HYPOTHYROIDISM INDUCED BY A TRANSPLANTED FIBROSARCOMA IN THE RAT

By
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ABSTRACT

Rats bearing a methylcholanthrene induced fibrosarcoma showed a decrease in thyroid weight, a decreased responsiveness to the goitrogenous effect of PTU and a significant decrease in 24 hour thyroidal $^{131}$I uptake. The thyroid of the tumourous rats was also shown to have a marked inability to synthesize thyroid hormones. The effects on the thyroid were found to accumulate proportionally to the time during which the tumour grew in the rat, and lead to a fibrous and degenerative invasion of the thyroid gland.

In the rats bearing tumours, 1) the plasma and the pituitary TSH concentrations were within normal range; 2) large doses of exogenous TSH were able to stimulate the thyroid; 3) injection of saline extracts from the tumour elicited thyroid changes similar to those produced by the tumour. It is concluded from these data that the tumour produces an unusual hypothyroidism in the rat, which is caused by a substance elaborated by the tumour. The substance destroys progressively the thyroid tissue, leaving less and less thyroid parenchyma to respond to TSH and to secrete thyroid hormones. The substance seems to interfere with TSH at the thyroid level.

The purpose of this investigation was to elucidate the pathogenesis of the hypothyroidism of rats bearing a transplanted fibrosarcoma induced by methylcholanthrene. A chance observation showed that these rats displayed a small, yellowish and fibrous thyroid gland. Their goitrogenic response to propylthiouracil administration was less marked than in non-tumourous rats;

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their 24 hours radioiodine thyroidal uptake was decreased, as well as the concentration of radioiodinated plasma iodothyronine (Claus et al. 1962).

The results obtained in these experiments show that the effect of the fibrosarcoma upon the thyroid is due to the secretion by the tumour of a substance that has a direct effect upon the thyroid, or induces the formation of an endogenous substance that will in turn act upon the thyroid, and modify its response to endogenous thyrotrophin.

MATERIALS AND METHODS

I. Animals

Male Sprague Dawley rats (Holtzman Co., Houston, Texas and Cheek Jones Farms, Houston, Texas) weighing 125 to 200 g were used throughout these studies.

The transplanted fibrosarcoma has been described previously. The successive transplanted tumours were labelled as follows: MT. A (original tumour transplant), MT. B (original tumour transplant kept at the Memorial Hospital Vivarium, Houston), MT. C (original tumour transplant kept at Cheek Jones Farms). The tumour was transplanted into the right groin of the rat.

II. Diet

Unless otherwise indicated, the rats were maintained on Purina Laboratory Chow and tap water ad libitum. In order to induce a goiter, they were fed a Remington Diet containing 0.15 per cent propylthiouracil (General Biochemical, Chagrin Falls, Ohio). This was subsequently referred to as the goitrogenic diet.

III. Treatments

1. Thyrotrophin* (TSH) was injected subcutaneously (s.c.) in doses of 0.5 USP units in 1 ml of saline. In one experiment the injections were made on the 24th, 25th, 26th, 27th and 28th day following the tumour transplantation while in another, day 24 was omitted. The rats included in the second experiment received 10 μc** 131I 24 hours before sacrifice.

2. Myleran*** was injected intraperitoneally (i.p.) as suggested by Haddow & Timmis (1953) in doses of 1.5 mg per kg body weight, 7 days after the transplantation of the tumour.

3. Cytoxan**** in saline (100 mg in 20 ml) was injected i.p. (45 mg and 34 mg per kg body weight), on days 7 and 14 after tumour transplantation (Arnold et al. 1958; Lane 1960).

4. Tumour Extracts of three kinds were prepared. Chopped frozen tumour was homogenized in the cold with a Waring Blendor, in 50 ml of acetone per gram of tissue (Keller & Bloch 1960) and stirred overnight. The powdered, air-dried residue

* Thyrotrophin, Thyrotropar, Armour Pharmaceutical Company.

** μc = microcurie.

*** Busulfan (1-4 dimethanesulfonxybutane), courtesy of Borrroughs Welcome Comp.

**** Cyclophosphamide (N-Nbis (betachloroethyl) N'O propylene phosphoric acid ester diamide), courtesy of Mead Johnson Company.
was extracted with 2 N acetic acid and lyophilized. An aliquot of 2.4 g fresh tumour was suspended in 1 ml of 10 per cent USP gelatin in saline and was injected s.c. twice daily for 8 days.

