CLINICAL AND EXPERIMENTAL STUDIES
OF SIMPLE AND MALIGNANT GOITRE

BY

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Thesis submitted to the University of Edinburgh
for the degree of Doctor of Medicine
1961.
## CONTENTS

### VOLUME I

<table>
<thead>
<tr>
<th>Section</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREFACE</td>
<td>1</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>3</td>
</tr>
<tr>
<td>PART I. CLINICAL STUDIES OF SIMPLE GOITRE</td>
<td>11</td>
</tr>
<tr>
<td>1. Methods</td>
<td>12</td>
</tr>
<tr>
<td>2. Simple goitre in Sheffield and Ormiston</td>
<td>35</td>
</tr>
<tr>
<td>3. The effect of supplementary iodine on simple goitre in school children in Oxfordshire and Wiltshire</td>
<td>62</td>
</tr>
<tr>
<td>4. Observations on iodine metabolism in simple goitre in Sheffield</td>
<td>79</td>
</tr>
<tr>
<td>PART II. EXPERIMENTAL STUDIES OF FACTORS INFLUENCING THYROID SIZE</td>
<td>132</td>
</tr>
<tr>
<td>5. Effect of season on thyroid size</td>
<td>133</td>
</tr>
<tr>
<td>6. Goitrogenic activity of milk</td>
<td>145</td>
</tr>
<tr>
<td>7. Aspects of iodine metabolism in experimental goitre</td>
<td>184</td>
</tr>
</tbody>
</table>

### VOLUME II.

<table>
<thead>
<tr>
<th>Section</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PART III. ANTITHYROID ACTIVITY AND METABOLISM OF GOITROGENS</td>
<td>195</td>
</tr>
<tr>
<td>8. Factors influencing the activity of antithyroid compounds</td>
<td>196</td>
</tr>
<tr>
<td>9. Uptake and metabolism of $^{35}$S thiohydantoins by the thyroid and other tissues</td>
<td>225</td>
</tr>
<tr>
<td>10. Prolongation of activity of antithyroid compounds</td>
<td>239</td>
</tr>
</tbody>
</table>
**PART IV. CLINICAL AND EXPERIMENTAL STUDIES OF MALIGNANT GOITRE**

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>11. Biological characteristics of thyroid carcinoma in relation to the importance of previous hyperplasia as an aetiologica factor</td>
<td>248</td>
</tr>
<tr>
<td>12. Effect of previous irradiation on the response of the rat thyroid to stimulation by thyrotrophin</td>
<td>317</td>
</tr>
</tbody>
</table>

**SYNTHESIS AND SUGGESTED STUDIES**

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>325</td>
</tr>
</tbody>
</table>

**SUMMARY**

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>335</td>
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</tbody>
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**ACKNOWLEDGMENTS**

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
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<td>338</td>
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**BIBLIOGRAPHY**

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>340</td>
</tr>
</tbody>
</table>

**APPENDICES**

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Chemical estimation of iodine</td>
<td>361</td>
</tr>
<tr>
<td>2. Technique of autoradiography</td>
<td>371</td>
</tr>
<tr>
<td>3. Preparation of $^{35}$S labelled thiouydantoins</td>
<td>373</td>
</tr>
<tr>
<td>4. Example of calculation of total thyroid iodine</td>
<td>375</td>
</tr>
</tbody>
</table>
PART III

ANTITHYROID ACTIVITY AND METABOLISM
OF GOITROGENS
FACTORS INFLUENCING THE ACTIVITY OF ANTITHYROID COMPOUNDS

The activity of antithyroid compounds is commonly assessed by the depression of $^{131}$I uptake produced by a single dose or by the production of thyroid hyperplasia and enlargement following administration for some days or weeks. There is a general assumption that comparable results may be obtained by either method (Searle et al. 304). The importance of such compounds that occur in nature is their ability to produce goitre, but the only method of testing them in man is by the accumulation of $^{131}$I by the thyroid. It seemed necessary to make a comparison between these methods, and a large number of compounds had to be tested to form a valid comparison. As described in Chapter 6, derivatives of 2-thio-oxazolidone occur in many species of plants. This compound is strikingly similar in chemical structure to three other compounds, all of which inhibit organic binding of iodine by the thyroid. These are 2-thiouracil, 2-mercaptoimidazole, and 2-thiohydantoin, and their structures compared to 2-thio-oxazolidone are shown in Fig. 73. Derivatives of 2-thio-oxazolidone were not available, but Dr Elmore in the Department of Chemistry in Sheffield prepared a large number of derivatives of 2-thiohydantoïn. The antithyroid activity of these derivatives
Fig. 73. Structure of three thioureido compounds compared with 2-thiooxazolidone.
in rats was studied by measuring changes in thyroid uptake of $^{131}$I after a single dose, and by changes in weight, histological appearance and uptake of the thyroid after repeated doses. The relationship between structure and activity is also of importance in the study of antithyroid compounds. This is discussed with reference to previous work on derivatives of 2-thiouracil and 2-mercaptopimidazole, and compared to the findings with derivatives of 2-thiohydantoin.

**Antithyroid activity following single dose of drug**

The mean depression of $^{131}$I content of the thyroid glands for each substance tested is expressed as % of the control glands (Table 34). 2-thiohydantoin is very active, but thiohydantoinic acid, which is prepared from it by mild alkaline hydrolysis, is without activity. 1-methyl-2-thiohydantoin is similar to the parent compound. All the other compounds are 5-substituted thiohydantoins. High potency remains with ascending 5-alkyl substitution, until there is a sudden loss of activity with 5-$\alpha$-hexyl thiohydantoin. Ring substitution, illustrated by the 5-benzyl derivative, still gives high activity, but this disappears with 5-$\alpha$-hydroxy-benzyl derivative. This, and succeeding compounds in Table 34, show low activity or are inactive. They contain polar groups in the side-chain. 5-$\rho$-hydroxy-benzyl-2-thiohydantoin is still inactive.
Table — Antithyroid activity of various thiohydantoins in rats as tested by a single oral dose of 0.05 m.mole/kg.

The significance of differences in $^{131}$I uptake between treated and control animals was calculated using Student's $t$ test. The level of significance is indicated in the second column of the table, as follows:

- **P < 0.01;
- *0.05 > P > 0.01;
- N.S. P > 0.05.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mean Depression of $^{131}$I Content of Thyroid as % of Control Group</th>
<th>No. of Rats Treated</th>
<th>No. of Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Thiohydantoins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thiohydantoic acid</td>
<td>-26 N.S.</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>1-Methyl-</td>
<td>79**</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>5-Methyl-</td>
<td>82**</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>5-Ethyl-</td>
<td>80**</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>5-n-Propyl-</td>
<td>75**</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>5-iso-Propyl-</td>
<td>79**</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>5-n-Butyl-</td>
<td>82**</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>5-iso-Butyl-</td>
<td>86**</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>5-sec-Butyl-</td>
<td>86**</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>5-n-Hexyl-</td>
<td>18 N.S.</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>5-Benzyl-</td>
<td>90**</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>5-α-Hydroxybenzyl-</td>
<td>-18 N.S.</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>5-Carbamoylmethyl-</td>
<td>3 *</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>5-Carboxymethyl-</td>
<td>-24 *</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>5-Methylthioethyl-</td>
<td>31*</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>5-Methyisulphonylethyl</td>
<td>-24 N.S.</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>5-Indolylmethyl-</td>
<td>33*</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>5-(4'-Aminobutyl)-hydrochloride</td>
<td></td>
<td>18 N.S.</td>
<td>6</td>
</tr>
</tbody>
</table>
when given by intraperitoneal injection, suggesting that inactivity is not due to lack of absorption from the intestine.

Increasing the dose shows that some slight antithyroid activity of a thiohydantoin containing a polar substituent can be demonstrated. 5-ρ-hydroxy-benzyl-2-thiohydantoin produces 48% depression of 4 hr. 131I uptake, when the dose is increased tenfold, namely to 0.5 m.mole / kg.

**Comparative antithyroid activity by single dose technique**

Dose/response relationships for several thiohydantoins were studied and compared with that for 2-thiouracil. At least five doses were used for each compound and % depression of 4 hr. 131I uptake calculated by comparison with the mean uptake in a control group of rats. Regression slopes of the dose/response curves were calculated and relative potency of the substances compared at the level of 70% depression of 131I uptake (Table 35 and Figs. 74 & 75). The dose/response curves were reduced to linearity within the ranges of observations, by using logarithmic scales. The horizontal logarithmic scale is \( \log_{10} \) (dose in mg) and the vertical logarithmic scale is \( \log_{10} \)

\[
\left( \frac{1}{1} - \frac{\% \text{ depression}}{100} \right) \quad \text{or in terms of observation, } \log_{10} \left( \text{mean control uptake/mean trial uptake} \right).
\]
### Table 35. Regression slopes of dose/response curves for thiouracil and various thiohydantoins

<table>
<thead>
<tr>
<th>Compound</th>
<th>Regression Slope (Units in Mean Log Response/Unit of Log Dose) ± S.E.</th>
<th>Common Slope</th>
<th>Dose in mg. for 70% Depression of 131I Uptake (Accurate to -30, + 50%, See Text)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Thiouracil</td>
<td>0.764 ± 0.080</td>
<td></td>
<td>0.93</td>
</tr>
<tr>
<td>2-Thiohydantoin</td>
<td>0.741 ± 0.072</td>
<td>0.735</td>
<td>1.20</td>
</tr>
<tr>
<td>5-Benzyl- &quot;</td>
<td>0.699 ± 0.084</td>
<td></td>
<td>2.10</td>
</tr>
<tr>
<td>5-Methyl &quot;</td>
<td>0.457 ± 0.115</td>
<td></td>
<td>3.00</td>
</tr>
<tr>
<td>5-n-Propyl &quot;</td>
<td>0.452 ± 0.068</td>
<td>0.455</td>
<td>2.70</td>
</tr>
<tr>
<td>5-iso-Butyl&quot;</td>
<td>0.619 ± 0.085</td>
<td></td>
<td>3.00</td>
</tr>
<tr>
<td>1-Methyl &quot;</td>
<td>0.351 ± 0.080</td>
<td></td>
<td>1.50</td>
</tr>
</tbody>
</table>
Fig. 74. Calculated regression lines from dose/response curves for 2-thiouracil, 2-thiohydantoin, 5-benzyl-2-thiohydantoin and 5-iso-butyl-2-thiohydantoin. Horizontal logarithmic scale is $\log_{10}$ (dose in mg.). Vertical logarithmic scale is $\log_{10}$ (mean control uptake/mean trial uptake). $\pm$ s.e. of regression line is indicated for each substance. The left-hand regression line refers to 2-thiouracil.
Fig. 75. Calculated regression lines from dose/response curves for 2-thiouracil, 1-methyl-2-thiohydantoin, 5-n-propyl-2-thiohydantoin, and 5-methyl-2-thiohydantoin. Horizontal logarithmic scale is \( \log_{10} \) (dose in mg.). Vertical logarithmic scale is \( \log_{10} \) (mean control uptake/mean trial uptake). ± s.e. of regression line is indicated for each substance. The left-hand regression line refers to 2-thiouracil.
Regression slopes were then calculated by least squares.

An analysis of variance, corresponding to a goodness of fit test and a test of parallelism for the regression lines, is shown in Table 36. The means of groups of rats given the same dose did not deviate from the regression line more than would be expected from the within group variations; there was thus no indication either of non-linearity of regression or of some uncontrolled experimental error affecting all rats in a dose group, and the deviations gave no indication of statistical significance \( (F = 0.89) \).

The regression lines deviated significantly from a common regression slope \( (F = 3.41) \). Thus relative potency cannot be compared from these slopes, apart from comparison at a given % depression. However, common regression slopes could be ascribed to two groups of substances as within these groups there was no significant deviation from parallelism. Relative potency can be assessed without qualification within the group 2-thiouracil, 2-thiohydantoin, and 5-benzyl-2-thiohydantoin, and between 5-methyl-2-thiohydantoin and 5-\(^{\text{-}}\)propyl-2-thiohydantoin, but not between these groups or the other two thiohydantoins tested (see Table 35). 2-Thiouracil and 2-thiohydantoin have almost equal potency, and the latter is nearly twice as potent as its 5-benzyl derivative. Interpretation of the flatter slopes of 5-methyl, 5-\(^{\text{-}}\)propyl, 5-iso
Table 36. Analysis of variance to test for a common regression slope of dose/response curves and goodness of fit of regression lines

The double asterisk indicates significant at the 1% level

<table>
<thead>
<tr>
<th></th>
<th>Degrees of Freedom</th>
<th>Mean Square</th>
<th>F Criteria</th>
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<tbody>
<tr>
<td>Deviations of regression lines from parallelism</td>
<td>6</td>
<td>1,240</td>
<td>$F$ criterion = 3.41**</td>
</tr>
<tr>
<td>Deviations of dose level group means from regression lines</td>
<td>21</td>
<td>287</td>
<td>$F$ criterion = 0.89 N.S.</td>
</tr>
<tr>
<td>Variations within dose level groups</td>
<td>220</td>
<td>364</td>
<td></td>
</tr>
</tbody>
</table>
-butyl-2-thiohydantoin and 1-methyl-2-thiohydantoin is difficult and is discussed later. All the substances are compared at one particular % depression. It is difficult to give precise standard errors for the doses giving 70% depression, as the mean control uptake for each dose/response curve is based on observations from 7 to 10 rats. Unfortunately, there is significant variation in controls measured at different times, probably due to variations in iodine content of diet, but limits of error probably range from -30% to +50%. When the substances are compared in this way, 2-thiohydantoin is the most potent, and substitution of methyl, n-propyl or iso-butyl in the 5-position reduces activity about threefold.

**Duration of activity**

The durations of action of 2-thiohydantoin and 5-methyl-2-thiohydantoin are compared with 2-thiouracil (Table 37). 2-Thiohydantoin and its 5-methyl derivative produced greater depression of iodide uptake than 2-thiouracil, when uptake was measured from 1 to 5 hr. after the dose. These thiohydantoins were calculated to be more potent than 2-thiouracil from the dose/response curves. However, when the interval between dosing and giving 131I was lengthened to 4, 8 and 12 hr. this effect of the thiohydantoins became less than 2-thiouracil. This is suggestive evidence that the duration of action of these thiohydantoins was shorter than 2-thiouracil.
Table 37. The depression of $^{131}$I uptake produced by 2-thiohydantoin, 5-methyl-2-thiohydantoin, and 2-thiouracil. $^{131}$I given 1 hr., 4 hr., 8 hr., and 12 hr. after the drug and uptake measured 4 hr. later.

† Indicates a dose estimated to produce equal depression of $^{131}$I uptake at 1 hr.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Oral Dose† (mg./kg.)</th>
<th>% Depression of $^{131}$I Uptake 1 to 5 hr. after Dose</th>
<th>% Depression of $^{131}$I Uptake 4 to 8 hr. after Dose</th>
<th>% Depression of $^{131}$I Uptake 8 to 12 hr. after Dose</th>
<th>% Depression of $^{131}$I Uptake 12 to 16 hr. after Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Thiohydantoin</td>
<td>0.3</td>
<td>88</td>
<td>85</td>
<td>46</td>
<td>21</td>
</tr>
<tr>
<td>5-Methyl-</td>
<td>0.4</td>
<td>82</td>
<td>73</td>
<td>42</td>
<td>15</td>
</tr>
<tr>
<td>2-Thiouracil</td>
<td>0.1</td>
<td>72</td>
<td>67</td>
<td>53</td>
<td>35</td>
</tr>
</tbody>
</table>
Effects of prolonged administration

The effect of various thiohydantoins given daily for 42 days was studied by observing changes in growth rate, thyroid weight, histological changes in thyroid and 24 hr. $^{131}$I uptake (Table 38). 2-Thiohydantoin and four 5-alkyl derivatives were tested. The other three compounds, 5-benzyl, 5-methyl-thioethyl- and 5-carboxymethyl-2-thiohydantoin, were chosen to represent an aromatic derivative, and polar compounds. The substances were given by subcutaneous injection at two dose levels, 10 mg./kg. and 50 mg./kg. The smaller of these doses was approximately twice the dose of 0·05m.mole/kg. used in the single-dose technique. Control rats were given distilled H$_2$O subcutaneously. Depression of daily weight gain was only found with the 5-methyl, 5-iso-propyl, and 5-benzyl derivatives at the level of 50 mg./kg. and this was associated with changes in thyroid weight, histology, and 24 hr. $^{131}$I uptake. As judged by these criteria, each substance produced uniform and consistent effects. The one striking exception was 2-thiohydantoin which had been found, from the dose/response curves to single injections, to be the most potent of the thiohydantoins tested. After 6 weeks of administration there was no depression of weight gain, and only moderate increase of thyroid weight, compared with its 5-methyl iso-propyl and benzyl
Table 38. Effect of various thiohydantoins on growth rate, thyroid weight and histology and 24 hr. $^{131}$I uptake in rats given either 50 mg./kg. or 10 mg./kg. daily by subcutaneous injection for 42 days.

The significance of differences in daily weight gain, thyroid weight and $^{131}$I uptake between treated and control animals was calculated using Student's t-test. The level of significance is indicated as in Table 34.

<table>
<thead>
<tr>
<th>Compound</th>
<th>No. of Rats Dosed</th>
<th>Mean Daily Weight Gain as % of Mean Daily Weight Gain of 8 Control Rats</th>
<th>Mean Increase in thyroid Weight Compared with Mean Thyroid Weight (mg./100g./Rat) of 8 Control Rats</th>
<th>Histological Changes of Hyperplasia (2 Rats in Each Group) Classified According to Astwood</th>
<th>Mean Depression (2 Rats in Each Group) of 24 hr. $^{131}$I Content of Thyroid as % of Mean of 4 Control Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Thiohydantoin</td>
<td>4/4</td>
<td>95 N.S. 96 N.S.</td>
<td>3.4* 2.1*</td>
<td>+++ + +</td>
<td>72** 63**</td>
</tr>
<tr>
<td>5-Methyl</td>
<td>4/4</td>
<td>76* 93*</td>
<td>3.2** 0.0*</td>
<td>+++ +</td>
<td>63** 58**</td>
</tr>
<tr>
<td>5-Propyl</td>
<td>4/4</td>
<td>86 N.S. 100*</td>
<td>9.0** 2.0**</td>
<td>+++ + +</td>
<td>69** 37**</td>
</tr>
<tr>
<td>5-iso-Propyl</td>
<td>4/4</td>
<td>71** 103*</td>
<td>19.8** 7.8**</td>
<td>+++ + +</td>
<td>86** 45**</td>
</tr>
<tr>
<td>5-iso-Butyl</td>
<td>4/4</td>
<td>84 N.S. 105*</td>
<td>8.0** 1.6*</td>
<td>+++ + +</td>
<td>36** 18 N.S</td>
</tr>
<tr>
<td>5-Benzyle</td>
<td>4/4</td>
<td>56** 89*</td>
<td>27.2** 8.2**</td>
<td>+++ + +</td>
<td>83** 64**</td>
</tr>
<tr>
<td>5-Methyl-thioethyl-</td>
<td>4/4</td>
<td>97 N.S. 99*</td>
<td>1.5* 1.0</td>
<td>+ +</td>
<td>47* 35*</td>
</tr>
<tr>
<td>5-Carboxymethyl-</td>
<td>4/4</td>
<td>95 N.S. 108*</td>
<td>0.0 N.S. 0.1 N.S.</td>
<td>- -</td>
<td>-3 N.S. -21 N.S</td>
</tr>
</tbody>
</table>
Fig. 76. Thyroid gland from a control rat, used in estimating degree of hyperplasia produced by thiohydantoins. H. & E. x 125.

Fig. 77. High power of above gland, showing lack of hyperplasia. H. & E. x 500.
Fig. 78. Thyroid gland from another control rat, used in estimating degree of hyperplasia produced by thiohydantoins. H. & E. x 125.

Fig. 79. High power of above gland, showing lack of hyperplasia. H. & E. x 500.
Fig. 80. Thyroid gland from another control rat.  
H. & E. x 125.

Fig. 81. High power of above gland.  H. & E. x 500.
Fig. 82. Thyroid gland from a rat receiving 10 mg./kg. of 2-thiohydantoin s-c daily for 42 days. There is distinct increase in epithelial height and moderate loss of colloid. Grade ++ hyperplasia. H. & E. x 500.

Fig. 83. Thyroid gland from a rat receiving 10 mg./kg. of 5-iso-propyl-2-thiohydantoin s-c daily for 42 days. There is striking epithelial hyperplasia and almost complete loss of colloid. Grade +++ . H. & E. x 500.
Fig. 84. Thyroid gland from a rat receiving 50 mg./kg. of 5-methyl-2-thiohydantoin s-c daily for 42 days. There is striking epithelial hyperplasia and loss of colloid. Grade ++ .

H. & E. x 125.

Fig. 85. Thyroid gland from a rat receiving 50 mg./kg. of 5-n-propyl-2-thiohydantoin, showing almost maximal hyperplasia and loss of colloid.

Grade ++++. 

H. & E. x 125.
Fig. 86. Thyroid gland from a rat receiving 50 mg./kg. of 5-iso-butyl-2-thiohydantoin s.c. daily for 42 days. There is maximal hyperplasia and complete loss of colloid. Grade ++ + + +. H. & E. x 125.

Fig. 87. High power of above gland. Grade ++ + + +. H. & E. x 500.
Fig. 88. Thyroid gland from a rat receiving 10 mg./kg. of 5-benzyl-2-thiohydantoin s.c. daily for 42 days. There is maximal hyperplasia and complete loss of colloid. Grade ++++. H. & E. x 125.

