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STUDIES ON THE TREATMENT OF THYROTOXICOSIS WITH RADIOACTIVE IODINE (^{131}I) AND ANTITHYROID DRUGS.

BY

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RELEVANT PRESENTATIONS AND PUBLICATIONS

A number of presentations to learned societies and publications related to the studies in this thesis have been made and are listed below.

Presentations to Learned Societies

Connell, J.M.C., Alexander, W.D., McCruden, D.C. & Hilditch, T.E. Transient hypothyroidism following radioiodine therapy: A reversible defect of organification?
Scottish Society for Experimental Medicine, May 1982.

McCruden, D.C., Low, L., Connell, J.M.C. & Alexander, W.D. What is the correct dose of Methimazole? A dose response curve in Man.
European Thyroid Asociation, September 1982.

Ferguson, M.M., Alexander, W.D., Connell, J.M.C. & Younger, A. Accumulation of antithyroid drugs by human white blood cells.
Thyroid Club, 1982.

Connell, J.M.C., McCruden, D.C., Hilditch, T.E. & Alexander, W.D. Carbimazole pretreatment does not affect the kinetics of therapeutic radioiodine (^{131}I).
Scottish Society for Experimental Medicine, May 1985.

Connell, J.M.C., Hilditch, T.E., McCruden, D.C. & Alexander, W.D. The radioprotective action of carbimazole is not a consequence of altered thyroidal iodide kinetics.
Thyroid Club, 1986.

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Connell, J.M.C., McCruden, D.C., Ferguson, M.M. & Alexander, W.D. Accumulation of thiourylene antithyroid drugs in mouse salivary gland. Journal of Endocrinology, (1983), **96**, 91-96.

Connell, J.M.C., Ferguson, M.M., Chang, D.S.C. & Alexander, W.D. Influcence of sodium perchlorate on Thiourylene antithyroid drug accumulation in mice. Journal of Endocrinology, (1983), **98**, 183-187.

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Ferguson, M.M., Alexander, W.D., Connell, J.M.C., Lappin, A.G., McCruden, D.C., McLure, R., Mairs, R.J. & Younger, A. Peroxidase activity in relation to iodide, 17β-oestradiol, and Thiourylene drug uptake in human polymorphoneutrophils. Biochemical Pharmacology, (1983), **33**, 757-762.

Connell, J.M.C. & Alexander, W.D. Selection of therapy in thyrotoxicosis. The Physician, (1983), **4**, 214-217.

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McCruden, D.C., Connell, J.M.C., Hilditch, T.E. & Alexander, W.D. ^{123}I-iodine uptake and discharge kinetics in studies of binding inhibition by antithyroid drugs. Acta Endocrinologica, (1986), (In Press).

Connell, J.M.C., Hilditch, T.E. & Alexander, W.D. Effect of chronic Carbimazole treatment on thyroidal kinetics of radioiodine. Submitted for Publication.

Connell, J.M.C., McCruden, D.C., Hilditch, T.E. & Alexander, W.D. Effects of Radioiodine (^{131}I) treatment on thyroid iodide handling in patients with thyrotoxicosis. Submitted for Publication.

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The design and performance of the studies described in the thesis, and the subsequent analysis of data were primarily my own responsibility. However, the assistance of all the above named individuals was a major factor in completion of this work.

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John M.C. Connell March 1986.

SUMMARY

Radioactive iodine (^{131}I) is widely used in the treatment of thyrotoxicosis: an estimated 500,000 patients have been given this form of therapy since its widespread availability in the late 1940s. Despite this experience there is still uncertainty about the response of patients to ^{131}I, particularly in the first year after treatment; the lag between ^{131}I administration and biochemical response can cause problems in patient management. There are also surprisingly few data on the effects of ^{131}I on thyroid physiological processes: much of what is known was published before the availability of accurate measurement of thyroid hormone levels and thyroid stimulating hormone. This thesis attempts to address these points. The effects of antithyroid drug treatment on response to ^{131}I are also examined, and the possible reasons for interactions between antithyroid drugs and ^{131}I treatment explored.

In the first part of the experimental section of the thesis the early effects of radioiodine (^{131}I) treatment in patients with thyrotoxicosis were examined. In 50% of patients serum concentrations of thyroxine (T4) and tri-iodothyronine (T3) rose 48 hours after ^{131}I administration, although in none was this associated with clinical deterioration. A similar finding was noted in patients who had been given carbimazole treatment for a minimum of three months before ^{131}I administration. There were no major changes in serum thyroglobulin or TRAb levels following ^{131}I treatment, and no relationship between changes in these variables and thyroid hormone concentrations was observed.

The effect of ^{131}I therapy on early (20 minute) uptake of ^{123}I following intravenous injection of radioisotope was examined serially in 55 patients with thyrotoxicosis: 24 of these had been treated before ^{131}I with carbimazole. In all subjects 20 minute uptake of ^{123}I, which was taken to represent iodide trapping by the thyroid, fell by four weeks after ^{131}I administration. In those patients who subsequently developed permanent hypothyroidism within the next few months uptake values remained low. In contrast, a small rise in uptake measurements following the initial fall occurred in those patients who were biochemically euthyroid one year after ^{131}I administration. Patients who were still thyrotoxic one year after ^{131}I administration had a higher 20 minute uptake of ^{123}I before ^{131}I

administration ($p < 0.05$), and showed only a small fall in uptake after ^{131}I treatment. Thus, by four weeks after treatment 20 minute uptake in those patients who were euthyroid one year after treatment was higher than in those who became hypothyroid, and lower than in those who remained thyrotoxic (both $p < 0.05$). All patients whose uptake of ^{123}I was 4% or less four weeks after ^{131}I administration subsequently became either euthyroid or hypothyroid, while all whose uptake was greater than 8% at this time failed to enter remission. The maximum change in uptake of ^{123}I occurred within four weeks of ^{131}I therapy, and it is suggested that early iodide uptake measurements may be used to predict outcome after ^{131}I administration.

Six patients had an episode of transient hypothyroidism following ^{131}I therapy. In two of these patients, in whom measurements were made, this was shown to be associated with reversible defects of iodide organification (measured by perchlorate discharge). In three others ^{123}I uptake measurements 20 and 60 minutes after intravenous administration of radioisotope were consistent with a defect of iodide organification. It appears, therefore, that permanent hypothyroidism is a consequence of major impairment of iodide trapping; in contrast, if trapping is not irreversibly damaged, and there is a defect of iodide organification present, the tendency for such defects to recover means that the associated hypothyroidism may be transient. If early iodide uptake after ^{131}I treatment is $>2\%$ in a patient with hypothyroidism, an iodide organification defect may be partially responsible for the hypothyroidism.

Iodide organification was formally studied in 24 patients given ^{131}I treatment using an intravenous perchlorate discharge test. Nine had evidence of an organification defect within three months after treatment. In all patients there was a tendency for these defects to improve with time, and there was no evidence that iodide organification impairment had a major influence on thyroid biochemical function.

A major theme of the thesis is the interaction between carbimazole and ^{131}I treatment. Of 79 patients studied, 36 were made euthyroid with carbimazole before ^{131}I administration. Carbimazole pretreatment (drug stopped at least 72 hours before ^{131}I) caused a reduction in the incidence of hypothyroidism during the first twelve months after ^{131}I treatment (19% v 42%, $p < 0.05$). As carbimazole treatment can cause intrathyroidal iodide depletion, the possibility that this apparent "radio-protective" effect of

carbimazole was a consequence of a reduced thyroidal effective half life of ^{131}I was studied. Carbimazole pretreatment (for a minimum of three months) was, however, shown not to affect the biological half life, the effective half life of ^{131}I, or the estimated radiation dose to the thyroid. In a study of the effect of methimazole treatment on thyroid histology and autoradiographic distribution of ^{125}I in rat thyroid, stopping the drug 48 hours before sacrifice was shown to result in near normalisation of thyroid histological appearance and iodide distribution. Thus, the effects of carbimazole on early outcome after ^{131}I treatment are unlikely to be due to alteration of thyroid distribution or retention of ^{131}I. It is suggested that scavenging of radiation-produced free radicals by either drug or metabolite may account for the radioprotective effect following ^{131}I treatment.

In the final part of the thesis the phenomenon of antithyroid drug accumulation by thyroid tissue was examined, with particular reference to potential interactions between antithyroid drugs and iodide. In mouse thyroid, antithyroid drug (methimazole and propylthiouracil labelled with ^{35}S) accumulation was partly inhibited by acute blockade of the anion trap by perchlorate. These drugs were also shown to be concentrated within mouse salivary gland (submandibular): tissue localisation was similar to that of ^{125}I-iodide, being in the intralobular duct and convoluted granular tubule. Using histochemical techniques, peroxidase activity, and glucose-6-phosphate dehydrogenase, which is necessary for hydrogen peroxide generation, were localised to the same areas. Perchlorate again caused a slight fall in the amount of drug taken up by tissue. Finally, ^{35}S-propylthiouracil was also shown to be concentrated by phagocytosing human polymorphonuclear cells, which contain myeloperoxidase. Drug uptake was not affected by perchlorate or ouabain, both of which inhibit specific membrane bound anion trapping, but was inhibited by iodide, and unlabelled methimazole and propylthiouracil at concentrations which also inhibited myeloperoxidase activity (measured by chemiluminescence). These studies suggest that antithyroid drug accumulation by tissue is not dependent on a specific anion trapping mechanism, but on the presence of tissue peroxidase. Interaction between peroxidase and drug results in inhibition of iodide organification and leads to drug oxidation: it is suggested that drug oxidation is key to its intracellular accumulation, with transfer of drug across the cell membrane being a passive process regulated by the concentration of free, non-metabolised drug within the cell.

Decreased thyroid iodide content limits drug oxidation, and it is possible that this secondarily reduces accumulation within tissue, accounting for the effects of perchlorate in mouse thyroid and salivary tissue described.

The action of antithyroid drugs to inhibit iodide organification, the accumulation of antithyroid drugs within target tissue, and the radioprotective effect of these agents following ^{131}I may all be consequences of the chemical oxidative potential of the drugs which allows interaction with hydrogen peroxide/peroxidase systems and tissue free radicals.

CHAPTER ONE - REVIEW

Section One

1.1. Normal Thyroid Function

1.1.1. Historical background

Although the description of endemic cretinism is attributed to Paracelsus in 1603, it was not until the mid 19th Century that the relationship between thyroid dysfunction and ill health was fully appreciated (Iason 1946). The first clinical descriptions of thyrotoxicosis were made by Parry in 1825 and Graves some 10 years later (Graves 1835; Parry 1825): neither, however, realised that thyroid enlargement was of pathogenic significance. The primary role of the thyroid gland in the genesis of the syndrome now recognised as thyrotoxicosis was not established until the 1880s, when the earliest subtotal thyroidectomies for the condition were performed (Werner 1978a). Since that time there have been major advances in the understanding of normal thyroid physiology, and in the pathophysiology of thyroid dysfunction. Although of only passing interest to this thesis, the demonstration of the high iodine content of thyroglobulin by Oswald (1899), the description of the chemical structure of thyroxine by Harrington (1926) and the discovery of tri-iodothyronine by Gross and Pitt-Rivers (1954) have been milestones in the development of the current state of knowledge. Similarly, the definition of the thyroid-pituitary feedback loop in 1949 (Hoskins 1949) and the isolation of immunoglobulin-derived, long-acting thyroid stimulator in patients with thyrotoxicosis by Adams and Purves (1956) have been major conceptual steps in the understanding of normal and abnormal thyroid function. However, despite the accumulation of this large body of knowledge, there remain areas of uncertainty and empiricism still prevalent in the management of thyrotoxicosis.

1.1.2. Normal thyroid anatomy and physiology

The normal thyroid gland is a bilobed structure lying anterior to the trachea inferior to the thyroid cartilage. The two lobes are joined by a central isthmus which is anterior to the second and third cartilaginous rings. Microscopically the gland is seen to be composed of follicles which are approximately spherical structures with an outer shell of a single layer of

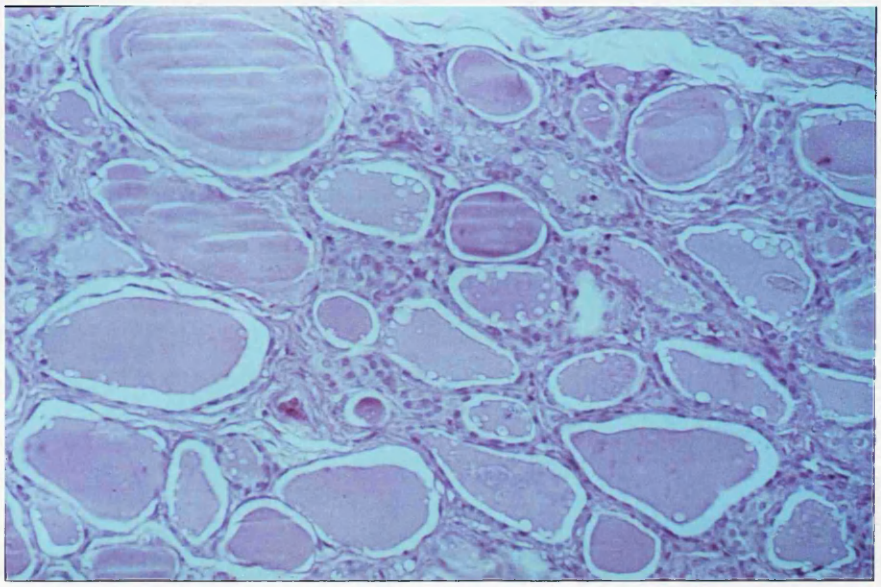


Fig 1

HISTOLOGICAL SECTION (HAEMATOXYLIN AND EOSIN, x300) OF NORMAL HUMAN THYROID.

HOMOGENOUS COLLOID ENCLOSED BY FOLLICULAR CELLS COMPRISE THE BASIC UNIT, THE FOLLICLE. NOTE VARIATION IN FOLLICULAR SIZE. IODIDE IS TRAPPED FROM THE BASAL (OUTER) BORDER OF THE FOLLICULAR CELL, AND TRANSPORTED TO THE INNER BORDER (CELL/COLLOID INTERFACE) WHICH IS THE SITE OF THYROID HORMONE SYNTHESIS.

epithelial cells enclosing a homogeneous colloid composed of thyroglobulin, an iodinated glycoprotein. The follicle is enclosed by a basement membrane; thyroid gland stroma - connective tissue with a rich capillary and lymphatic network - lies between follicles. A much smaller number of secretory cells are enclosed by the basement membrane, but do not communicate with the lumen of the follicle and are known as parafollicular (C) cells (Wolfe, Voelkel and Tashjian, 1974). These secrete calcitonin. A representative histological section of normal human thyroid is shown in Figure 1.

