that intake of food and of MTU was the same in the lesioned and control animals. Therefore, only the evidence obtained in these latter two groups seems convincing. The occurrence of definite goitre inhibition in lesioned females (non-spayed) as well as males (after castration) makes it highly unlikely that there is a basic difference between the two sexes in the hypothalamic control of TSH secretion, such as exists for control of secretion of luteinizing hormone (Harris, 1964).
REFERENCES