Fig. 89. High power of above gland. Grade ++++. H. & E. x 500.
Fig. 90. Thyroid gland from a rat receiving 50 mg./kg. of 5-methylthioethyl-2-thiohydantoin s-c daily for 42 days. There is only slight epithelial hyperplasia. Grade +. H. & E. x 500.

Fig. 91. Thyroid gland from a rat receiving 50 mg./kg. of 5-carboxymethyl-2-thiohydantoin s-c daily for 42 days. There is no evidence of hyperplasia when compared with control rats (Figs. 76-81). Grade - H. & E. x 500.
derivatives. However, it still produced pronounced depression of 24 hr. $^{131}$I uptake at the end of the 42-day period of administration. The degree of histological change produced by 2-thiohydantoin seemed to correlate with increase of thyroid weight, as it appeared to do for all other compounds tested. Also striking were the differences in effects produced by the two propyl derivatives. 5-Methyl-thioethyl-2-thiohydantoin was weakly active when tested by the single dose technique and remained so with this method. The compound with the polar carboxyl group, 5-carboxymethyl-2-thiohydantoin, had no activity by either method. Representative photomicrographs are shown in Figs. 76 - 91, graded using the classification given by Astwood (11).

Apart from the effects described above, all the rats remained well and active during administration of these compounds. Liver, kidneys, adrenal glands and testes showed no change in gross weight and no histological changes compared with those from control rats at the end of the 6 weeks period.

Discussion

Several different methods are available for the assessment of antithyroid activity, and for the study of the relation between structure and activity. Such methods fall roughly into two groups. The substance to be tested is given by mouth, or by injection, or in the diet for some days, and antithyroid activity is assessed by the degree of hyperplasia on histo-
logical examination (Astwood, 11), increase in thyroid weight (Astwood et al., 17); Jensen and Kjerulf-Jensen, 166) decrease in thyroidal iodine concentration (Bywater, McGinty, and Jenesel, 53). A combination of these measurements has also been used (Astwood et al., 17); Seifter and Ehrich, 308). Alternatively, antithyroid activity is measured after a single dose of the test substance, using uptake of radioiodine (Larson, Keating, Peacock, and Rawson, 191). Many compounds were tested in this way by McGinty et al. (212) and later by Searle et al. (304). These observers used experimental animals, and thyroidal radiiodide was measured on dissected thyroids. Stanley and Astwood (327), using in vivo counting and single-dose technique, have assayed drugs for antithyroid activity in man. However, there has been little work on the correlation of results obtained using single or repeated doses and there is a general assumption that comparable results may be obtained by either method (Searle et al., 304, 305). The results of the present investigation on thiohydantoins do not support this assumption. Using the single-dose technique, dose/response curves indicate that 2-thiohydantoin is almost equipotent with 2-thiouracil, and that 3-methyl, 5-propyl, 5-butyl and 5-benzyl derivatives of 2-thiohydantoin are less active than the parent substance. On the other hand, after daily
administration and on assessing the response by increase in thyroid weight and hyperplasia histologically, 2-thiohydantoin appears to be much less active than these derivatives. These results on chronic administration resemble those found by Astwood et al. (17) for 6-substituted derivatives of 2-thiouracil. Propyl and benzyl derivatives were about ten times as potent, and among the alkyl substituted compounds, peak activity was found with the propyl-derivative. It was also found that activity was greatly diminished in 6-n-hexyl-2-thiouracil, and it is of interest that 5-n-hexyl-2-thiohydantoin has no activity on acute testing in the present study. This striking relationship between activity and number of carbon atoms on the side-chain of 6-substituted thiouracils was confirmed by McGinty and Bywater (210,211), Vanderlann and Bissell (348) and Anderson, Halverstadt, Miller, and Roblin (7). The relationship for alkyl substitution in the 5-position of 2-thiouracil is not so apparent (Astwood et al., 17; Anderson et al., 7).

Much work has also been done with derivatives of 2-mercaptoglyoxaline, which is structurally very similar to 2-thiohydantoin. Searle et al. (304), using the single-dose technique, did not find any increase of activity with 4-alkyl substitution on 2-mercaptoglyoxaline, and they compared this with the results obtained by Astwood et al. (17) on chronic
testing. Searle *et al.* (304) do not appear to have studied dose/response curves with all their 4-alkyl derivatives. It is possible that varying rates of metabolism or excretion may account for differences found between single dose testing and more chronic administration of compounds. However, no great difference was found between 2-thiohydantoin and its 5-methyl derivative when the rate of return of thyroid activity to normal was measured, and the activity of both of these was of shorter duration than that of 2-thiouracil. Lawson, Rimington, and Searle (192) noted that the duration of action of 2-mercaptoglyoxalines was apparently less than that of 2-thiouracil. This problem of destruction and excretion requires further study. Jackman *et al.* (162) studied some 4-substituted mercaptoglyoxalines and 5-substituted thiohydantoins, but related their activity to 2-thiouracil and not to the parent compounds. In agreement with the present results, they found no change in activity with varying numbers of carbon atoms in the 5-alkyl side chain of 2-thiohydantoins. Activity is severely diminished by the presence of a polar substituent at C(5) and in the 2-thiohydantoin ring. This is clearly illustrated by a comparison of the antithyroid properties of 5-benzyl and 5-p-hydroxybenzyl-2-thiohydantoin. The largest effect arises from groups such as $-\text{NH}_3^+$ and $-\text{COO}^-$ on the one hand and $-\text{CO.NH}_2 -\text{OH}$ on the other.
Compounds containing any of these substituents might be bound by plasma proteins, the former by electrovalent linkages and the latter by hydrogen-bonds. Such binding would prevent the drug from reaching the thyroid in a significant concentration. A group such as \( \text{SCH}_3 \) could be bound rather weakly by an ion-dipole interaction, and it is noticeable that 5-methylthioethyl-2-thiohydantoin has a weak but significant antithyroid activity. Alternatively, interaction between drug and plasma proteins may not be important, and the result may be a true representation of activity within the thyroid. A similar correlation between chemical structure and antithyroid properties has been observed with 6-substituted-2-thiouracils (Astwood, 11; Astwood et al., 16, 17; McGinity and Bywater, 210, 211; Vanderlann and Bissell, 348; Miller, Dessert, and Anderson, 246; Horner, Kimmig, and Schreiner, 156), and substituted 2-mercaptoglyoxalines (Astwood, 11; Astwood et al., 17; Searle et al., 304, 305).

All the thiohydantoins tested in the present work contain the thioureido group, and this is also common to thiouracils and mercaptoglyoxalines, which have been studied by others. The mechanism of action of compounds containing this group is still not certain, but it is likely that some enzyme system catalysing oxidation of iodide to iodine in the thyroid...
is blocked (Astwood, 12). It is therefore disturbing to find significant deviations from parallelism of dose/response curves for different thiohydantoins. The flatter slopes for 5-\text{-}n\text{-}propyl, 5\text{-}m\text{-}ethyl, 5\text{-}iso\text{-}butyl and 1\text{-}methyl\text{-}2\text{-}thiohydantoin may be due to the observations being taken higher up the shoulder of a response curve with a lower limiting \% depression. This can occur with enzyme inhibitors of the partially competitive type (Dixon and Webb, 90). Similar behaviour has also been noted by Astwood (12) for 2-thiouracil and its derivatives, and he concluded that quantitative comparisons could only be made at some selected level of response. Similar treatment is required for the thiohydantoins and they have been compared here at the level of 70\% depression of $^{131}$I uptake. The central portion of the dose/response curve using this method approximates to a straight line. Larson et al. (191) and Searle et al. (304) suggest that comparison of potency should be made between the limits of 20\% and 80\%.

It is clear from these results on derivatives of 2-thiohydantoin that comparative activities of antithyroid compounds are dependent on the method of testing. Thus the activity of a natural antithyroid substance to depress $^{131}$I accumulation gradient may be different from the activity to produce thyroid enlargement. This difference may be related to
variations in metabolic degradation rates and rates of excretion. The relationship between lack of anti-thyroid activity and the presence of a polar group on the side chain is discussed fully in the following chapter.

Summary

1. The antithyroid activity of 2-thiohydantoin and 18 derivatives was measured in rats by depression of 4 hr. $^{131}$I uptake after a single dose. The presence of polar groups in the 5-substituent was associated with reduction or complete loss of activity at the dose level of 0.05 mole/kg. by mouth.

2. Dose response curves for 2-thiohydantoin and 6 derivatives were measured by the single dose technique, and activities also measured by effect of chronic administration for 42 days on thyroid weight and histological appearance. By the single dose technique, 2-thiohydantoin was more potent than its 5-alkyl derivatives. This order of activity was reversed by the method of assessment based on thyroid weight.
The thioureido group is common to 2-thiouracil, 2-mercaptoimidazole, 2-thiohydantoin and their substituents. It is also found in 2-thio-oxazolidone with the replacement of one nitrogen atom by an oxygen atom. The antithyroid activity of all these compounds has been attributed to this group (14, 272). This antithyroid activity is greatly decreased when a polar group is present on the side chain of substituents as shown by Astwood et al. (17) for 2-thiouracil, by Searle et al. (304) for 2-mercaptoimidazole, and for 2-thiohydantoin as described in the previous chapter. It seemed of interest to elucidate the mechanism whereby activity was lost by such minor changes in structure. Shulman (310) showed that thiourea, which was one of the first antithyroid compounds described (11, 219, 290, 291), was metabolised by the thyroid, as after $^{35}$S thiourea some of the radioactivity in the thyroid was in the form of sulphate. This work was confirmed by Maloof and Soedak (222). This suggested that comparing the metabolism by the thyroid of a non-polar thiohydantoin which had antithyroid activity and a polar thiohydantoin without such activity, might provide an explanation for their differing activities.
The compounds selected were the parent compound 2-thiohydantoin, and a polar substituent in the 5-position, 5-carboxymethyl-2-thiohydantoin. They were prepared from ammonium thiocyanate labelled with $35S$ and the appropriate amino-acid. The details of the preparation are given in Appendix 3.

**Uptake and metabolism of $2\left[35S\right]$-thiohydantoin and 5-carboxymethyl-$2\left[35S\right]$-thiohydantoin by the rat thyroid and other tissues**

Young male albino rats (150 g.) were injected intraperitoneally with $2\left[35S\right]$-thiohydantoin (70 μmoles/kg.) or 5-carboxymethyl-$2\left[35S\right]$-thiohydantoin (130 μmoles/kg.). The dose of the parent non-polar compound was known to produce almost complete inhibition of $131I$ uptake by the rat thyroid. The rats were kept in groups of 3 and groups killed at intervals during the 24 hr. after injection. Urine was collected in vessels from which light was excluded, as these compounds are unstable. Samples of blood, and bones, liver were taken and the thyroid glands removed. The total radioactivity was measured and expressed as % of the dose/gm. of wet tissue, except red blood cells and bone where dry weight was used. The results for $2\left[35S\right]$-thiohydantoin are shown in Table 38. The radioactivity of serum and all tissues except the thyroid was maximal within 1 hr. and then declined.
Table 38. Mean content of total radioactivity of rat tissues following $^{35}S$-2-thiohydantoin

<table>
<thead>
<tr>
<th>Time after administration</th>
<th>0.5 hr.</th>
<th>1 hr.</th>
<th>3 hr.</th>
<th>6 hr.</th>
<th>12 hr.</th>
<th>24 hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Serum (% dose/gm.)</td>
<td>0.76</td>
<td>0.87</td>
<td>0.49</td>
<td>0.32</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>Red blood cells (% dose/gm.)</td>
<td>2.94</td>
<td>2.09</td>
<td>1.27</td>
<td>0.71</td>
<td>0.24</td>
<td>0.22</td>
</tr>
<tr>
<td>Liver (% dose/gm.)</td>
<td>0.88</td>
<td>0.90</td>
<td>0.55</td>
<td>0.29</td>
<td>0.15</td>
<td>0.10</td>
</tr>
<tr>
<td>Bone (% dose/gm.)</td>
<td>0.52</td>
<td>0.33</td>
<td>0.23</td>
<td>0.20</td>
<td>0.10</td>
<td>0.06</td>
</tr>
<tr>
<td>Thyroid (% dose/gm.)</td>
<td>1.4</td>
<td>1.4</td>
<td>3.2</td>
<td>5.1</td>
<td>7.0</td>
<td>7.6</td>
</tr>
<tr>
<td>Urine (% dose/gm.)</td>
<td>-</td>
<td>9.2</td>
<td>23.6</td>
<td>33.9</td>
<td>50.9</td>
<td>64.5</td>
</tr>
</tbody>
</table>
steadily during the following 23 hr. This fall correlated with the radioactivity in the urine and after 24 hr. nearly 65% of the dose had been excreted in the urine. Only 30 min. after injection the thyroid total radioactivity was twice that of serum, and the thyroid content of 35S continued to increase, so that at 24 hr. the thyroid contained 7.6% of dose/g. which was sixty times the serum concentration at 24 hr. This shows a very high concentrating activity of 35S by the thyroid when 2[35S]-thioydantoin is given. The results following the injection of the polar compound 5-carboxymethyl-2[35S]-thioydantoin are given in Table 39. Contents of radioactivity of serum and all tissues 30 min. after injection were similar to the non-polar compound. The exception was 35S content of bone which was only one-third of the value following 2-[35S]-thioydantoin. During the 24 hr. period, serum and tissue contents of 35S fell more rapidly than with the non-polar compound, and this was associated with rapid excretion in the urine, so that almost all of the dose had been excreted after 24 hr. compared to 65% with 2[35S]-thioydantoin. The content of thyroid radioactivity was strikingly different, in that no progressive increase occurred during 24 hr. as with 2-[35S]-thioydantoin. The thyroid content of 35S 30 min. after 5-carboxymethyl-2[35S]-thioydantoin was twice the corresponding serum content, and though a progressive decrease
Table 39  Mean content of total radioactivity of rat tissues following 35S -5-carboxymethyl-2-thiohydantoin

<table>
<thead>
<tr>
<th>Time after administration</th>
<th>0.5 hr.</th>
<th>1 hr.</th>
<th>3 hr.</th>
<th>6 hr.</th>
<th>12 hr.</th>
<th>24 hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Serum (% dose/gm.)</td>
<td>0.72</td>
<td>0.44</td>
<td>0.11</td>
<td>0.10</td>
<td>0.10</td>
<td>0.04</td>
</tr>
<tr>
<td>Red blood cells (% dose/gm.)</td>
<td>2.0</td>
<td>0.72</td>
<td>0.39</td>
<td>0.19</td>
<td>0.21</td>
<td>0.20</td>
</tr>
<tr>
<td>Liver (% dose/gm.)</td>
<td>0.87</td>
<td>0.29</td>
<td>0.16</td>
<td>0.12</td>
<td>0.06</td>
<td>0.05</td>
</tr>
<tr>
<td>Bone (% dose/gm.)</td>
<td>0.15</td>
<td>0.15</td>
<td>0.07</td>
<td>0.04</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>Thyroid (% dose/gm.)</td>
<td>1.3</td>
<td>7.7</td>
<td>4.6</td>
<td>0.6</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>Urine (% dose)</td>
<td>-</td>
<td>47.0</td>
<td>63.9</td>
<td>75.9</td>
<td>84.3</td>
<td>99.1</td>
</tr>
</tbody>
</table>
occurred, the thyroid content remained higher than the serum content throughout the 24 hr. period.

Chromatography of $^{35}\text{S}$ radioactivity
in thyroid and urine

The chromatographic separation of a mixture of $^{35}\text{S}$ sulphate and $2\left[^{35}\text{S}\right]$-thiohydantoin is shown in Fig. 92. Two thyroid glands from the group of 3 rats killed 24 hr. after $2\left[^{35}\text{S}\right]$-thiohydantoin were each homogenised with 0.1 ml. of water, and the supernatant was placed on paper. The radiochromatogram, as illustrated by Fig. 93, showed only radioactive sulphate from both thyroids and no other radioactivity. Thiohydantoins absorb ultra-violet light and inspection of the chromatogram under such light showed no absorbing spots. Thus the concentrations of radioactivity by the thyroid after $2\left[^{35}\text{S}\right]$-thiohydantoin was due to $^{35}\text{S}$ sulphate as a metabolic product from the parent compound, and no concentration of this compound occurred. There was not enough radioactivity in the thyroids 24 hr. after 5-carboxymethyl-$2\left[^{35}\text{S}\right]$-thiohydantoin to produce any radioactive spots by autoradiography, and inspection under ultra-violet light showed no thiohydantoin spot.

A radiochromatogram of urine 24 hr. after $2\left[^{35}\text{S}\right]$-thiohydantoin is shown in Fig. 94. About 8% of the radioactivity was present as sulphate and 62% as unaltered $2$-thiohydantoin, which showed absorption under u-v light. There were two other radioactive
Fig. 92. Separation of a mixture of $[35S]$ sulphate and $[35S]^{-2}$-thiohydantoin, shown on a radiochromatogram.
Fig. 93. Radiochromatogram of the supernatant fluid of a homogenised rat thyroid gland, 24 hr. after injection of $[^{35}S]$-2-thiohydantoin.
Fig. 94. Radiochromatogram of urine collected from three rats for 24 hrs., following injection of $^{2}{_{35}}$-thiohydantoin.
Fig. 95. Radiochromatogram of urine collected from three rats for 24 hrs following injection of 5-carboxymethyl-2[35S]-thiohydantoin.
spots indicated in Fig. 94 as $X_1$ and $X_2$, which have not been identified. $X_2$ absorbed u-v. light and therefore has the thiohydantoin structure. It is a reasonable suggestion that it may be 2-methylthiohydantoin, but this compound is not available for comparison purposes. $X_1$ is almost certainly inorganic in nature as it did not absorb u-v light, or move with acidified butanol. However $X_1$ is not sulphide, thiocyanate or thiosulphate, as they have been found to have different Rf values.

Chromatography of urine 24 hr. after 5-carboxy-

methyl 2 $^{35}$S -thiohydantoin is shown in Fig. 95

About 65% of the parent compound was found and 15% of radioactivity was present as $^{35}$S sulphate. $X_1$ was also found but $X_2$ was not present. There was a suggestive spot of radioactivity to the right of the parent compound, but it did not absorb u-v light.

The metabolism of these two thiohydantoins is similar in that sulphate. $X_1$ and injected compound were found in the urine.

**Discussion**

These results show an active metabolism of thiohydantoins by the rat thyroid, with the production of sulphate within the gland, and are similar to the thyroid metabolism of thiourea reported by Shulman (310) and Maloof and Soodak (222). There was no concentration by the thyroid of the $^{35}$S labelled thiohydantoins, which corresponds to the
lack of thyroid concentration of either thiourea or
2-thiouracil noted by Maloof and Soodak (222). As
the levels of these two thiohydantoins in liver and
red blood cells were similar 30 min. after injection,
it seems unlikely that any significant degree of
plasma protein binding of the polar compound occurred,
and the suggestion given in the last chapter that
plasma protein binding might account for the lack of
activity of polar group compounds by preventing ade¬
quate tissue concentrations may be dismissed. It is
tempting to suggest that activity of these compounds
is dependent on some intermediate substance produced
by thyroid metabolic activity, and that the polar
group compound is inactive as it is not metabolised
and hence desulphurized to the same degree as the
non-polar compound. One other study of the metabol¬
ism of non-polar and polar group antithyroid compounds
was that by Van Erkelens (352), using 4-methyl
thiouracil and 4-carboxythiouracil (thio-orotic acid)
labelled with $^{35}$S. The latter was shown by Astwood
et al. (17) to have very low antithyroid activity,
associated with the carboxyl polar group. As the
dose of each compound was not stated it is difficult
to extract comparable information from this paper
by van Erkelens but concentration of sulphate after
4-methyl thiouracil was at least 10 times higher than
after the polar compound. Using thyroid slices
Maloof and Specter (224) and Maloof and Soodak (223)
have demonstrated that the sulphur of thiourea can be split and found that protein-bound sulphur, thiosulphate, sulphate and an unknown compound was produced. Maloof and Soodak claimed that thiouracil was also desulphurized, but their earlier work (222) showed no concentration of sulphate in the thyroid after giving thiouracil to rats. Their work clearly demonstrated that inorganic sulphate was not concentrated by the thyroid gland when given as such, and was only found after the administration and metabolism of thiourea.

The compounds found in urine after 2-[35S]-thiohydantoïn did not include thiosulphate but an unidentified compound (X1) which incorporated 35S and was probably inorganic in nature. The suggestion that X2, which has the thiohydantoïn ring structure, is 2-methyl-thiohydantoïn, is based on the report of Sarcione and Sokal (298) that 2-thiouracil is methylated in rats to 2-methylthiouracil. Unfortunately a pure preparation of 2-methyl-thiohydantoïn is not yet available.

These experiments demonstrate the ability of the thyroid tissues to break the carbon-sulphur bond of the thioureaïdo group, and it seems a definite possibility that the cleavage of this bond, which appears to be essential for the sulphur-containing antithyroid compounds, underlies their specific anti-
thyroid activity. Obviously the polar group compounds are not metabolised to the same extent and this may reflect some linkage of such a group and thyroid enzymes, which prevents the cleavage of the thioureido group.

Antithyroid compounds are also metabolised by other tissues and experiments concerning the influence of other substances on their metabolism will be described in the next chapter.

**Summary**

1. 2-[35S] thiohydantoin was metabolised by the rat thyroid to sulphate, and an increasing concentration of sulphate was found in the thyroid during the 24 hrs. after injection. Of 35S injected, there was 7.6%/g. of thyroid and 0.12%/ml. of serum after 24 hr. There was no concentration gradient between thyroid and serum for 2-thiohydantoin.

2. 5-carboxymethyl-2-[35S] thiohydantoin, which has little antithyroid activity because of the polar group, was not metabolised to sulphate to any extent. The corresponding values after 24 hr. were 0.6% dose/g. of thyroid and 0.04% dose/ml. of serum.