On electron microscopy the follicular cells are noted to be polarised, with the cell nucleus at the basal part of the cell; the apex of the cell is orientated towards the lumen of the follicle. Projecting from the apical portion of the cell into the lumen of the follicle are microvilli, and the surface area of the cell in contact with colloid material is therefore greatly increased. The thyroid follicular cell is responsible for the manufacture of the colloid glycoprotein, thyroglobulin, on which is produced thyroid hormones (thyroxine and tri-iodothyronine). Briefly, iodide of dietary origin, circulating in the blood stream, is concentrated within the follicular cell by means of a highly efficient iodide trap. The 30-fold concentration which results is necessary in view of the scarcity of iodine in the environment (Stanley and Astwood, 1949). Iodide trapping, which occurs at the basal membrane of the follicular cell, is not specific for iodide: related molecules of the halide group such as bromide, complex anions such as perchlorate and pertechnetate and pseudo-halides such as thiocyanate are also concentrated within follicular cells by this mechanism (Wolff, 1963). Iodide transport across the follicular cell membrane is an active, energy dependent process linked to membrane bound sodium/potassium ATPase (Wolff and Halmi, 1963).

Following transport within the cell, iodide is incorporated into organic form in a series of chemical reactions. It is likely that the initial step involves the oxidation of iodide to iodine ($2 \times I^- \rightarrow I^2 + 2e$): this is catalysed by the haemprotein enzyme, thyroid peroxidase (Taurog, 1970), which is localised on the apical margin of the cell at the cell membrane/colloid interface (Tice and Wollman, 1974). There remains some controversy about the exact nature of the physical state of the oxidised species of iodine. Molecular iodine (I_2), enzyme-bound iodinium (I^+) or enzyme-bound iodine free radical ($I^\bullet$) are all theoretically capable of reacting with the amino acid tyrosine to form mono- and diiodotyrosines (Taurog, 1978). Tyrosine residues

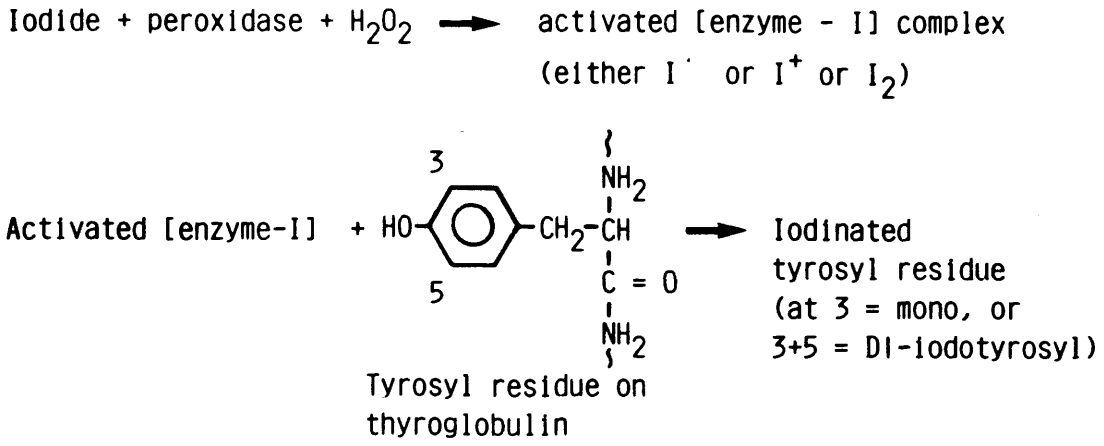
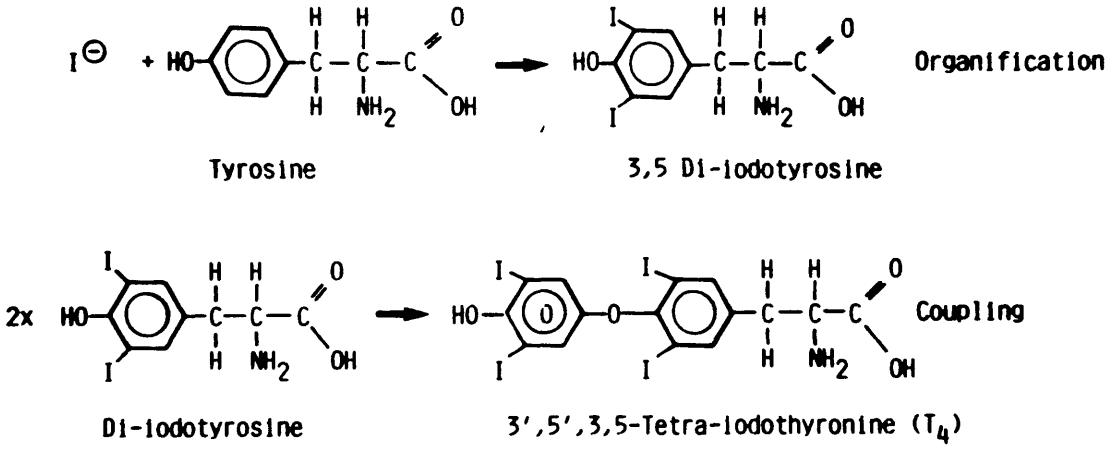


Fig 2

(a) + (b) SCHEMATIC REPRESENTATION OF IODOTHYRONINE FORMATION

TYROSINE RESIDUES ON THE THYROGLOBULIN MOLECULE ARE IODINATED AT EITHER 3', OR 3' AND 5' POSITIONS TO GIVE MONO OR DI-IODOTYROSYL RESIDUES. THIS IS CATALYSED BY THYROID PEROXIDASE /H_2O_2 (2a, ABOVE).(THE PRECISE IODINATING SPECIES IS UNCERTAIN). COUPLING OF IODOTYROSYL RESIDUES ON THE THYROGLOBULIN MOLECULE GIVES THYROID HORMONES (2b BELOW).



are part of the polypeptide structure of thyroglobulin, and the iodination of tyrosine also occurs at the cell/lumen interface of the follicle (Ekholm and Wollman, 1975). Steps leading to the production of iodotyrosines are shown schematically in Figure 2.

Subsequent coupling of iodotyrosine residues in a thyroid-peroxidase catalysed reaction leads to production of thyroid hormone bound to thyroglobulin: the native structure of thyroglobulin is necessary for this reaction to proceed most efficiently (Lamas, Taurog, Salvatore, et al., 1975). Coupling of one molecule of monoiodotyrosine with one of diiodotyrosine produces tri-iodothyronine; or two molecules of diiodotyrosine will give tetraiodothyronine (thyroxine) (Figure 2) (Harington and Barger, 1927). Thyroid hormones remain stored within the colloid as peptide-linked iodinated amino acids on the thyroglobulin molecule. Release of free-thyroid hormone into the circulation therefore requires initial proteolysis of thyroglobulin. Small droplets of colloid are engulfed by the apical portion of the follicular cell in a process of endocytosis; these droplets then fuse with lysosomes which contain proteases (Seljelid, Reith and Nakken, 1970). The liberated thyroid hormones are released into the circulation via the basal portion of the follicular cell: iodinated amino acids liberated from thyroglobulin at the same time are dehalogenated, and the iodide derived from this process is recirculated within the pathway summarised above (Stanbury, 1974).

1.1.3. Control of thyroid gland secretion

The observation that the pituitary glands of some cretins were enlarged (Reichlin, 1978) led eventually to the cybernetic theory of thyroid/pituitary feedback control expressed by Hoskins (1949). The importance of pituitary regulation in maintenance of normal thyroid function is demonstrated by the development of thyroid gland atrophy and clinical hypothyroidism in hypophysectomised subjects (Burger and Patel, 1977). Thyroid stimulating hormone (TSH) produced by the thyrotroph cells of the anterior pituitary is a 30,000 dalton glycoprotein consisting of 2 sub-units: an alpha sub-unit which is common to other pituitary hormones, such as luteinizing hormone and FSH, and a beta sub-unit which is responsible for biological expression of TSH activity (Pierce, 1971). TSH binds to a specific receptor on the basal portion of the follicular cell, and exerts a number of effects on follicular cell function. Many of these effects are secondary to

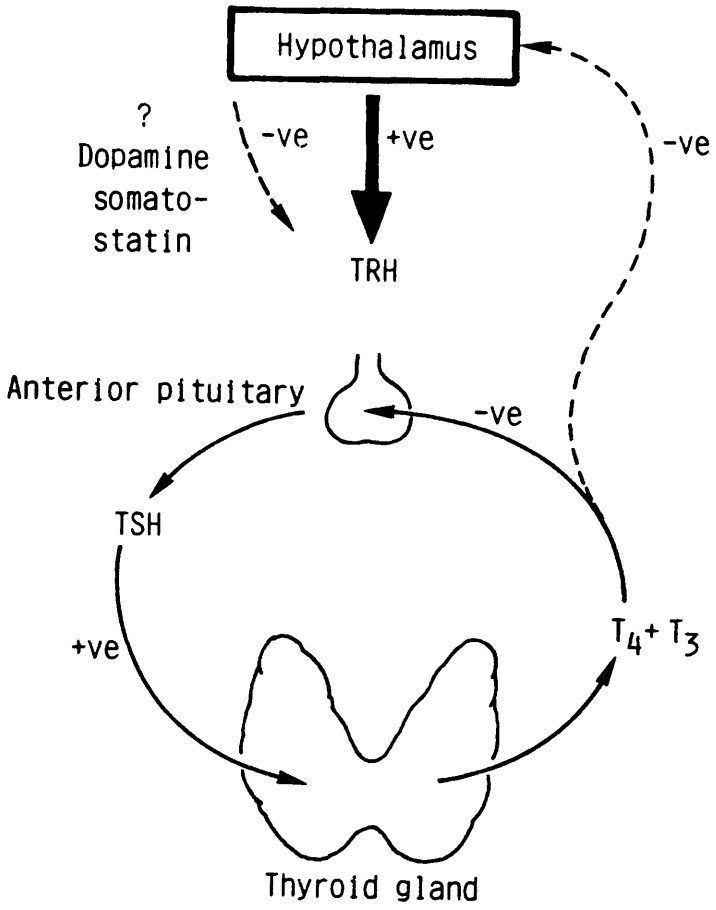


Fig 3

HYPOTHALAMIC/PITUITARY/THYROID AXIS

THYROTROPHIN-RELEASING HORMONE, PRODUCED IN THE HYPOTHALAMUS IS TRANSPORTED VIA THE PORTAL CIRCULATION TO THE ANTERIOR PITUITARY, TO TONICALLY REGULATE THYROTROPHIN (TSH) RELEASE. TSH STIMULATES IODIDE UPTAKE, ORGANIFICATION AND THYROID HORMONE RELEASE BY THE THYROID GLAND. THYROID HORMONE INHIBITS SYNTHESIS AND RELEASE OF TSH BY THE PITUITARY, AND MAY ALSO INFLUENCE HYPOTHALAMIC TRH SECRETION. OTHER HYPOTHALAMIC FACTORS SUCH AS DOPAMINE AND SOMATOSTATIN MAY ALSO MODULATE TSH SECRETION.

stimulation of adenylate cyclase and affect production of cyclic AMP (Dumont, 1971); more recently the importance of phospholipid turnover, generation of calcium as a second messenger and of the calcium dependent enzyme, calmodulin in intracellular transduction of the TSH signal has been demonstrated (Brown, Walker and Tomlinson, 1985; Ollis, MacNeil, Walker, et al., 1983).

The earliest effect of TSH is to increase colloid droplet formation at the apical brush border, leading to release of preformed thyroid hormone into the circulation (Tonoue, Tong and Stolc, 1970). TSH leads to an initial rise in the efflux of iodide from the follicular cell, followed, after a lag period of a few hours, by a sustained increase in iodide trapping (Halmi, 1961). As this is the rate-limiting step in thyroid hormonogenesis, the result is accelerated thyroid hormone production (Ingbar, 1978). However, the increased rate of hormone production appears also to arise from increased organification and coupling of iodotyrosines, which is seen even when iodide trapping is blocked by perchlorate, indicating a TSH-mediated increase in thyroid peroxidase activity (Tong, 1966). Finally, TSH has marked effects on follicular cell carbohydrate, lipid, protein and nucleic acid metabolism, which result in thyroid cell division and thyroid gland growth (Begg and Munro, 1965).

1.1.4. Hypothalamic/pituitary/thyroid axis

The thyroid and pituitary participate in a classical negative feedback loop, so that the secretory products of the thyroid gland (thyroxine and tri-iodothyronine) inhibit pituitary TSH synthesis and release (Cotton, Gorman and Maybury, 1971) (Figure 3). Conversely, when T4 and T3 fall, TSH secretion is enhanced. In turn, maintenance of normal TSH secretion depends on thyrotrophin releasing hormone (TRH) synthesis and its release from hypothalamic peptidergic neurones into the hypophyseal portal circulation (Burgus, Dunn, Desiderio, et al., 1970). Thus, if the pituitary stalk is sectioned, pituitary TSH secretion falls and secondary hypothyroidism ensues. Other hypothalamic substances which may potentially influence TSH synthesis and release are (inhibitory) somatostatin and dopamine, and (stimulatory) alpha adrenoreceptor agonists (Scanlon, Rees-Smith and Hall, 1978). It is likely that there is thyroid hormone feedback influencing hypothalamic activity in man, although the importance of this is controversial (Feek,

Sawers, Brown, et al. 1980).

1.1.5. Circulating thyroid hormones

The principal secretory product of the thyroid gland is thyroxine (T4). The mean daily T4 secretion rate is about 90 ug, which represents about 10% of the extrathyroidal T4 pool (Chopra, 1978). T4 circulates in plasma largely (> 99%) bound to carrier proteins: the high affinity, low capacity thyroxine binding globulin, and the low affinity high capacity thyroxine binding prealbumin (Nicoloff, 1978). These carrier proteins act as buffers, damping the effect of sudden alteration in thyroid gland secretion on free thyroid hormone concentrations: it has also been suggested that carrier proteins may serve to regulate the rate of uptake of free thyroid hormone by organs such as the placenta (Ekins, Sinha, Woods, et al., 1983; Pharoah, Connolly, Ekins, et al., 1984). Carrier protein bound thyroxine is in equilibrium with free thyroid hormone in plasma.

