THE ANTI-THYROID ACTION OF PARA-AMINO-SALICYLIC ACID

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SUMMARY

1. Para-amino-salicylic acid (PAS sodium salt) given to rats under the experimental conditions described produces a fall of radio-iodine uptake to 10% of the control value at the end of 16 days. After 30 days this low level is unchanged.
2. Oxygen consumption falls gradually to 12% below the control value at the end of 30 days' treatment with PAS.
3. Thyroxine with PAS abolishes the fall in oxygen consumption and raises the radio-iodine uptake slightly above that given with PAS alone.
4. Methyl thiouracil with PAS does not depress the oxygen consumption level below that obtained with PAS alone. The radio-iodine uptake is raised slightly above that found with PAS.
5. After 15 weeks' administration of PAS radio-iodine uptake is more than double that of the control value.
6. The rise in radio-iodine uptake when thyroxine and methyl thiouracil are given with PAS and when PAS treatment is prolonged, is accompanied by structural changes in the thyroid gland indicative of an increased output of thyrotrophic hormone.
7. After 29 weeks' treatment with PAS signs of exhaustion of the thyroid gland appear. Complete degeneration of the follicular epithelium was observed in some animals at this time.
8. The structural changes in the gland are reversible even after 25 weeks' treatment with PAS at a dose level of 1 mg/g body weight/day.

The administration of para-amino-salicylic acid (PAS) in therapeutic doses for a period of 10 days has been found to produce a 'moderate thyroid hyperplasia' in rats. This change was prevented by thyroxine but not by sodium iodide and was not enhanced by methyl thiouracil [Kjerulf-Jensen & Wollbrandt, 1951]. Hanngren [1952] showed that in man the rate of uptake of $^{131}$I by the thyroid gland was reduced by an intravenous injection of PAS. This evidence of an immediate anti-thyroid action by the drug was of some interest as hypothyroidism arises but rarely as the result of PAS therapy for tuberculosis and then only after prolonged administration. In such cases when the drug was withdrawn the signs of hypothyroidism disappeared [Clausen & Kjerulf-Jensen, 1951; Komrower, 1951; Librach, 1952]. It appeared possible that the immediate anti-thyroid effect was reversed as treatment proceeded, and that the late appearance of signs of hypothyroidism was due to 'exhaustion' of the gland, which however recovered full function on withdrawal of the drug. The experiments described below were designed to study the immediate and more remote effects of PAS on the structure and function of the thyroid gland of albino rats.

METHODS

The experiments were divided into two series. The first consisted of short-term experiments during which PAS was administered for varying periods up to 30 days. The animals in this series were male albino rats of a single strain weighing between 180 and 250 g at the start of the experiment. They had been adapted to live at an
environmental temperature of 29.5°C for a period of at least 5 weeks before the experiment began and were maintained at this temperature during its course [Beattie & Chambers, 1953]. They were fed *ad lib.* on a stock cubed diet which contained approximately 850 µg iodine/kg [Thomson, 1936]. In the second series of experiments the rats were kept in an animal house the temperature of which varied between 17 and 22°C. The experimental periods lasted for between 15 and 34 weeks. Feeding was identical with that in the first series.

*Determination of oxygen consumption.* This was done by the method of Bargeton & Krumm-Heller [1949]. The detailed technique employed has been published elsewhere [Beattie & Chambers, 1953]. In order to be able to compare oxygen consumption levels of animals of differing weight and of the same animal as its weight increased, this level was expressed as Cal./m²/24. The method of calculating heat production from the oxygen consumption values observed has been described by Beattie & Chambers [1953]. Determinations of oxygen consumption were carried out on animals of the first, but not of the second series of experiments, owing to the variations in the environmental temperature referred to above.

*Uptake of ¹³¹I.* This was determined 4 hr after the injection of a known amount of the isotope [McGinty, Rawson & Thompson, 1948; Raben, 1950]. In the earlier experiments in both series of animals the dose of the isotope was 20 µc. contained in 0.5 ml. water and was given by intraperitoneal injection. The isotope was in the form of iodide and was substantially carrier-free containing between 0.0005 and 0.002 µg of ¹²⁷I/ml of the injection solution [Boursnell, 1953]. A dose of nembutal (50 mg/kg) was given by intraperitoneal injection 3½ hr after the injection of the isotope solution. Precisely 4 hr after injection the thyroid gland was removed and dropped into a tube containing 5 ml. of hot (100°C) 10% (w/v) sodium hydroxide solution. When the gland had dissolved, the tube was cooled to room temperature and the pH adjusted to 7.5-8.0 with hydrochloric acid, and made up to 20 ml. with distilled water. The activity of the solution was determined on duplicate aliquots (0.1 ml.) by a Geiger-Müller counter and appropriate probe and scaler units. The activity of each sample was expressed as a percentage of the activity contained in the volume of solution injected.