3. Sulphate and the appropriate thiohydantoin were found in urine after injection. Two unidentified 35S compounds were present after 2-[35S]-thiohydantoin. X1 was a thiohydantoin derivative and X2 was inorganic, but not sulphide, thiosulphate or thiocyanate. The latter was also present after 5-carboxymethyl-2-[35S]-thiohydantoin.
In recent years much interest has been aroused by the demonstration that many foreign substances are metabolised by enzyme systems present in microsomes within liver cells, and that these systems may be inhibited by certain other compounds (24, 45, 77). By this inhibition, the duration of action of many drugs can be prolonged, and often the degree of activity is increased. Thus the duration of action of hexobarbitone can be increased 15 times in mice and 37 times in rats (24, 45, 77). Several substances can produce such inhibition but most work has been done with β-diethylaminoethyldiphenylpropylacetate whose code name is SKF 525-A. When given alone this compound produces no pharmacological action in the doses used to inhibit the metabolism or biotransformation of various drugs.

Thiouracil and, as shown in the last chapter, thiohydantoins are metabolised by desulphurization from the thioureido group, and appearance of sulphate in the urine. It seemed of interest to investigate whether the action of antithyroid substances could be increased or the duration of action prolonged by SKF 525-A, and thus show whether these substances were metabolised by these microsomal enzyme systems.
Effect of SKF 525-A on the antithyroid activities of thiohydantoins.

Antithyroid activity was measured in rats by the standard method of effect on 4 hr. uptake of $^{131}$I. The dose of any compound had to be selected with care, so that it produced significant depression of 4 hr. uptake, but this depression was less than 50%, so that any increase in activity due to inhibition of metabolism of the compound by SKF 525-A could be recognised. After many trials, the standard procedure developed was to measure 4 hr. uptake of $^{131}$I between 8 and 12 hr. after the antithyroid compound, SKF 525-A having been injected intraperitoneally 40 min. before the antithyroid compound was given by mouth. The results with 2-thiohydantoin, two substituents on the 5-position and 1-methyl-2-thiohydantoin are shown in Table 41. Striking and significant increases in activity occurred with each of these compounds after SKF 525-A. It should be pointed out that the control group used to estimate depression of uptake produced by antithyroid compound and SKF 525-A, were also given SKF 525-A. However no significant depression occurred from SKF 525-A alone. Dose-response curves for all these thiohydantoins were given in Chapter 8, but their ranges only cover the depressions of $^{131}$I uptake produced with and without SKF 525-A in the case of 2-thiohydantoin and 5-benzyl-2-thiohydantoin. Plotting the depressions on these
Table 41. Effect of β-diethylaminoethyl diphenylpropylacetate (S.K.F. 525 A) on the antithyroid activities of thiohydantoins

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg./100g. body wt.)</th>
<th>Time of 131I uptake after dose (hrs.)</th>
<th>Mean depression of 131I content of thyroid as % of control group</th>
<th>No. of rats treated</th>
<th>No. of controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Thiohydantoin</td>
<td>0.50</td>
<td>8 - 12</td>
<td>30 *</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>&quot; + S.K.F.</td>
<td></td>
<td></td>
<td>60 **</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>5-Methyl-2-thiohydantoin</td>
<td>0.50</td>
<td>&quot;</td>
<td>18 *</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>&quot; + S.K.F.</td>
<td></td>
<td></td>
<td>52 **</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>5-Benzyl-2-thiohydantoin</td>
<td>0.50</td>
<td>&quot;</td>
<td>50 **</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>&quot; + S.K.F.</td>
<td></td>
<td></td>
<td>72 **</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>1-Methyl-2-thiohydantoin</td>
<td>0.40</td>
<td>&quot;</td>
<td>12 N.S.</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>&quot; + S.K.F.</td>
<td></td>
<td></td>
<td>51 **</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

The dose of S.K.F. was 5 mg./100 gm. body wt. intraperitoneally 40 min. before antithyroid compound was given.

** P < 0.01; * 0.05 > P > 0.01; N.S. P > 0.05
curves, shows that SKF 525-A administration was equivalent to increasing the dose of 2-thiohydantoin by a factor of 2.1, and 2.4 in the case of 5-benzyl-2-thiohydantoin. Assuming that the slopes of the dose-response curves can be used to extend the range covered, SKF 525-A was equivalent to increasing the dose of 5-methyl-2-thiohydantoin at least 5 times, and at least 10 times for 1-methyl-2-thiohydantoin. It is interesting that the dose-response curves for these two pairs of compounds had significantly different slopes as described in Chapter 8.

**Effect of SKF 525-A on the antithyroid activities of 2-thiouracil and mercapto-imidazoles.**

Using the same procedure for testing as with thiohydantoin, the effect of SKF 525-A on other antithyroid compounds was measured and the results are shown in Table 42. The activity of 2-thiouracil was increased after SKF 525-A, and this was equivalent to increasing the dose by a factor of 1.8, using dose-response data given in Chapter 8. The dose-response curve of 2-thiouracil was not different from the two thiohydantoins, whose activities, as shown above, were also increased approximately 2 times by SKF 525-A. The parent 2-mercaptoimidazole and two substituents, 1-methyl-2-mercaptoimidazole and 1-methyl-2-carbethoxyimidazole also showed increased
Table 42. Effect of β-diethylaminoethyl diphenylpropylacetate (S.K.F. 525A) on the antithyroid activity of mercaptoimidazoles and thiouracil.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg./100g. body wt.)</th>
<th>Time of 131I uptake after dose (hrs.)</th>
<th>Mean depression of 131I content of thyroid as % of control group</th>
<th>No. of rats treated</th>
<th>No. of controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbimazole</td>
<td>0.05</td>
<td>1 - 5</td>
<td>42 **</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>&quot; + S.K.F. &quot;</td>
<td></td>
<td>&quot;</td>
<td>67 **</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Carbimazole</td>
<td>0.05</td>
<td>8 - 12</td>
<td>28 *</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>&quot; + S.K.F. &quot;</td>
<td></td>
<td>&quot;</td>
<td>49 **</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Carbimazole</td>
<td>0.10</td>
<td>12 - 16</td>
<td>29 *</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>&quot; + S.K.F. &quot;</td>
<td></td>
<td>&quot;</td>
<td>63 **</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>2-Mercaptoimidazole</td>
<td>0.05</td>
<td>8 - 12</td>
<td>4 N.S.</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>&quot; + S.K.F. &quot;</td>
<td></td>
<td>&quot;</td>
<td>44 **</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>5-Methyl-2-Mercaptoimidazole</td>
<td>0.05</td>
<td></td>
<td>43 **</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>&quot; + S.K.F. &quot;</td>
<td></td>
<td>&quot;</td>
<td>63 **</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>2-Thiouracil</td>
<td>0.10</td>
<td></td>
<td>47 **</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>&quot; + S.K.F. &quot;</td>
<td></td>
<td>&quot;</td>
<td>67 **</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

The dose of S.K.F. was 5 mg./100 gm. body wt. intraperitoneally 40 min. before antithyroid compound was given.
antithyroid activity after SKF 525-A. Comparison of effect of SKF 525-A on carbimazole, when 4 hr. uptake was measured 1-5 hr. and 8-12 hr. after the same dose of carbimazole, showed no significant difference in the increases produced. Increase in activity could be produced with SKF 525-A, even when uptake was measured between 12-15 hr. after carbimazole.

Discussion

The weight of evidence on the mechanism of action of SKF 525-A (24, 45, 77, 46) suggests that the increased activity and prolongation of activity of thiohydantoin, 2-thiouracil, and mercaptoimidazole is due to inhibition of their metabolic degradation in the liver to inactive compounds. As the thioureido group appears to be essential for their antithyroid activity, it is likely that this inhibition acts on the desulphurization of this group. This metabolic pathway has been described (322, 381). Thus a decrease of $^{35}$S as sulphate would be expected after giving SKF 525-A and $^{35}$S labelled antithyroid compound. A preliminary experiment has been done on these lines with $^2\big[^{35}\text{S}\big]$-thiohydantoin, and the results support this hypothesis. However there is considerable variation in sulphate excretion between individual animals, and more results are required before being definite on this point. This is similar to the work of Sarcione and Sokal (298) who found that $^{35}$S sulphate
excretion after $2^{35S}$-thiouracil varied considerably in 3 rats.

In Chapter 8 it was shown that two groups of thiohydantoins were differentiated by differing common slopes of dose-response curves, and that the slope of 2-thiouracil was parallel to one of these groups. A hypothesis which might be tested is that these differing slopes are due to differing rates of degradation by a liver enzyme system. It has been shown that the activities of these two groups of thiohydantoins are selectively increased by SKF 525-A, which suggests that this transforming system is capable of being selectively inhibited.

This biotransformation system is of great interest in relation to the effect of substances foreign to an organism, and considerable evidence has developed concerning its role in the different rates of metabolism of drugs shown between species. Brodie (45) has shown that the activity of the microsomal enzyme system against hexabarbitone is different in various species, and that the activities correlate with duration of action of hexabarbitone in these species. He has also shown that a similar explanation can account for strain differences in response to drugs. Of great interest has been the demonstration that drugs may be destroyed at different rates on the basis of sex differences. Certain barbiturates produce longer sleeping times in female
rats compared to male rats (153, 257). Brodie (45) has shown that this is due to different microsomal enzyme activities between the sexes, and that it can be reversed by appropriate treatment with oestradiol or testosterone. It has already been demonstrated that antithyroid compounds are found naturally in certain foods. When these are isolated it will be interesting to study their metabolic degradation rates in man, particularly for any difference between sexes. This is of particular importance in relation to the observed higher incidence of simple goitre in women.

Summary

1. The depression of $^{131}$I uptake by the rat thyroid due to thiohydantoins, mercaptoimidazoles and 2-thiouracil is increased by prior administration of SKF 525-A.

2. SKF 525-A produced a differential effect on the activity of thiohydantoins, which correlated with the slopes of their dose-response curves.

3. Suggestive evidence is given that production of $^{35}$S sulphate following $2[35S]$-thiohydantoin is less after prior treatment with SKF 525-A.
PART IV

CLINICAL AND EXPERIMENTAL STUDIES
OF MALIGNANT GOITRE
11.

BIOLOGICAL CHARACTERISTICS OF THYROID CARCINOMA, IN RELATION TO THE IMPORTANCE OF PREVIOUS HYPERPLASIA AS AN AETIOLOGICAL FACTOR

In 1928 Wegelin (373) reported that thyroid carcinoma was ten times more frequent in Berne in Switzerland compared to Berlin, and he related this difference to the high incidence of simple goitre in Switzerland. Since then there have been many reports that there is an association between simple goitre and thyroid cancer, based on two kinds of evidence. The high incidence of carcinoma in nodular goitres at operation has been quoted as evidence that carcinoma arises more frequently in such goitres (74, 75, 78, 150). Further evidence that there is a geographical variation in incidence of thyroid cancer which correlates with simple goitre incidence, has seemed to strengthen this suggestion (220, 72). Hyperthyroidism represents a situation where extreme thyroid hyperplasia occurs, but it is striking that thyroid carcinoma has been thought to develop only rarely in such hyperplastic glands. (30, 78, 241). Ionising radiations have been shown to be aetiologically important in cancer and evidence has been given that they might play a part in the genesis of thyroid carcinoma (94, 66, 312). In an attempt to elucidate the
importance of these factors in the development of thyroid carcinoma, a review of patients with thyroid carcinoma in Sheffield has been carried out. Particular attention has been paid to the biological characteristics of the different histological types of thyroid carcinoma, and to the association of carcinoma with simple goitre, hyperthyroidism and previous exposure of the neck to ionizing radiations.

**Patients studied**

The series comprises the patients referred to the Sheffield National Centre for Radiotherapy between 1946 and 1956. In all there were 103 patients, but three were excluded when the histological findings were reviewed, leaving a total of 100 (Table ). Forty-six were alive in 1955; 40 of these have been seen and examined for any recurrence of growth, and their state as regards thyroid function assessed. The other six patients were unable to attend for review, but a report was obtained on their condition. Survival statistics are given for the period from the date of treatment to the end of 1960. Tissue for histological examination was not obtained from 22 patients. There were strong clinical grounds for diagnosing thyroid carcinoma; and because the purpose of this review is to discuss a series of cases of thyroid carcinoma which are not selected in any way, apart from being referred to a Radiotherapy Centre, and since the omission of 22 per cent. of a series would at once produce a selection
Table 43. The distribution of 100 cases of thyroid carcinoma by sex, histological confirmation, and other thyroid disease

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole series</td>
<td>100</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>With histological evidence of</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>thyroid carcinoma</td>
<td>78</td>
<td>17</td>
<td>61</td>
</tr>
<tr>
<td>Without histological evidence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>of thyroid carcinoma</td>
<td>22</td>
<td>3</td>
<td>19</td>
</tr>
<tr>
<td>With history of previous</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>goitre for more than 5 years</td>
<td>18</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>With coexistent hyperthyroidism</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>and thyroid carcinoma</td>
<td>7</td>
<td>-</td>
<td>7</td>
</tr>
</tbody>
</table>
bias, these patients have been included, but considered separately whenever possible.

**Criteria for diagnosis of carcinoma.** The only reasonably certain evidence of carcinoma of the thyroid is spread of the tumour tissue outside the confines of the gland. Local invasion of surrounding structures, such as muscle, trachea, and oesophagus, may be found at operation, but this finding is sometimes confused with chronic thyroiditis. Local recurrence of a mass is strong evidence of malignancy, especially if histological examination of the original tissue suggested carcinoma. Though a thyroid adenoma may recur, histological findings, particularly the lack of invasion of capsule and veins, help to establish the diagnosis. Finally, the presence or development of metastases is clear evidence that the tumour is malignant. Although each of these points makes the presence of a carcinoma reasonably certain, histological examination of tissue is required to confirm the origin of the tumour. By considering together the pathological spread and the microscopic appearances, the series may be divided into the following groups.

1. **Carcinoma, thyroid in origin.** In this group, 67 per cent. of the total number of cases, at least one of the above features of malignancy was present, and histological examination confirmed the belief that
the tumour arose in the thyroid.

2. **Probably carcinoma, thyroid in origin.** In 11 cases the diagnosis of carcinoma was made from the examination of tissue removed from a swelling in the thyroid. The histological criteria for this diagnosis will be considered later, but thyroid carcinoma is notoriously difficult to diagnose solely on histological grounds (Graham, 120). Thus the findings in these cases are only presumptive of carcinoma, pending the development of more certain evidence of malignancy.

3. **Carcinoma, probably thyroid in origin.** This group comprises the 22 cases in which no tissue for histological examination was obtained. They all showed local recurrence or metastases during the course of the disease, and the evidence of malignancy is thus very strong, but the evidence of a thyroid origin is entirely clinical.

**Clinical features**

**Age and sex distribution.** Women comprised 80 per cent. of the whole series (Table 43). Harnett (141) and Sloan (319) found the same ratio of female to male patients. Beahrs, Pemberton, and Black (30) found a lower ratio (2:1), and Pemberton (267), reviewing 774 cases, noted a ratio of only 1.7:1. McDermott, Morgan, Hamlin, and Cope (208) observed a striking difference in sex incidence only among patients with differentiated tumours; this
point will be considered more fully in discussing the features of histological types of thyroid carcinoma. The mean age of the whole series of patients was 54.9 years, with no difference between the sexes (Table 44). The mean age of the patients in whom no histological evidence of the thyroid origin of the malignant disease was obtained was higher than the mean age of the whole series.

Presence of other thyroid disease. A number of patients showed evidence of thyroid disease apart from carcinoma (Table 43). Previous goitre was only recorded if the patient gave a history of thyroid swelling for more than five years before the development of symptoms suggestive of carcinoma. Similarly, coexistent hyperthyroidism was only diagnosed when certain strict criteria were fulfilled. Both of these associations will be described fully in later sections. In addition there was one case of hypothyroidism and carcinoma. This occurred in a girl of 27 years, who from an early age had had numerous operations for goitre. She had developed hypothyroidism, and some years later a thyroid swelling recurred, which was found to be a follicular carcinoma.

Initial symptoms. The first symptoms noted by each patient are shown in Table 45. Patients with a previous goitre are treated separately, since local symptoms are often neglected when the patient already has a thyroid swelling. Symptoms referable to the
<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>All patients</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole series</td>
<td>100</td>
<td>54.9 ± 1.5</td>
<td>54.0 ± 3.7</td>
<td>55.1 ± 1.7</td>
</tr>
<tr>
<td>With histological evidence of thyroid carcinoma</td>
<td>78</td>
<td>51.5 ± 1.4</td>
<td>50.8 ± 3.3</td>
<td>51.7 ± 1.6</td>
</tr>
<tr>
<td>Without histological evidence of thyroid carcinoma</td>
<td>22</td>
<td>66.8 ± 2.7</td>
<td>..</td>
<td>65.9 ± 2.8</td>
</tr>
<tr>
<td>With previous goitre for more than five years</td>
<td>18</td>
<td>53.7 ± 3.6</td>
<td>..</td>
<td>55.2 ± 3.7</td>
</tr>
</tbody>
</table>
Table 45: Initial symptoms in 100 cases of thyroid carcinoma

<table>
<thead>
<tr>
<th></th>
<th>Without previous goitre</th>
<th>With previous goitre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
</tr>
<tr>
<td>Total number</td>
<td>18</td>
<td>64</td>
</tr>
<tr>
<td>Swelling of thyroid</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>Increase in size of previous goitre</td>
<td>15</td>
<td>52</td>
</tr>
<tr>
<td>Swelling of cervical lymph-glands</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Pressure symptoms in neck</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Symptoms referable to hyperthyroidism</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Symptoms referable to osseous metastases</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>
neck predominated. In only five per cent. of the whole series was the first symptom remote from the thyroid, and these five patients all had pain or pathological fracture from a bony metastasis. In six per cent. enlargement of one or more cervical lymph-glands was the first complaint. These cases correspond to those formerly classified as lateral aberrant tumours, but a primary thyroid carcinoma was always present. Pressure symptoms have been striking by their rarity, especially in patients without previous goitre. The majority of patients with associated hyperthyroidism also complained of a swelling in the neck.

**Clinical diagnosis of thyroid carcinoma.** The present series illustrates the fact that in many cases, which later prove to be malignant, a clinical diagnosis of thyroid cancer cannot be made. Suspicious features are a hard and irregular swelling, fixation to surrounding structures, rapid increase in swelling, especially if producing pressure symptoms, and vocal cord paralysis. More certain criteria are cervical gland enlargement and evidence of remote metastases. The 22 cases lacking histological corroboration of the thyroid origin of the tumour all showed the above local features, and many showed evidence of local or remote metastases. In 31 of the remaining 73 cases, however, the initial clinical diagnosis was a benign lesion, but after surgical treatment histological examination
revealed a carcinoma. In 20 cases the diagnosis was first suggested at operation, by the discovery of local invasion and metastases in cervical glands. Thus the finding of a thyroid swelling which is not hard, irregular, or fixed is of no practical value in excluding thyroid carcinoma.

Metastases. The incidence and sites of metastases are shown in Table  . They are divided into metastases found when the patient was first seen, and those developing thereafter. Slightly more than half of the total series of patients showed evidence of metastases, and this proportion is not significantly changed when related to histological evidence of thyroid cancer or to the presence of previous goitre. Spread to cervical lymph-glands was commonly present when the patient was first seen, and developed in an appreciable number thereafter. Remote metastases were not common initially, but became evident later, especially in the lungs and bones.

Characteristics of different histological types

Classification. The histological types of tumour from the 78 patients from whom tissue had been obtained by biopsy, at operation, or at subsequent autopsy, were classified according to the system of Willis (1953). Tissue for such re-assessment could be obtained in only 70 cases. In the remaining eight
Table 46  Cases of thyroid carcinoma with metastases

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Patients</th>
<th>Cervical glands</th>
<th>Pulmonary</th>
<th>Osseus</th>
<th>Other sites</th>
<th>Total number with metastases</th>
<th>Cervical glands</th>
<th>Pulmonary</th>
<th>Osseus</th>
<th>Hepatic</th>
<th>Cerebral</th>
<th>Other sites</th>
<th>Total number with metastases</th>
<th>Number of patients with metastases at any time</th>
</tr>
</thead>
<tbody>
<tr>
<td>With histological evidence of malignancy</td>
<td>78</td>
<td>17</td>
<td>2</td>
<td>4</td>
<td>..</td>
<td>23</td>
<td>9</td>
<td>12</td>
<td>9</td>
<td>4</td>
<td>3</td>
<td>9</td>
<td>30</td>
<td>47</td>
</tr>
<tr>
<td>Without histological evidence of malignancy</td>
<td>22</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>8</td>
<td>1</td>
<td>3</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>4</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Without previous goitre</td>
<td>82</td>
<td>18</td>
<td>2</td>
<td>4</td>
<td>..</td>
<td>23</td>
<td>8</td>
<td>13</td>
<td>8</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>29</td>
<td>48</td>
</tr>
<tr>
<td>With previous goitre</td>
<td>18</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>..</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>..</td>
<td>4</td>
<td>5</td>
<td>11</td>
</tr>
</tbody>
</table>
cases the initial microscopic reports described a carcinoma, and the features diagnostic of malignancy, mentioned above, were also present; the original reports on the histological examination were accordingly used in classifying them. It is unlikely that such a small percentage would change the overall picture. It was found that all cases fitted into the following classification, which is a simplification of that given by Willis (333).