Until the 1950s it was thought that all the effects of thyroid hormone were mediated through T4. With the discovery of tri-iodothyronine (T3) by Gross and Pitt-Rivers (1954) it became apparent that the action of thyroid hormone could not be soley attributed to T4. It now seems likely that all the actions of thyroid hormone at a cellular level are mediated through T3 (Brown, Chopra and Cornell, 1974). Although some circulating T3 is derived from thyroidal secretion, the principle source of T3 is from peripheral deiodination of T4: this occurs mainly in liver and kidney (Braverman, Ingbar and Sterling, 1970). In all cells, some receptor bound T3 is derived from intracellular conversion of T4 to T3: the proportion of T3 derived in this way varies from tissue to tissue. Anterior pituitary gland tissue has a high obligatory intracellular conversion rate of T4 to T3 (Siwa, Dick and Larsen, 1978). A fall in circulating T4 concentrations will, therefore, lead to a shortage of intracellular T3 in pituitary tissue, while other tissues which do not depend upon intracellular T4 deiodination to the same extent will not be affected. It is in this way that the pituitary cell may sense the need for increased thyroid hormone production and so increase TSH secretion at a time when other tissues have an adequate supply of thyroid hormone at nuclear receptor level.

Coupling of T3 with its specific nuclear receptors has a genomic effect leading to an increase in mRNA synthesis, which controls synthesis of

new protein (Oppenheimer, Koerner and Schwartz, 1972). It is possible, but by no means certain, that all the cellular actions of T3 are mediated in this way. These actions include the stimulation of energy-dependent transport of amino acids and electrolytes across plasma membranes, enzyme synthesis and increased cell mitotic activity (Ismail-Beigi and Edelman, 1971; Sokoloff and Kaufmann, 1961.

Section Two
1.2. Clinical Aspects of Thyrotoxicosis

1.2.1. Introduction

Thyrotoxicosis is the clinical state produced by increased thyroidal release of hormone, leading to sustained elevation of plasma thyroid hormone levels (occasionally ingestion of thyroid hormone can mimic features of thyrotoxicosis: thyrotoxicosis facitata). Common symptoms of thyrotoxicosis include fatigue, anxiety, palpitations, weight loss, heat intolerance and excess sweating (Werner, 1978b). Crooks and Wayne evaluated symptoms and signs of the disease in a group of patients with thyrotoxicosis in an attempt to improve diagnostic discrimination from patients with non-thyroid illness (Crooks, Wayne and Robb, 1960). In this study, heat intolerance and weight loss were shown to be good discriminators, while clinical examination findings of value included hyperkinesis, tachycardia, tremor and warm extremities.

Some degree of thyroid gland enlargement is usual: in young patients the gland tends to be diffusely enlarged and vascular, often with an overlying bruit. In older subjects, or in subjects with a long history of goitre, the gland may be firm and even nodular. In up to 16% of patients the gland may be impalpable especially, in older patients (Greenwood, Daly and Himsworth, 1985).

Thyrotoxicosis is often associated with abnormality in the appearance of the eyes. Ocular changes can very widely: in all subjects with thyroid hormone excess upper eyelid retraction may occur, imparting a "staring" appearance to the eyes. In immunologically-mediated thyroid disease the specific ophthalmopathy, first described by Graves, may develop. This may include periorbital swelling, proptosis (unilateral or bilateral), and conjunctival oedema. Infiltration and later fibrosis of the intra-orbital muscles can lead to diplopia. In some instances there may be a rise in intra-ocular pressure and in severe cases sight may be threatened (Werner, 1977).

1.2.2. Epidemiology of thyrotoxicosis

The true incidence and prevalence of thyrotoxicosis is difficult to assess. In a community survey in the North of England, Tunbridge and colleagues reported a prevalence of thyrotoxicosis of 1.1 - 1.6%, with a male

to female ratio of 1/10 Tunbridge, Evered and Hall, et al., 1977). This would lead to an expected annual incidence of 2-3 cases per 1000 females per year. These figures accord with an earlier survey of G.P. practices reported by Logan and Cushion (1958). It is likely that the prevalence of thyrotoxicosis is influenced by prevailing dietary iodine intake: in some countries where programmes to increase dietary iodine intake have been conducted, an increased incidence of thyrotoxicosis has been documented (Connolly, Vidor and Stewart, 1970; Ek, Johansson and Von Porat, 1963). The high proportion of such cases in a study in Tasmania were shown to have measurable long acting thyroid stimulator (LATS) concentrations, suggesting that the previous iodine deficiency was responsible for maintenance of euthyroidism in the presence of abnormal thyroid stimulation (Vidor et al, 1973). An effect of iodine itself on production thyroid immunological stimulators is, however, difficult to exclude.

Thyrotoxicosis is found in all races: true differences in prevalence among racial or geographical groups are difficult to define in view of the effect of iodine availability discussed above. The incidence of the disorder in Japan, where iodine intake is high, has been estimated as 8 cases per 1000 females per year (Hoffenberg, 1973).

The disease is uncommon in children and adolescents. Although thyrotoxicosis has traditionally been seen as a disease of middle life, a study in Denmark has suggested that the disorder may be commoner than previously thought in the elderly (Ronnov-Jessen and Kirkegaard, 1973). One feature of this study, which showed a hospital bias of elderly (> 65 years) to younger patients of 6:1 was the atypical presentation in older patients.

The majority of patients with thyrotoxicosis have immunologically mediated thyroid disease (Graves' disease): the immunological abnormalities will be discussed later. A small number of patients have a solitary hyperfunctioning nodule responsible for excess hormone secretion. Lastly, in some patients with thyrotoxicosis a multinodular goitre appears responsible for the disorder. It has been suggested that in such patients a long standing non-toxic goitre escapes from the usual feedback control mechanism, and that hormone secretion becomes autonomous (Studer, 1982). In this situation iodine availability may assume greater importance in setting the level of thyroid hormone secretion, and intermittent or chronic thyrotoxicosis may supervene. However, the recent discovery of antibodies which promote

thyroid growth has suggested that immunological factors may be of aetiological significance in the development of non-toxic goitre (Valente, Vitti, Rotella, et al., 1983), and it is possible that the pathogenesis of toxic multinodular goitre more closely resembles that of Graves' disease than has been previously thought.

In the United States it has been suggested that as many as 30% of patients with thyrotoxicosis have a subacute "silent" thyroiditis, characterised by self-limiting thyroid hormone excess associated with low thyroid iodide uptake measurements (Klein and Levey, 1982). However, interpretation of the significance of low iodine uptake measurements is difficult without knowledge of prevailing dietary iodine intake, and estimate of absolute thyroid iodine uptake. Histological examination of thyroid tissue from such patients shows lymphocytic infiltration, and the relationship of this disorder with classical Graves' disease is unclear. The incidence of this disorder in the United Kingdom is not known.

1.2.3. Genetic factors in thyrotoxicosis

It has been recognised for some time that autoimmune thyroid disease occurs more frequently in first and second degree relatives of affected subjects than in the general population (Roitt and Doniach, 1958; Doniach, 1975). The exact mode of inheritance remains unclear: it has been suggested that an autosomal recessive mode with reduced penetrance might be present, although alternatives have been suggested (Fraser, 1963; Martin, 1945). More recently, it has become clear that Graves' disease is inherited in genetic linkage with certain cell-surface histocompatibility antigens: in Caucasian subjects HLA B-8 and DRw3 are increased in frequency (Svejgaard, Platz and Ryder, 1984), while in a Japanese population Bw35 has been shown to be increased in frequency (Kawa, Nakamura and Nakazawa, et al., 1977). These markers may be of value in predicting the likelihood of development of a remission of thyrotoxicosis during antithyroid drug treatment (McGregor, Rees-Smith, Hall, et al., 1980).

1.2.4. Pathological features

In classical Graves' disease the thyroid gland is diffusely enlarged and more vascular than usual. Microscopically there is hyperplasia of the follicles with relative loss of colloid; the follicular cells become elongated

and there is often evidence of increased lymphoid tissue within the gland. On electron microscopy there is evidence of increased metabolic and synthetic activity with hypertrophy of golgi apparatus and an increased number of mitochondriae (Meissner, 1978).

In toxic nodular goitre the microscopic appearance is essentially that of non-toxic multinodular goitre. Heterogeneity of follicle size, with some large follicles distended with colloid but others showing marked involution, is evident. Areas of fibrosis is seen between groups of follicles, giving rise to the nodular appearance and texture of the gland. The spectrum of follicular size reflects the non-homogeneous nature of metabolic and synthetic activity within the gland (Studer, Peter and Gerber, 1985).

1.2.5. Immunological abnormalities in Graves' disease

Indirect evidence that abnormal function of the immune system is involved in the pathogenesis of Graves' disease includes the histological appearance of lymphocytic infiltration of the gland, the enlargement of the thymus which is known to be present in some patients with Graves' disease, and the association between Graves' disease and other organ-specific autoimmune disorders such as Addisonian pernicious anaemia and autoimmune adrenalitis (Irvine, 1967). More direct evidence that the pathogenic abnormality in the condition was an antibody directed against, and stimulatory to the TSH receptor came in the late 1950s with the description of long-acting thyroid stimulator (LATS) isolated from the immunoglobulin fraction of serum of patients with thyrotoxicosis (long-acting is in comparison with the duration of the action of TSH) (Adams and Purves, 1956). LATS could be shown to be present in some, but not all, patients with Graves' disease (Hardisty, Hanford, Humphries, et al., 1981; Ollis, Tomlinson and Munro, 1985). Arnaud and colleagues showed that LATS was capable of stimulating normal thyroid tissue (Arnaud, Kneubuhler, Seiling, et al., 1965); further, a correlation could be demonstrated between LATS activity and radioiodine turnover in the thyroids of thyrotoxic subjects, and these data strongly supported the suggestion that LATS was a direct cause of thyroid hormone excess (Carneiro, Dorrington and Munro, 1966).

Adams and Kennedy (1967) subsequently described another gamma globulin-derived substance which could block LATS binding to the TSH receptor but which was devoid of stimulatory activity in the mouse thyroid

(LATS protector, LATS-P). In untreated thyrotoxic patients LATS-P levels are generally higher than those of LATS; either LATS or LATS-P have been shown to be present in sera of up to 86% of thyrotoxic subjects (Hardisty et al., 1981).

Since the initial discovery of such thyroid stimulating antibodies, a number of other techniques for detection and quantification of antibody directed against the TSH receptor have been developed. Evidence of thyroid stimulation can be obtained using in vitro measurement of cyclic AMP or T3 generation from standardised thyroid tissue (Atkinson and Kendall-Taylor, 1981; Bidey, Marshall and Ekins, 1981), detection of the ability of antibodies to bind to the TSH receptor can be performed by measuring inhibition of binding of labelled TSH to purified thyroid membranes (Rees-Smith, Pyle, Peterson, et al., 1977). It is not always clear, however, whether these varied assay systems are detecting the same antibodies: it is probable that there is a spectrum of thyroid directed antibodies in Graves' disease. Recently the presence of antibodies which bind to the TSH receptor, but which do not stimulate cyclic AMP generation have been described, and these clearly differ from those which stimulate thyroid function (Endo, Kasagi and Konishi, 1978). It is possible that these TSH receptor binding, non-stimulating antibodies are involved in the pathogenesis of some immunologically mediated hypothyroidism: it has recently been suggested that these may account for some cases of neonatal hypothyroidism in mothers with Graves' disease (Matsuura, Yamada, Nohara, et al., 1980). A further recent discovery has been the description of antibodies which stimulate thyroid growth but not hormonogenesis (Valente et al., 1983). This may account for the long recognised discrepancy between thyroid size and clinical and biochemical severity of thyrotoxicosis: it is possible that goitre development in thyrotoxicosis is at least partly dependent on thyroid growth stimulating immunoglobulin. It remains to be explained how antibody directed against the same receptor can stimulate separately functions subserved by the one trophic hormone (TSH).

As was initially demonstrated to be the case with LATS, not all patients with Graves' disease can be shown to be positive for one single type of antibody activity. Thus, only about 75% of patients with the disorder can be shown to have antibody which blocks the binding of TSH (TRAb) to its receptor (Rees-Smith et al., 1977). However, in a study where a battery of

different antibody detection assays were performed in a group of patients with Graves' disease, 100% of patients were shown to have one or other type of antibody present, although no correlation was seen between the various assays (Hardisty, Kendall-Taylor, Atkinson, et al., 1983).

Other autoantibodies directed against thyroid follicular cell components, such as antimicrosomal or antithyroglobulin antibodies are present in varying titre in subjects with Graves' disease (Mori and Kriss, 1971). These are unlikely to be of direct pathological significance.

The cause of production of antibodies directed against the TSH receptor in Graves' disease remains unclear. Antibody production by the B-lymphocyte is partly controlled by T-lymphocytes which may either promote (helper cell) or inhibit (suppressor cell) antibody production. It has been suggested that production of autoantibody is due to development of a normally suppressed "forbidden" clone of B-lymphocytes, as a consequence of a defect in suppressor T-cell function (Strakosch, Wenzel, Row, et al., 1982; Thielmans, Van Haelst, DeWaele, et al., 1981). Abnormalities of suppressor/helper T-cell ratio have been demonstrated in thyroid-derived lymphocytes in Graves' disease (Ludgate, McGregor, Weetman, et al., 1984). Thus, while the exact mechanism is unclear, it seems likely that excess thyroid hormone production is due to abnormal stimulation of the TSH receptor by autoantibodies which may be produced as a consequence of abnormal T-lymphocyte modulation of B-lymphocyte function.

The role of non-humoral immune effector mechanisms in the pathogenesis of Graves' disease is uncertain. Plasma cells, complement and immune complexes have been demonstrated by immunofluoresence studies in thyroid glands from patients with thyrotoxicosis (Feldman, Becker and Montsopoulos, 1976) as was the presence of cell dependent cytotoxic antibody (Werner, Wegelius, Fierer, et al., 1972; Tomasi, 1978). Such abnormalities would be more in keeping with an inflammatory response (e.g. chronic thyroiditis). However, if Graves' disease and Hashimoto's thyroiditis are, as seems likely from clinical and epidemiological studies, opposite ends of the spectrum of autoimmune thyroid disease, it would be expected that, in immunopathological studies, a continuum of humoral and cellular immunological abnormalities affecting thyroid tissue would be present. Thus, in an individual with clinical thyrotoxicosis, immunopathological features of chronic thyroiditis may also be present, just as evidence of abnormal humoral

immunological activity may be seen in some patients with primary hypothyroidism.