The aqueous acetone solution obtained after precipitation of the acetone powder was evaporated, then partitioned with hexane. The hexane-soluble fraction was evaporated to an oily residue, diluted to 8 ml with peanut oil, and used as a lipid extract. Each milliliter of the lipid extract was suspended in 19 ml of 10 per cent USP gelatin in saline. Intact rats were injected i.p. twice daily for 8 days with 1 ml of this preparation which represented an aliquot of 2.4 g fresh tumour.

A saline extract and a hydrolyzed saline extract were also prepared. Each gram of chopped frozen tumour was homogenized in the cold with 3 ml of saline. A few crystals of ethylene diamine tetraacetic acid were added. The soluble fraction was lyophilized. An aliquot of the lyophilized powder was hydrolyzed by refluxing for 16 hours with 5.7 N HCl (Haurowitz 1963). The lyophilized and the hydrolyzed lyophilized extracts were each dissolved in warm saline and filtered (Putnam 1958). Each 2 ml of the filtered solutions was an aliquot of 3.99 g fresh tumour, and was injected s.c. to 2 groups of rats for 10 consecutive days.

IV. Histology

Animals, unless otherwise stated, were killed with ether and the thyroid glands were removed attached to the trachea and kept in 10 per cent formalin at least 8 days before dissection. The organs fixed in 10 per cent formalin were embedded in paraffin and stained with haematoxylin and eosin. The microphotographic fields were taken in such a manner as to include peripheral and central regions of each section. All the photographs were taken at the same magnification (× 145).

V. Parameters of Thyroid Function

1. The thyroid iodine uptake was evaluated by gamma counting on dissected thyroids 24 hours after i.p. injection of 10 μc or 3 μc of 131I, in a Picker Transistorized Spectroscaler III. The PB131I* was evaluated 20 or 24 hours following i.p. injection of 10 μc 131I by the procedure described by Ackerman et al. (1961).

2. Assessment of TSH secretion was obtained by evaluating the plasma and pituitary TSH by the bioassay originally described by McKenzie (1958) and modified by Sakiz & Guillemin (1964). Saline extracts of 5 hypophyses were injected at 3 dose levels (1/1800, 1/600 and 1/200 dilutions) in volumes of 0.3 ml. For the measurement of plasma TSH levels, plasma pooled from 5 rats was injected at a single dose level (0.5 ml for rats on a normal diet and 0.1 ml in a volume of 0.5 ml for rats on a goitrogenic diet), saline and two doses of USP thyrotrophin reference standard, 0.6 and 0.2 milliunits, were injected in 0.5 ml volume.

The comparison of the TSH activity of the pituitary gland from a normal versus tumourous rat was made with a 6 point assay (Bliss 1952). The adjusted responses obtained in the mice were compared to those of saline with the multiple comparison test of Dunnnett (1955). Approximate potencies of the plasma samples, expressed in milliunits TSH reference standard per 100 ml, were further calculated in multiple 3 point assays (2 doses of standard, 1 dose of unknown) as originally described by Gaddum (1953) with confidence limits calculated for P = 95 per cent.

* PB131I = Plasma radioactive protein bound iodine.
VI. Presentation of Results

In all the tables the chapter headings are identical unless otherwise stated. The abbreviations and symbols used are as follows:
- Treatment = 0 (intact), MT (tumourous).
- N = number of rats in the group.
- Days = number of days after tumour transplantation.
- Goitrogen = number of days given the goitrogenic diet (see text).
- ± = standard error from the mean.
- F = ratio of the variances (mean square deviation from the mean) between groups and inside groups (Lison 1958).
- P = probability of significant difference.
- = not significant.
- * = 0.05 level of significance.
- ** = 0.01 level of significance.
- *** = 0.001 level of significance.
- r = correlation coefficients calculated according to Snedecor (1956).
- Thyroid c. p. m. = gamma counts per minute obtained, divided by 100.
- Adrenal weight = the weight of both adrenal glands.
- Thyroid weight = the weight of the thyroid removed from the trachea after having been kept 8 days in 10 per cent formalin.
- Rat Body Weight = rat and tumour weight, when present.