In later experiments the dose of the isotope was reduced to 5 µc. and the above procedure followed. Using a fluid counter it was possible to estimate the activity of two aliquots each of 9 ml.

As there was a possibility that the uptake of isotope by the thyroid gland might vary with the dose level, a series of control experiments were done to establish the mean isotope uptake in normal animals. In twelve rats which had lived for 8 weeks at the high constant environmental temperature of 29.5°C the mean uptake after a dose of 20 µc. was 6.04% (s.e. ± 0.23), and 5.55% (s.e. ± 0.15) in twenty-seven animals after a dose of 5 µc. In twenty-four non heat-adapted rats given a dose of 20 µc. the uptake was 9.71% (s.e. ± 0.35), and in eleven rats injected with a dose of 5 µc. it was 9.33% (s.e. ± 0.27). These results showed that there was no significant difference between the uptake measured with a large dose of isotope and that with a smaller one.

To enable comparisons to be made between rats of different weights the isotope uptake determined was corrected for a standard body weight of 250 g. Thyroid weight
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has been shown to be proportional to body weight over the weight range of animals used in these experiments [Donaldson, 1924]. It was assumed that the isotope uptake was proportional to the thyroid weight.

**Drugs.** In the first series all drugs were administered in solution by stomach tube as a single dose each day at 5 p.m. At the same time the control animals were given an equal volume of normal saline, also by stomach tube. The solutions were made up within 30 min of being given. The dose levels per gram of body weight/day were: PAS (sodium salt), 0.5 mg; thyroxine, 0.05 μg; 6-methyl 2-thiouracil, 1000 μg.

The rats of group A of the second series were given the drugs in their food. The cubed diet was crushed and drugs incorporated by mixing. It was estimated that each animal ate 10 g/100 g body weight/day. On this basis the daily dose levels per gram body weight were: PAS (sodium salt), 1.0 mg; thyroxine, 0.05 μg; 6-methyl 2-thiouracil, 2000 μg; sodium iodide, 0.0012 and 0.0024 μg. In groups B–E the drugs were administered by stomach tube once a day at the above dose levels, and control animals received identical volumes of saline by stomach tube at the same time.

**Histology.** In each group of animals, the thyroid gland of one or two rats was removed and fixed in Susa's solution or, after removal, the gland was divided into two equal halves along the middle line and one half was used for 131I determinations and the other for histological examination. Sections of 5 μ thickness were stained with haematoxylin and eosin.

**RESULTS**

**Series 1 (heat-adapted animals given PAS up to 30 days).** The control level of oxygen consumption was determined for each experimental group before drug administration was begun. In addition, a control group of ten rats was kept under observation during the experimental period. After administration of PAS the oxygen consumption declined progressively with time until it reached a level of 11.5% below the control value at the end of 30 days' treatment (Table 1, groups A–C). When 0.05 μg/day of thyroxine was given with PAS for 20 days, after a previous 10 days' treatment with PAS alone, it was found that the level of oxygen consumption was within the control range at the end of the experiment (Table 1, group D). The same dose of PAS was used for 10 days and was followed by 20 days' treatment with methyl thiouracil and PAS. The level of oxygen consumption was not depressed below that found after 30 days' treatment with PAS alone (Table 1, group E).

In the control group the mean 131I uptake was 6.24% (s.e. ± 0.19). After 16 days' treatment with PAS the isotope uptake had fallen to 1.13% (s.e. ± 0.14), giving a fall of 80% below the control value (Table 2, group B). There was no further decline after 30 days' treatment (Table 2, group C). When thyroxine was given with PAS for 20 days, following 10 days' treatment with PAS alone, the uptake was 2.49% (s.e. ± 0.14). There was thus a significant difference between the uptake in this group and that found after 30 days' treatment with PAS alone (Table 2, group D). When PAS and methyl thiouracil were given together the isotope uptake was 2.2% (s.e. ± 0.13), which was significantly different from that found with PAS alone.