1.2.6. Natural history of Graves' disease

Since the advent of effective treatment for thyrotoxicosis, it has been difficult to study the natural course of untreated disease. In the era before thyroid surgery it was estimated that mortality of the condition was between 8 and 12% (Sattler, 1952). In the remainder, the course of Graves' disease varied from a single short-lived self-limiting episode, through repeated episodes of spontaneouosly remitting relapses to chronic unremitting disease. The percentage of patients expected to enter spontaneously long term remission was around 25% (Sattler, 1952).

In more recent times, information about the natural history of the condition may be obtained from studies of patients treated with beta adrenoreceptor blocking drugs. Propranolol has been shown not to effect titres of TSH receptor directed antibodies, and so is unlikely to have any affect on the underlying course of the disease (McGregor, Peterson, McLachlan, et al., 1980); remission rates in patients treated only with propranolol are, therefore, likely to reflect spontaneous remission rates. As, however, elderly subjects and subjects with more severe disease tend not to be included in such studies, such information may over-estimate the true remission rate. In 28 subjects given propranolol, McLarty reported a remission rate over a 16 month period of just under 18% (McLarty, Brownlie, Alexander, et al., 1973), in an earlier study examining the fall in uptake of tracer radioiodine, response was seen in 22% of patients (Pimstone, Joffe, Pimstone, et al., 1969). This is in general agreement with other studies, and suggests that the true spontaneous remission rate over a 1-2 year period is less than 25%. This is likely to be affected by, amongst other things, availability of dietary iodide.

SECTION THREE
1.3. RADIOACTIVE IODINE IN THE TREATMENT OF THYROTOXICOSIS

1.3.1. Introduction

External irradiation of the thyroid was used as a treatment for thyrotoxicosis with some success (around 50% response rate) in the 1920s. Internal irradiation of the thyroid was, however, only made possible by the production of radioactive isotopes of iodine. The first man made radioisotope of iodine (^{128}I) was produced by neutron bombardment by Fermi (1934); it was demonstrated that this isotope (^{128}I) was accumulated and distributed in the same manner as stable (^{127}I) iodide in thyroid tissue. These fundamental observations paved the way for the use of radioactive iodine in the treatment of thyrotoxicosis, first reported by Hertz and Roberts (1942) using the short-lived isotope ^{130}I (decay half-life of 12 hours). Intensive investigation into this novel therapeutic approach followed, and Chapman and Evans (1946) described the results of the use of ^{131}I in 22 thyrotoxic patients. In that same year, ^{131}I was released by the Atomic Energy Commission in the United States for national distribution, and the first large scale trials of the use of that isotope began.

In the United Kingdom, the use of ^{131}I in the treatment of thyrotoxicosis was first reported by Blomfield and co-workers in Sheffield in 1951: 20 patients had been treated since 1949, and in this initial report all cases responded to therapy, although some required more than one treatment. No patient was reported to have become hypothyroid (Blomfield, Jones, McGregor, et al., 1951).

Since these early days a large number (probably in excess of 500 000) patients have been given ^{131}I for thyrotoxicosis. Some of the relevant experience reported will be discussed below.

1.3.2. Radiobiological aspects

Like stable iodide, all radioisotopes of iodine are well absorbed from the gut (Halnan, 1964). The efficiency of the thyroid iodide trap, particularly in thyrotoxicosis, ensures that a large proportion of iodide administered orally is accumulated within the gland and incorporated in thyroid hormone, linked to molecules of thyroglobulin. As thyroid hormone in

this form is stored and located principally within the colloid of the gland, it follows that radioactive iodine is also localised there. The therapeutic efficacy of radioactive iodine therapy depends on this high concentration of isotope within the gland so that radiation damage to the thyroid follicle cell can occur without a significant effect on neighbouring or distal structures.

^{131}I has been the radioisotope used principally in radioiodine therapy. The radioisotope decays with a physical half-life of 8.05 days. The principal decay products are beta particles (electrons) released when nuclear neutrons convert into protons, and gamma rays, which are given off as transitions occur between the excited and ground state of the nuclei. In this process of decay, ^{131}I is converted to stable ^{131}xenon (Barnes, Rhodes and Wagner, 1978). The beta particles emitted by the radioisotope during decay are responsible for about 95% of the tissue radiation absorbed by the gland and hence for the therapeutic effect. These beta particles, of mean energy 0.19 Mev, are absorbed within a relatively short distance (maximum range in tissue = 2100 μM). This, however, is considerably greater than the length of the follicular cell (10 μM) and, indeed, of follicular diameter (150-200 μM) (Greig, McDougall and Halnan, 1972). Thus, ^{131}I deposited in the colloid of one follicle can irradiate not only the cells of that follicle but also cells of surrounding follicles. Although, therefore, the distribution of ^{131}I in hyperplastic thyroid tissue may be patchy (Kreutzer, Miller, Soley, et al. 1958), the emission characteristics of ^{131}I probably ensure that a significant dose of radiation is delivered to all follicles.

The exact mechanisms of radiation induced cell damage responsible for the therapeutic action of ^{131}I are not fully understood. It is possible that the energy absorbed by the tissue from the beta emission results in the generation of free-oxygen (superoxide) radicals: these free radicals may cause chemical damage to the cell, leading to impairment of biological function (Phil and Edjarn, 1958). This damage is not confined to the aspects of cell function involved in thyroid hormonogenesis: after treatment with ^{131}I cell lifespan is reduced (in high dose radiation induced-cell death will, of course, result) (Freeburg, Kurland and Blumgart, 1953). There is often impairment of reproductive ability, so that during mitosis the cell dies (Al-Hindawi and Wilson, 1965). Thus, following a sublethal dose of ^{131}I, although cell function may persist for some time, the effects of therapy on the capacity of cells to replicate ensure eventual cell death (Walinder, Jonsson and Sjoden,

1972).

It has been established from experimental studies involving irradiation of rat thyroid tissue, and follow-up of patients treated with ^{131}I, that the cell mechanisms involved in replication are more sensitive to radiation damage when compared with the relative radioresistance of cell metabolic function (Philp, 1966). Following ^{131}I therapy, therefore, the gland may be capable of continued thyroid hormone synthesis. When cell division is stimulated, however, the cell is unable to undergo normal mitosis and dies. In the normal thyroid gland most cells are in interphase (i.e. not dividing), although in a gland stimulated by either TSH or immunological stimulators the rate of mitosis is increased (Greig et al., 1969). Thus, gradual loss of thyroid cell number may occur late after ^{131}I therapy is given, leading to eventual thyroid failure. In a similar manner, the goitrogenic properties of antithyroid drugs after ^{131}I therapy in experimental animals are limited because of mitotic cell death (Doniach, 1953). Thus the immediate effect of ^{131}I treatment on gland function will depend upon a number of cellular physiological processes. Assuming that immediate cell death does not occur, thyroid hormone secretion following ^{131}I administration will be a function of the effects of treatment on thyroid trapping, iodide oxidation and incorporation into thyroglobulin, and on thyroid hormone release. In the long term, the ability to maintain hormone secretion will also depend upon the ability to maintain thyroid cell number through cell division. The implication of these concepts in clinical aspects of thyroid function will be discussed later.

Gamma emission from decay of ^{131}I contributes little to tissue damage. However, this emission allows external gamma counting over the thyroid to be performed, giving accurate measurement of radioisotope uptake by the gland.

1.3.3. Histological changes

The effects of ^{131}I therapy on thyroid gland histology have been reported in a number of studies. Within two months of administration of the isotope, cell necrosis, breakdown of follicular structure, interstitial oedema and interstitial fibrosis are seen (Andrews, Kniseley, Bigelow, et al., 1954; Dobyns, Vickery, Maloof, et al., 1953). In animal studies evidence of chromosomal damage has been noted (Moore and Colvin, 1966). There is

evidence that some of the above effects are dose related. Although in the rat significant damage is caused by small doses of ^{131}I (240 RAD) (Speight, Baba and Wilson, 1968), a conventional therapy dose of ^{131}I in man will deliver around 5000 RADS.

Despite these abnormal histological findings, thyroid tissue in such studies has been shown to retain the ability to trap iodide (Andrews et al., 1954): this is further evidence of the relative resistance of the metabolic functions of the follicular cell to the effects of radiation.

1.3.4.Choice of isotope in radioiodine therapy

Ease of production, suitable spectra of beta and gamma emission, and early clinical experience led to the widespread use of ^{131}I in the treatment of thyrotoxicosis in the 1950s and 1960s. It became apparent, however, that there was a high incidence of iatrogenic hypothyroidism in such patients, and this appeared to be an inevitable consequence of the emission characteristics of this radioisotope, leading to radiation damage of the thyroid follicular cell nucleus with consequent cell sterilization. In vitro studies of fibroblasts in culture showed that cytoplasmic irradiation had no effect on cell division, while irradiation of the nucleus resulted in cell sterilization (Munro, 1970). The mean distance of travel of electrons emitted by decay of ^{131}I is many times longer than the length of the thyroid follicular cell, and nuclear irradiation is therefore inevitable. These concepts led to attempts to evaluate an alternative isotope of iodine (^{125}I) in the treatment of thyrotoxicosis. ^{125}I decays by emission of low energy X-rays (27-35 Kev) and low energy electrons (3-34.5 Kev) (Anon, 1985a); the larger proportion of radiation dose to the thyroid is derived from the latter. These low energy electrons have a maximum range in tissue of 25 μM and since the follicular cell nucleus is 10 μM from the apex cell/colloid interface, it was argued that it would largely escape radiation damage from ^{125}I. Thus the effects of radiation on cell division would be reduced, with consequent reduction in later hypothyroidism (Ertl, Feinendegen and Heiniger, 1970; Greig, McDougal and Halnan, 1972). In theory, the only part of the follicular cell to be substantially irradiated would be the area adjacent to the cell/colloid interface, which is a site of iodide organification.

Initial animal studies supported this hypothesis. In the rat, reports suggested that ^{125}I did not significantly affect nuclear DNA synthesis or the

basally sited iodide transport mechanism (Berdjis, Byers and Bice, 1972; Vickery and Williams, 1971).

McDougall and co-workers were the first to report results of ^{125}I therapy in patients with thyrotoxicosis, in a study demonstrating that the isotope was effective in treating the condition (McDougall, Greig and Gillespie, 1971). In a subsequent report of the effects of ^{125}I treatment in 297 subjects, followed for a minimum of 6 months, the same group found that optimal results were obtained with an estimated dose of 300 μCi/g of thyroid tissue (Bremner, McDougall and Greig, 1973): comparing three doses of ^{125}I, these authors found that subjects given the lowest dose had a reduced incidence of hypothyroidism in comparison with the other two groups: 64% of the subjects in the low dose group were euthyroid. Other initial studies were encouraging: in a report from New York, three of six patients had an episode of reversible hypothyroidism in the early months after therapy (Werner and Johnson, 1971). However, in longer term follow-up of patients given ^{125}I, it became apparent that the incidence of hypothyroidism increased steadily with time, as had already been demonstrated with ^{131}I (Weidger, Johnson and Werner, 1974). The incidence of hypothyroidism appeared to be dose related, and a high percentage of patients required more than one treatment of ^{125}I. In a follow-up study, 33% of patients given ^{125}I became hypothyroid (McDougall and Greig, 1976). It therefore appeared that ^{125}I treatment offered no major advantage when compared with ^{131}I, despite the promising early animal and human studies (Chapman, 1971; McDougall and Greig, 1976). As ^{131}I had been in use for than 20 year without evidence of other harmful effect, it remained the isotope of choice.

1.3.5. Early response to radioiodine therapy

As might be expected, the response of the thyrotoxic patient to ^{131}I treatment is not absolute: rather, there is a spectrum of outcome after treatment both in terms of time and magnitude of response. This leads to an element of uncertainty with regard to early response to ^{131}I. Considerable effort has been expended in analysis of factors which influence the outcome after treatment: it is intended to consider these below. In this section the response examined is that occurring early (within the first 12 months) after therapy. The problem of late response (i.e. that of cumulative permanent hypothyroidism) will be discussed later.

The early changes in thyroid physiology after ^{131}I administration are not well documented: this is largely a reflection of the dependence in vivo on tracer isotope studies which are technically difficult to perform in subjects given therapeutic doses of ^{131}I. Evidence of a mild radiation thyroiditis is seen in a number of patients who complain of discomfort in the neck several days after treatment (Einhorn, Einhorn, Fagraeus, et al., 1967). Associated with this may be a rise in circulating thyroid hormone concentrations, probably due to leakage of preformed thyroid hormone across damaged cell membrane (Lamberg, Hernberg, Wahlberg, et al., 1958). In some patients this has been reported to be associated with deterioration in clinical condition (Lamberg, 1959). Thus, in 1959, Lamberg reported that thyroid crisis was seen in 5 of 144 patients shortly after ^{131}I was given: in view of the date of the report there was no corroborating biochemical data to confirm the probable rise in thyroid hormone concentrations in these patients. The same group reviewed their experience of a further 387 patients treated with 131I and reported that "impending thyroid crisis" occurred in four (Viherkoski, Lamberg, Hernberg, et al., 1970). Insufficient data are available, however, to ascertain whether these four subjects manifest different biochemical changes from the 383 in whom no clinical deterioration was seen. Shafer and Nuttal (1975) examined T3 and T4 concentrations after ^{131}I administration. They demonstrated that in most subjects a small rise in T3 and T4 occurred, but that this was not related to dose. The rise in T3 appeared to be greater, possibly reflecting the smaller extracellular pool of T3. In this study the authors suggested that clinical deterioration associated with the rise in circulating thyroid hormone levels occurred around 5 days after treatment. In contrast, Creutzig and colleagues, in a study of 46 patients given ^{131}I treatment, reported only a small and inconsistent rise in T3 within the first week after treatment (Creutzig, Kallfelz, Haindl, et al., 1976). More recently, Tamagnia reported that in a group of 14 patients given Propranolol along with ^{131}I, no major change in T4 or T3 was seen (Tamagna, Levine and Hershmann, 1979). In three subjects a parallel rise in both hormones did occur 10-12 days after treatment, but was not associated with any clinical deterioration. No relationship was seen between hormone change after treatment and pretreatment hormone levels or gland size.

Despite this rather conflicting evidence, the occurrence of thyroid

storm or clinical deterioration after ^{131}I treatment is widely acknowledged. Some of the reports of its occurrence are, however, rather anecdotal (Anon, 1972): in a number of cases intercurrent clinical problems seem equally likely to be responsible for the deterioration as ^{131}I treatment. However, Parker and Lawson (1973) in a retrospective study reported the occurrence of 33 deaths in patients with thyrotoxicosis, 20% of whom had had ^{131}I treatment in the preceding three weeks. Although there was insufficient clinical or biochemical data in individual cases to come to any conclusions, it is possible that, in some of these subjects, deterioration in clinical state resulted from hormone release after ^{131}I administration. Patients with major intercurrent illness may be especially at risk.