RESULTS

The results of preliminary experiments were published earlier (Claus et al. 1962). They showed that rats bearing a methylcholanthrene induced fibrosarcoma had a decrease in thyroid weight, a decrease responsiveness to the goitrogenous effect of PTU and a significant decrease in 24 hour thyroidal 131I uptake. The thyroid of the tumourous rats was also shown to have a marked inability to synthesize thyroid hormones. The effects on the thyroid were found to accumulate proportionally to the time during which the tumour grew in the rat, and lead to a fibrous and degenerative invasion of the thyroid gland.

The results obtained in these experiments are reported here.

I. Effect of Large Doses of TSH

The results reported in Table 1 A show that 5 doses of 0.5 USP units of TSH suppressed the inhibitory effect of the tumour upon goitrogenesis. In the untreated tumorous rat on a normal diet, four injections of 0.5 USP units increased the thyroid 131I uptake in the tumorous rat from 35 to 78 per cent of the normal control and the PB131I from 31 to 105 per cent of the control (Table 1 B).

II. Correlation Between Tumour Weight, Thyroid Weight and Thyroid Function

Early experiments showed that ablation of tumours of 20 to 30 days of age
Table 1.
Effect of large doses of TSH.

A. Upon goitrogenesis in normal and tumourous rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Days</th>
<th>Other treatment</th>
<th>Thyroid weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>0</td>
<td>Goitrogen 10 days$^\circ$</td>
<td>$41.9 \pm 0.7$ F = 7.740$^\circ$</td>
</tr>
<tr>
<td>MT. B2</td>
<td>10</td>
<td>30</td>
<td>Goitrogen 10 days</td>
<td>$33.2 \pm 0.2$</td>
</tr>
<tr>
<td>MT. B2</td>
<td>10</td>
<td>30</td>
<td>Goitrogen 10 days</td>
<td>$33.2 \pm 0.2$  F = 31.1$^{**}$</td>
</tr>
<tr>
<td>MT. B2</td>
<td>10</td>
<td>30</td>
<td>Goitrogen 10 days + TSH</td>
<td>$40.2 \pm 1.9$</td>
</tr>
</tbody>
</table>

B. Upon the thyroid function of normal and tumourous rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Days</th>
<th>Other Treatment</th>
<th>Thyroid radioactivity (cpm)</th>
<th>Plasma PB$^{131}$I (cpm per 1 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6</td>
<td>6</td>
<td>-</td>
<td>$1473 \pm 101$</td>
<td>$621 \pm 58$</td>
</tr>
<tr>
<td>MT. B3</td>
<td>6</td>
<td>30</td>
<td>-</td>
<td>$542 \pm 89$</td>
<td>$196 \pm 37$                     F = 19.30$^{<strong>}$ F = 214$^{</strong>}$</td>
</tr>
<tr>
<td>MT. B3</td>
<td>6</td>
<td>30</td>
<td>TSH</td>
<td>$1149 \pm 104$</td>
<td>$661 \pm 53$</td>
</tr>
</tbody>
</table>

$^\circ$ The goitrogenic diet was started 20 days following the transplantation of the tumour.

was lethal most of the time, thus anti-tumour drugs were used to obtain a partial suppression of the tumour in order to study the relation between tumour mass, tumour growth, and thyroid function. The results are shown in Table 2 A and B.

Calculation of correlation coefficients showed no correlation between the body weight and the $^{131}$I thyroid uptake or PB$^{131}$I in the intact rat. There was no correlation between the weight of the tumour and the decrease in $^{131}$I uptake, but there was an inverse correlation between the weight of the tumour and the PB$^{131}$I values (Table 2 C).

III. Effect of the Tumour Upon Pituitary TSH

The 6-point assay shows no difference in TSH activity between the prepara-
Table 2.
Correlation between tumour weight, thyroid weight and thyroid function.
A. Effect of antitumour agents upon body weight, thyroid weight and thyroid function of the intact rat.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Days</th>
<th>Body weight g</th>
<th>Thyroid weight mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>10</td>
<td>-</td>
<td>230 ± 3.9</td>
<td>17.8 ± 0.93</td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>Myleran</td>
<td>233 ± 4</td>
<td>15.4 ± 0.60</td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>-</td>
<td>230 ± 3.9</td>
<td>17.8 ± 0.93</td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>Cytoxan</td>
<td>195 ± 10</td>
<td>16.3 ± 1.23</td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>-</td>
<td>855 ± 62</td>
<td>729 ± 40</td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>Myleran</td>
<td>837 ± 54</td>
<td>629 ± 70</td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>-</td>
<td>855 ± 62</td>
<td>729 ± 40</td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>Cytoxan</td>
<td>762 ± 109</td>
<td>655 ± 56</td>
</tr>
</tbody>
</table>

Results from pituitaries of intact control (A) or tumourous (B) rats; the Potency Ratio, B/A, was 1.133, with Confidence Limits of 1.492 – .864.