The structural changes in the thyroid gland after 30 days' treatment with PAS were: (1) disappearance of colloid from most of the follicles in the central part of the gland leaving (2) a ring of peripheral follicles still containing appreciable amounts of colloid, (3) absence of any cellular proliferation, and (4) increase in cell height of the
Table 1. Series 1. Oxygen consumption in heat-adapted rats (short-term experiments)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>Treatment</th>
<th>Duration (days)</th>
<th>No. of observations</th>
<th>Means ± s.e.</th>
<th>% change from control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>5th week control</td>
<td>—</td>
<td>30</td>
<td>692 ± 2.59</td>
<td>—</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>10th week control</td>
<td>—</td>
<td>30</td>
<td>695 ± 3.36</td>
<td>—</td>
</tr>
<tr>
<td>A</td>
<td>6</td>
<td>Control</td>
<td>7</td>
<td>12</td>
<td>706 ± 3.49</td>
<td>—</td>
</tr>
<tr>
<td>B</td>
<td>5</td>
<td>Control</td>
<td>7</td>
<td>10</td>
<td>692 ± 6.17</td>
<td>—</td>
</tr>
<tr>
<td>C</td>
<td>6</td>
<td>Control</td>
<td>7</td>
<td>12</td>
<td>695 ± 4.68</td>
<td>—</td>
</tr>
<tr>
<td>D</td>
<td>6</td>
<td>Control</td>
<td>7</td>
<td>12</td>
<td>688 ± 4.10</td>
<td>—</td>
</tr>
<tr>
<td>E</td>
<td>6</td>
<td>Control</td>
<td>7</td>
<td>12</td>
<td>712 ± 3.70</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>PAS</td>
<td>10</td>
<td>6</td>
<td>667 ± 3.80</td>
<td>—3.1</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>PAS + thyroxine</td>
<td>20</td>
<td>6</td>
<td>697 ± 4.49</td>
<td>+1.3</td>
</tr>
</tbody>
</table>

Table 2. Series 1. Uptake of $^{131}$I/4 hr in heat-adapted rats (short term experiments)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>Treatment</th>
<th>Duration (days)</th>
<th>No. of observations</th>
<th>Means (%) ± s.e.</th>
<th>% change from controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>—</td>
<td>—</td>
<td>10</td>
<td>6-24 ± 0.19</td>
<td>—</td>
</tr>
<tr>
<td>B</td>
<td>5</td>
<td>PAS</td>
<td>16</td>
<td>4</td>
<td>1-13 ± 0.14</td>
<td>—82</td>
</tr>
<tr>
<td>C</td>
<td>6</td>
<td>PAS</td>
<td>30</td>
<td>5</td>
<td>1-25 ± 0.14</td>
<td>—80</td>
</tr>
<tr>
<td>D</td>
<td>6</td>
<td>PAS</td>
<td>10</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>PAS + thyroxine</td>
<td>20</td>
<td>5</td>
<td>2-49 ± 0.15</td>
<td>—60</td>
</tr>
<tr>
<td>E</td>
<td>6</td>
<td>PAS</td>
<td>10</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>PAS + methyl thiouracil</td>
<td>20</td>
<td>5</td>
<td>2-2 ± 0.13</td>
<td>—65</td>
</tr>
</tbody>
</table>

Follicular epithelium in the central part of the gland (Pl. 1, fig. 2). When a small dose of thyroxine (0.025 µg/g) was given with PAS for 20 days, the thyroid gland showed a great increase in colloid content over that seen after 30 days' treatment with PAS alone. The colloid was well vaculated, and the follicular epithelium had increased in height (Pl. 1, fig. 3). When the dose of thyroxine was doubled (series 1, group D), all the follicles were filled with colloid and the epithelial cells were flattened. Vacuolation of the colloid was found only in isolated spots within a few widely separated follicles. There was evidence of cellular proliferation (Pl. 1, fig. 4).

After treatment with methyl thiouracil and PAS (series 1, group E) two dissimilar patterns often existed together within the same gland. One pattern (Pl. 1, fig. 5) consisted of small follicles tightly packed together with no interfollicular cells and containing little or no colloid. When colloid was present it had large vacuoles. The height of the cells of the follicular epithelium was markedly increased. This pattern appeared to be an exaggeration of that found after 30 days' treatment with PAS (Pl. 1, fig. 2). The second pattern (Pl. 1, fig. 6) showed follicles containing little or no colloid lying between larger follicles containing vacuolated colloid. Many cells were
seen outside organized follicles, but these cells appeared to arrange themselves to form new follicles. Many of these cells were undergoing division (Pl. 2, fig. 1).