Support for the concept of disruption of thyroid cell integrity leading to release of hormone comes from evidence of a rise in circulating thyroglobulin concentrations shortly after ^{131}I treatment (Gardner, Rothman and Utiger, 1979): it is possible that this parallels a rise in T3 and T4 levels, although there are no data on this point.

Apart from this rather non-specific "injury" pattern which may occur after ^{131}I therapy, subsequent thyroid hormone production will be determined by the effects of ^{131}I on the individual components of hormonogenesis: iodide trapping and iodide organification. If cell death occurs, both trapping and organification fail, and hypothyroidism ensues rapdily.

Studies of the effect of ^{131}I on iodide transport and organification in vivo rely on tracer radioisotope studies, either radioiodide or radioactive materials which are trapped by the thyroid in a similar manner, such as ^{99m}Tc-Pertechnetate. In two early studies Myant (1953 a + b) examined 2-hour uptake of ^{131}I in a small group of patients given therapeutic ^{131}I. He found that three weeks after treatment uptake values were, in the majority of subjects, lower than subsequent values suggesting a temporary inhibition of iodide uptake. However, there are considerable technical difficulties in measuring ^{131}I uptake in a gland with a high background count of the same radioisotope, and the accuracy of these data may be open to question. It is of interest that Myant noted the persistance of high uptake values in some patients clinically euthyroid after ^{131}I. He concluded that "cells have the ability to trap iodide, but that hormone secretion is abnormal". This would be consistent with a defect of iodide organification and preservation of trapping function by the gland.

In a major study of tracer radioisotope behaviour in relation to the treatment of thyrotoxicosis Larsson (1955) included 3- and 24-hour uptake studies of ^{131}I and also examined urinary ^{131}I excretion. He showed that in patients who attained clinical euthyroidism the "ability of the gland to accumulate ^{131}I was often depressed to a subnormal level during the first few months after treatment, followed by a recovery during the following months". He also reported a lag between change in radioisotope uptake and clinical and biochemical responses, and suggested that radioisotope studies might have some predictive value. Like Myant (1953a), he also showed that uptake measurements were elevated in some patients who were clinically euthyroid after treatment. As the uptake measurements in this study were all "late" (i.e. greater than 3 hours after administration of isotope), no definite conclusions can be drawn about the effect of ^{131}I treatment on the discrete functions of iodide trapping and organification.

Einhorn and Hastad (1961) examined early (11 minute) uptakes of ^{132}I in 41 patients, three and eight weeks after ^{131}I therapy. They showed that in the majority of patients early iodide uptake had fallen by three weeks after treatment: in subjects who entered remission this fall was greater than in subjects who failed to respond. This study, in which a very early uptake was measured, suggests that the initial fall in uptake seen after ^{131}I treatment is a consequence of radiation inhibition of iodide trapping. As this step is rate-limiting in thyroid hormonogenesis (Ingbar, 1978), this will have a major influence on subsequent biochemical function.

In 1973, Sagel and co-workers reported, in a retrospective study, that a 6-hour uptake which failed to fall after ^{131}I treatment was a predictor of relapse of thyrotoxicosis (Sagel, Epstein and Jackson, 1973). Sequential uptake data were not included in this study.

The finding that changes in thyroid iodide handling precede altered hormone secretion has been confirmed by other workers: Franco showed that while uptake values had fallen within two weeks of ^{131}I treatment, changes in T3 and T4 occurred several weeks later (Franco, Coppler and Kovaleski, 1970). Using ^{99m}Tc, Gray (1975) showed that a 15 min uptake fell in most, but not all, patients given ^{131}I and who subsequently became hypothyroid or euthyroid within two months of treatment. In some patients who failed to enter remission a small fall in uptake was also seen, but this was not a consistent finding. As this isotope is not organified in human thyroid, such

changes in uptake should reflect ^{131}I related effects on iodide trapping. It is of interest that in patients given ^{125}I therapy, ^{99m}Tc uptake fell within two days after treatment.

Gray (1975) also studied the effect of ^{131}I and ^{125}I treatment on iodide organification using an intravenous perchlorate discharge test. He demonstrated that in the first 3 months 33% of patients had a defect of iodide organification after ^{131}I treatment, but, at later periods after therapy, such defects were uncommon. In contrast, organification defects were seen in 64% of patients given ^{125}I therapy, and this high incidence persisted over the next few months. This would tend to support the hypothesis, discussed above, that ^{125}I irradiates principally the portion of the cell responsible for iodide organification, in contrast to ^{131}I which delivers a more uniform cell radiation dose.

There have been no other specific studies of the effect of ^{131}I treatment on iodide organification. However, a number of authors have reported that patients who became euthyroid were abnormally sensitive to iodide induced myxoedema, and that the doses of iodide necessary to cause this were unusually small. This may be indirect evidence of a partial organification block in such subjects, although no direct measurements of iodide organification were reported (Braverman, Woebert and Ingbar, 1969; Braverman, Ingbar, Vagenakis, et al., 1971).

In summary, therefore, there are surprisingly few data on the early effects of ^{131}I on thyroid physiological processes: many of the available findings were published before the widespread availablity of accurate measurement of T3, T4 and TSH. Most studies of thyroid iodide handling in this situation have not been designed to separate iodide trapping from iodide organification. There is general agreement that uptake of tracer radioisotope falls to a variable extent after the administration of ^{131}I. In a proportion of patients uptake values may subsequently return towards pretreatment levels. The studies of Gray (1975) and the early uptake studies of Einhorn and Hasted (1961) suggest that most of the changes seen in uptake are a consequence of an effect on iodide trapping, and that iodide organification is relatively unimpaired. These concepts are examined further in the experimental section of this thesis.

Data on other physiological variables, such as intermediary metabolism or control of thyroid hormone release are not available. From the foregoing

discussion it is clear that clinical and biochemical responses to ^{131}I treatment are dependent upon the effect of therapy on thyroid iodide handling.

1.3.6. Outcome and clinical response following ^{131}I therapy

The spectrum of biochemical and clinical response to ^{131}I reflects the variability of the effect of therapy on thyroid physiological processes: division of patients into discrete categories of thyroid status (e.g. hypothyroid, euthyroid, thyrotoxic) is based on biochemical assessment using rather arbitrary ranges of circulating thyroid hormone concentrations. The classification is, however, clinically convenient and allows comparison of results of therapy to be made from one series to another. Before the development of accurate radioimmunoassay techniques for measurement of thyroid hormone concentrations, biochemical definition of thyroid status was less reliable. This is especially so of patients with mild hypothyroidism, and it is likely that in early series the incidence of this was underestimated.

In general terms, thyrotoxic patients given ^{131}I treatment may remain thyrotoxic or become euthyroid within a variable period of time. Subsequently, euthyroid patients may have a relapse of thyrotoxicosis, remain euthyroid or become hypothyroid. In a few instances subjects who have become hypothyroid recover normal thyroid hormone secretion (transient hypothyroidism). As discussed previously, the development of hypothyroidism after a long period of stable euthyroidism (late hypothyroidism) is thought to be a consequence of gradual thyroid failure resulting from radiation induced cell sterilization and death. This will be discussed later.

During the first two weeks after ^{131}I treatment there is mild radiation thyroiditis which may be accompanied by a rise in circulating thyroid hormone concentrations (1.3.5.). In some patients this may be accompanied by a variable degree of local tenderness and pain on swallowing. Early estimates suggest that these symptoms are limited to less than 10% of patients treated although specific enquiry may reveal a higher incidence (Becker and Hurley, 1971).

Following the administration of ^{131}I there is a lag period before biochemical response becomes evident (Larsson, 1955), accounted for by the stores of preformed thyroid hormone contained within the colloid of the gland which have a biological half life of 14 days. The elimination half-life

of thyroxine in plasma is approximately seven days and even if ^{131}I therapy causes a cessation of all new thyroid hormone synthesis, there will be an inevitable delay of several weeks before the biochemical and clinical manifestations of hypothyroidism are evident (Nicoloff, Low, Dussault, et al., 1972).

The extent of the biochemical response to ^{131}I will depend upon the residual capacity of the gland to secrete thyroid hormone. The aim of treatment has usually been to render the patient euthyroid, and hypothyroidism has been seen as a disadvantage. Because of this, studies were conducted to see if low doses of ^{131}I could reduce the incidence of hypothyroidism (Glennon, Gordon and Sawin, 1972; Hagen, Oullette and Chapman, 1967; Hardisty, Smith, Bethell, et al., 1981). In most the incidence of early hypothyroidism was reported to be dose related: it appears, however, that this occurred at the expense of failure of a greater proportion of patients than normal to respond adequately to treatment so that multiple doses of ^{131}I become necessary. In some series in which lower than usual doses of ^{131}I have been given, response rates are difficult to evaluate, as ^{131}I has been given in combination with long-term potassium iodide or antithyroid treatment (Cevallos, Hagen and Maloof, 1974; Rapoport, Caplan and De Groot, 1973; Sridama, McCormick, Kaplan, et al., 1984). Summarised data from a representative selection of series, illustrating the effect of dose on response rate and incidence of early hypothyroidism, are shown in Table 1.

Of the patients who fail to respond within a few months to the initial dose of ^{131}I, the majority become euthyroid following a second dose: a small number of patients require three doses or more. Most patients respond to ^{131}I treatment within six months, and, in most series, there is therefore little difference in response rate at six months compared to that at 12 months (Dworkin, 1974). Similarly, the majority of cases of early hypothyroidism are detected within the first six months of treatment (Nofal, Beierwaltes and Patno, 1966).

In summary, the response to ^{131}I occurs early after treatment: the dose of ^{131}I partly determines this response. Hypothyroidism as a result of therapy can affect between 8 and 90% of patients, in the first year, depending on the dose of ^{131}I given. There is some evidence that the incidence of early hypothyroidism is increasing as a result of a tendency

TABLE 1

COMPARISON OF INCIDENCE OF EARLY HYPOTHYROIDISM* WITH DIFFERENT DOSES OF ^{131}I

AUTHORS	DOSE OF ^{131}I (MEAN, mCi)	INCIDENCE (%) OF HYPOTHYROIDISM AT 1 YEAR	COMMENTS
SMITH + WILSON (1967)	2.8	4.3	HIGHER RATE OF PERSISTENT THYROTOXICOSIS
GLENNON et al (1972)	3	3.7	
RAPOPORT, CAPLAN et al (1973)	3.4	7	
CEVALLOS, HAGEN et al (1974)	3.6	9	RATE OF DEVELOPMENT OF HYPOTHYROIDISM AFTER 1st YEAR SIMILAR TO HIGH DOSE GROUP
SMITH + WILSON (1967)	5	9	YEARLY RATE OF ONSET OF HYPOTHYROIDISM AFTER 1 YEAR NOT DOSE-RELATED
CUNNIEN, HAY et al (1982)	7.3	40	
CEVALLOS et al (1974)	7.4	39	
GREIG (1973)	7.5	10	
VON HOFE, DOREMANN et al (1978)	10	69	
SAFA et al (1974)	12	90	
CUNNIEN et al (1982)	13.8	96	COMPARE WITH EXPERIENCE USING LOWER DOSE TREATMENT ABOVE

* IN 1st YEAR AFTER TREATMENT

towards administration of higher doses of ^{131}I (Alevizaki, Alevizaki and Ikkos, 1985). In the experience of the Mayo Clinic over the last 30 years, the incidence of early hypothyroidism in the most recent years is greater than 90% (Cunnien, Hay, Gorman, et al., 1982).

1.3.7. Transient hypothyroidism

Occurrence of hypothyroidism, which is reversible within the first few months after treatment, has been reported in several studies of patients given ^{131}I (Chapman, Maloof, Maisterrena, et al., 1954). The incidence of this varies from series to series. Segal et al. reported data on more than 1600 patients, and found that 7% had an episode of transient hypothyroidism after treatment (Segal, Silver, Yokalem, et al., 1961). McGirr found that 11% of patients in a smaller series developed this complication (McGirr, Thomson and Murray, 1964); more recently, Sawers and colleagues detected the phenomenon in 5% of subjects given ^{131}I (Sawers et al., 1982). It is of interest, therefore, that some authors make no mention of the phenomenon. The mechanism of recovery of thyroid function has not been adequately explained, nor have methods of differentiating potentially transient hypothyroidism from permanent loss of thyroid function been described. Dorfmann has suggested that if hypothyroidism occurs in a subject shortly after ^{131}I therapy, in whom little change in goitre size has occurred, there is a possibility that recovery of thyroid function may occur (Dorfmann, Young and Caretta, 1977). The failure of some authors to detect transient hypothyroidism suggests that in some series the incidence of early, permanent hypothyroidism may be increased owing to the inclusion of subjects with potentially reversible thyroid failure. The phenomenon of transient hypothyroidism and the experience in the present work are examined more fully later.

1.3.8. Factors which influence the early response to ^{131}I therapy

(a) Goitre size In most series of patients given ^{131}I, calculation of the administered dose has included a correction factor for goitre size, resulting in patients with large goitres receiving greater doses of ^{131}I than those with smaller glands. In a review of 850 patients treated over 16 years, Nofal concluded that hypothyroidism occurred with increasing frequency in patients with small goitres (Nofal et al. 1966). This may either

reflect a real tendency for patients with small glands to be more susceptible to ^{131}I treatment, or may indicate that the accuracy of most estimates of goitre size is poor, with a tendency for clinicians to under-estimate the mass of large goitres. However, the finding of a higher incidence of hypothyroidism in patients with small goitres was also noted by Segal in a review of 1600 treated patients (Segal et al. 1961). In patients with an estimated gland mass of less than 30 g, 12% developed hypothyroidism whilst only 4.4% of those with an estimated gland mass of greater than 60 g did so.

(b) Nodularity of goitre Most patients with thyrotoxicosis have Graves' disease, and thus have relatively diffuse goitres. Thyroid nodularity results in relative resistance to the effects of ^{131}I treatment, so that the incidence of early post treatment hypothyroidism is reduced (Miller, 1971), and a higher percentage of patients require more than one dose of ^{131}I. This resistance to the effects of ^{131}I may be a consequence of the non-homogeneous distribution of ^{131}I which can be demonstrated using autoradiography (Kreutzer et al. 1950), so that the functional mass of thyroid tissue irradiated is less. The incidence of late onset hypothyroidism in patients with nodular goitres given ^{131}I may not, however, be reduced (Fontana, Curti, Biggi, et al., 1980).