Calculation of the approximate plasma TSH concentration does not show any difference between samples from intact control or tumourous rats. It shows a significant increase in the plasma TSH of the goitrous rats. Results are presented in Table 3.

IV. The Effect of the Tumour Upon the Histology of the Thyroid Gland
The effect of the tumour upon the histology of the thyroid gland is illustrated and described in Figs. 1, 2 and 3. Generally speaking, the presence of the tumour lead to a pattern of thyroid inactivation and its invasion by fibrous tissues. Following ablation of the tumour, the thyroid gland appeared active again.

V. Effect of Tumour Extracts Upon the Thyroid and Glands of Normal Rats
1. Results obtained after eight bi-daily injections of acetone powder-acetic acid extract and lipid extract are reported in Table 4 A. The body weights
B. Effect of antitumour agents upon tumour weight, thyroid weight and thyroid function of the intact rat.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Days</th>
<th>Body weight g</th>
<th>Tumour weight g</th>
<th>Thyroid weight mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT. B4</td>
<td>10</td>
<td>-</td>
<td>272 ± 6.4</td>
<td>61.2 ± 4.9</td>
<td>13.7 ± 0.6</td>
</tr>
<tr>
<td>MT. B4</td>
<td>10</td>
<td>Myleran 236 ± 19</td>
<td>29.9 ± 3.6</td>
<td>13.4 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>MT. B4</td>
<td>10</td>
<td>-</td>
<td>272 ± 6.4</td>
<td>61.2 ± 4.9</td>
<td>13.7 ± 0.6</td>
</tr>
<tr>
<td>MT. B4</td>
<td>10</td>
<td>Cytoxan 226 ± 8.7</td>
<td>13.8 ± 3.6</td>
<td>14.2 ± 0.74</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Days</th>
<th>Thyroid radioactivity cpm</th>
<th>Plasma PB(^{131})I (cpm/(\text{1 ml}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT. B4</td>
<td>10</td>
<td>-</td>
<td>609 ± 59</td>
<td>340 ± 52</td>
</tr>
<tr>
<td>MT. B4</td>
<td>10</td>
<td>Myleran 452 ± 68</td>
<td>410 ± 49</td>
<td></td>
</tr>
<tr>
<td>MT. B4</td>
<td>10</td>
<td>-</td>
<td>609 ± 59</td>
<td>340 ± 52</td>
</tr>
<tr>
<td>MT. B4</td>
<td>10</td>
<td>Cytoxan 502 ± 68</td>
<td>520 ± 56</td>
<td></td>
</tr>
</tbody>
</table>

C. Correlation between body or tumour weight and thyroid function.

<table>
<thead>
<tr>
<th>Intact rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight and thyroid radioactivity</td>
</tr>
<tr>
<td>r = 0.17, no correlation</td>
</tr>
<tr>
<td>Body weight and PB(^{131})I</td>
</tr>
<tr>
<td>r = 0.02, no correlation</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tumourous rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour and thyroid radioactivity</td>
</tr>
<tr>
<td>r = 0.298</td>
</tr>
<tr>
<td>(t_c = 1.604)</td>
</tr>
<tr>
<td>no correlation</td>
</tr>
<tr>
<td>Tumour and PB(^{131})I</td>
</tr>
<tr>
<td>r = 0.5746</td>
</tr>
<tr>
<td>(t_c = 3.630^{**})</td>
</tr>
<tr>
<td>no correlation</td>
</tr>
</tbody>
</table>

were decreased by the injections of both extracts. The weight of the thyroid was reduced but not the uptake of \(^{131}\)I in the rats treated with the acetone powder acetic acid extract. There was no difference in thyroid weight or \(^{131}\)I uptake between the groups injected with the solvent or the lipid extract.
Table 3.
Effect of the tumour (MT. C 6, 20 days old) upon plasma TSH of normal and goitrous rats.