Series 2 (non heat-adapted animals given PAS for 15–29 weeks). Oxygen consumption levels were not determined on these animals. In group A to which PAS had been given for 15 weeks, the control $^{131}$I uptake was 9.71% (s.e. ± 0.35), and that of the animals receiving PAS was 5.12% (s.e. ± 0.38) (Table 3, group A). After 29 weeks’ treatment with PAS the isotope uptake was not significantly different from that of the corresponding control group (Table 3, group B). When PAS had been given for 25 weeks and then withdrawn, the isotope uptake 9 weeks after the last dose of PAS was again not significantly different from the corresponding control level (Table 3, group C).

Table 3. Series 2. Uptake of $^{131}$I/4 hr in non heat-adapted rats (long-term experiments)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>Experiment</th>
<th>Duration (days)</th>
<th>No. of observations</th>
<th>Uptake of $^{131}$I/250 g body wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Treatment</td>
<td></td>
<td></td>
<td>% change from controls</td>
</tr>
<tr>
<td>A</td>
<td>24</td>
<td>Control</td>
<td>105</td>
<td>21</td>
<td>9.71 ± 0.35</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>PAS</td>
<td>105</td>
<td>23</td>
<td>9.71 ± 0.35 ± 0.64 + 131</td>
</tr>
<tr>
<td>B</td>
<td>5</td>
<td>Control</td>
<td>203</td>
<td>5</td>
<td>9.5 ± 1.12</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>PAS</td>
<td>203</td>
<td>3</td>
<td>8.1 ± 0.64 186 15</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>Control</td>
<td>238</td>
<td>3</td>
<td>10.1 ± 1.15</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>PAS</td>
<td>175</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>No PAS for 9 weeks</td>
<td>63</td>
<td>4</td>
<td>10.7 ± 1.2 + 6</td>
</tr>
<tr>
<td>D</td>
<td>3</td>
<td>Control</td>
<td>105</td>
<td>21</td>
<td>9.71 ± 0.35 ± 0.38 + 46</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>NaI</td>
<td>16</td>
<td>3</td>
<td>5.12 ± 0.38</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>NaI + PAS</td>
<td>16</td>
<td>3</td>
<td>7.5 ± 0.69 6 + 23</td>
</tr>
<tr>
<td>E</td>
<td>3</td>
<td>NaI</td>
<td>16</td>
<td>3</td>
<td>4.4 ± 0.54 65 55</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>NaI + PAS</td>
<td>16</td>
<td>3</td>
<td>6.1 ± 1.04 37 37</td>
</tr>
</tbody>
</table>

When sodium iodide was given in the drinking water to provide 1.2 μg/kg/day for 16 days the $^{131}$I uptake fell from a control value of 9.71% (s.e. ± 0.35) to 5.12% (s.e. ± 0.38). When PAS was given simultaneously with this amount of sodium iodide the $^{131}$I uptake was 7.5% (s.e. ± 0.69) (see Table 3, group D). The addition of PAS had produced a significant rise in the uptake of $^{131}$I. When the amount of sodium iodide in the drinking water was doubled the uptake of isotope was reduced to 4.4% (s.e. ± 0.54), but when PAS was given simultaneously with the sodium iodide the uptake was 6.1% (s.e. ± 1.04) (Table 3, group E).

After 15 weeks’ treatment with PAS (1 mg/g body weight/day) the thyroid gland showed two different structural patterns. One of these was indistinguishable from that shown in Pl. 1, fig. 6, except for the presence of small cells with darkly staining nuclei in the colloid of some of the follicles. These cells were seen to arise from the follicular epithelium. Many cells were present between the follicles and some of these were forming new follicles. In these interfollicular regions there was active cell division. The second pattern was that shown in Pl. 2, fig. 2. The follicular epithelium was increased in height, and the follicles were filled with non-vacuolated colloid. Occasional mitotic figures were seen.

After 15 weeks’ treatment with PAS a group of five rats were given thyroxine (0.05 μg/g/day) with PAS for 2 weeks. The gland structure (Pl. 2, fig. 3) was inter-
mediate between that shown in Pl. 1, figs. 3 and 4. This experiment was repeated with the substitution of methyl thiouracil (20 \( \mu \)g/g day) for thyroxine. The gland structure (Pl. 2, fig. 4) resembled that found after 20 days' treatment with PAS and methyl thiouracil after a preliminary period of 10 days on PAS alone (Pl. 1, fig. 6). The follicles were small, filled with vacuolated colloid and showed no increase in cell height. On the other hand, the cells lying between the follicles and derived from the follicular epithelium showed active cell division.