(c) Other treatment of thyrotoxicosis In his major retrospective review of 1600 patients given ^{131}I, Segal reported that pretreatment of patients with antithyroid drugs was associated with a lower incidence of hypothyroidism (4.3% versus 9.4%) (Segal et al. 1961). The effect of pretreatment with the antithyroid drug methylthiouracil on response to ^{131}I treatment was prospectively studied by Crooks et al., who compared a group of patients given the drug until five days before ^{131}I with a similar group given no pretreatment, and showed that the single dose cure rate was reduced in the former group (Crooks, Buchanan, Wayne, et al., 1960). In a follow-up study, Buchanan showed that this effect was not seen in patients pretreated with potassium perchlorate (Buchanan, Keutras, Crooks, et al., 1965). In a study of the effect of pretreatment with carbimazole on response to ^{131}I therapy, Goolden and Fraser (1969) were unable to demonstrate any effect on outcome over one year. However, treatment with carbimazole both before and after ^{131}I therapy was reported to lessen the incidence of early hypothyroidism in comparison with ^{131}I alone (Aro,

Huttunen, Lamberg, et al., 1981; Bliddal, Hansen, Rogowski, et al., 1982; Reynolds and Kotchen, 1979). In these studies antithyroid drugs were stopped 48 hours before ^{131}I was given and restarted 48 hours after treatment. In a much smaller study Steinbach and co-workers reported that concurrent treatment with propylthiouracil or methimazole reduced the incidence of early hypothyroidism compared with ^{131}I alone, although the numbers involved in this study (11 patients in each group) make the significance of this report uncertain (Steinbach, Donoghue and Goldman, 1979).

These data suggest that the effect of ^{131}I is reduced in patients given thiourylene antithyroid drugs both before and after ^{131}I treatment, while the finding of Crooks and colleagues (1960) suggests that this also occurs in patients given antithyroid drugs only before ^{131}I. The mechanism of this effect is unclear. All of the drugs shown to have an effect are members of the thiourylene group, whereas perchlorate, which had no demonstrable effect in the study of Buchanan et al. (1965) is chemically and pharmacologically dissimilar. Thiourylene drugs are accumulated and undergo metabolic degradation within the thyroid gland. It is possible, therefore, that the effect of these drugs on response to ^{131}I is a consequence of a pharmacological interaction between drug and thyroid gland. Drugs of this class act by inhibiting iodide organification (Maloof, Smith and Soodak, 1969), and it may be that the reduction of response to ^{131}I treatment is a consequence of impairment of retention of radioisotope within the thyroid gland. The only study which has examined this point is that of Crooks et al. (1960) who reported that the biological half-life of therapeutic ^{131}I was not affected by pretreatment with methylthiouracil. The mechanism of the radio-protective effect of antithyroid drug treatment is examined further in this thesis.

Previous thyroid surgery or previous ^{131}I treatment increase the incidence of post treatment hypothyroidism (Segal et al. 1961), presumably by decreasing the functional mass of thyroid tissue to be irradiated.

(d) Individual sensitivity From the discussion above it will be evident that the early response to ^{131}I therapy is difficult to predict and is influenced by a number of factors. The contribution of individual sensitivity to irradiation in determining the response is difficult to determine; this is partly because of problems inherent in accurate measurement of the dose of irradiation deposited per unit of thyroid tissue following ^{131}I administration.

It seems likely, however, that there are interindividual factors which also influence the extent of tissue damage caused by ^{131}I.

1.3.9. Late hypothyroidism

The concept that hypothyroidism, occurring "late" after ^{131}I therapy is a consequence of cell sterilization, has been widely accepted (Philp, 1966). There is in vitro evidence that thyroid cells are unable to replicate after relatively small doses of ^{131}I, when cellular metabolic function is unimpaired (Al-Hindaawi and Wilson, 1965; Munro, 1970), and this effect is irreversible. It is therefore reasonable to suggest that a similar mechanism in vivo accounts for gradually developing, late-onset thyroid failure.

It has been suggested that immunological factors might play a part in the development of hypothyroidism. Green and Wilson (1964) demonstrated that the incidence of hypothyroidism after subtotal thyroidectomy was related to histological evidence of thyroiditis in excised tissue, and suggested that, in a similar manner, the occurrence of hypothyroidism after ^{131}I therapy might be related to immunological disturbance. Burke and Silverstein (1969) reported that thyroid autoantibodies were found, in higher titre, in patients becoming hypothyroid in the first year after ^{131}I therapy, but did not extend the study to examine hypothyroidism occurring later. In a retrospective study of patients followed for up to 12 years, Lundell and Holm (1980) demonstrated that hypothyroidism was significantly more common in patients who had high titres of antibody to thyroglobulin and cytoplasmic antigen before treatment. In patients who developed such antibodies after treatment hypothyroidism also occurred more commonly. However, closer examination of these data suggests that the increase in hypothyroidism was a phenomenon occurring in the first 12 months after treatment, and the annual incidence of hypothyroidism thereafter was similar in patients who were antibody positive or negative. From the above studies, therefore, there is no convincing evidence that late hypothyroidism is directly related to immunological factors.

In all series of patients followed for a number of years after ^{131}I treatment, the percentage of patients who become hypothyroid has been noted to increase with time. Greig (1973) found that 15 years after treatment, nearly 80% of patients were hypothyroid, while Nofal et al. (1966) in the United States reported a 70% incidence at 10 years after treatment.

In comparison, a surgically treated group had a 10 year incidence of hypothyroidism of 43%. This late incidence of hypothyroidism is rather higher than reported in early series, and this is likely to be a consequence of the longer period of follow-up in later studies. The annual rate of development of hypothyroidism after the first year is reported in most series to be between 2 and 4% per year (Dworkin, 1971; Greig 1966; Toft, Irvine, Seth, et al., 1975). This appears to be unrelated to the dose of ^{131}I administered: Glennon et al. (1972), in a review of patients given between 3 and 4 mCi of ^{131}I reported an annual incidence of hypothyroidism of 3-4%, similar to the figure quoted in the study by Smith and Wilson (1967) in the United Kingdom in which a range of doses of ^{131}I were administered. Thus, although dose of ^{131}I influences the early development of hypothyroidism, late onset thyroid failure appears to be an inexorable, dose independent phenomenon.

Patients with multinodular goitre, or autonomously functioning single nodules have been thought to be relatively resistant to the effects of ^{131}I Miller, 1971). However, in a carefully analysed series using life table method of analysis, the cumulative incidence of hypothyroidism 20 years after treatment was found to be 44% (Fontana et al. 1980). In other studies of patients with either multiple or single nodules given ^{131}I, the occurrence of hypothyroidism was similar to that seen in patients with diffuse goitres (Hamburger, Kadian and Rossin, 1967), and may reflect the low dose of radiation necessary to cause cell sterilization (Walinder et al. 1972).

As might be expected, the development of hypothyroidism late after treatment is a gradual process with insidious clinical onset. Toft has characterised the biochemical features of late onset hypothyroidism in a series of papers (Toft, Irvine, Hunter, et al., 1974; Toft et al., 1975; Toft, Irvine, Seth, et al., 1978). He demonstrated that only those patients who were euthyroid (normal T4 and T3) but with an elevated concentration of TSH develop hypothyroidism over a relatively short period of follow-up. In any 12 months period, after the first year, between 2 and 6% of such patients will become hypothyroid, which is in general agreement with other annual rates discussed above. Patients with normal basal TSH concentrations did not become hypothyroid over this time, although a few developed high basal TSH levels. This predictability of development of thyroid failure has implications for the organisation of long-term follow-up of patients given

^{131}I therapy (discussed below).

1.3.10. Subclinical hypothyroidism

This term is used to describe the biochemical syndrome of a high basal concentration of TSH associated with circulating T3 and T4 levels within the normal reference range. While there is no dispute that patients with biochemical hypothyroidism require thyroid hormone replacement therapy, the true thyroid status of patients who are subclinically hypothyroid is a matter of some debate (Evered, Ormston, Smith, et al., 1973; Evered, Young, Tunbridge, et al., 1975; Toft, Kellet, Sawers, et al., 1982). The problem only became recognised with the development of accurate and widely available assays for TSH. It is clear from the studies of Toft, however, that a proportion of patients will develop subclinical hypothyroidism following ^{131}I therapy, and that around 2-6% of such patients will become frankly hypothyroid over 12 months of follow-up.

It is likely that the elevated basal TSH reflects a small fall in free thyroid hormone concentrations. This stimulates the thyroid to secrete more hormone in an attempt to maintain euthyroidism. In the TSH stimulated gland the ratio of monoiodotyrosine to di-iodotyrosine is increased and, following coupling, T3 is therefore secreted in greater than usual amounts, while T4 concentrations may not change (Taurog, 1978). The unique requirement of pituitary tissue for a high proportion of intracellular T3 to be derived from local monodeiodination of T4 means that the TSH induced rise in circulating T3 does not suppress the increased TSH secretion (Siwa et al. 1978). Thus, increased thyroid hormone secretion may be maintained without desensitising the thyroid/pituitary feedback loop. As other body tissues are able to utilise circulating T3 for thyroid hormone needs, it is likely that tissue euthyroidism is maintained. Studies of cardiac contractility in patients with subclinical hypothyroidism, have shown that T4 treatment produces no demonstrable change in cardiac function (Ridgway, Cooper, Walker, et al., 1980). If this implies that there is no hypothyroidism at tissue level (other than the pituitary) there may be no justification for routine use of thyroid hormone replacement in such patients, especially if more than 90% of this group are likely to have unchanging biochemical thyroid function during short-term follow-up. However, it has been suggested that subclinical hypothyroidism is a risk factor for ischaemic heart disease. Tunbridge and

colleagues reviewed 105 patients treated previously with ^{131}I therapy, and showed that those with raised basal TSH concentrations but normal T3 and T4 levels had elevation of serum cholesterol (Tunbridge, Harsoulis and Goolden, 1974). Follow-up one year later showed that no major change in biochemical function had occurred. This elevation of cholesterol might be an arguement in favour of treating such patients with thyroxine. It is therefore of some interest that retrospective studies of patients with ischaemic heart disease have suggested that the incidence of mild abnormalities of thyroid function (positive thyroid autoantibodies or borderline elevation of TSH) is greater than normal (Tieche, Lipi, Gutzwiller, et al., 1981). This is an important question which remains unresolved at present.

1.3.11. Adverse effects of ^{131}I treatment

Apart the inexorable development of hypothyroidism, ^{131}I treatment is remarkably free of side effects. In a small number of patients mild radiation thyroiditis results in local tenderness over the gland, and the possible exacerbation of thyrotoxicosis due to a transient rise in T3 and T4 concentrations following ^{131}I administration has been discussed previously.

(a) Salivary gland function The salivary glands in man are able to trap iodide in a similar manner to the thyroid, although no significant organification of the anion subsequently occurs, and iodide is secreted in saliva (Brown-Grant and Taylor, 1963; Fletcher, Honour and Rowlands, 1956; Lazarus, 1972). ^{131}I is therefore accumulated by salivary glands and, although retention of the isotope will be relatively short-lived, some radiation effects might be expected. In patients given ^{131}I in large doses for thyroid cancer some impairment of salivary gland function has been reported (Doniach, 1978), resulting in dryness of the mouth. There are, however, no reports of this occurring as a permanent consequence of conventional therapeutic doses of ^{131}I given for thyrotoxicosis.

(b) Parathyroid function The proximity of the parathyroid glands to the thyroid suggests that radiation induced impairment of parathyroid function might occur after ^{131}I administration. Adams and Chalmers (1965) suggested that 10% patients given ^{131}I have impaired parathyroid reserve, based on abnormalities in the mobilization of calcium from bone after administration of the calcium chelator EDTA. Harden and colleagues demonstrated increased urinary phosphate excretion in patients following ^{131}I

treatment, and also suggested that parathyroid function might be diminished (Harden, Harrison and Alexander, 1963). There are, however, very few documented cases of gross biochemical hypoparathyroidism with hypocalcaemia following ^{131}I treatment (Orme and Connolly, 1971; Jialal, Pillay and Asmal, 1980).

The other important hormone involved in calcium homeostasis is calcitonin, secreted by the parafollicular (C) cells of the thyroid. Again, it would be reasonable to suspect that calcitonin deficiency might be a consequence of ^{131}I therapy. The effects of calcitonin deficiency on serum calcium and bone turnover rates are not clear (Smith, Fraser and Wilson, 1973). However, it has been suggested that the reduction in total bone mass seen in thyroidectomised subjects is a consequence of calcitonin deficiency (McDermott, Kidd, Blue, et al., 1983). In a study of bone density in patients with treated and untreated thyrotoxicosis, Fraser and colleagues showed that patients treated with ^{131}I had a lower bone density than those given antithyroid drugs or surgery (Fraser, Anderson, Smith, et al., 1971). This may reflect calcitonin deficiency, although the available data on calcitonin levels before and after ^{131}I treatment in thyrotoxic subjects suggest that secretion of the hormone is maintained (Fraser and Wilson, 1971).

1.3.12. Effect on dysthyroid eye disease

Exacerbation of existing ophthalmopathy in patients with Graves' disease has been reported after ^{131}I treatment (Hamilton, Schultz and De Gowin, 1960). The true risk of this potentially serious adverse reaction is difficult to assess, as the course of ophthalmopathy associated with Graves' disease does not always parallel that of the thyroid disorder. Jones and colleagues in 1969 reported the results of serial measurements of exophthalmos following ^{131}I treatment, and demonstrated that the degree of proptosis increased in the first year after therapy, with the maximum rate of increase occurring in the early months (Jones, Munro and Wilson, 1969). However, other authors have reported that exacerbation of eye signs occurs with all forms of treatment for Graves' disease and is not confined to patients given ^{131}I (Bartels and Irie, 1961): in an early study, Soley (1942) suggested that 50% of patients treated surgically showed some evidence of deterioration of ophthalmopathy.