<table>
<thead>
<tr>
<th>Approximate TSH concentration in milliunits of TSH/100 ml plasma</th>
<th>Confidence limits at $P = 0.05$ as in multiple 3-point assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Without tumour</td>
<td>17.17</td>
</tr>
<tr>
<td>B. With tumour</td>
<td>12.64</td>
</tr>
<tr>
<td>C. With goiter</td>
<td>626.80</td>
</tr>
<tr>
<td>D. With goiter and tumour</td>
<td>526.80</td>
</tr>
</tbody>
</table>

2. Results obtained after 10 daily injections of saline tumour extract and hydrolyzed saline tumour extract are shown in Table 4 B. The body weight was the same in all groups, the thyroid weight and the $^{131}$I uptake was significantly decreased in the rats treated with the saline extracts whereas the $^{131}$I uptake was slightly elevated in the group that received injection of the hydrolyzed saline extract. Thus, there is a greater difference between the effect of the saline tumour extract before and after hydrolysis than between the saline tumour extract and the saline control. Histological examination of thyroid sections stained with haematoxylin and eosin showed that the thyroid of the rats who received the saline tumour extracts were quiescent (Fig. 4).

**DISCUSSION**

Rats bearing a methylcholanthrene induced fibrosarcoma show a decreased thyroid weight, a low response to the goitrogenic effect of PTU, a decreased $^{131}$I 24 hours thyroid uptake and a marked inability to synthesize thyroid hormones. The effects of the tumour were found to accumulate proportionally to the time during which the tumour grew in the rat and led to the fibrous and degenerative invasion of the thyroid.

This hypothyroidism was not accompanied by significant changes in the concentration of pituitary or plasma TSH. The syndrome, overcome by administration of large doses of TSH or by surgical removal of the tumour was induced in the normal rat by multiple injections of a saline extract of the tumour.

Presence of a hypothyroid state in the presence of adequate TSH can be explained in the rats that had the fibrosarcoma for more than 25 days as being due to the replacement of thyroid secretory tissue by fibrous and degenerative tissue. Thyroidal inactivation at early stages of the growth of the tumour or following multiple injections of extracts from the tumour in intact rats cannot be attributed to the same mechanism.
Fig. 1.
Effect of the tumour upon the histology of the thyroid gland.
A. The thyroid of rat on a normal diet.

*Plate 1.* Thyroid section from a normal intact rat. In the periphery there are a few follicles with flat epithelium and dark nuclei, but most of the follicles are the same as in the center: they have a high epithelium surrounding a light coloured colloid and the cells have a granulous appearance.

*Plate 2.* Thyroid section from a rat with a 58 day old tumour (MTA4). There are very large follicles in the periphery. They have a flattened epithelium and are filled with colloid. The rest of the peripheral zone and the center of the gland are invaded by fibrous and cellular tissue with undifferentiated chromatic nuclei. There are also calcium deposits.

*Plate 3.* Thyroid section from a rat with a 30 day old tumour (MTA4). In the periphery there are follicles with a flattened epithelium and abundant colloid. In the center of the gland the follicles have the same appearance except for a few that have a higher epithelium. There is some invasion by fibrous tissue.

*Plate 4.* Thyroid section from a rat with a 20 day old tumour (MTC6). The periphery and the center of the thyroid have follicles with a flat epithelium that are filled with colloid. In the center there are some rare follicles with higher epithelial cells and granulous colloid.
Fig. 2.
Effect of the tumour upon the histology of the thyroid gland.
B. The goiter of the PTU fed rats.
Plate 5. Thyroid section from an intact rat fed a goitrogenic diet for 27 days. There is only one type of follicle either in the periphery or the center. It has very large epithelial cells bulging inward, leaving little or no acinar space. There is a complete absence of colloid.
Plate 6. Thyroid section from a rat with a 76 day old fibrosarcoma fed a goitrogenous diet for 70 days (MTA4). In the periphery some follicles with colloid and a flat epithelium are present but most of the glandular structure is disorganized by fibrous and cellular tissue with chromatic nuclei.
Plate 7. Thyroid section from an intact rat fed a goitrogenic diet for 20 days. The same pattern is observed as in Plate 5.
Plate 8. Thyroid section from a rat with a 20 day old fibrosarcoma fed a goitrogenic diet for 20 days (MTC6). In the periphery there are follicles with large acinar spaces, either empty, filled with colloid or with retracted colloid. In the center the follicles have the typical aspect of PTU effect: there are follicles with no acinar space due to the bulging of epithelial cells.
Fig. 3.
Effect of the tumour upon the histology of the thyroid gland.
C. The thyroid of the rat after tumour ablation.