When treatment with PAS was continued for 20 weeks the follicles varied greatly in size (Pl. 2, fig. 5). Some of the larger follicles seemed to be in process of subdivision by the formation of septa. Their colloid contents were markedly vacuolated. Many follicles had become filled with cells derived from the follicular epithelium. Cellular proliferation appeared to be directed towards the filling-up of existing follicles, rather than to the formation of new follicles. In some animals the gland consisted entirely of filled-up follicles, but the cells in these had become necrotic leaving only a very few normal cells in the large peripheral follicles. The stroma in these glands was increased in amount (Pl. 2, fig. 6).

**Discussion**

In the short-term experiments (series 1) the great depression of the \( ^{131} \)I uptake by the PAS treatment with a relatively small fall in the level of oxygen consumption suggests that the primary effect of PAS is to inhibit the iodide-concentrating power of the thyroid gland. This inhibition is accompanied by changes in the structure of the gland which indicate a marked increase in the output of thyrotrophic hormone (TH). This increased output of TH cannot be attributed solely to a fall in the blood thyroxine (as indicated by the small reduction in oxygen consumption), for when thyroxine is given with PAS and the oxygen consumption level is restored to the control level, the \( ^{131} \)I uptake is not reduced further but rather tends to increase though it does not reach the control level. Moreover, when methyl thiouracil is given with PAS the oxygen consumption is not depressed below that found with PAS alone, yet the uptake of \( ^{131} \)I is increased. There is good evidence of a still greater rise in TH output (increased cell height and the presence of mitoses in the follicular epithelium of the thyroid gland). The depression of the \( ^{131} \)I uptake, found when the daily intake of sodium iodide is increased, is not further augmented when PAS is given simultaneously but is diminished. The thyroid structure when PAS and sodium iodide are given together shows evidence of a high TH output. All these observations suggest that PAS not only inhibits the iodide-concentrating power of the thyroid gland but also stimulates directly an increased production of TH with consequent structural changes in the thyroid gland.

In the long-term experiments (series 2) \( ^{131} \)I uptake, after 15 weeks' treatment with PAS, had risen to double that of the control level. The thyroid gland showed changes indicating high TH production. At this time the early inhibition of the iodide-concentrating power of the gland had been overcome, presumably by a high level of TH production. As the growth curves of these animals (already recorded by Bavin & James [1952]) differed in no way from those of the corresponding control animals, it may be presumed that thyroxine production and utilization had not been affected by the PAS treatment. After 29 weeks' treatment with PAS the isotope uptake fell to the control level. The gland structure showed some evidence of 'exhaustion', though
when the PAS was withdrawn and the animals given no drug for 2 weeks the gland structure had reverted to the normal state. The finding of one gland in an advanced state of degeneration in the 29 weeks’ group suggested that had PAS treatment continued much longer an irreversible hypothyroidism might have resulted.

Although the nature of the iodide-concentrating mechanism is unknown, Rosenberg [1952] has shown that ‘most classes of anti-thyroid substances are either competitive substrates or inhibitors of peroxidase’. In a series of phenolic substances investigated he found that PAS was a peroxidase inhibitor, while Randall [1946] concluded that the thiouracil type of compound was a competitive substrate. Dempsey [1949] and Astwood [1949] had suggested that peroxidase might exist within the thyroid gland and provide a mechanism for the production of iodine or periodate from iodide; either of these would act as an iodinating agent for tyrosine within the thyroglobulin molecule.

While the possibility that a peroxidase mechanism may exist within the thyroid gland cells cannot be excluded, the histological observations recorded here suggest that the rate of production of thyroglobulin may be reduced by PAS. The PAS effect may therefore be due to a reduction in the amount of protein produced by the gland cells and which is capable of being iodinated. For example, after 30 days’ treatment with PAS the colloid content of the gland was extremely small, while at the same time $^{131}$I uptake had been greatly reduced, and after 15 weeks’ treatment when the isotope uptake was double that of the control value the gland contained much colloid. Means [1951] suggested that the iodide-concentrating power of the gland may be due to the presence of a specific iodine-binding protein.