A. Differentiated tumours:

(1) Papillary carcinoma
(2) Follicular carcinoma

B. Undifferentiated tumours:

(1) Small-cell carcinoma
(2) Large-cell carcinoma

C. Rare tumours:

(1) Epidermoid carcinoma
(2) Fibrosarcoma

The sections were assessed in the following manner. All were examined by the author, without knowing the previous histological diagnosis, and classified as above. The results were then compared with the previous opinions. Cases in which evidence, other than histological, of malignancy existed, and in which the two opinions were the same, were then accepted as classified. The histological slides from the remaining cases were then examined by Professor D.H. Collins. They comprised the 11 cases without other evidence of malignancy, the cases in which the two opinions differed, and the cases of co-
existent thyrotoxicosis and thyroid carcinoma. In the final assessment only 10 cases were placed in a different histological group, so that substantial agreement was reached with the original opinions. The tumours were classified according to the predominant histological type seen in the primary lesion in the thyroid. Like so many other tumours, thyroid carcinoma may present a variable histological picture in the same tumour, and occasionally metastatic deposits may differ from the primary thyroid tumour. In the present series the tumour was graded according to the histological appearance of a metastasis only in the two cases in which tissue from the primary tumour was not available.

As already stated, in 11 cases there was no evidence of malignancy other than a swelling within the thyroid, from which sections had been taken. Such lesions present great difficulties in diagnosis when the histological picture is papillary in type. Most pathologists follow the opinion of Willis (383) that papillary adenoma cannot be distinguished from papillary adenocarcinoma on histological grounds. Graham (120) suggested the criteria of capsular invasion and invasion of blood-vessels for deciding malignancy in such lesions, and this principle was also stressed by Vaux (355). Warren (366) has raised doubt about the validity of such criteria. In the final estimate these 11 cases were accepted as examples of carcinoma
because there was gross capsular invasion and the usual morphological criteria of carcinoma cells were satisfied. Three other cases, rejected from the series, showed retention of the tumour within a capsule, and the appearance of the cells was not definitely carcinomatous.

Relative incidence (see Table 47 and Fig. 100). Papillary carcinoma (Fig. 96) comprised the largest group (45 per cent.) among the 78 cases. This has been the usual finding in most large series which have been reported, such as those of Frazell and Foote (114) and Cope, Dobyns, Hamlin, and Hopkirk (76).

As no difference in any characteristic examined was found between small-cell and large-cell undifferentiated carcinomas, these have been grouped together, and comprise 30 per cent. of the cases (Fig. 97). Follicular carcinoma (Fig. 96) was the smallest of the main groups (23 per cent.). At variance with these results, and with most other series, are those of Alhadeff, Scott, and Taylor (5), who found follicular carcinoma to be the commonest type.

Sex incidence. The female preponderance in the series as a whole is retained in each histological type (Table 47). The female : male ratio is increased in follicular carcinoma, and decreased in undifferentiated carcinoma. This finding in the latter type of tumour has been frequently noted (McDermott, Morgan, Hamlin, and Cope, 208).
Table 47. Classification of 78 cases of thyroid carcinoma by histological type of tumour related to age, sex, and survival

<table>
<thead>
<tr>
<th>Predominant histological type of tumour</th>
<th>Number of patients</th>
<th>Number with evidence other than histological, of carcinoma</th>
<th>Male: female ratio</th>
<th>Mean age at diagnosis (yrs)</th>
<th>Standard error</th>
<th>Number dead as percentage of total</th>
<th>Average survival time of those dead (years)</th>
<th>Expected survivors %</th>
<th>Crude 5-year survival rate % and standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papillary</td>
<td>35</td>
<td>28</td>
<td>1:4</td>
<td>44.3±2.6</td>
<td></td>
<td>23</td>
<td>3.1</td>
<td>93.6</td>
<td>73.5±7.5</td>
</tr>
<tr>
<td>Follicular</td>
<td>18</td>
<td>16</td>
<td>1:8</td>
<td>55.2±2.5</td>
<td></td>
<td>67</td>
<td>4.8</td>
<td>91.7</td>
<td>54.5±11.7</td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>23</td>
<td>21</td>
<td>1:2.3</td>
<td>60.1±2.2</td>
<td></td>
<td>70</td>
<td>1.3</td>
<td>86.7</td>
<td>16.7±7.8</td>
</tr>
<tr>
<td>Epidermoid</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rare</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrosarcoma</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 96. Papillary carcinoma of the thyroid without colloid formation, coexistent with hyperthyroidism. H. & E. x 170.

Fig. 97. Undifferentiated carcinoma of the thyroid. H. & E. x 170.
Fig. 98. Follicular carcinoma of the thyroid, with well-developed follicles and solid alveoli. The carcinoma was coexistent with hyperthyroidism. H. & E. x 170.

Fig. 99. Metastasis of papillary carcinoma of the thyroid in lung, coexistent with hyperthyroidism. H. & E. x 170.
The mean age is strikingly different in each group (Table 47). The age incidence of papillary carcinoma is significantly different from that found in the follicular and undifferentiated types. Comparing the papillary and follicular groups, \( t = 2.6 \), and \( P < 0.02 \). When the papillary and undifferentiated groups are compared, \( t = 3.90 \) and \( P < 0.001 \). The fact that thyroid carcinoma may occur in young persons is well known, but it is obvious that such patients usually have a papillary carcinoma. Of the whole series of 100 patients, 18 were 40 years old or younger, and in 16 of these the growth was papillary in type. The fact that young people develop papillary carcinoma is also well attested by Black (36) and Crile (83). This type is not found exclusively, however, in the younger age-groups, for 19 of the patients with papillary growths were over the age of 40 years. The mean ages of the groups with follicular and undifferentiated carcinoma fall into the usual age range for cancer.

The survival of different groups cannot be discussed except in relation to the survival data of the whole series, and to the results of different forms of treatment. The five-year survival rate has been used for purposes of comparison, and for this comparison the patients fall into the usual groups: 'alive without evidence of tumour,' 'alive but with tumour present,' and 'dead with tumour present.' No patient died apparently free from tumour, so that deaths from
intercurrent diseases are not considered. The expected five-year survival rates of different groups have been calculated from life expectancy tables (Annual Abstract of Statistics, 1948) and are shown in Tables 47 and 43. Table 46 gives the results, as regards survival, in the whole series and in particular groups. Patients who survived for five years and died later are also included, so that the final column shows the number surviving for a variable period after five years. In Fig. 100 all the cases except the two rare tumours are included, and the survival of the patients is shown up to the end of 1955. The mean survival times of patients who died have been calculated for the various groups, and are shown in Table 47.

Fifty-three patients began treatment before the end of 1950 (Table 48). Twenty-five survived for more than five years, and 22 (41.5 per cent.) were alive and showed no evidence of the presence of tumour. Of the 25 patients, however, who were alive five years after treatment, thirteen died later with a tumour present. Thus it is clear that five-year survival figures are of little value in assessing the treatment of thyroid carcinoma. This fact has been emphasised by McDermott, Morgan, Hamlin, and Cope (208), and Ward (366). When the results from different groups are considered, there are wide divergencies in survival rates. The five-year survival
Table 48. Survival rates of 53 patients with thyroid carcinoma

<table>
<thead>
<tr>
<th>Possible survivors after 5 years</th>
<th>Actual number surviving after 5 years</th>
<th>Surviving after 5 years without tumour</th>
<th>Expected 5-year survivors (%)</th>
<th>Died after 5 years with tumour</th>
<th>Number surviving to date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole series</td>
<td>53</td>
<td>25</td>
<td>22</td>
<td>41.5±4.9</td>
<td>88.1</td>
</tr>
<tr>
<td>With histological evidence of thyroid tumour</td>
<td>45</td>
<td>23</td>
<td>20</td>
<td>44.5±5.6</td>
<td>91.4</td>
</tr>
<tr>
<td>Papillary</td>
<td>15</td>
<td>13</td>
<td>11</td>
<td>73.5±7.5</td>
<td>93.6</td>
</tr>
<tr>
<td>Follicular</td>
<td>11</td>
<td>6</td>
<td>6</td>
<td>54.5±11.7</td>
<td>91.7</td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>18</td>
<td>4</td>
<td>3</td>
<td>16.7±7.8</td>
<td>86.7</td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Without histological evidence of thyroid tumour</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td>25.0±9.2</td>
<td>77.0</td>
</tr>
<tr>
<td>Treated by operation and deep X-ray therapy</td>
<td>25</td>
<td>14</td>
<td>13</td>
<td>52.0±7.4</td>
<td>90.5</td>
</tr>
<tr>
<td>Treated by deep X-ray therapy</td>
<td>22</td>
<td>5</td>
<td>4</td>
<td>18.2±6.8</td>
<td>82.4</td>
</tr>
<tr>
<td>Others</td>
<td>6</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>
Fig. 100. Survival time of individual patients grouped according to the histological type of thyroid carcinoma.

○ = Alive, with no clinical evidence of tumour
□ = Alive, but with evidence of tumour present
■ = Dead, with tumour present.
rate for patients with papillary carcinoma was 73.5 per cent., and the mean survival time for those who had died was 3.1 years. This result is in striking contrast to the outcome among patients with undifferentiated tumours, whose five-year survival rate was only 16.7 per cent., and mean survival time, for those who had died, only 1.3 years. Cases of follicular carcinoma were intermediate between these two groups in respect of five-year survival, but the mean survival time was longer than in papillary carcinoma. The differences between these three groups are significant at the 5 per cent. level. When the expected five-year survival from life tables is taken into account, the difference between cases of papillary and follicular carcinoma is on the borderline of significance. It is striking that those patients who died some time after 5 years usually had follicular or undifferentiated tumours. The fate of patients with undifferentiated tumours was very similar to that of patients in whom histological corroboration of thyroid carcinoma was lacking. The five-year survival rate in the latter group was 25 per cent., and the mean survival time of those who had died was only 0.8 year. The majority of patients having undifferentiated tumours, or tumours not histologically examined, died within 12 months of treatment (Fig. 100). Those surviving this period lived for variable periods during the
following few years. The similarity suggests that the majority of the patients in whom no histological evidence was obtained had in fact undifferentiated tumours. The differences in survival according to histological type of tumour are also reflected in the survival results after different forms of treatment, as shown in Table 49. The majority of patients were treated either by operation followed by deep X-ray therapy, or by deep X-ray therapy alone. The low five-year survival rate after treatment by deep X-ray therapy is explicable when it is seen that, in 84 per cent. of the patients so treated, either the tumour was undifferentiated or its type was not known. No comparison of treatments can be made when such a bias of selection has been present. Deep X-ray therapy was used alone when the patient was too ill, or the tumour too advanced, for operation. To a large extent this fact would also account for the failure to obtain tissue for histological examination.

The above findings as regards survival are similar to previous reports. McDermott, Morgan, Hamlin, and Cope (203), Frazell and Foot (114), and Dunhill (95) have stressed the high survival rates for papillary carcinoma, and the very poor results for undifferentiated tumours.

It is impossible to ascertain how far these results are due to treatment, as there are no control
Table 49 Treatment of 100 patients with thyroid carcinoma, classified by histological type of tumour

<table>
<thead>
<tr>
<th></th>
<th>Operation and deep X-ray therapy</th>
<th>Deep X-ray therapy</th>
<th>Operation</th>
<th>$^{131}I$</th>
<th>No treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of patients</strong></td>
<td>46</td>
<td>32</td>
<td>11</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td><strong>5-year survival per cent</strong></td>
<td>52</td>
<td>18</td>
<td>..</td>
<td>..</td>
<td>..</td>
</tr>
<tr>
<td><strong>Type of tumour:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papillary</td>
<td>27</td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>..</td>
</tr>
<tr>
<td>Follicular</td>
<td>7</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>12</td>
<td>9</td>
<td>2</td>
<td>..</td>
<td>..</td>
</tr>
<tr>
<td>No histological</td>
<td>..</td>
<td>18</td>
<td>..</td>
<td>..</td>
<td>4</td>
</tr>
<tr>
<td>examination</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>..</td>
<td>1</td>
<td>1</td>
<td>..</td>
<td>..</td>
</tr>
</tbody>
</table>
groups for comparison. Only patients with papillary carcinoma have a good expectation of life. But it is this particular type of tumour which creates the greatest difficulties for the pathologist in deciding whether it is malignant. Ten patients out of 15 with papillary carcinoma survived more than ten years. Seven of these patients showed evidence, other than histological, of malignant disease, at time of diagnosis, and even in them the survival rate was high. Sloan (319) reported similar differences in survival according to the type of tumour, but also found that the chance of survival with a particular type decreased with increasing age at diagnosis. The results from the present series do not suggest this tendency, but the groups are smaller than those reported by Sloan.

Occurrence of metastases. No striking differences in the development of metastases were noted between the histological types of tumour, with the possible exception of metastases in bone. Eleven patients with morphological evidence of thyroid carcinoma developed osseous metastases. Five of these, or nearly 50 per cent., had follicular carcinoma (Fig. 98). This predilection of follicular carcinoma to produce bony metastases was reported by Frazell and Foote (114).

Relation to previous goitre

Eighteen patients (18 per cent.) gave a history of previous swelling in the region of the thyroid gland for at least five years. In only three had the
swelling been present for less than 10 years; in two
the tumours were thought to be simple non-toxic
goitres, and carcinoma was found when operation was
performed: the third patient had thyrotoxicosis when
she was first seen, and is described with the other
cases of coexistent hyperthyroidism and carcinoma.
The incidence of previous goitre should be taken as
the minimal level, as an unknown number of those
patients who are now dead were not questioned speci-
ically about this point. Figures quoted in this
respect have varied widely, and have usually been
higher than in the present series. Harnett (141)
reported that 50 per cent. of patients gave a history
of previous goitre, and 40 per cent. had had goitre
for more than five years. In our series the propor-
tion of male patients was smaller in the group with
previous goitre (11 per cent.) than in the group with-
out goitre (22 per cent.), a finding which is to be
expected from the known sex distribution of goitre
(Table 50). The mean ages of the groups with and
without goitre are similar, and it is of interest to
compare the incidence of goitre in the general popu-
lation in Sheffield from the data given in Chapter 2.
The incidence of visible goitre in women over 40 and
a mean age of 57.3 yr. is 18.6 per cent., which is
almost identical with the incidence of previous
goitre in this series of patients with thyroid cancer.
The corresponding value for men, with a mean age of 54.2 years, is 5.1 per cent., which is not significantly higher than in the carcinoma series. The first symptoms in patients with previous goitre were similar to those found in the whole series: 14 (73 per cent.) complained of local symptoms referable to the neck. Nine complained of increase in size of their previous goitre, and the other five of pressure symptoms, such as dysphagia and dyspnoea, or swelling of cervical lymph-glands. Two of the remaining four patients had hyperthyroidism, and symptoms referable to bony metastases were present in the other two cases. The incidence of the different types of carcinoma in this group with previous goitre was not strikingly different from that found in the non-goitrous group. Sixteen of the tumours were examined histologically, and papillary carcinoma (six cases) and follicular carcinoma (six cases) were commonest. There were four cases of undifferentiated carcinoma. McDermott, Morgan, Hamlin, and Cope (208) found that the incidence of previous goitre was much higher in cases of follicular carcinoma. Similarly, the survival among this group with goitre did not differ significantly from that of patients without goitre. The crude five-year survival rate in the former group was 44.5 per cent. of nine cases, and in the latter it was 41 per cent. of cases. This result is at variance with the
Table 50. Cases of thyroid carcinoma with previous goitre compared by age, sex, and survival to those without previous goitre.

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>Total</th>
<th>No previous goitre</th>
<th>Previous goitre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>18</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Female</td>
<td>64</td>
<td>16</td>
<td>16</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mean age ± s.e. (yrs.)</th>
<th>No previous goitre</th>
<th>Previous goitre</th>
</tr>
</thead>
<tbody>
<tr>
<td>54.0 ± 1.8</td>
<td>53.7 ± 3.6</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Duration of goitre</th>
<th>No previous goitre</th>
<th>Previous goitre</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 - 10 yrs.</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>10 + yrs.</td>
<td></td>
<td>15</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>With histological evidence of thyroid carcinoma</th>
<th>No previous goitre</th>
<th>Previous goitre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>62</td>
<td>16</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Without histological evidence of thyroid carcinoma</th>
<th>No previous goitre</th>
<th>Previous goitre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Survival</th>
<th>No previous goitre</th>
<th>Previous goitre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alive</td>
<td>36</td>
<td>8</td>
</tr>
<tr>
<td>Dead</td>
<td>46</td>
<td>10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Crude 5-year survival rate %</th>
<th>No previous goitre</th>
<th>Previous goitre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>41</td>
<td>44.5</td>
</tr>
</tbody>
</table>
series reported by Harnett (141), in which five-year survival was much higher in the group with previous goitre, 53.2 per cent. as compared with 27.1 per cent.

Relation to hyperthyroidism

There were seven cases (7 per cent.) of carcinoma with coexistent hyperthyroidism. Usually the rarity of coexistence of the two conditions is stressed (Means, 241). The incidence in most reviews of thyroid carcinoma is low, but variable. Thus Pemberton (267) reported an incidence of 1.3 per cent. in a total series of 774 cases, Ward (365), 0.6 per cent. in 168 cases, Horn, Welty, Brooks, Rheads, and Pendergrass (155) 3 per cent. in 71 cases, and Clute and Warren (73) 1.7 per cent. in 226 cases. On the other hand, Friedell ( ) found 13.8 per cent. in 412 cases, Cole, Slaughter, and Rossiter (75) 13 per cent. in 38 cases, and Ward, Hendrick, and Chambers (367) 26 per cent. in 112 cases. Similarly, Piercey (270) found seven (12 per cent.) cases of carcinoma and toxic goitre in a total of 58 cases with carcinoma. Obvious reasons for these differences are the relatively small number of cases in each series, and the lack of uniformity, or even of any details, about the criteria for accepting the diagnosis of hyperthyroidism. In the present series the criteria for diagnosing hyperthyroidism have been
strict. In five cases the clinical diagnosis was corroborated by radiiodine investigation. Otherwise clinical diagnosis was accepted only if there was obvious objective improvement with antithyroid medication, either by iodine or the thiouracil-like drugs. Thyrotoxicosis was suspected clinically in at least three other cases, but, as these criteria were not satisfied, they were not included in this group. Increase in basal metabolic rate was not accepted, as this may occur with any carcinoma. These seven cases also fulfilled rigid criteria for the diagnosis of carcinoma. They were all examined histologically, and all showed evidence of extension of tumour outside the thyroid, in the form of direct local invasion of surrounding structures or of lymphatic or haematogenous metastases.

The initial symptoms in all cases were due to hyperthyroidism, and had been present for periods varying from a few months to six years (Table 51). Carcinoma was obvious on the first examination in hospital only in Case T1, and this was principally due to finding multiple pulmonary opacities in the chest radiograph. Another patient (Case T6) had immediate operation for dysphagia, and there were grounds for a clinical diagnosis of carcinoma, as the thyroid swelling was very hard. The other five patients were all given medical or surgical treatment for hyperthyroidism, without any suspicion of the
### Table 51. Clinical and pathological features of seven cases of coexistent hyperthyroidism and thyroid carcinoma

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>Clinical features</th>
<th>4-hr protein-bound activity (%/litre)</th>
<th>Criteria for diagnosis of hyperthyroidism</th>
<th>Criteria for diagnosis of thyroid carcinoma</th>
<th>Fate after diagnosis of carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>F</td>
<td>53</td>
<td>Loss of weight, sweating, nervousness, and swelling of neck for 6 years. Hyperkinesis, tachycardia, and lid retraction. Thyroid enlarged, with hard mass in right lobe. Chest X-ray showed multiple rounded opacities at both lung bases</td>
<td>70 4·1</td>
<td>Follicular carcinoma</td>
<td>Pulmonary &amp; vertebral proved at autopsy</td>
<td>Died at 2·5 yrs.</td>
</tr>
<tr>
<td>T2</td>
<td>F</td>
<td>60</td>
<td>Loss of weight, sweating, and palpitations for 1 yr. Tachycardia, tremor, and hyperkinesis. Smooth swelling in right lobe of thyroid. Hyperthyroidism treated with 131I. Death 6 months later from cerebral metastases</td>
<td>67 1·55</td>
<td>Papillary carcinoma</td>
<td>Cerebral &amp; pulmonary, proved at autopsy</td>
<td>Died at 0·75 yrs.</td>
</tr>
<tr>
<td>T3</td>
<td>F</td>
<td>59</td>
<td>Nervousness, loss of weight, palpitations, and swelling of neck for 6 months. Hyperkinesis, tachycardia, and tremor. Smooth swelling in left lobe of thyroid. One year later dysphagia and stridor. Thyroid enlarged and hard.</td>
<td>0·40</td>
<td>Follicular carcinoma</td>
<td></td>
<td>Alive at 1 year</td>
</tr>
<tr>
<td>T4</td>
<td>F</td>
<td>56</td>
<td>Nervousness, loss of weight, palpitations, and sweating, with swelling of neck for 5 months. Dysphagia for 2 months. Tremor, tachycardia, and large diffuse swelling of thyroid. Improved after two weeks on iodine, and operated on for hyperthyroidism</td>
<td></td>
<td>Undifferentiated carcinoma</td>
<td>Invading neck-muscles at operation</td>
<td>Alive at 1·8 yrs.</td>
</tr>
<tr>
<td>T5</td>
<td>F</td>
<td>31</td>
<td>Loss of weight and swelling of neck for 1 year. Remained well for 2 years, after treatment with thionamides for 5 mths. Relapsed with nervousness, prominent eyes, and loss of weight. Tremor, tachycardia, and thyroid swelling with glands on either side.</td>
<td>60 0·41</td>
<td>Papillary carcinoma</td>
<td>Cervical lymph-glands, proved at operation</td>
<td>Alive at 2·9 yrs.</td>
</tr>
<tr>
<td>T6</td>
<td>F</td>
<td>53</td>
<td>Goitre for at least 10 yrs. Nervousness, loss of weight, sweating, and increase in size of swelling. Hyperkinesis, tachycardia, and hard nodular swelling of thyroid. Operation was performed for dysphagia.</td>
<td>62 1·35 but after partial removal of thyroid</td>
<td>Undifferentiated carcinoma</td>
<td>Invading neck-muscles at operation</td>
<td>Alive at 5·5 yrs.</td>
</tr>
<tr>
<td>T7</td>
<td>F</td>
<td>60</td>
<td>Loss of weight, nervousness, and sweating, with swelling of neck for 2 years. Tremor, tachycardia, and firm enlargement of thyroid. Improved after 10 days on iodine, and operated on for hyperthyroidism.</td>
<td></td>
<td>Follicular carcinoma</td>
<td>Recurrence in neck 2 years after operation</td>
<td>Died at 7 years</td>
</tr>
</tbody>
</table>

presence of a carcinoma. In the three patients treated surgically, tumour tissue was found at operation, and carcinoma confirmed histologically. Treatment of the other two was by thiouracil and radioiodine. In the patient treated with thiouracil carcinoma was diagnosed from the later development of cervical lymphatic metastases. The other patient (Case T2) was treated with $^{131}$I for hyperthyroidism, and within three months her toxic symptoms were much improved. Six months after treatment she developed signs of cerebral metastases, and died two months later. At autopsy a papillary carcinoma of the thyroid was found, with cerebral and pulmonary metastases (Fig. 99) p. 264. It is conceivable that the tumour developed only after treatment, but from analogy with the other cases it is probable that it was present when radioiodine was given. Thus, in five of the seven patients, carcinoma was not diagnosed when they were first seen in hospital. Three, however, had been referred to surgeons, and the diagnosis resulted from the selection of surgical treatment for the hyperthyroidism. If, as was possible, they had been treated like the other two patients by medical means, the diagnosis of carcinoma would have been made only from the development of other manifestations.