Potential causes of worsening of eye disease after ^{131}I treatment are

not fully understood. Immune factors have been thought to be important: Hetzel reported that only patients who had detectable concentrations of LATS which rose after ^{131}I treatment showed progression of eye signs (Hetzel, Mason and Wang, 1968), consistent with findings from an earlier study in the United States in which it was suggested that eye disease could develop for the first time in patients given ^{131}I in whom LATS measurement became positive (Kriss, Pleshakov, Rosenblum, et al., 1967). In untreated thyrotoxicosis there is, however, no close relationship between degree of ophthalmopathy and level of thyroid directed antibody (Feldt-Rasmussen, Kemp, Bech, et al., 1981). It may be that LATS is not responsible for the progression of the eye signs, but acts as a marker of some other immunological event. MacDougall and Kriss (1974) suggested that after ^{131}I treatment there was release of thyroid antigenic material. In the presence of antibodies (i.e. antithyroglobulin or antimicrosomal) immune complexes may form, which could be deposited in retro-orbital tissue. This could then be associated with lymphocytic infiltration and increased inflammatory response, and consequent exacerbation of the condition. It may be of relevence that the lymphatic drainage of the orbit and thyroid are connected (Kriss, Konishi and Herman, 1975). Although this theory is plausible, there is little direct evidence in its support. However, during the 1960s attempts were made to treat thyroid ophthalmopathy by extirpation of the thyroid either by surgery or radioiodine ablation to abolish the source of thyroid antigenic material. This potentially hazardous treatment showed no major benefit in improvement of eye disease (Boyle, Greig, Thomson, et al., 1969), and the practice fell into decline. More recently the existence of a separate antibody directed against extra-ocular muscle tissue has been identified in patients with Graves' disease, and it may be that disturbance in the formation of this antibody accounts for some exacerbation of eye disease following treatment of thyrotoxicosis (Atkinson, Holcombe, Taylor, et al., 1984; Faryna, Nauman and Gardas, 1985).

It is thought possible that dysthyroid eye disease is worsened by iatrogenic hypothyroidism, possibly associated with TSH excess (Bolonkin, Tate, Luber, et al., 1975). The reason for this is unknown. Interestingly, Jones et al. (1969) suggested that the most marked deterioration in eye signs following ^{131}I treatment occurred in those subjects with rapid weight gain. These subjects may have had hypothyroidism although biochemical data are

not available.

Thus, although worsening of dysthyroid eye disease may occur after ^{131}I treatment, the problem may also be associated with other forms of therapy. The cause of the deterioration is not known, although may be associated with changes in thyroid or thyroid associated immunological factors as a consequence of ^{131}I therapy.

1.3.13. Carcinogenesis

In the early days of ^{131}I treatment, there was concern that a late consequence might be thyroid carcinogenesis. In children given low dose external radiation to head and neck and followed-up many years later thyroid nodule formation and thyroid carcinoma have been reported in a number of studies (Hemelmann, Hall, Phillips, et al., 1975; Rooney and Powell, 1959). Similarly, in the populations of Hiroshima and Nagasaki exposed to radiation from nuclear explosions there is a clearly increased incidence of thyroid cancer (Wood, Tanagaki, Neuski, et al., 1969), and in animal studies thyroid tumours can be induced by irradiation (Doniach, 1958). One feature of all these studies, however, has been the relatively low level of radiation to the thyroid, estimated by Doniach (1953) to be in the region of 200 Rad, and it has even been suggested that radiation doses of as low as 9 Rad to the thyroid might increase the incidence of thyroid cancer (Anon, 1985). In contrast, conventional doses of ^{131}I will deliver around 5000 Rad to the thyroid (Halnan, 1964). This high dose causes cell sterilization, and cell death during mitosis precludes carcinogenesis. It is reasonable to expect that sublethal doses of radiation could, however, cause chromosomal damage which would lead to the development of carcinoma. It appears, therefore, that the apparently inescapable cumulative late hypothyroidism as a consequence of ^{131}I is an outcome which protects patients from the risk of iatrogenic carcinogenesis.

Despite these theoretical arguements the development of thyroid cancer in patients given ^{131}I therapy has occasionally been described: 7 cases have been reported in literature (Hamburger and Meier, 1971; Sheline, Lindsay, McCormack, et al., 1962). This, however, is probably less than the expected incidence of thyroid cancer in a population the size of that given ^{131}I (Halnan, 1985; Hoffman, 1984). In one series it was reported that thyroid nodules developed in 3 out of 18 patients aged less than 20 years

given ^{131}I therapy, and in one patient a low grade carcinoma was demonstrated (Sheline et al. 1962). These patients were given low doses of ^{131}I (less than 2 mCi). In view of the evidence that low level radiation to the head and neck in children can predispose to subsequent development of thyroid cancer, there appears to be little justification for giving smaller than conventional doses of ^{131}I in the treatment of thyrotoxicosis.

1.3.14. Leukaemogenesis

There have been sporadic reports of leukaemia developing in subjects given ^{131}I for thyrotoxicosis (Dobyns, Sheline, Workman, et al., 1974; Pochin, 1960). However, both in the U.K. and in the United States, the follow-up of large numbers of patients has not demonstrated an increased incidence of either acute or chronic leukaemia attributable to ^{131}I. The theoretical radiation dose to the bone marrow would be around 2 Rad, which is half the dose expected to double the spontaneous mutation rate. In a recent review Halnan (1985) concluded that there was no risk of leukaemogenesis from the doses of ^{131}I given for thyrotoxicosis.

1.3.15. Genetic damage

Blood borne ^{131}I, and ^{131}I excreted in the urine, will cause gonadal irradiation, and there has been concern that this might cause damage to germ cells in ovary and testes. In male subjects given large doses of ^{131}I for thyroid carcinoma, azoospermia and dose-dependent elevation of serum FSH have been documented (Handelsman and Turtle, 1983). However, such effects are only apparent with doses of ^{131}I in excess of 50 mCi. Robertson and Gorman (1976) calculated that following a 10 mCi dose of ^{131}I, the radiation dose delivered to the gonads is around 3 Rads, which is similar to that from abdominal X-rays such as intravenous pyelography. In a large series of patients given ^{131}I treatment, children conceived subsequently showed no evidence of genetic damage (Chapman, 1971b). In view of this, current recomendations are that ^{131}I may be given to patients of childbearing age, without fear of long term damage to germ cell tissue (Halnan, 1985).

1.3.16. Irradiation of the foetus

Iodide crosses the placenta: if pregnant women are given ^{131}I, the

foetus will be exposed to radiation, and from about 12 weeks gestation, the foetal thyroid is capable of trapping iodide. ^{131}I has not been given to pregnant women as a matter of policy but despite this a surprisingly high number of patients, especially in the United States, have been given ^{131}I during pregnancy, normally in the first trimester. In a survey of a large number of centres in the United States, Stoffer and Hamburger (1976) discovered 237 cases given ^{131}I during the first trimester. There was no increase in the spontaneous abortion rate or in perinatal mortality, and the congenital malformation rate was not different from that expected in a similar group of pregnant women not given ^{131}I therapy. Six infants were, however hypothyroid, probably as a consequence of accumulation of ^{131}I in developing thyroid tissue. In a separate case report one infant of a mother given 103 mCi of ^{131}I with thyroid cancer during the first trimester had hypothyroidism and hypoparathyroidism (Richards, Brewer, Conley, et al., 1981). Thus, although there appears to be no major hazard to the foetus when the mother is accidentally given a conventional dose of ^{131}I in the early stage of pregnancy, it is prudent to avoid this form of treatment in pregnant patients.

1.3.17. Effect of radioiodine therapy on circulating thyroid autoantibodies

The relationship of thyroid autoantibodies to the development of hypothyroidism after ^{131}I treatment has been discussed previously (1.3.9.). There is now considerable evidence that ^{131}I treatment may itself modify autoantibody synthesis. In a number of studies it has been shown that the titre of antibodies directed against the TSH receptor rises within the first few months after ^{131}I administration, and that levels subsequently decline (Atkinson, McGregor, Kendall-Taylor, et al., 1982; Bech and Madsen, 1980). Patients who are antibody negative before ^{131}I treatment may become positive. This phenomenon has also been demonstrated with antibodies directed against thyroid cell cytoplasmic components and thyroglobulin (Einhorn, Fagraeus and Jonsson, 1965; O'Gormon, Staffurth and Ballentyne, 1964). It seems unlikely that this rise in antibodies is of major pathogenic significance in the events following ^{131}I administration (Bech, Blundal, Nielson, 1982; Lundell and Holm, 1980). The cause of the rise is not entirely clear. It is possible that the recognised effects of irradiation on T-lymphocyte subset populations, leading to a fall in the ratio of suppressor

to helper cells, results in enhanced antibody production (McGregor, McLachlan, Rees-Smith, et al., 1979). Release of thyroid antigenic material following radiation disruption of cell integrity may also be of relevance. Whether such immune events can be involved in potential exacerbation of dysthyroid eye disease after ^{131}I is a matter of speculation (Atkinson et al. 1984; McDougall and Kriss, 1974).

1.3.18. Clinical use of radioiodine

Early fears about genetic hazards from ^{131}I treatment led to a tendency in the United Kingdom to limit this form of therapy to patients over childbearing age. In practice, this meant that treatment was not given to patients below 40 years of age (Halnan, 1985). It is now accepted that there is no discernible genetic or carcinogenetic hazard from ^{131}I therapy, and most recent recommendations suggest that patients younger than 40 years can be treated with safety (Halnan, 1985). In the United States of America this has been practice for a number of years. There is still a natural reluctance to give this form of treatment to children in view of the evidence of the development of thyroid tumours years after low dose external irradiation of the neck (Hempelman et al. 1975). However, there is no objective contraindication to giving ^{131}I treatment to children, and in the United States this has been proposed as the treatment of choice (Hamburger, 1985).

There are few specific contraindications to ^{131}I therapy in thyrotoxicosis. Clearly, thyroid surgery is the treatment of choice where a large goitre causes pressure symptoms. Large glands may be less likely to shrink in the short term after ^{131}I therapy, particularly where there is an element of nodularity, and there may be cosmetic arguements in favour of surgery in patients with this form of disease. Such patients may require two or more doses of ^{131}I to effect cure (Miller, 1971), and in this situation surgical treatment may offer a more rapid and effective alternative.

In view of the uncertainty about the effect of ^{131}I treatment on the course of dysthyroid eye disease, some authorities advocate avoiding this form of therapy in patients with moderate to severe eye signs (Bartels and Irie, 1961). As discussed in a earlier section, evidence on this point is rather inconclusive.

The effect of pretreatment with antithyroid drugs on response to ^{131}I

has been discussed previously. The major benefit to patients is that potential for immediate worsening of the condition after ^{131}I administration may be avoided: the main disadvantage is that the possible radio-protective effect of antithyroid drugs may lead to delay before final cure is achieved. This may be overcome by increasing the dose of ^{131}I given to such patients (Crooks et al. 1960). Where antithyroid drugs are given immediately after ^{131}I administration, the biochemical response to radiation will be obscured, and on discontinuation of drug treatment the patient may be at risk of relapse of thyrotoxicosis. In comparison, beta blocker therapy given as concurrent treatment with ^{131}I may be effective in controlling symptoms of thyrotoxicosis until treatment takes effect (Hadden, Montgomery, Shanks, et al., 1968; Sterling and Hoffenberg, 1971). With these drugs reliable assessment of the effect of ^{131}I on thyroid hormone levels can be made. Beta blockers will not, of course, have any effect on the release of preformed hormone from the gland immediately after ^{131}I is given.

Evidence that the early response of the thyroid to ^{131}I therapy is dose-related has been presented earlier. As the annual cumulative incidence of late hypothyroidism appears to be independent of dose, it has been suggested that all patients should receive a large enough dose to ensure euthyroidism, even if this implies treating the majority of patients from an early stage with thyroid hormone replacement (Wise, Aahmad and Burnet, 1975). In studies where attempts have been made to control the radiation dose delivered to the thyroid, formulae for calculating the oral dose of ^{131}I to be administerd have been derived. Most of these include a measurement of iodide uptake by the gland using tracer radioisotopes of iodide and an estimate of gland size (Beierwaltes, 1978). The kinetics of tracer doses of ^{131}I within the thyroid are probably similar to those of therapy doses, and the assumptions made about the uptake and retention of therapy doses of ^{131}I based on tracer studies appear to be generally correct (O'Conner, Cullen and Malone, 1979). The major inaccuracy in dosimetry arises from estimate of thyroid mass, which is usually performed clinically. More accurate estimate of mass can be had by scintigraphic estimation of thyroid surface area (Brown and Spencer, 1978) or from the use of thyroid ultrasound (Rasmussen and Hjorth, 1974). Even with these more objective techniques, however, there has been little attempt to validate the mass estimates with actual gland size measured after surgical removal of the thyroid.

There have been few attempts to confirm the accuracy of dosimetry calculations by directly measuring the dose taken up and retained by the gland, and the uncertainty about the value and accuracy of dosimetry has led some authors to recommend the use of more empirical doses of ^{131}I which eliminate the need for complex measurements (Wise et al. 1975).

1.3.19. Follow-up of patients given ^{131}I

In the early months after ^{131}I treatment, patients are reviewed as often as the clinical course dictates. Thereafter, when patients have become euthyroid, the main development to be anticipated is late onset hypothyroidism. As has been discussed previously (1.3.9.), the annual incidence of this in a large number of series is between 2 and 6% (Greig, 1966; Toft et al., 1975; Segal et al., 1961). Toft has demonstrated that in subjects who have responded to ^{131}I only those with elevation of basal TSH run a risk of hypothyroidism in the short term (Toft et al. 1974) and has suggested that such patients do not require to be reviewed at more than yearly intervals. Other patients with completely normal biochemistry may be seen even less frequently. Additionally, patients who have become hypothyroid and are taking thyroid hormone replacement may need some form of intermittent review to ensure that the dose of thyroid hormone is appropriate (Beckett, Kellet, Gow, et al., 1985).

The number of patients given ^{131}I, and the long term nature of follow-up, means that the total number of patients requiring some form of regular review is large. In an attempt to improve efficiency and cost effectiveness of such follow-up, computer assisted patient follow-up schemes have been devised. In Scotland, the Scottish Automated Follow-up Register (SAFUR) has been established for this purpose. The system involves the patient's general practitioner, who is responsible for patient contact and dispatch of blood samples to a central laboratory. Centralised assessment of laboratory results and patient data is maintained, whilst the local thyroid clinic is responsible for patient recall or other management change if results are unsatisfactory (Jones, Hedley and Curtis, 1982). Although proof that such a system is better in terms of quality of health care is lacking (Toft, 1983), there is some evidence that automated follow-up registers are cost effective and more efficient than traditional outpatient follow-up based on a hospital clinic (Hedley, Bewsher and Edwards, 1984).