Whatever the nature of the iodide-concentrating mechanism may be, there is no doubt that it is inhibited by PAS. This inhibition is reversible. It must be pointed out that even when inhibition of iodide-concentrating power by PAS is marked, changes in the structure of the secretory epithelium of the thyroid gland compatible with an increased output of TH are present.

It is unlikely that PAS produces its effect through a pituitary-adrenal mechanism as PAS and hydroxy-salicylic acid do not bring about any depletion of adrenal ascorbic acid [Cronheim, King & Hyder, 1952]. Moreover, both ACTH and cortisone lower the plasma-bound iodine and the $^{131}$I uptake of the thyroid gland, [Wolfson, Beierwales, Robinson, Duff, Jones, Krarpp, Siemieni & Eya, 1951]. There was no evidence in any animals of either series that there was any increase in adrenal activity.

We wish to thank Dr H. B. Fell, F.R.S., for providing the facilities to carry out this investigation, and the Medical Research Council for a grant towards the expenses of the work.

Our thanks are due to Mr E. M. Bavin of Herts Pharmaceuticals Ltd., who put the animals contained in groups A, B and C of series 2 at our disposal for radio-iodine uptake determinations and histological examination, and who provided us with samples of pure para-amino-salicylic acid. We also wish to thank Dr J. Boursnell and Mrs V. Rizk, Biochemical Laboratory, Cambridge, for their assistance in making some of the radio-iodine determinations, and Mr V. C. Norfield for some of the photomicrographs. We are grateful to Dr R. Pitt-Rivers for her interest in this investigation and for much helpful advice.
REFERENCES


Bourass, J. [1953]. (Personal communication.)


DESCRIPTION OF PLATES

PLATE 1. All magnifications × 250.

Fig. 1. Thyroid gland from a rat after living for 10 weeks at an environmental temperature of 29.5° C.

Fig. 2. Central part of the thyroid gland of a rat after 30 days' treatment with 0.5 mg PAS/g body wt./day (Group C, series 1). The follicles are small, contain little colloid and have a columnar epithelium.

Fig. 3. Central part of the gland from an animal in group B, series 1; 10 days' treatment with PAS, 20 days with PAS and 0.025 mg/day thyroxine. The colloid content of the follicles is increased, but many small follicles containing no colloid still remain.

Fig. 4. Central part of the gland from an animal in group D, series 1. After 10 days' treatment with PAS and 20 days with PAS and 0.05 mg thyroxine/g/day, the follicles are well filled with colloid and the epithelium has become flattened. Cellular proliferation is present.

Fig. 5. Central part of the gland from an animal in group B, series 1, after 10 days' treatment with PAS and 20 days with PAS and methyl thiouracil. Only small masses of vacuolated colloid remain. The cells are columnar and highly granular.

Fig. 6. Central part of the gland from an animal in group E, series 1. All follicles contain vacuolated colloid. The interfollicular cellular material shows many mitotic figures. Some of the cells are arranging themselves in alveoli. The epithelium is not so columnar as in Fig. 5.

PLATE 2. Except Fig. 1 (× 415), all magnifications × 250.

Fig. 1. A portion of the section shown in Pl. 1, fig. 6. A dividing cell can be seen near the centre of the figure. Note the columnar epithelium of the follicle.

Fig. 2. A portion of a gland taken from an animal after 15 weeks' treatment with 1.0 mg PAS/g/day. Note the high columnar epithelium and the absence of interfollicular cells in this part of the gland. (Group A, series 2.)

Fig. 3. After 15 weeks' treatment with PAS followed by 2 weeks' treatment with PAS and thyroxine. Note the small number of interfollicular cells. The follicles have enlarged and are filled with colloid which is vacuolated around the periphery. The epithelial cells are flattened (group E, series 2).

Fig. 4. After 15 weeks' treatment with PAS and 2 weeks' treatment with PAS and methyl thiouracil. The larger follicles show vacuolation of the colloid. Between the follicles there is much cellular proliferation, and in this region mitotic figures, as in fig. 1 above, were frequent (group F, series 2).

Fig. 5. After 29 weeks' treatment with PAS (group C, series 2). The larger follicles are being invaded by septa made up of epithelial cells. Some follicles are filled with these cells. The colloid present is vacuolated.

Fig. 6. Part of a gland from group C, series 2. The follicles are filled with cells of the epithelial type but in an advanced stage of degeneration. The nuclei are pyknotic. Only small remnants of colloid remain in the gland. The fibrous stroma of the gland is much thickened.