In these seven cases of coexistent carcinoma and toxicity there was no association with any particular type of tumour. In three cases the predominant structure was follicular adenocarcinoma, and of
the other patients two had a papillary carcinoma and two an undifferentiated solid growth. Black (36) reported that, of 112 patients who had papillary adenocarcinoma, 15 per cent. had hyperthyroidism, which is a high incidence of such coexistence. An even higher incidence, of 36 per cent., was given by Chesky, Dreese, and Hellwig (64) in a total of 25 cases with Hürthle-cell tumours.

**Relationship to previous radiation**

An attempt was made to find the incidence of exposure of the neck to ionizing radiation before the development of thyroid carcinoma. All the 46 patients who were alive at the end of 1955 were questioned about this point, and in addition the relatives of two patients, who had been under the age of 40 years when the diagnosis was made, and had died, were interrogated. The relatives of deceased patients who were over 40 years when diagnosed were not questioned, as it was unlikely that they could be certain about any previous radiation therapy. A history of any previous treatment by X-rays, radium, or any form of lamp treatment, to any part of the body was sought from patients and relatives. Details of any such therapy were then amplified from the hospital or clinic where this treatment had been given. Eight patients were under the age of 35 years when
thyroid carcinoma was diagnosed. Three of them had had previous therapy by ionizing radiation to the neck or neighbouring regions for various benign conditions. In addition to this series of 100 patients with thyroid carcinoma, six patients have been seen with a history of previous treatment to the neck by ionizing radiation. In four the diagnosis of thyroid carcinoma was established, and in two there was an adenomatous condition of the thyroid presenting some neoplastic features. This gives a total of nine cases with thyroid carcinoma or adenoma, where there was previous treatment with radiation. These are described in detailed case reports, Cases 1, 5 and 6 being those from the series of 100 patients referred to the Sheffield Radiotherapy Centre. The details of all cases regarding irradiation dose, latent period, and nature of the neoplasm are summarized in Table 52.

In six patients the irradiation was given in infancy or childhood for the treatment of cutaneous lesions in the neck region, and the details of the original treatment are available. Except in Case 6, the site of irradiation is known from the position of the scar of the original lesion. A calculation of the amount of irradiation received by the thyroid gland has been attempted, and the assumptions on which this has been based are described in the case reports. In three patients (Cases 1, 2, and 6) this did not exceed 300 r.
Table 52 Details of Cases of thyroid carcinoma following previous irradiation.

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age at Time of Irradiation</th>
<th>Diagnosis and Site of Irradiation</th>
<th>Method of Irradiation</th>
<th>Estimated Maximum dose to thyroid</th>
<th>Age at Surgical biopsy of thyroid</th>
<th>Interval between Irradiation and first sign of goitre</th>
<th>Type of neoplasm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>2 months</td>
<td>Naevus on right mandible</td>
<td>Radium</td>
<td>130r</td>
<td>10 yrs.</td>
<td>9 yrs.</td>
<td>Papillary carcinoma</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>3 &quot;</td>
<td>Naevus on right side of neck</td>
<td>X-ray</td>
<td>100r 160r</td>
<td>12 &quot; 8&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>5 months</td>
<td>Naevus on front of neck</td>
<td>Radium</td>
<td>2,700r</td>
<td>19 &quot; 18&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>3 &quot;</td>
<td>Naevus on left side of neck</td>
<td>&quot;</td>
<td>1,400r</td>
<td>22 &quot; 75&quot;</td>
<td>&quot;</td>
<td>Papillary and follicular carcinoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 &quot;</td>
<td></td>
<td>&quot;</td>
<td>1,300r</td>
<td></td>
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<tr>
<td>5</td>
<td>M</td>
<td>12 years</td>
<td>Keloid on right side of neck</td>
<td>&quot;</td>
<td>1,000r</td>
<td>25 &quot; 8&quot;</td>
<td>&quot;</td>
<td>Papillary carcinoma</td>
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<td>13 &quot;</td>
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<td>1,700r</td>
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<tr>
<td>6</td>
<td>F</td>
<td>14 &quot;</td>
<td>Eczema of neck</td>
<td>X-ray</td>
<td>150r</td>
<td>31 &quot; 17&quot;</td>
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<td></td>
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<td>16 &quot;</td>
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<tr>
<td>7</td>
<td>F</td>
<td>26 &quot;</td>
<td>Thyrotoxicosis</td>
<td>&quot;</td>
<td>2,000r</td>
<td>63 &quot; 37&quot;</td>
<td>&quot;</td>
<td>Anaplastic carcinoma</td>
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<tr>
<td>8</td>
<td>F</td>
<td>3 months</td>
<td>Naevus on left side of neck</td>
<td>Radium</td>
<td>1,000r</td>
<td>27 &quot; 17&quot;</td>
<td>&quot;</td>
<td>Papillary carcinoma</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>21 years</td>
<td>Thyrotoxicosis</td>
<td>X-ray</td>
<td>2,000r</td>
<td>43 &quot; 22&quot;</td>
<td>&quot;</td>
<td>Adenoma</td>
</tr>
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</table>
The interval between the irradiation and the observation of a swelling in the thyroid probably affords the best indication of the latent period, as there was usually a considerable delay before the diagnosis was finally established by surgical exploration and histological examination. In the patients receiving irradiation in the first year of life and subsequently developing a carcinoma the latent period in three (Cases 1, 2, and 4) was under 10 years, but in Case 3 the swelling was apparently not observed until she was 18 years old. In Cases 5 and 6 the irradiation was given in adolescence, and the latent periods were 8 and 17 years. In all these patients irradiated in childhood the carcinoma was predominantly papillary in type, though some follicular arrangement was apparent in Case 4. In four (Cases 1, 2, 4, and 5) the skin lesion was localized on one side of the neck, and in them the thyroid swelling first appeared and the spread of malignant tissue was most extensive in the lobe of the gland immediately adjacent to the irradiated area. In one patient (Case 3) the cutaneous lesion was in the midline and the carcinomatous nodule was situated behind the scar. Case 6 had extensive irradiation of the front of the neck and there was involvement of both sides. The tumours from these patients are illustrated in Figs. 101-107.

The seventh patient with a carcinoma differed greatly from the others. She was irradiated in adult
Fig. 101. Case 1. Thyroid carcinoma following irradiation. Section shows papillary carcinoma of the thyroid with no colloid formation, in a cervical lymph-gland. H. & E. x 200.

Fig. 102. Case 5. Thyroid carcinoma following irradiation. Section shows a papillary carcinoma with mitoses and no colloid formation. H. & E. x 310.
Fig. 103. Case 2. Thyroid carcinoma following irradiation. Section shows solid areas of compact acini and also some follicular formation with scanty colloid. H. & E. x 138.

Fig. 104. Case 2. Thyroid carcinoma following irradiation. This high power section shows solid acini and slight follicular formation with some colloid. H. & E. x 310.
Fig. 105. Case 3. Thyroid carcinoma following irradiation. Section shows a papillary carcinoma with slight follicular formation and scanty colloid production.  

Fig. 106. Case 4. Thyroid carcinoma following irradiation. Section shows a mixture of follicular and papillary formation, with scanty colloid production.
Fig. 107. Case 6. Thyroid carcinoma following irradiation. Section shows papillary carcinoma with no follicular formation or colloid production. H. & E. x 150.
life for the treatment of thyrotoxicosis, and 37 years later developed an anaplastic carcinoma in contrast to the predominantly papillary type seen in the younger patients.

The available details regarding the irradiation of the initial lesion, and the operative findings and histological features leading to the diagnosis of carcinoma, are described in the following case reports.

**Case 1**

Female born on March 10, 1933. Treatment with radium was given to a capillary naevus overlying the right mandible, beginning on May 4, 1933, and extending over a period of a year. On each of five occasions irradiation was given with five 5-mg. needles; total radium, 25 mg. for 30 minutes. The needles were applied close to the skin, but the size of the treated area is not precisely known. It is assumed that the area was 4 sq. cm. and the distance of the needles from the skin 0.5 cm. It is thought that the nearest part of the thyroid lay between 1.5 cm. and 3 cm. from the treated area. Assuming average size and development during the first year of life, the naevus received approximately 400 r and the thyroid between 50 and 130 r.

Nine years later, in January, 1947, a swelling in the neck was noted by her mother. On August 24, 1948, a surgical exploration was carried out. The left lobe of the thyroid appeared normal. The right
lobe was hard and irregular, and contained a fairly well circumscribed nodule about 1 cm. in diameter. There was some enlargement of the cervical lymph nodes on the right side, and several were removed. Histologically there was a papillary carcinoma of the thyroid with metastases in the lymph nodes (Fig. 101).

Deep X-ray therapy up to 3,100 r was given to the neck between September 13 and October 15. Several enlarged lymph nodes were present after treatment, but they remained constant in size until 1952, when they began to enlarge. Block dissection of the left side of the neck was carried out on April 9, 1953, and of the right side on June 23. Enlarged lymph nodes on both sides contained papillary carcinomatous metastases.

Case 2

Female born on May 29, 1944. A small naevus was present on the lower part of the right side of the neck (Figs. 103, 104) and this was treated on two occasions by X-irradiation. On August 22 the naevus was treated with 400 r, 4 cm. focus skin distance, 45 kV, 1 mm. Al filter, 2.5 cm. diameter. Treatment was repeated on August 21, 1946, when the naevus received 400 r, 15 cm. focus skin distance, 100 kV, 1 mm. Al filter, 3 cm. diameter. Assuming that the nearest part of the thyroid was 2 cm. distant from the point of application of the beam, it is possible that a small fraction of the right lobe received a
dose of as much as 260 r, made up of 100 r from the first treatment and 160 r from the second. Some of the rest of the thyroid, including the posterior part of the left lobe, may have received a dose of up to 200 r, made up of 80 r from the first treatment and 120 r from the second, but the majority of the gland probably received a dose of less than 100 r.

In 1952 a swelling in the region of the thyroid was noted by her mother, and this slowly increased in size, particularly on the right side. Some tracheal compression was evident on X-ray examination. At operation in November, 1956, the sternothyroid was adherent and bound to a nodular fixed thyroid. The nodules were vascular and hard, and spread extensively into the surrounding structures, especially on the right side. Two biopsies of the gland were carried out, but extensive excision was not possible. The microscopical appearance of both nodules was that of a carcinoma, predominantly of papillary type but showing also some more solid areas of compact acini of thyroid epithelium enclosing no colloid. The larger nodule (right lobe) was almost wholly papillary in structure and had very little capsule of fibrous or normal thyroid tissue. The smaller nodule (left lobe) was enclosed by a fibrous capsule, but there was a definite invasion of venules. Mitotic figures, though present, were not very frequently seen (Figs. 103 & 104).
Case 3

Female born on October 20, 1938. A large naevus on the front of the neck, measuring approximately 5·5 cm. in diameter, was treated with radium. On March 18, 1939, 10 1-mg. needles were inserted for 72 hours. The radium plane consisted of an ellipse 5 by 4 cm., seven needles being placed in an outer ring and three in an inner ring. The radium probably covered most of the neck between the jaw and the upper border of the clavicles. Assuming some of the thyroid tissue was in a plane 0·5 cm. below the radium plane and the area was 15 sq. cm., the dose in 72 hours on a basis of 302 mg. hr./l,000 r was approximately 2,300 r. At 1 cm. deep to the radium plane on a basis of 546 mg. hr./l,000 r, the dose was approximately 1,300 r. The irradiation scar in the patient was over and below the thyroid swelling. The dose at the upper limit of the ring of radium needles and 1 cm. deep to the skin may have been 20 per cent greater than quoted above. It may be assumed that the dose on the anterior part of the gland was between 1,300 and 2,700 r.

A swelling was noted in the thyroid in 1956. At operation on May 12, 1957, a round nodular swelling about 3 cm. in diameter was excised from the isthmus and right lobe of the thyroid. Section revealed a pale, fleshy tumour with a few areas of recent haemorrhage. On histological examination
there was a fairly well circumscribed papillary adenocarcinoma of the thyroid. The epithelial cells were mainly cuboidal, but a certain amount of pleomorphism was evident. They formed a single layer around the vesicles and fronds, but in places they were heaped and occasional mitoses were seen. Colloid was restricted to the marginal areas of the tumour. There had been some haemorrhage. The capsule consisted of fibrous tissue and compressed normal gland (Fig. 105).

**Case 4**

Female born on June 1, 1935. A large naevus was present on the left side of the neck, measuring approximately 7 by 3 cm. Two treatments with radium were given.

On August 27, 1935, radium needles were inserted - six 2-mg. and two 1-mg.; total radium, 14 mg. for 36.5 hours. The area of implant was 20 sq. cm. The following estimates and assumptions have been made. (1) The distance from the affected skin to the main mass of the left lobe of thyroid was 8 mm. and to the extreme edge of the right lobe 22 mm. (2) The needles lay within 5 mm. of the left lobe and were 8 mm. distant from the main mass of the lobe. On this basis the estimated dose to the nearest point of the left thyroid lobe was 1,400 r, to the main mass of the lobe 1,000 r, and to the extreme edge of the gland 400 r.

On December 19, 1936, radium needles were again inserted - eight 2-mg. and three 1-mg.; total radium,
19 mg. for 42.5 hours. The area of implant was 32 sq. cm. It is estimated that on this occasion the nearest part of the left thyroid lobe was 8 mm., the main mass 10 mm., and the extreme edge of the right lobe 40 mm. from the needles. The estimated dose to the nearest point of the left thyroid lobe was 1,300 r, to the mass of the lobe 970 r, and to the extreme edge of the right lobe 230 r.

The total doses from the two exposures to irradiation therefore probably lay in the following range - left lobe 2,000-2,700 r, and right lobe 600-700 r.

A swelling in the left lobe of her thyroid gland was noted in early childhood, probably during the period 1941-3, though no precise date can be given. There was a slow increase in the size of the swelling, particularly during 1937. At operation on September 25, 1957, a hard nodular irregular swelling was present in the left lobe of the thyroid gland and was closely adherent to the strap muscles on the left side of the neck. Metastases were present in the lymph nodes in the left supraclavicular fossa. The tissue was friable and covered with numerous thin-walled vessels. As much as possible was removed, but dissection beneath the strap muscles was extremely difficult, and was abandoned. The right lobe appeared essentially normal.
Histologically there was a mixture of follicular and papillary highly cellular thyroid tissue. A small proportion of the acini contained densely staining colloid, but the cell pattern was highly irregular and many mitotic figures were present. The appearances were those of a fairly well differentiated adenocarcinoma, and the neoplasm merged with a thin peripheral rim of normal thyroid tissue. (Fig. 106).

Case 5

Male born on June 5, 1923. He was burnt on the right side of the neck when 3 years old and the resultant keloid scar was excised in 1932 and again in 1935. This second excision was followed by radium therapy. Two applications were made externally in sorbo-rubber tubes. On August 1, 1935, 41.5 mg. was applied for 15 hours, and on March 17, 1936, 79 mg. for 13 hours. Assuming the near lobe of the thyroid lay 1 cm. beneath the skin at the point of application of the radium, the right lobe received approximately 1,000 r on the first occasion and 1,700 r on the second. The left lobe received 150 r and 250 r respectively.

A swelling was noted on the right side of the neck in 1944, but this was left undisturbed for four years. On September 21, 1948, a lymph node deep to the right sternomastoid was excised; histology showed a papillary carcinomatous metastasis from the thyroid (Fig. ). On October 6, 1948, a right hemithyroid-
ectomy was carried out together with removal of three more slightly enlarged lymph nodes on the right side of the neck. A nodule about 2 cm. in diameter was embedded in the right lobe of the thyroid gland, and histologically this was a papillary carcinoma.

Case 6

Female born on April 26, 1922. On April 3, 1936, she was treated by X-rays for eczema of the neck, and this was repeated on June 9, 1938. On each occasion the total dose was 300 r by unfiltered X-rays of 75 kV. It is estimated that the anterior surface of the thyroid received approximately 150 r on each occasion and that the dose to the thyroid tissue 2 cm. from its anterior surface was 60 r.

In 1950 she noticed a swelling in the region of the thyroid gland and was given methylthiouracil for about five months. In November, 1952, this swelling increased in size: she developed some prominence of the eyes and had symptoms suggestive of hyperthyroidism. The swelling on both sides of the neck continued to enlarge. On April 7, 1953, a biopsy of a lymph node from the right side of the neck revealed a papillary carcinoma. At operation on July 1, 1953, the thyroid gland felt extremely hard throughout. The left lobe was removed apart from a thin slice posteriorly. The right lobe was very irregular and was attached to the trachea. The whole right lobe was removed together with a chain of enlarged lymph nodes.
Histologically the right lobe contained a papillary carcinoma and the lymph nodes were replaced by this tumour. The left lobe was partially replaced by a papillary carcinoma (Fig. 107).

**Case 7**

Female born on April 4, 1893. During the period 1918–22 repeated X-ray therapy was given to the thyroid for the treatment of thyrotoxicosis. No details of dosage are available, but gross atrophy, scarring and telangiectasia of skin of neck was produced, particularly over the thyroid isthmus. From what is known of current practice at that time and from the conspicuous cutaneous reaction it is probable that the thyroid gland received more than 2,000 r.

Thereafter she remained well but with some residual enlargement of the gland until 1955, when she noticed an increase in the swelling on the right side. On examination there was a hard nodular enlargement of the isthmus and right lobe. At operation on April 15, 1956, the right lobe of the thyroid was enlarged and adherent to surrounding structures. The left lobe appeared normal. The whole thyroid was removed with the exception of some tissue firmly adherent to the carotid sheath. Histologically there was an undifferentiated anaplastic carcinoma with striking pleomorphism of cells and very numerous mitoses.
Case 8

Female born on August 4, 1930. When aged 3 months she had treatment with radium needles for a naevus overlying the tendinous head of the left sternomastoid muscle. The needles were left in for 36 hours, but other details of the treatment are not available. There is a conspicuous irradiation scar, and comparison with the other similar cases suggests that the thyroid received at least 1,000 r. When aged about 17 years a goitre was noted by her doctor. This slowly increased in size. In December, 1957, a large irregular swelling was present in the isthmus and right lobe of her thyroid gland. At operation on December 19, 1957, the strap muscles and fascia of the neck stripped clearly, revealing the right lobe to be three or four times normal size, soft but not cystic in consistency, and with a normal vascular supply. This enlargement included the isthmus and ended abruptly at the junction with the left lobe. The right lobe and isthmus were excised.

On section of the excised tissue two fleshy nodules were seen, the larger 3 cm. and the smaller 2 cm. in diameter. On histological examination two adenomatous areas were seen compressing the surrounding thyroid tissue and possessing rudimentary and incomplete fibrous capsules. Their structure was that of actively hyperplastic thyroid tissue; the epithelium was cuboidal and surrounded colloid-filled
vesicles of the normal range of size. Infolds of epithelium were occasionally seen. A microfollicular structure predominated in parts. Mitoses in the epithelial cells were fairly frequent. A papillary pattern of growth was seen in some parts of both adenomas but the papillary processes usually enclosed colloid vesicles and were invested with a single layer of regular and uniform cells. The thyroid tissue outside the adenomas was more normal, but here, too, mitotic figures were usually frequent in the epithelial cells. A marginal area in one of the sections showed intermingling of thyroid acini, fibrous tissue, and striped muscle, but this probably did not indicate neoplastic invasion. The general features and the absence of anaplasia, vascular invasion, and infiltration of the surrounding tissues preclude a definite histological diagnosis of carcinoma, but the process does seem to be an adenomatous neoplastic condition rather than a simple goitre.

Case 9
Female born in 1914. She developed a thyroid swelling when she was about 15 years old. When 21 years of age she became unduly nervous, lost weight, and had a rapid pulse rate. A diagnosis of thyrotoxicosis was made, and in 1935 she received about four treatments with X-rays to the neck. Unfortunately it has not been possible to trace the records of this treatment, and the dose is not known. From
what is known of practice at the time it probably exceeded 2,000 r.