Plate 9. Thyroid section from a rat with a 36 day old fibrosarcoma (MTB4). In the periphery there are a few follicles with flat epithelium. The gland is invaded by a granulous substance and shows zones of degeneration and necrosis.

Plate 10. Thyroid section from a rat with a 26 day old fibrosarcoma surgically removed 13 days before sacrifice* (MTB4X). In the periphery and the center the follicles show a high epithelium bulging within the acini which is filled with a light stained colloid. There is one zone of abnormal tissue rich in nuclei of various shapes. Some of the granulous substance found in the section seen on Plate 9 is also seen.

* The weight of the tumour at time of sacrifice was 272 g. The parameters of thyroid function for the rat with the excised tumour were compared to the ones with the tumour: the weight of the thyroid was 19 mg vs 12 mg, the 24 hours radioiodine uptake, in per cent of the dose given, 0.29 % vs 11 % and the PBI^{31}I in cpm/ml 30 vs 102.
Normal ranges of pituitary and plasma TSH together with a decreased neo-biogenesis of thyroid hormones may be explained by the presence of normal amounts of stable iodothyronines in the plasma of tumourous rats (Claus et al. 1962). These secreted hormones were probably sufficient to insure a normal feedback to the hypophysis and the thyroid.

The aetiology of this hypothyroidism does not seem related to any of the ones classically described (Means et al. 1964).

Money (1955) and Begg (1958) suggested that rats with hypothyroidism in-
Effect of multiple injections of saline tumour extract, hydrolyzed saline tumour extract and saline upon the histology of the thyroid gland of intact rats.

Plate 11. Thyroid section from a rat injected with saline. The peripheral follicles have a low epithelium and are filled with colloid but the ones in the central part have a cuboid epithelium and contracted colloid.

Plate 12. Thyroid section from a rat injected with saline tumour extract. All the follicles of the periphery have a flattened epithelium and are filled with colloid. The central follicles have the same appearance except that some have a cuboid epithelium.

Plate 13. Thyroid section from a rat injected with hydrolyzed saline tumour extract. The periphery of the thyroid gland as well as the center shows follicles with cuboid epithelium. In the central follicles the colloid has either disappeared or was contracted.
duced by a tumour had a shift of pituitary secretion from TSH to corticotrophin. Our results show that this shift did not occur.

Reichlin (1957) described that starvation led to hypothyroidism, but Begg & White (1956) showed that the limited response to goitrogens observed in rats with Walker 256 fibrosarcoma was not related to inanition.

Iodine trapping by the tumour may have created a shortage in iodine supply: this specific fibrosarcoma was not concentrating appreciable amounts of iodine (Claus et al. 1962).

The plasma of the tumourous rats may have contained an abnormal iodinated compound with an inhibitory effect upon the thyroid (Money et al. 1959). However, radioautographic studies of the plasma of tumourous rats failed to detect any such produce (Claus et al. 1962).

The tumourous rats may have a decreased responsiveness to TSH. This was suggested by the occurrence of thyroidal activation after injection of large doses of TSH into tumour bearing rats and by the lack of activity observed in the thyroid of the rats following multiple injections of tumour extracts.

If the effects of the fibrosarcoma upon the thyroid are reproduced by injection of tumour extracts in the intact rat, the agent responsible for the hypothyroidism must be elaborated by the tumour.

The mechanism of the hypothyroidism which appears in the rats as a result of the growth of a methylcholanthrene induced fibrosarcoma can be described as follows: a substance X destroys progressively the thyroid tissue leaving less and less thyroid parenchyma to respond to TSH and to secrete triiodothyronine and thyroxine. Substance X is present in saline tumour extract but not in the acetic acid extract of a tumour acetone powder or in the hydrolyzed saline tumour extract; thus, it is likely a sensitive molecule which does not withstand strong acid hydrolysis. In early stages X seems to interfere with TSH and to act as a TSH antagonist.

Many non-endocrine tumours were found to induce hormone like effect in their host and in experimental animals injected with extracts from these tumours (Lipsett et al. 1964). There is no report of substances elaborated by non-endocrine malignant tumours that has an anti-hormone like effect.

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REFERENCES

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