In 1955 a lump was noted in the right side of the neck which gradually increased in size. In October, 1957, there was a hard enlargement of the right lobe of the thyroid, narrowing the trachea and displacing it to the left. There was no clinical evidence of hyperthyroidism, and a $^{131}$I tracer test showed normal thyroid function.

At operation on October 23, 1957, the strap muscles and fascial layers stripped easily off the underlying thyroid. The left lobe was approximately of normal size and texture; the right lobe was enlarged four or five times, with a particularly exaggerated upper pole. It was elastic to palpation, with averaged-sized vessels and without evidence of an extracapsular spread. At its lower pole was a cyst about 1 cm. in diameter. Following ligation of the superior and inferior thyroid arteries a right hemithyroidectomy was performed. The left lobe was left undisturbed. On section the resected portion of the right lobe consisted of several ovoid or spherical masses from 0.5 to 3 cm. in diameter. These were surrounded by a fibrous capsule, sometimes fairly dense; in several parts, however, the capsule consisted only of compressed but otherwise normal-looking thyroid tissue. The structure of the nodules varied greatly, ranging from relatively solid masses
of polyhedral cells to large colloid-filled follicles lined by cuboidal or slightly flattened epithelial cells. Colloid was relatively scanty in the follicles and in the stroma. The epithelial cells in the nodules had large nuclei, often with prominent nucleoli, and pyknosis was widespread. Mitoses were not infrequent, particularly in the parts where the follicles were small or where the cells formed solid columns. Few intrafollicular projections were present, and scalloping of the colloid was confined to a few follicles. Several large scars and deposits of calcium were present in the stroma. Though no pathological features were found to suggest that the condition was definitely carcinomatous, the adenomata showed a fairly active hyperplasia.

Discussion

The present series confirms the majority of previous reports concerning the biological characteristics of the different types of thyroid carcinoma. The three main types are papillary, follicular, and undifferentiated carcinoma. They show striking differences in incidence, sex ratio, age of onset, and survival, and the type of tumour appears to be the main determining factor with respect to survival.

There has been considerable discussion about the importance of previous hyperplasia in the development of thyroid carcinoma. The evidence for hyper-
plasia as a precancerous state is both clinical and experimental. It is a common belief that thyroid carcinoma develops more frequently in diffuse and nodular goitres. Three main points have been marshalled as evidence for the malignant potentialities of such glands. In most series of thyroid carcinoma a high incidence of previous thyroid enlargement, either diffuse or nodular, has been noted, but has varied widely in the different reports. Harnett (141) described the incidence as 47.5 per cent, Horn, Welty, Brooks, Rhoads, and Pendergrass (155) as 42 per cent, and Sloan (319) as 24 per cent. In the present series 18 per cent of patients had goitre for more than five years before the diagnosis of carcinoma was made. An immediate difficulty in the interpretation of these findings is the lack of knowledge regarding the prevalence of goitre in the population from which cases of thyroid cancer have been drawn. This information, unfortunately, is not available for any region. Hazard and Kaufman (142), however, from 403 autopsies in adult patients, found 17 per cent. of thyroid glands to be over 35 gm. in weight, and half of these were nodular goitres. Crile (84) has suggested that 10 per cent. of middle-aged women in the Great Lakes area of the United States have nodular goitres, and Sloan (319) thought that the incidence in the general population might not be very different from that found in his cases of carcinoma. The survey
of a general practice in Sheffield reported in Chapter 2, might be taken as representative of the findings in the general population of the Sheffield region. Nearly 19 per cent. of women over 40 had visible thyroid enlargement, which is very close to the incidence of previous goitre (18 per cent.) in the present series of patients with thyroid cancer. This suggests that thyroid carcinoma does not arise more frequently in goitrous glands compared to normal glands. The difficulty of interpretation, however, is shown by the series of 51 patients with thyroid carcinoma, who all had goitre, reported by Kearns, Davis, and Balkin (1955). It has already been stressed that many patients with carcinoma are subjected to operation for goitre with no clinical suspicion of malignancy, so that any patients then found to have carcinoma have had a previous goitre. A selection bias in favour of a high incidence will thus be present in any clinic where goitre is treated surgically. Thyroid carcinomas may enlarge very slowly, and many of these previous goitres may have been carcinomatous from the beginning (Frazell and Foote, 114). Sloan (319) has emphasized the fact that most patients with thyroid carcinoma have no previous goitre. His report is most significant in this respect, since the non-malignant thyroid tissue was examined histologically, and the presence of diffuse or nodular goitre in 24 per cent. was confirmed. The weight of evidence is
against the belief that the majority of thyroid cancers arise in adenomatous goitres.

The importance of goitre in producing carcinoma has also been stressed by considering the incidence of carcinoma in diffuse and nodular goitres, though the published figures show considerable variation. Representative of such evidence are the incidences of 10.1 per cent. (Cope, Dobyns, Hamlin, and Hopkirk, 78), 5.6 per cent. (Majarakis, Slaughter, and Cole, 220), 17.1 per cent. (Cole, Majarakis, and Slaughter, 74), 4.5 per cent. (Zimmerman, Wagner, Perlmutter, and Amromin, 396), 12.5 per cent. (Hermanson, Gargill, and Lesses, 150), 9.1 per cent. (Cattell and Colcock, 61), 4.8 per cent. (Beahrs, Pemberton, and Black, 30), 5.8 per cent. (Cloud and Branch, 72), 7.6 per cent. (Hinton and Lord, 152), and 5.4 per cent. (Horn, Welty, Brooks, Rheads, and Pendergrass, 155). A selection factor is obviously present, since all cases of goitre are not treated surgically, and these figures give no indication of the actual incidence of carcinoma in goitre. Zimmerman and Wagner (397) have shown that the incidence of carcinoma found in nodular goitres in U.S.A. increased steadily between 1916 and 1949, and that similar increases were described from particular surgical clinics during this period. This was probably a relative increase in carcinoma, as the incidence of
nodular goitre in America has greatly decreased over this period. Another factor may be that the histological criteria for the diagnosis of carcinoma have changed. Majarakis, Slaughter and Cole (220) and Cloud and Branch (72) have pointed out that the large variation may be correlated with geographical regions, the proportion being higher where endemic goitre is present. The high incidence of thyroid carcinoma in autopsies as reported by Wegelin (373) for Switzerland and the apparent fall in death rate from thyroid cancer in Zurich, since the reduction of simple goitre by iodized salt, reported by Wespi-Eggenberger (330) would seem to confirm this. In other countries, however, endemic goitre has strikingly decreased without any apparent decrease in deaths from thyroid carcinoma, as reported by Zimmermann et al. (397). In Finland Saxen and Saxen (300) found no difference in mortality rates for thyroid cancer in rural areas, where goitre was either rare or common. Another method of approach is to consider the incidence rate of thyroid cancer in the general population. Extensive data are given by Sokal (321) for various parts of U.S.A. and the mean value is 2.5 new cases per 100,000 population per year, and 0.7 per cent. incidence of thyroid cancer as a percentage of all cancer. Data from the Cancer Records Bureau of the Sheffield Region for 1957 and 1958 give corresponding mean values of 0.8 new cases per
100,000 population per year, and 0.5 per cent. incidence of thyroid cancer related to all cases of cancer. Comparison is difficult as standards of registration may not be similar, but it seems that Sheffield, with a high incidence of simple goitre, has a lower incidence of thyroid cancer than the U.S.A., and a similar incidence to Greater London, where thyroid cancer was 0.7 per cent. of all cancer in 1938-39 (Harnett, 141). Another method of expressing this rate is to assume that all these cases of thyroid cancer in Sheffield will not survive, which is a gross over-estimate as already shown. This will give a calculated death rate from thyroid cancer of 0.8 per 100,000 which is similar to the mean value of 0.7 given by the Registrar General's Reports between 1939-59 (237). It is doubtful whether this problem of thyroid cancer in relation to simple goitre can be solved with existing data, but currently the weight of evidence suggests that if there is an increased likelihood of thyroid cancer developing in goitrous glands, it is extremely small.

Many observers have stressed the fact that carcinoma is commoner in goitre with a localized nodularity than in multinodular goitre (Cope, Dobyns, Hamlin, and Hopkirk, 78; Majarakis, Slaughter, and Cole, 220; Cole, Majarakis, and Slaughter, 74; Hermanson, Gargill, and Lesses, 150), though this
difference was not found by Cloud and Branch (72).
The chance of developing carcinoma might be expected to increase with multiple nodules, so that the observed incidence would suggest some difference between solitary and multiple nodules. Thus it is possible that such solitary nodules are carcinomatous from the beginning. In interpreting these differences, however, it should be remembered that there is a striking lack of correlation between clinical estimation of thyroid nodularity and pathological findings (Mortensen, Woolner, and Bennett, 254).

Hyperplasia of the thyroid is most striking in hyperthyroidism, but the evidence that it is associated with an increased incidence of thyroid carcinoma is meagre. Cope, Dobyns, Hamlin, and Hopkirk (73) found 0.8 per cent. of cases of thyroid cancer in patients with diffuse toxic goitre. Beahrs, Pemberton, and Black (30) found 0.5 per cent. This incidence is doubled when hyperthyroidism occurs with nodular goitre (Cole, Majarakis, and Slaughter, 74; Beahrs, Pemberton, and Black, 30). Thus, compared with the results already quoted for goitre without hyperthyroidism, the incidence appears to be much lower when hyperthyroidism is present. As already mentioned, very variable figures have been reported as regards the incidence of hyperthyroidism in patients with thyroid carcinoma. It does not appear to be so rare as has
been suggested (Means, 241). In 7 per cent. of the present series there was reliable evidence of hyperthyroidism and carcinoma. Much of the variation may be explained by lack of definite evidence to support the diagnosis of hyperthyroidism. Confirmation of such diagnosis by the basal metabolic rate (Friedell, 115) is unreliable, as this rate may be raised in any type of malignant disease. In assessing the significance of the coincidence of these two conditions in 7 per cent. of the present series, there is one main selection factor which must be considered. Hospitals in the region, but outside Sheffield, only send patients with thyroid carcinoma to Sheffield Radiotherapy Centre if the tumour is likely to concentrate radiiodine. If the patient had coexistent thyrotoxicosis, radiiodine investigations would almost certainly be sought. In fact this condition applies only to Case T3, and the high incidence cannot be explained by this manner of selection. Selection due to Sheffield being a centre for the treatment of thyrotoxicosis by radiiodine must also be considered. Normally only patients over 45 years old are treated in this way, and thus more are likely to have coexistent carcinoma than would be found in the usual age distribution of patients treated for thyrotoxicosis. Only one patient, however, (Case T2) was referred for this purpose, and she was given radiiodine with no clinical suspicion of carcinoma at the
time of treatment. Thyrotoxicosis is commoner in the Sheffield region, and this factor would inevitably increase the probability of the two conditions occurring together. A similar effect could result from an increased frequency of thyroid carcinoma in this region, but as already mentioned, this has not been found.

There is strong evidence that the hyperthyroidism is only very rarely due directly to the production of thyroid hormone by the carcinoma. Cunningham, Hilton, and Pochin (35) have found the iodine-concentrating activity of thyroid carcinomas to be usually less than 10 per cent. of that of normal thyroid tissue. This is confirmed by Fitzgerald (104). Thus, even with the most differentiated thyroid tumours that have been investigated, about one kilogram of tumour tissue would be required to produce the average tenfold increase in thyroid-hormone production observed in hyperthyroidism. In only one of our patients was there evidence of such a large amount of tumour tissue, and this particular carcinoma did not concentrate radioiodine. The conclusion seems inescapable that the hyperthyroidism was due to excessive function of non-malignant thyroid tissue. A similar conclusion was reached by Berlin and Gargill (31) in a review of the coexistence of diffuse toxic goitre and malignancy of the thyroid.

Experimental evidence of the tendency of
hyperplastic thyroid glands to develop malignant tumors has been provided by producing chronic thyroid hyperplasia in several ways. As an analogy with the increased incidence of thyroid carcinoma in endemic goitre regions, Axelrad and Leblond (21) have demonstrated the occurrence of thyroid carcinoma in rats maintained on an iodine-deficient diet. The tumors, however, could not be successfully transplanted. Prolonged administration of antithyroid agents, in particular thiouracil, has also been shown to produce thyroid carcinoma in rats (Purves and Griesbach, 279; Money, Fitzgerald, Godwin, and Rawson, 249). Metastasizing tumors occur only after administration of goitrogens for about 20 months, which is half the lifetime of a rat. The development of such carcinomas is hastened by simultaneous administration of the carcinogen 2-acetylaminofluorene (Bielschowsky, 92), and by radioiodine (Doniach, 92). Successful transplantation of carcinoma from thiouracil administration in rats has been achieved by Purves, Griesbach, and Kennedy (280).

Thiouracil therapy for hyperthyroidism in man has not produced an increased incidence of thyroid carcinoma, and undue significance cannot be attributed to the development of thyroid cancer after only a few months of thiouracil administration, as described by Payne, Crane, and Price (264).
The development of a carcinoma of the thyroid many years after exposure to ionizing radiation constitutes no evidence of a causal relationship; this must be sought on other lines. Most evidence is available with regard to the association between irradiation in childhood and the development of a neoplasm in the thyroid. In reviewing all cases since 1946 of proved carcinoma of the thyroid at the hospitals in Sheffield from which the cases have been drawn, there were 12 patients developing the condition while under 35 years of age. In six a history of ionizing irradiation of the neck region in childhood was obtained. There is no available information regarding the frequency of exposure of the neck region to irradiation in the general public, but it seems highly improbable that it reaches the level of 50 per cent. In attempting to obtain the past history it is most important that the parents should be interviewed, as the patient may be unaware of previous therapy if it was given in early life for a condition leaving no scar. One patient included in the non-irradiated group developed a thyroid carcinoma when 29 years of age. He gave a history of treatment with "rays" for swollen glands in the neck while in Holland 16 years previously. No details have been traced, but the description of the apparatus given by the parents suggested that the treatment was by ultra-violet rather than X-irradiation. A similar high incidence of previous exposure to
ionizing radiation among younger patients with thyroid carcinoma has been reported by others (Duffy and Fitzgerald, 94; Clark, 66; Fetterman, 163). A further indication of a possible association between the irradiation and the development of the carcinoma is that in the five patients with a localized cutaneous lesion the site of the initial and maximal development of the carcinoma was invariably in the underlying part of the thyroid receiving the greatest exposure. In Case 8 the thyroid nodules were situated in the isthmus and right lobe, while the naevus overlaid the tendinous head of the left sternomastoid. As, however, the disposition of the radium needles is not known, no calculation of the exposure of different parts of the thyroid to radiation has been possible in this patient. More definite evidence has been obtained by following the subsequent medical history of infants subjected to X-irradiation of the thymus. Simpson and Hempelmann (312) found 10 cases of thyroid carcinoma and 7 cases of thyroid adenoma among 1,502 irradiated children, while in 1,933 untreated siblings there was no carcinoma and only one with an adenoma. Their study thus provided strong circumstantial evidence that irradiation in infancy may be an aetiological factor in the pathogenesis of thyroid cancer. Their cases were irradiated for supposed enlargement of the thymus, the present cases chiefly for skin disorders.
particularly naevi. It is, of course, remotely possible, though highly improbable, that the various conditions for which the irradiation was given are all associated directly with an increased liability to develop cancer. Two further points are noteworthy in our cases of carcinoma following irradiation in childhood. Firstly, the calculated dose to the thyroid gland was low and in three cases did not exceed 300 r. In Case 1 the dose was slightly below the minimum of 180 r reported by Simpson and Hempelmann (312).

Secondly, the latent period in the children is frequently shorter than that seen in carcinoma following irradiation in adult life (Medical Research Council, 242).

The two patients (Cases 8 and 9) presenting nodular adenomas in the thyroid following irradiation are of considerable interest. The histological features suggested considerable cellular hyperplasia: mitosis was frequently seen. However, in the absence of local invasion and metastases the histological appearances alone did not justify a diagnosis of carcinoma. Nevertheless more innocent-looking thyroid adenomas have often been the origin of distant metastases. It is clear from Simpson and Hempelmann's (312) study that there is an increase in the incidence of thyroid adenomas as well as carcinomas following irradiation. The histology of the two adenomas reported certainly suggests that they should be regarded
as premalignant conditions.

In contrast to the information now available concerning children there is little to suggest that irradiation of the thyroid in adult life may lead to the development of a carcinoma. Indeed in Case 7 the irradiation for thyrotoxicosis in 1919 and the anaplastic thyroid carcinoma in 1956 might be regarded as fortuitous. Although X-irradiation was a recognized method of treating thyrotoxicosis over 40 years ago, reports of cases of thyroid carcinoma following this procedure are remarkably few (Quimby and Werner, 264). No accurate information is available regarding the frequency of the use of this method of treatment of thyrotoxicosis. In Sheffield it was employed particularly during the years 1931-43 and 99 patients were treated. The latent period for the development of a carcinoma following irradiation in adult life is at least 20 years (Medical Research Council, 242), and the available evidence in the case of the adult thyroid suggests that it may be much longer. It is thus possible that an association may be noted more often in future years.

It is clearly important to review present practice with regard to exposure of the thyroid to ionizing radiation. It is evident that the increased risk incurred by any individual patient as a result of exposure is very small. It is apparent only when a large survey of irradiated individuals is carried out
or when actual cases of carcinoma in younger patients are studied. Zimmerman and Wagner (397) have produced suggestive evidence that an absolute increase in thyroid carcinoma has occurred during the last 30 years. Doses apparently sufficient to induce neoplastic change are surprisingly small and may be exceeded in certain diagnostic and therapeutic procedures in childhood. On the average a test dose of 131I resulting in an uptake of 15 microcuries distributed evenly throughout a gland weighing 15 g. or less would deliver a dose to the thyroid of at least 100 r. As, however, the distribution is probably irregular, certain regions would receive a dose considerably in excess of this amount. There is no evidence so far that the use of radiiodine, either for diagnosis or for therapy has led to the development of thyroid carcinoma (194, 38, 39), but an association with acute leukaemia has been suggested in patients who have been treated for hyperthyroidism (2, 275, 276) or treated for thyroid carcinoma with much larger doses of 131I (37, 307). As the latent period may be larger in adults, this lack of evidence in relation to thyroid carcinoma must still be accepted with reservation, and an association may yet be found. From the evidence available, however, it appears that an association between thyroid carcinoma and previous radiation is found only in the younger
age groups. The experimental evidence concerning this association will be discussed in the following chapter.

Summary

1. One hundred cases of thyroid carcinoma, referred to a radiotherapy centre over a period of 10 yr. have been reviewed.

2. The major factor in relation to age of onset and survival was the histological type of tumour. Papillary tumours occurred in younger patients and prognosis was good. Older patients had undifferentiated tumours and a poor prognosis. Follicular tumours were intermediate between the other two types.

3. The relationship between simple goitre and subsequent development of a carcinoma has been considered. The majority of thyroid carcinomas arise in normal thyroid glands, and the risk of such a tumour developing in a goitrous gland can only be slightly greater than from a normal gland.

4. In seven cases of thyroid carcinoma, there was coexistent hyperthyroidism.

5. An association between previous treatment to the neck region by ionizing radiations and subsequent development of thyroid carcinoma has been clearly demonstrated.

Seven such cases are described, and in six the thyroid had been exposed to irradiation in infancy.
or childhood, during the treatment of cutaneous lesions. The dose varied from 130 r to 2,700 r and the latent period from 5 to 18 years. The carcinoma developed in that part of the thyroid which received the greatest dose.

Exposure to ionizing radiation in childhood predisposes to the development of thyroid tumours. The association following irradiation in adult life is less certain.
EFFECT OF PREVIOUS IRRADIATION ON THE
RESPONSE OF THE RAT THYROID TO STIMUL-
ATION BY THYROTROPHIN

It is accepted that ionising radiation can
induce malignant change due to the direct effects of
the energy on the irradiated tissue (54, 242). There
is experimental evidence that this applies to
thyroid malignancy, as Goldberg and Chaikoff (113)
have produced both thyroid carcinomas and adenomas in
rats 12 - 18 months after a single dose of 400 μc. of
$^{131}$I, with an average thyroid radiation dose of
100,000 rads. Thyroid tumours in rats can be produced
by much smaller doses of radiation, when goitrogens
are given following the radiation. Thus Doniach (92)
has found thyroid tumours after only 30 μc. of $^{131}$I,
when methylthiouracil is given. It appears that hyper-
plasia due to high thyrotrophic hormone secretion will
increase the number of tumours produced as a result
of radiation damage. This also applies to other
carcinogens, such as 2-acetylaminofluorene
(Bielschowsky, 34). Usually thyrotrophin secretion
has been stimulated by the administration of goitro-
gens, producing low serum thyroxine levels and hence
pituitary stimulation. As described in the previous
chapter, thyroid tumours in rats have been produced
by goitrogens alone (279, 249), and are transplant-
able to thyroxine deficient rats (253, 280, 383).
It is possible that this synergistic effect of thyrotrophin and carcinogens is also important in the development of thyroid tumours in man. Excessive thyrotrophin secretion will occur, when the thyroid is limited in its ability to increase thyroxine secretion on demand, and to maintain its secretion when some inhibitor is given. Skanse (315) and Abbatt et al. (1) have demonstrated that the response of the rat thyroid is limited after 10 μc. of $^{131}$I. This gives a large radiation dose of approximately 5,000 rads. As shown in the last chapter, thyroid tumours have developed after much smaller doses of radiation, when this was given in childhood. It seemed of interest to determine whether comparable doses of the order of 200 - 500 rads would limit the response of the rat thyroid to some stimulus. If such happened, it is probable that excessive secretion of thyrotrophin would occur in an attempt to overcome this limited response. The stimulus chosen was the administration of a goitrogen, as used by Skanse (315), Doniach and Cogothetopoulos (93) and Abbatt et al. (1).

**Effect of $^{131}$I on response of rat thyroid to carbimazole**

Young rats (60 - 80 g.) were given doses of $^{131}$I ranging from 20.0 μc. to 1.25 μc. in groups of 20 rats each. After 24 hrs, 5 rats from each group
were killed and thyroid uptake of $^{131}\text{I}$ measured. This value was used in calculating the radiation dose received by the remainder of each group. After 6 months, carbimazole was given in drinking water for 28 days, so that the daily dose was 4 mg. for each rat. The ratio of concentration of $^{131}\text{I}$ as iodide between thyroid and serum, as described by Vanderlann and Vanderlann (351) was measured at the end of this period, by collecting blood by heart puncture, 1 hr. after giving 2 $\mu$e. of $^{131}\text{I}$. The rats were killed immediately after blood collection when thyroid weight and content of $^{131}\text{I}$ were measured. The results are shown in Table 53. Large goitres were produced in the control rats by carbimazole, with a mean of 14·3 mg./100 g. body weight. The thyroid/serum ratio of $^{131}\text{I}$ in the control rats was very high, and about 10 times the value expected in rats not treated with carbimazole. This confirms previous findings that thyroid/serum ratios are much increased by chronic goitrogen treatment (351, 139). There was significant reduction in thyroid/serum ratios in the groups given 20, 10 and 5 $\mu$e. of $^{131}\text{I}$ 6 mths. before, compared to the control group, which had no radiation. Significant reductions in weight increase occurred even in the group given 2·5 $\mu$e. of $^{131}\text{I}$. Thus increase in both thyroid/serum ratio and weight in response to goitrogen treatment was reduced
Table 53. Effect of radio-iodine 6 mths. before on the response of rat thyroid to carbimazole

<table>
<thead>
<tr>
<th>Amount of $^{131}I$ (µc.)</th>
<th>Mean thyroid weight (mg./100 g. body wt) ± s.e.</th>
<th>Mean thyroid/serum ratio of $^{131}I$ (% dose /mg. /% dose /0.1 ml.) ± s.e.</th>
<th>No. of rats treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.0</td>
<td>6.13 ± 0.31</td>
<td>174.9 ± 5.3</td>
<td>15</td>
</tr>
<tr>
<td>10.0</td>
<td>8.22 ± 0.46</td>
<td>226.5 ± 6.2</td>
<td>15</td>
</tr>
<tr>
<td>5.0</td>
<td>11.69 ± 0.57</td>
<td>249.3 ± 7.0</td>
<td>15</td>
</tr>
<tr>
<td>2.5</td>
<td>12.65 ± 0.68</td>
<td>261.8 ± 7.5</td>
<td>15</td>
</tr>
<tr>
<td>1.25</td>
<td>13.38 ± 0.83</td>
<td>272.3 ± 8.2</td>
<td>15</td>
</tr>
<tr>
<td>Control</td>
<td>14.31 ± 0.89</td>
<td>275.8 ± 8.7</td>
<td>30</td>
</tr>
</tbody>
</table>

Carbimazole was supplied in drinking water so their daily dose was 4 mg., and given for 28 days.
in these irradiated thyroids. The values in the group given 1.25 μc. are also decreased even though these are not significant by student's t-test.

The radiation dose delivered was calculated as follows. The mean 24 hr. uptake of the original dose was approximately 15%. The biological half-life of 131I was assumed to be 3.3 days from the data given by other workers (100, 221, 306), and the rat thyroid gland to be 12 mg. when body weight was 70 - 80 g. Using the formulae given by Mine and Brownell (151), these values provide a total beta and gamma radiation dose of 310 rads/μc. of 131I given. This value is very similar to those given by Skanse (315), Feller et al., (100), Maloof et al., (221) and Abbatt, et al. (1). Thus the rat thyroid gland given a total radiation dose of under 500 rads 6 months previously, was unable to respond by the same increase in thyroid weight and thyroid/serum ratio as a normal gland to a goitrogenic stimulus. Radiation doses greater than this produced correspondingly greater reductions in response. The threshold dose is in the region of 500 rads.

**Discussion**

The results seem to indicate that the capacity of the rat thyroid to hypertrophy is affected by much smaller radiation doses than previously described. The cause of the hypertrophy in response to
a goitrogen is increase in hypophyseal secretion of thyrotrrophic hormone, as it is prevented by hypophysectomy (87, 137). This stimulation of thyrotrophin is dependent on a lowered thyroxine level in the blood, and is again prevented by giving thyroxine with the goitrogen (87, 134). Thyrotrophin produces increase in weight and an increase in thyroid/serum ratio is a most sensitive index of increased thyrotrphin secretion. (328, 350, 351). Thus the inability of these irradiated glands to respond in this way suggests that a greater increase of thyrotrophin will occur and the glands will be subjected to higher thyrotrophin levels. The evidence that tumours may be produced in the rat thyroid by increased thyrotrphin secretion in response to goitrogens has already been described (249, 279). The weight of evidence suggests that the irradiated gland will be more liable to develop tumours when goitrogens are given due to an even greater stimulation by thyrotrphin than in the non-irradiated gland. Such a mechanism may underlie the association of thyroid carcinoma and previous irradiation described in the last chapter. It is of interest that the radiation dose of 800 rads shown to affect the rat thyroid gland is similar to the doses given to the children who developed thyroid carcinoma. Previous evidence has suggested that the rat thyroid is more resistant
to radiation than the human thyroid. Thus Warren (369) described resistance and Golberg and Chaikoff (117) found normal thyroid function in rats 5–8 mths after 300 μc. of $^{131}$I. However their indices of function were coarse. Skanse (315) noted effects after 50 μc. $^{131}$I, and his results seem to show decreased response to thiouracil after 10 μc. $^{131}$I. Abbatt et al. (1) showed a similar decreased response to 30 μc. and possibly to 10 μc.$^{131}$I. Strikingly they noted impairment after much smaller doses of external radiation, and the threshold for this type appeared to be between 500 and 1,000 rads, and that inhibition was nearly complete after 1,000 rads. They suggested temporal and spatial factors to account for this difference between external radiation and internal from $^{131}$I, and equated 30 μc. $^{131}$I and 1,000 rads of external radiation. From the present results the effect produced by 10 μc. $^{131}$I would equate with 1,000 rads from their results. This gives a ratio of 3:1 for radiation doses; a ratio of 10:1 was found by Abbatt et al. (1). It seems clear from the present results that 2.5 μc. of $^{131}$I produces a limited response of the rat thyroid 6 months later.

**Summary**

1. The radiation dose delivered to the rat thyroid gland by $^{131}$I was shown to be approximately 310 rads/μc.
2. Increase in thyroid weight and thyroid/serum ratio of iodide was limited 6 months after $^{131}\text{I}$ irradiation and significant effects were found after 2.5 μc. $^{131}\text{I}$. A simple dose-response relationship was observed in the dose range 1.25 - 20 μc. $^{131}\text{I}$ which was 400 - 6,000 rads.

3. The importance of this limited response of the irradiated thyroid gland in the eventual production of thyroid tumours is discussed.
SYNTHESIS AND SUGGESTED STUDIES

In this thesis the results of many investigations on patients with simple goitre, and of experiments on animals designed to demonstrate and clarify the role of goitrogenic substances have been described. It is proposed to harvest the conclusions which can be reached, and to discuss them in relation to the three main causes of goitre in man; these being deficiency of dietary iodine, inherited disturbances of thyroid hormone synthesis, and goitrogenic substances.

The incidence of simple goitre in Great Britain is not uniform, and large regional differences have been demonstrated. All degrees of thyroid enlargement are commoner in all age groups in Sheffield compared to Ormiston in Scotland. The incidence of thyroid enlargement in school children from Oxfordshire and Wiltshire is similar to Sheffield and also greater than Ormiston. These findings, which confirm observations during the past century that such regional differences did exist, suggest that inherited defects of thyroid hormone synthesis cannot be the complete explanation of simple goitre. It seems very unlikely that the incidence of such defects will vary greatly within a small, densely populated country such as Britain and any such variation would probably have a racial basis, where marriage within the group was encouraged. This has already been demonstrated
by McGirr (215), where a high incidence of goitre was demonstrated in a group of tinkers, and they were shown to have defective hormone synthesis on a genetic basis. There is no reason to suppose that the differing incidences in Sheffield and Ormiston are due to any selection factors such as race or creed. However, it is possible that other factors may uncover such inherited defects. Thus it is possible to postulate that iodine deficiency or goitrogenic substances might cause goitre, where a partial defect of hormone synthesis was present. The lack of a familial incidence of thyroid enlargement in Sheffield is against this postulate and suggests that inherited defects are not important in the causation of simple goitre in the general population.

A seasonal appearance of goitrogenic activity has been demonstrated in milk from Sheffield and Yorkshire, by the production of thyroid enlargement in rats. This does not demonstrate that such milk can produce thyroid enlargement in human beings, but an antithyroid effect of such milk is demonstrable in man by the depression of radiiodine accumulation. The degree of thyroid enlargement produced in rats by 25 ml. of milk daily is approximately equivalent to 50 - 100 µg. of carbimazole. Thus 500 ml. of milk represents an intake of 1-2 mg. of
carbimazole, and MacGregor and Miller (217) have demonstrated that such doses produce almost complete inhibition of the accumulation of radioiodine by the human thyroid gland. This suggests that the goitrogenic substance in milk has an activity considerably less than carbimazole in man, as only slight to moderate inhibition of accumulation was produced in patients by 500 ml. of milk. The measurement of antithyroid activities of a large number of antithyroid compounds has demonstrated that the correlation between ability to depress $^{131}$I uptake and to produce thyroid enlargement may be poor for certain compounds, probably due to different rates of degradation and excretion. Thus the assessment of the importance of this goitrogenic material in milk in the production of human thyroid enlargement is difficult. It awaits the isolation and description of the pharmacological properties of the pure substance. Supplementary iodine partially inhibits the thyroid enlargement produced by milk and the corresponding amount of carbimazole. It is therefore probable that ingestion of small amounts of goitrogenic substances will produce thyroid enlargement, when there is also a deficient intake of iodine. The evidence that iodine deficiency is rarely the sole cause of goitre has been reviewed in Chapter 4, and this dual causation is an attractive hypothesis. A variable intake of
goitrogenic substances would explain the variable development of goitre with the same iodine intake, which has been amply demonstrated by other workers. Preliminary results suggest that goitrogenic substances are present in greater amounts in milk from Sheffield compared to Ormiston, and may account entirely for the observed difference in goitre incidence. However the effect of ingestion of a greater amount of goitrogens in Sheffield would be accentuated by a deficient iodine intake, and clearly both factors must be evaluated to obtain a complete aetiological picture of simple goitre. Further, a variable sensitivity to the pharmacological action of most substances is well recognised, compared to a variable response to deficiency of an essential element. Such a variable sensitivity to goitrogens might explain the development of goitre in only a proportion of people living in regions where deficiency of iodine is present, and for the investigative findings of iodine deficiency in people from these regions who have no thyroid enlargement. The enzyme system which metabolises antithyroid compounds has been shown in the present work to be itself capable of being inhibited by other substances. It will be important to investigate the metabolism of any goitrogen in milk which can be isolated, to find out whether a variable sensitivity is demonstrable and to correlate this sensitivity with
Supplementary iodine does not appear to have decreased the incidence of thyroid enlargement in school children from Oxfordshire or Wiltshire. This is surprising as both iodine deficiency or goitrogens are inhibited as causes of goitre by added iodine either completely or partially. This investigation was not ideal, as the iodine intake of the control children was not measured and assumed to be less than that of the children taking school lunches with supplementary iodine. It is also conceivable that the intake of goitrogens might be higher in the children taking school meals, as milk and milk products form a large part of such meals, and emphasises that any investigation of simple thyroid enlargement must take into account both iodine intake and ingestion of goitrogens. The higher incidence of thyroid enlargement in Oxfordshire compared to Wiltshire must be considered against the known regional variations in goitre incidence that occur, and the effect of seasonal influences. Thus goitrogenic material is found in highest amounts in milk during the spring, and an increase in thyroid size in sheep during the spring is shown in the present work. The children in these two counties were examined in the spring and autumn respectively. Such influences show that season must be considered in future goitre surveys, and that the demonstration of a seasonal variation in goitre
incidence in the same group of children will clarify the importance of goitrogens in the production of human goitre.

Higher uptakes of $^{131}\text{I}$ are a constant feature of simple goitre in man, and are confirmed in the present work. The paradoxical situation of demonstrating the acute effect of goitrogens by depressing uptake of $^{131}\text{I}$ and postulating their importance in the production of simple goitre is resolved by describing effects of chronic administration of small amounts of antithyroid compounds such as carbimazole to rats. High uptakes can be produced and are inhibited by supplementary iodine. Further it is shown that a constant action of such compounds is the blockage of hormone synthesis between the stage of iodotyrosines and iodothyronines, so that larger amounts of monoiodotyrosine are found in the thyroid glands. This also emphasises that the finding of increased iodotyrosines and decreased iodothyronines in human simple goitre is not specific for inherited defects of hormone synthesis as has been suggested (Pitt-Rivers, et. al. 274). Obviously goitrogenic substances will also produce this finding, and it has also been demonstrated where iodine deficiency has been present (Querido et al. 281). The latter workers did not assess the role of goitrogens in their investigations of endemic goitre. The present work shows that this
action of goitrogens may be found when no inhibition of organic binding of iodine occurs. Thus the lack of any such defect of organic binding does not exclude the ingestion of significant amounts of goitrogens.

The findings using radiiodine and chemical iodine measurements on human simple goitre are confirmatory of previous work. They include high uptake of $^{131}$I, protein-bound iodine levels that are lower than normal, and decreased concentration of iodine in the goitrous thyroid gland. The normal renal clearance of iodine and normal or increased biological half-life of $^{131}$I show that increased loss of iodine does not occur. These findings occur in iodine deficiency, and some degree of this must be present. However, a demonstration of decreased iodine intake or excretion, which was not attempted, does not show that this is the sole cause of the goitre. These findings are also consistent with the ingestion of small amounts of goitrogenic substances acting with iodine deficiency. The finding of increased total thyroid iodine contents of human goitrous thyroid glands may be a reflection of a block in hormone synthesis and a relatively small store of thyroid hormone due to the action of goitrogenic substances, with increased amounts of iodosylbromides.

Carcinoma of the thyroid is often accepted as a complication of simple goitre, especially when the stage of nodular goitre is reached. A reconsideration
of this problem by the study of a large group of patients with thyroid cancer is given. Manifestly, the majority of the thyroid tumours arise in otherwise normal thyroid glands, and by appraisal of known incidences of simple goitre and thyroid carcinoma, it seems probable that simple goitre does not predispose to the development of thyroid cancer. It is suggested that the misapprehension has arisen due to selection factors before patients with goitre are considered for operation. Previous ionising radiation to the neck of young children is shown to be associated with the later development of thyroid cancer, and experimental results show the severe limitation of response of the thyroid produced by small and moderate amounts of ionising radiation.

The present work has not produced an answer to the question of the aetiology of simple goitre, and was not expected considering the complexity of the problem and the amount of work on this problem during the past fifty years. It has demonstrated the presence of regional differences in goitre incidence in Britain, that the ingestion of goitrogenic substances may be an important factor in the production of goitre and that currently, the weight of evidence suggests that such regional differences may best be explained by the concerted action of some degree of deficient intake of iodine along with the ingestion of goitrogens.
It has shown that such substances are related to season, and that other seasonal influences such as light and temperature must be considered in the aetiology of goitre, when some degree of iodine deficiency is present. Further it has shown that the chronic ingestion of goitrogenic substances in animals can produce thyroid enlargement with high uptake of radiiodine and impaired synthesis of thyroid hormone. It has suggested many further studies, and the following are briefly considered.

Isolation of goitrogenic substance in milk

This is of great importance, as its pharmacological actions cannot be studied completely until the pure substance is available. In particular the assessment of its ability to produce goitre cannot be studied. It is unlikely that this can be done in man by attempting to produce goitre, but its acute effect, duration of action, metabolism and excretion can be investigated. More than one such compound may be present and this may vary with pasture and therefore regionally. The ability to produce goitre can be studied on some animal, which is naturally prone to goitre, and the sheep is an obvious choice. The production of simple goitre with all the features of human goitre, could then be attempted by varying administrative regimes, and combined with deficient and supplemented intakes of iodine.
Assessment of aetiology of simple goitre

The relative importance of iodine deficiency and ingestion of goitrogens should be assessed regionally. This can be done by assessing intake of iodine by dietary surveys, accompanied by measurement of iodine of sample diets, and measuring excretion of iodine in urine. The author has found the latter measurement to be difficult and a reliable and accurate method is required. The amount of goitrogens could be compared by assays, such as those used in the present work. This would give a more complete picture of goitre in Britain, and should be combined with regional clinical surveys, and investigation of the possibility of seasonal variations in goitre incidence.
SUMMARY

1. Thyroid enlargement was more frequent in both sexes and all age groups in Sheffield compared to Ormiston, East Lothian. History of goitre, and incidence of hyperthyroidism and thyroidectomy were significantly higher in Sheffield, but there was no objective evidence of a familial incidence of goitre in either place.

2. The use of iodized salt in Oxfordshire and Wiltshire schools produced no significant decrease in incidence of thyroid enlargement after 18 months. The supplement was shown to be significant as the average iodine content of the school lunches in Wiltshire was 120 μg.

3. Increased uptake of $^{131}$I, low serum protein-bound iodine, normal renal clearance of $^{131}$I, and biological half-life of $^{131}$I which was not decreased were features of simple goitre in Sheffield. No defect in organic binding of $^{131}$I was demonstrable. Concentration of iodine in the goitrous thyroid was constantly lower than normal, but mean total iodine was within normal limits. There was an inverse correlation between thyroid size and concentration of iodine in the thyroid, and a direct correlation of thyroid size and total iodine content of the thyroid.
4. A single dose of cow's milk depressed accumulation of $^{131}$I in man and rats, and produced thyroid enlargement without depression of uptake after prolonged administration. This effect was found with liquid milk in Sheffield only from February to May each year, and was maximal during this period when dried milk from Yorkshire was examined. The goitrogenic effect of milk was partially inhibited by iodide. Preliminary results showed that milk from Ormiston has less antithyroid activity than Sheffield milk.

5. A three-fold increase in size of sheep thyroid glands was found to coincide with the appearance of goitrogenic material in milk.

6. The antithyroid activity of milk was not due to the iodine, calcium, or thiocyanate contents of the milk, but it was shown that impairment of organic binding of $^{131}$I occurred after single large doses of milk, which suggested that the activity was due to a substance similar to the thioureido compounds.

7. Increased uptake of $^{131}$I and evidence of inhibition of thyroid hormone synthesis after the stage of monoiodotyrosine was found after prolonged administration of small amounts of carbimazole. No inhibition of organic binding of $^{131}$I occurred with this dose. Under specific conditions carbimazole produced thyroid enlargement with a normal or increased uptake of $^{131}$I.
8. Antithyroid activities measured by depression of $^{131}$I uptake and goitre production did not always correlate using thiohydantoins as typical of thioureido compounds.

A polar group on the side chain in the 5-position of thiohydantoins caused loss of antithyroid activity, and was associated with greatly reduced metabolism to sulphate by the thyroid. The antithyroid activities of thioureido compounds were increased by SKF 525-A due to inhibition of the desulphurisation of these compounds by the liver.

9. The risk of thyroid tumours developing in goitrous thyroid glands was not clearly greater than in normal glands. Treatment of children and young people by ionising radiation to benign lesions of the neck was definitely associated with the later development of thyroid carcinoma. The ability of the rat thyroid to respond to a goitrogenic stimulus was limited by more than 500 rads of radiation from $^{131}$I given six months previously.
ACKNOWLEDGMENTS

The work for this thesis could not have been done without the help and encouragement of many persons. I am grateful in particular to Professor G.M. Wilson and Dr G.W. Blomfield for advice and for allowing me to study patients under their care. Professor R.P. Jepson and A.W. Kay were extremely kind and tolerant in arranging dates of operations to suit the studies on thyroid total iodine measurements.

The examinations of all patients in the general practices were performed by Dr M. Rushbrooke and Dr E.S.B. Wilson in Sheffield, and Dr J.S. Milne in Ormiston, and I am exceedingly grateful for their help and cooperation. The surveys in Oxfordshire and Wiltshire were carried out with Dr W.T.C. Berry and Dr J. Horne, and though only my own results are given, I am indebted to them for much discussion on the problems involved in these surveys.

Dr D.T. Elmore prepared all the thiohydantoins, while working in the Department of Chemistry in Sheffield and I am grateful to him for supplying me with these compounds. The Research Fund of the University of Sheffield provided funds for the purchase of $^{35}$S potassium thiocyanate and I would like to acknowledge this financial help. Dr G.B. Broadhead prepared the $^{35}$S labelled thiohydantoins with my assistance and I duly acknowledge his considerable skill in these preparations.
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I should like to thank all the volunteers who gave blood for estimation of protein bound iodine, and the patients who were so tolerant with these studies.

I wish to thank Mr S. Whiteley who efficiently organised the collection of sheep thyroid glands from the Sheffield abattoir.

Finally I should like to thank Mrs A.M. Robinson for her extreme patience and skill in typing this thesis and for giving up so much of her spare time.

Much of the material in Chapters 8 and 11 of this thesis has been published previously (173, 174, 364).
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APPENDIX 1.

Chemical estimation of iodine

a) Serum protein-bound iodine. The method described below incorporates several modifications of the alkaline incineration method of Acland (3).

Technical precautions. Elementary iodine must be excluded from the room where analyses are performed and this room is used for this estimation alone. It has been found that even with these precautions, there may be occasional cross-contamination from a room where thyroid iodine is measured. Thus these two estimations may have to be separated by a considerable distance. Glassware is kept scrupulously clean. When not in use, glassware is immersed in an aqueous solution of detergent ("Teepol", B.D.H.Ltd.), which effectively removes traces of iodine. It is rinsed with tap-water, and then washed at least six times with resin-filtered water. The latter is prepared by passage of water, distilled from a metal still, through a column of Amberlite Monobed MB-1 ion-exchange resin. All glassware is dried in a hot air oven sited in the same room at 90°.

Reagents. All chemicals are Analar grade except hydrochloric and sulphuric acids ("microanalytical reagent", Hopkin and Williams Ltd.) and ceric ammonium sulphate ("laboratory reagent low in other rare earths", B.D.H. Ltd.). The potassium iodide reference solution contains 130.8 mg. of the
dessicator-dried salt/litre (= 100 μg. of I/ml.), and is unchanged for several months. Ceric ammonium sulphate solution contains 19.5 g. of salt in 1 litre of 3.5 N H₂SO₄, and requires renewal after three months. Arsenious acid is prepared by dissolving 3.8 g. of AS₂O₃ in 50 ml. of warm N NaOH. To the cooled solution is added 50 ml. of water and 3.5 N H₂SO₄ to 500 ml.

Precipitation of P.B. I. Pipette 1 ml. of serum into "Pyrex" tubes, and add to each 1 ml. of water, followed by 1 ml. of 100% (w/v) ZnSO₄·10 H₂O and 1 ml. of 0.5 N NaOH, stirring between each addition. Allow to stand for 3 hr. and then centrifuge at 3,000 r.p.m. for 10 min. Wash the precipitate three times with resin-filtered water, and finally suspend in 1 ml. of 4N Na₂CO₃ by stirring, and washing last traces of precipitate into each tube by adding 1 ml. of water down the rod.

Ashing. Precipitate dried overnight at 95-98°, and then ashed in muffle furnace for 3½ hr. at 600 ± 10°. "Pyrex" tubes are discarded after three ashings, due to severe etching.

Estimation of released iodine. After cooling, add 1 ml. of 2N HCl and mix with a glass rod. Add 6 ml. of water to each tube and stand for 30 min., apart from three stirrings. Transfer to centrifuge tubes and spin for 20 min. at 3,000 r.p.m.

A 3 ml. sample of the supernatant is pipetted, and to this add 0.5 ml. of arsenious acid solution,
1 ml. of 3.5 N H₂SO₄ and 1 ml. of water. Calibration standards have 1 ml. of various iodide solutions added instead of water at this point. The tubes are placed in a water-bath at 37 ± 0.1°, and after 10 min. 0.5 ml. of ceric ammonium sulphate solution at 37° is added, mixed quickly and replaced in bath. Complete mixing is very important. This is done at 1 min. intervals to 12 tubes. Exactly 12.5 min. after this addition, the extinction at 415 μ in a 1 cm. cuvette is measured in a spectrophotometer (SP.600 Unicam), and all 12 tubes measured seriatim. Blanks are carried through the entire procedure using water in place of serum. A calibration line is constructed adding iodide solutions containing 0.02, 0.04, 0.06, 0.08 and 0.10 μg. of I'/ml. to blanks at the point indicated above. A blank reading provides the zero point for this calibration line, which is straight when the log. E 1cm is plotted against iodide concentration. Using the above concentration of ceric ammonium sulphate, which is half that given by Acland (3), the optical density of normal sera is around 0.75. This gives considerable gain in accuracy, as the optimal range of optical density for greatest accuracy is 0.2 - 0.8 (Lothian, 196).

Calibration control

Variable blanks have occurred with most published methods for estimating protein-bound iodine, probably due to atmospheric contamination. Rigid calibration control was set up to deal with this variable, and to achieve a gain
The properties of a calibration line are the height, the slope and the linearity. All of these can be checked by measuring three widely separated points on the line. Optimally these are the zero point (0), the mid-point (M) and the end of the line (I). These are therefore a blank reading, reading from 0.05 μg. I/ml. and from 0.10 μg. I/ml., the optical density of the latter being approximately 0.2. Initially estimations were performed in batches of 12 tubes, nine being unknown sera and three blanks. M and I were obtained by using two of the blank tubes. Duplicates were then obtained by repeating all of these in the following batch. Thus from each batch, the characteristics of the line were obtained, and a mean of the differences between 12 duplicates from each pair of batches.

This scheme was adapted after some months, so that more sera were estimated, with little sacrifice of control. Normally 11 sera and M was measured in each batch, so that height of the line was always checked. Every fourth week, 0 and M were measured in one batch, and M and I in the following, both with 10 sera. Thus the slope and linearity were checked and this scheme is outlined in Tables 54 and 55.

The four characteristics of height, slope, linearity, and mean of 11 or 12 duplicates between
Table 54. Example of calibration control for measuring height of line only

<table>
<thead>
<tr>
<th>Serum or Standard</th>
<th>Log observations</th>
<th>Difference x</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Date 1</td>
<td>Date 2</td>
<td>Mean</td>
</tr>
<tr>
<td>J.B.</td>
<td>0.4969</td>
<td>0.5263</td>
<td>0.5116</td>
</tr>
<tr>
<td>K.H.</td>
<td>0.6803</td>
<td>0.6693</td>
<td>0.6748</td>
</tr>
<tr>
<td>R.G.</td>
<td>0.7324</td>
<td>0.7292</td>
<td>0.7308</td>
</tr>
<tr>
<td>A.N.</td>
<td>0.8865</td>
<td>0.8867</td>
<td>0.8876</td>
</tr>
<tr>
<td>W.W.</td>
<td>0.7924</td>
<td>0.7846</td>
<td>0.7885</td>
</tr>
<tr>
<td>P.W.</td>
<td>0.7443</td>
<td>0.7308</td>
<td>0.7376</td>
</tr>
<tr>
<td>R.H.</td>
<td>0.6893</td>
<td>0.6875</td>
<td>0.6884</td>
</tr>
<tr>
<td>M.L.</td>
<td>0.6160</td>
<td>0.6149</td>
<td>0.6154</td>
</tr>
<tr>
<td>K.B.</td>
<td>0.5478</td>
<td>0.5575</td>
<td>0.5526</td>
</tr>
<tr>
<td>A.L.</td>
<td>0.9638</td>
<td>0.9731</td>
<td>0.9684</td>
</tr>
<tr>
<td>A.E.</td>
<td>0.9355</td>
<td>0.9460</td>
<td>0.9408</td>
</tr>
<tr>
<td>M (= 0.05 µg.)</td>
<td>0.7372</td>
<td>0.7160</td>
<td>0.7266 = M</td>
</tr>
<tr>
<td>Total</td>
<td>8.8224</td>
<td>8.8239</td>
<td>8.8290 = M</td>
</tr>
<tr>
<td>Mean</td>
<td>0.7352</td>
<td>0.7353</td>
<td>0.7353</td>
</tr>
</tbody>
</table>

\[ D = \text{Mean (Date 1)} - \text{Mean (Date 2)} \]
\[ = 0.7352 - 0.7353 \]
\[ = -0.0001 \]

Estimated Height is
\[ \bar{M} + \frac{1}{2} D = 0.7267 \]
\[ \bar{M} - \frac{1}{2} D = 0.7265 \]
Table 55. Example of calibration control for measuring height, slope and linearity of line.

<table>
<thead>
<tr>
<th>Serum or Standard</th>
<th>Date 1</th>
<th>Log Observations Date 2</th>
<th>Mean</th>
<th>Difference x</th>
</tr>
</thead>
<tbody>
<tr>
<td>D.A.</td>
<td>0.7185</td>
<td>0.7316</td>
<td>0.7250</td>
<td>0.0131</td>
</tr>
<tr>
<td>J.M.</td>
<td>0.7597</td>
<td>0.7818</td>
<td>0.7708</td>
<td>0.0221</td>
</tr>
<tr>
<td>J.S.</td>
<td>0.7332</td>
<td>0.7513</td>
<td>0.7422</td>
<td>0.0181</td>
</tr>
<tr>
<td>E.D.</td>
<td>0.9128</td>
<td>0.9345</td>
<td>0.9236</td>
<td>0.0217</td>
</tr>
<tr>
<td>M.S.</td>
<td>0.9042</td>
<td>0.9279</td>
<td>0.9160</td>
<td>0.0237</td>
</tr>
<tr>
<td>A.B.</td>
<td>0.9523</td>
<td>0.9731</td>
<td>0.9627</td>
<td>0.0208</td>
</tr>
<tr>
<td>B.J.</td>
<td>0.7672</td>
<td>0.7924</td>
<td>0.7798</td>
<td>0.0252</td>
</tr>
<tr>
<td>W.B.</td>
<td>0.7559</td>
<td>0.7796</td>
<td>0.7678</td>
<td>0.0237</td>
</tr>
<tr>
<td>K.G.</td>
<td>0.8751</td>
<td>0.9143</td>
<td>0.8947</td>
<td>0.0392</td>
</tr>
<tr>
<td>R.A.</td>
<td>0.7441</td>
<td>0.7528</td>
<td>0.7484</td>
<td>0.0087</td>
</tr>
</tbody>
</table>

\[
\begin{align*}
M \ (\text{mg}./\text{I}) &= M_1 = 0.7226 \quad M_2 = 0.7160 \quad \bar{M} = 0.7193 \quad \Delta x = 0.0066 \\
0 \text{ or I} &= 0 = 1.0864 \quad I = 0.3464 \quad 0.10\text{mg}./\text{I} \\
\text{D} &= \text{Mean (Date 1)} - \text{Mean (Date 2)} \text{ omitting 0 and I} \\
&= 0.8041 - 0.8232 \\
&= 0.0191
\end{align*}
\]

Estimated Height is \( \bar{M} + \frac{1}{2}D = 0.7293 \)  
\( \bar{M} - \frac{1}{2}D = 0.7093 \)

\[
\begin{align*}
0 - M_1 &= 0.3638 \\
M_2 - I &= 0.3696
\end{align*}
\]

Slope = \( \frac{1}{2} \left[ (0 - M_1) + (M_2 - I) \right] = 0.367 \)

Linearity = \( \frac{1}{2} \left[ (0 - M_1) - (M_2 - I) \right] = -0.003 \)
batches are plotted on control charts by date. A moving average of 13 results was kept, which included six results before and after any particular date. This was done for height of the calibration line and for the slope, and showed the trend with time. An analysis of variance gave two estimates of control of accuracy. The first $\sigma_0$ was the square root of half the long-run within-pair-of-trials variance of differences between duplicates. Deviations of height of the line, which was measured by the 13 date moving average of $M$ were checked by finding whether $M$ lay within $\pm 2 \sigma_0 \left( \frac{1}{2S} + \frac{1}{2} Z \right)$ of the last available average, where $S$ was the number of pairs averaged. Departures from linearity, measured every 4th week, were suspected if value lay outside $\pm 2 \sigma_0$. The second estimate was $\sigma^*$, which was the square root of half the long-run mean squared difference. Mean of two duplicates in different trials was accurate to within $\pm 2 \sigma^*$. Both $\sigma_0$ and $\sigma^*$ were revised from time to time, when more results had been obtained. The results for unknown sera were calculated, using the trend values for height and slope of calibration line. Where either showed that the method was out of control, the results of that date were discarded. The formula used was

$$Y = 0.05 - \frac{X}{(Slope)} - \text{Height of line}$$
Table 56. Comparison of present method with some published methods for protein-bound iodine

<table>
<thead>
<tr>
<th>Authors</th>
<th>Method</th>
<th>S.D. or Coeff. of variation for a single estimation</th>
<th>Mean difference between duplicates (μg.I/100ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gottschalk &amp; Riggs (119)</td>
<td>Man, Smirnow, Gildea &amp; Peters (225)</td>
<td>± 0.46 μg./100ml.</td>
<td>-</td>
</tr>
<tr>
<td>Stanbury et al. (324)</td>
<td>Barker (27) Modified</td>
<td>± 0.39 μg./100ml.</td>
<td>0.42</td>
</tr>
<tr>
<td>Sobel &amp; Sapsin (320)</td>
<td>Chaney (63)</td>
<td>± 4% *</td>
<td>0.15</td>
</tr>
<tr>
<td>Caraway (56)</td>
<td>Barker et al. (27)</td>
<td></td>
<td>0.50</td>
</tr>
<tr>
<td>Skanse &amp; Hedenskog (317)</td>
<td>Barker et al. (27) Modified</td>
<td>± 0.22 μg./100ml.</td>
<td>-</td>
</tr>
<tr>
<td>Lamberg et al. (185)</td>
<td>Barker et al. (27)</td>
<td>± 0.79 μg./100ml.</td>
<td>-</td>
</tr>
<tr>
<td>Thompson et al. (341)</td>
<td>Barker et al. (27) Modified</td>
<td>± 5.7% †</td>
<td>-</td>
</tr>
<tr>
<td>Acland (3)</td>
<td>Barker et al. (27) Modified</td>
<td>± 0.17 μg./100ml.</td>
<td>-</td>
</tr>
<tr>
<td>Kilpatrick</td>
<td>Barker et al. (27) Modified</td>
<td>± 0.23 μg./100ml.</td>
<td>0.27</td>
</tr>
</tbody>
</table>

*Calculated from the 95% range for the mean of duplicate estimations as given by the authors (± 5.6% and ± 10.9%).

†Calculated from the coefficient of variation for the mean of triplicate estimations as given by the authors (± 3.3%).

**Estimations were always in duplicate, and s.e. for mean of 2 replicates was ± 0.16 μg./100 ml.
where \( Y = \mu g. \) of iodide in tube

\[ X = \text{Mean of log observations of optical density readings in two different batches.} \]

Dilution was corrected and result expressed as \( \mu g. I/100 \text{ ml.} \) by multiplying \( Y \) by \( \frac{700}{3} \). The values of \( \sigma \) and \( \sigma^* \) were 0.0079 and 0.0076, for period of 3 yr.

That the calibration control was run. On converting to terms of added iodide, mean of two duplicates in different trials was accurate to within \( \pm 2 \sigma^* \) i.e. 0.32 \( \mu g. \). A comparison of the present method, using calibration control, with some published methods of determining serum protein-bound iodine is given in Table 56.

b) Iodine content of thyroid glands. The whole or part of the thyroid gland was dried at 95\(^o\) for 48 hr. and then ground in a mortar. Fat was extracted by petroleum ether in a Soxhlet apparatus for 48 hr. 0.2 g. of powdered gland was mixed with 2 ml. saturated potassium nitrate and 4 ml. saturated sodium carbonate in a pure nickel crucible. After drying at 95\(^o\) the crucible was heated to 600\(^o\) in a muffle furnace. Residue was dissolved in 2 ml. saturated potassium nitrate and 2 ml. saturated sodium carbonate and then drying and ashing stages repeated. Final residue was dissolved in water and washed into a 500 ml. conical flask. Six drops of bromine water
were then added, followed by enough concentrated sulphuric acid to bring solution to pH 2.0 using thymol blue as indicator, and allowed to stand for 1 hr. Bromine was then boiled off and residual traces removed by adding 100 mgm. of salicylic acid. After cooling, about 2 g. of solid potassium iodide was added, and liberated iodine titrated using 0.005 N sodium thiosulphate with starch as indicator.
APPENDIX 2

Technique of autoradiography

1. Three adjacent pieces of the thyroid gland were treated as follows:
   a) $^{131}I$ content estimated in well-type scintillation counter, for assessing exposure time.
   b) Second piece was fixed in 10% formalin in 0.85% NaCl for histology to compare with the autoradiograph.
   c) Third piece was fixed in pure ethyl alcohol for autoradiography. There is no loss of radioactivity during 24 hr. which is not so with most other fixatives.

2. After fixing, the third piece was mounted and processed in the routine way for the preparation of paraffin sections. Sections were cut at 5μ thickness.

3. Sections were mounted on gelatinised slides and some were pre-stained either with haematoxylin or by Masson's method, and some left unstained.

4. Sections were layered with Kodak A.R.10 autoradiograph stripping film, in the darkroom using a single Kodak Safelight, Series 1 (deep red). This stripping film was cut to size on its supporting glass, then stripped from the glass onto distilled water at 16-18°, and left to swell for
3 min. Section was then placed beneath this floating emulsion, and the latter was floated onto the section. It was then dried in a stream of cool air.

5. After drying, sections were placed in folders with sheets of lead between them, sealed in a light-tight box and stored in a refrigerator at 5°.

6. After 3 - 7 days, one or two sections were developed in Kodak D19b developer for 10 min. at 16 - 17° and fixed in hypo for 3 min. This was repeated at intervals up to 3 wk., until exposure was adequate, when remaining sections were developed.

7. Slides were dried to minimise tendency of the film to lift.

8. Pre-stained sections were cleared in xylene for 10-20 min., mounted in D.P.X. and dried at 37°.

9. Unstained sections were either left or stained with haematoxylin, dried, cleared in xylene, mounted and dried.
APPENDIX 3

Preparation of \[^{35}S\] labelled thiohydantoins

Both were prepared from ammonium \[^{35}S\] thio-
cyanate, supplied by the Radiochemical Centre at
Amersham with a specific activity of 10 mc/mM. The
amino acids and thiocyanate were dried in a vacuum
(0.01 mm.) over phosphorus pentoxide. Acetic acid
and freshly distilled acetic anhydride (1 : 9 v/v)
formed the condensing solvent.

\[2\,[^{35}S]-thiohydantoins\] Glycine (0.05 g.),
ammonium thiocyanate (0.025 g.) and ammonium \[^{35}S\]
 thiocyanate (0.025 g.) were mixed together and added
to 0.5 ml. of condensing solvent. The mixture was
heated until a dark yellow solution was obtained, and
then heated in a boiling water bath for 30 min. Water
(40 ml.) was added, mixture refluxed for 2 hr., and
then concentrated to approximately 1 ml. and allowed
to stand at 5° for 12 hr. The crystals were collected
by suction filtration and dried at 25 mm. pressure
over phosphorus pentoxide. A yield of 22.5% of
\[2\,[^{35}S]\]-thiohydantoin was obtained, with a melting
point of 227°. Chromatography of a solution showed
that 98% of the radioactivity was present as \[2\,[^{35}S]\]-
thiohydantoin. The resulting specific activity was
about 3.0 mc/mM.
5-Carboxymethyl-[\(^{35}\)S]-2-thiohydantoin. Asparagine (0.008 g.), ammonium thiocyanate (0.025 g.) and ammonium \([^{35}\text{S}]\) thiocyanate (0.025 g.) were mixed and added to 1 ml. of condensing solvent. The solution formed on heating was placed in a boiling water bath for 30 min. Acetic acid (40 ml. of 1.0 N) was then added and mixture refluxed for 6 hr. Solution was concentrated to approximately 1 ml., cooled to 5\(^\circ\), and a crystal of 5-carboxymethyl-2-thiohydantoin added. After 12 hr. the crystals were collected by suction filtration and dried in a dessicator over phosphorus pentoxide at 25 mm. pressure. A yield of about 60\% of 5-carboxymethyl-[\(^{35}\)S]-2-thiohydantoin was obtained, melting point 223\(^\circ\), was obtained by crystallisation from ethanol and on chromatography 98\% of the radioactivity was present as this compound. The resulting specific activity was about 5.0 mc/mM.
APPENDIX 4

Example of calculation of total thyroid iodine

Results B.L. Female. Simple Goitre for over 10 yr. 200 µc. of 131I orally.

<table>
<thead>
<tr>
<th>Time after 131I (dy.)</th>
<th>Thyroid content of 131I (% dose)</th>
<th>Serum Protein-bound 131I (% dose/litre)</th>
<th>Serum Protein-bound 127I (µg./100 ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>40.0</td>
<td>0.209</td>
<td>5.61</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>42.3</td>
<td>0.337</td>
<td>6.24</td>
</tr>
<tr>
<td>5</td>
<td>39.5</td>
<td>0.441</td>
<td>6.16</td>
</tr>
<tr>
<td>6</td>
<td>49.4</td>
<td>0.517</td>
<td>6.24</td>
</tr>
<tr>
<td>7</td>
<td>41.0</td>
<td>0.507</td>
<td>6.39</td>
</tr>
<tr>
<td>TSH given</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>40.8</td>
<td>0.725</td>
<td>8.26</td>
</tr>
<tr>
<td>9</td>
<td>40.9</td>
<td>0.749</td>
<td>7.60</td>
</tr>
</tbody>
</table>

Calculation of total thyroid iodine

a) Mean serum Pb127I before TSH = 61.0 µg./litre

Mean serum Pb131I of two days before TSH =

\[ \frac{0.517 + 0.507}{2} \]

= 0.512% dose/litre
Specific activity before TSH = $\frac{0.512}{61.0}$

$= 0.00839\% \text{ dose/µg.}$

$= 8.39\% \text{ dose/mg.}$

b) Serum PBI$^{127}$I on day after TSH = 81.9 µg./litre

∴ Serum PBI$^{127}$I released after TSH = 81.9 - 61.0

$= 20.9 \text{ µg./litre}$

Serum PBI$^{131}$I on day after TSH = 0.725% dose/litre

∴ Serum PBI$^{131}$I released after TSH = 0.725 - 0.512

$= 0.213\% \text{ dose/litre}$

Specific activity of hormone released after TSH = $\frac{0.213}{20.9}$

$= 0.0102\% \text{ dose/µg.}$

$= 10.2\% \text{ dose/mg.}$

c) As specific activity of released hormone = specific activity of hormone within gland and thyroid content of $^{131}$I when TSH given = 41.0 % dose

∴ Thyroid $^{127}$I content = \frac{\text{Thyroid $^{131}$I content}}{\text{Specific activity of released hormone}}

$= \frac{41.0}{10.2} \text{ mg.}$

$= 4.02 \text{ mg.}$