SECTION FOUR

1.4. ANTITHYROID DRUGS IN THE TREATMENT OF THYROTOXICOSIS

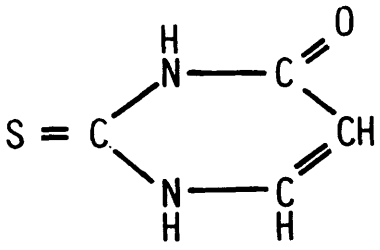
1.4.1. Introduction

Chemical agents which reduce thyroid hormone formation include ionic inhibitors of the anion trap, agents which reduce thyroid hormone release, and drugs which inhibit iodide organification. It is this last group which is of principle clinical value, and such agents will be considered in some detail. A review of the ionic inhibitors and other chemical agents which inhibit thyroid hormone secretion has been published elsewhere (Alexander, Connell and McCruden, 1981).

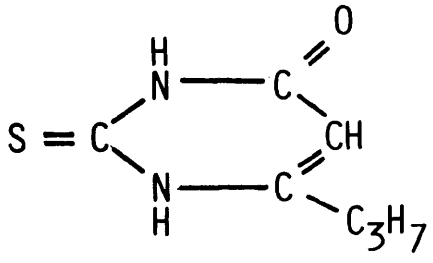
The first agents shown to inhibit thyroid hormone synthesis were sulphonamide drugs: in 1941, McKenzie and co-workers reported thyroid enlargement in rats given sulphaguanidine (McKenzie, McKenzie and McCallum, 1941): further investigation indicated that this was TSH dependent and associated with hypothyroidism. In subsequent studies, two major groups of compounds were shown to have antithyroid effects - 1. aniline derivatives, which include the sulphonamide drugs and 2. thiourylene agents which include all compounds in current medical use (Astwood, Bissell and Hughes, 1945). The sulphonamide drugs have not been shown to have major antithyroid effect in man, possibly because of limited dose and duration of administration. A small fall in serum thyroxine levels after administration of sulphamethaxazole to normal subjects has been demonstrated, but hypothyroidism has not been reported (Cohen, Beastall, Ratcliffe, et al., 1980). In animal studies, the potency of such drugs has been shown to be considerably less than the thiourylene agents such as methimazole or propylthiouracil (McKenzie and McKenzie, 1943), and this may be because the former group of drugs is not concentrated within thyroid tissue (Personal observations). Although the mode of action of sulphonamide drugs has not been fully investigated, it is likely that they interfere with iodide organification through an interaction with the thyroid peroxidase.

1.4.2. Action of thiourylene agents

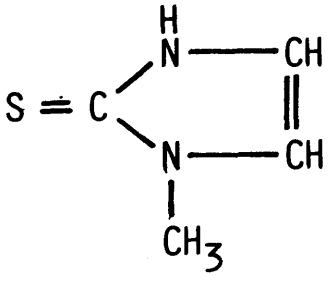
THIOURYLENE ANTITHYROID DRUGS



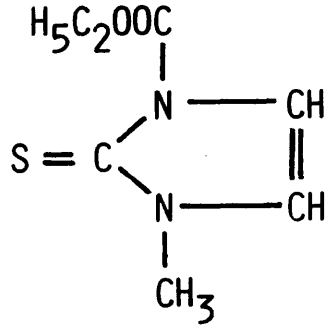
Thiouracil



Propylthiouracil



Methimazole



Carbimazole

Fig 4

STRUCTURE OF THIOURYLENE ANTITHYROID DRUGS

THE CHEMICAL STRUCTURES OF THIOURACIL, PROPYLTHIOURACIL, METHIMAZOLE AND CARBIMAZOLE ARE SHOWN. NOTE THAT ALL SHARE A COMMON S = C - N GROUPING WHICH IS ESSENTIAL FOR ACTION. IN VIVO CARBIMAZOLE IS RAPIDLY CONVERTED TO METHIMAZOLE.

Of the thiourylene drugs, thiourea and thiouracil were initially investigated by Astwood et al. (1945). These were subsequently replaced by less toxic agents such as propylthiouracil and methimazole. In the United Kingdom carbimazole has been used in place of methimazole: carbimazole is rapidly hydrolysed to yield methimazole in plasma or at alkaline pH (Nakashima and Taurog, 1979): in vivo this conversion may be enzymatic. There is no evidence that carbimazole has any antithyroid action other than that mediated by methimazole.

The chemical structures of representative thiourylene drugs are shown in Figure 4. It will be noted that all drugs contain the grouping S=C-N which appears to be essential for the antithyroid effect. These drugs reduce thyroid hormone synthesis by inhibition of intrathyroidal oxidation, and thus organification of iodide, leading to reduction in formation of iodotyrosine residues (Taurog, 1980). There is also evidence of inhibition of coupling of these residues on the thyroglobulin molecule by such drugs. This can be demonstrated in vitro using purified thyroid peroxidase and methimazole, but is difficult to demonstrate in vivo (Iino, Yamada and Greer, 1961). Such studies suggest that the coupling reaction is more sensitive to the action of antithyroid drugs than iodide organification itself.

There is some debate about the mechanism by which these drugs affect iodide oxidation and organification. This process is dependent on the enzyme thyroid peroxidase and a supply of hydrogen peroxide to act as an electron acceptor (Taurog, 1970). It is likely that the drugs interact with this enzyme. Taurog (1978) has suggested that normally iodide and peroxidase form a complex, with iodide being oxidised in the process by hydrogen peroxide. The chemical nature of oxidised iodide is unclear but may be either iodinium (I^+) or iodine free radicle. Tyrosine residues may then react with the enzyme/I complex to give rise to iodotyrosine (Fig. 2). Antithyroid drugs such as methimazole may compete with iodide for oxidation by thyroid peroxidase/hydrogen peroxide, so that the drug is oxidised and iodide remains in inorganic form. It has been suggested that oxidised drug may then interact with peroxidase to prevent irreversibly further enzyme action (Davidson, Soodak, Neary, et al., 1978). Davidson et al. (1978) demonstrated that in circumstances where iodide availability was high, iodide will be oxidised in preference to drug and that the enzyme iodide complex may then react with the drug to give rise to oxidised drug which will be a

disulphide product which will not inactivate peroxidase. In this process failure of organification of iodide will occur and free iodide will be released. Thus, it is possible that drug competes with either iodide or tyrosine residues as a substrate for oxidation by peroxidase/hydrogen peroxide or peroxidase/I complexes, and the availability of iodide may determine which oxidising species interacts with drug. Much of this speculation is based on results from in vitro studies using the drug, peroxidase and iodide, in a cell free system. The situation in the whole animal may differ significantly.

Thiourylene antithyroid drugs are concentrated within the thyroid glands of experimental animals (Desbarats-Schonbaum, Endrenyl, Koves, et al., 1972; Marchant, Alexander, Lazarus, et al., 1972) and probably also of man (Lazarus, Marchant, Alexander, et al., 1975). When radiolabelled antithyroid drugs are given (e.g. ^{35}S-methimazole) a thyroid/plasma gradient develops. Radiolabelled drug can be shown to be present in the colloid and follicular cells of the gland using autoradiography, with a pattern of distribution similar to that of tracer iodide (Ferguson, Marchant and Alexander, 1971). Drug accumulation and concentration appear to be active processes, being inhibited in vitro by agents such as dinitrophenol, which uncouple oxidative phosphorylation (Lang, 1982), and is enhanced in vivo by agents which stimulate iodide trapping such as TSH or LATS (Lees and Alexander, 1978). However, preliminary studies using thyroid lobes in vitro suggest that inhibition of iodide trapping with the perchlorate anion does not abolish drug accumulation (Lang, 1982), and it appears from such evidence that the process of drug accumulation is linked to, but not dependent upon, iodide metabolism.

The relationship between drug concentration and iodide uptake by the thyroid gland has been explored in a series of animal experiments by Marchant (1971), Lees (1976) and Lang (1982). In summary, there is clear evidence that dietary iodide depletion is associated with a fall in drug accumulation, and that dietary iodide excess enhances drug accumulation by the thyroid gland. Acute administration of iodide causes enhancement of drug accumulation at all levels of dietary iodide intake. It is unclear how the interaction of iodide availability and thyroidal accumulation of antithyroid drug affects clinical efficacy of the agents. It is possible, however, that the reported variations in response to antithyroid drug therapy reflect geographical differences in dietary iodide (Wartofsky, 1973; Azizi, 1985).

Metabolism of the thiourylene antithyroid drugs also occurs within the thyroid gland. Oxidation of the drugs by interaction with thyroid peroxidase/hydrogen peroxide initiates the metabolic breakdown of drug which gives rise to free sulphate and a number of intermediary metabolites (Marchant, 1971). These include methylthiohydantoin as a breakdown product of methimazole and a number of other unidentified metabolites (Skellern, Knight, Luman, et al., 1977). Some of these metabolites, such as methylthiohydantoin, may also possess antithyroid action, although animal studies suggest that these are much less potent than the parent compound (Skellern et al. 1977). As with accumulation of the drugs, intrathyroidal metabolism of these compounds is influenced by iodide availability. Thus, in animals maintained on a low iodide intake, metabolism of both methimazole and propylthiouracil is enhanced by acute iodide administration (Lang, Lees, Alexander, et al., 1983; Marchant, Lees and Alexander, 1978). The possible reason for this interaction between iodide availability and drug oxidation has already been discussed. The studies of Davidson et al. (1978) and Taurog (1970, 1978, 1980) suggest that, in conditions where a high drug/iodide ratio is present, drug is oxidised directly by thyroid peroxidase, and that oxidised drug irreversibly inactivates the enzyme: thus further drug oxidation is inhibited. In conditions of low drug/iodide ratio, iodide forms an oxidised complex with peroxidase, and this complex then oxidises drug. It is suggested that drug oxidised in this way does not inactivate thyroid peroxidase, and thus further scope for further drug metabolism exists. Although this hypothesis is attractive, there is no direct in vivo confirmatory evidence, although the data cited above on drug oxidation in vivo would be consistent with it (Lang, 1982; Lees, 1976; Marchant, 1971).

It has been suggested that intrathyroidal accumulation of antithyroid drug is important in the pharmacological specificity of such agents. In man, Jansson and colleagues, using a sensitive gas chromatography/mass spectroscopy assay for methimazole have shown that thyroid levels of the drug are higher than serum levels in subjects with Graves' disease (Jansson, Dahlberg, Johansson, et al., 1983). However, there was a wide variation in thyroid drug concentration between subjects, although within individuals, drug concentration throughout the gland appeared relatively homogeneous. Intrathyroidal drug levels appear to be similar no matter whether the last dose is taken shortly before, or 20 hours before, removal of thyroid tissue,

and it appears that intrathyroidal turnover of drug is slow. This contrasts with the relatively short half-life of methimazole in plasma (3 hours) and it seems likely that the plasma pharmacokinetic properties of such drugs are of little relevance (Skellern, Knight, Low, et al., 1980). The plasma half-life of propylthiouracil is around 90 minutes (Sitar and Hunninghake, 1975). Both propylthiouracil and methimazole are cleared more rapidly from the plasma of thyrotoxic subjects than euthyroid subjects (Alexander, Evans, McAulay, et al., 1975) and higher circulating drug levels are seen in patients with renal impairment. Propylthiouracil, but not methimazole impairs peripheral conversion of T4 to T3, and this may be of additional therapeutic benefit in thyrotoxicosis (Saberi, Sterling and Utiger, 1975).

1.4.3. Effects of antithyroid drugs on the course of Graves' disease

Antithyroid drugs such as methimazole and propylthiouracil are given to patients with Graves' disease to reduce thyroid hormone formation. There has been considerable speculation as to whether these agents might also affect the underlying immunological course of Graves' disease, and so induce remission of the disorder. This question has proved exceptionally difficult to answer: to demonstrate an effect it would need to be shown that the remission rate after treatment with antithyroid drugs was higher than would be expected from the natural course of the disorder. It does appear, however, that the majority of series in which antithyroid drugs have been given have reported a higher remission rate than would be expected (around 25%) over a similar period of follow-up: the reported remission rates after a single course of treatment with antithyroid drugs ranged from 33% to 77% (Weetman, McGregor and Hall, 1984). This variability may partly reflect differences in biochemical and clinical definition of remission and duration of follow-up. In long term surveillance of such patients treated only with anti-thyroid drugs, it has been suggested that a considerable percentage of those initially in remission may eventually relapse (Sugrue, McEvoy, Feely, et al., 1980). If this is so, it implies that antithyroid drugs may not influence the long term course of Graves' disease but may effect a relatively short lived change in some factor of pathogenic significance. There is indirect evidence that this might be the case from the studies of Alexander and co-workers, who demonstrated that in subjects who entered remission during antithyroid drug therapy a fall in iodide trapping by the gland was demonstrated

(Alexander, Harden and Shimmins, 1967; Alexander, McLarty, Robertson, et al., 1970). Antithyroid drug treatment has been shown to reduce the plasma concentration of thyroid stimulating immunoglobulins, and this did not occur in the same group of patients when treated with propranolol (McGregor et al. 1980). Similar findings have been reported from a number of other studies (McGregor, Rees-Smith and Hall, 1982), and it seems likely that thiourylene drugs act specifically to inhibit synthesis of thyroid directed antibodies (Fenzi, Hashizume, Roudebush, et al., 1979; Michie, Beck, Mahaffy, et al, 1967). In vitro, drugs can be shown to impair lymphocyte response to mitogenic and antigenic stimuli (Weiss and Davies, 1981), and this effect may be a consequence of action on monocytes (Ratanachaiyavong and McGregor, 1985): monocytes are responsible for uptake and processing of antigen which is then presented to the lymphocyte. Weetman and colleagues have reported that antithyroid drugs may act to scavenge superoxide radicals in monocytes (Weetman, Holt, Campbell, et al., 1983), a property which is likely to be of importance in the function of these cells. Finally, in patients with Graves' disease the ratio of helper to suppressor lymphocytes is abnormally high (Ludgate, et al. 1984), and this is returned to normal during antithyroid drug therapy (Sridama, Pacini and De Groot, 1982). There is, therefore, considerable in vitro and in vivo evidence that antithyroid drugs may influence the short-term course of Graves' disease by diminishing the synthesis of immunological stimulators directed against the TSH receptor: this may be mediated through a direct effect on T-lymphocytes or, more likely, other immunocompetent cells such as antigen presenting monocytes. This concept is discussed more fully in Chapter 4.

1.4.4. Clinical use of antithyroid drugs

Prolonged therapy with antithyroid drugs is usually given to patients in the hope that the disease will enter remission during treatment, and that ablative therapy with either surgery or ^{131}I can be avoided. This is most likely to happen in patients with small diffuse goitres and a short history of thyrotoxicosis (Alexander et al. 1981; McLarty, 1973). Where these latter treatments are indicated, however, antithyroid drugs may still be of value as preparatory treatment. Where rapid and permanent control of thyroid hormone excess is important (e.g. in the elderly or in patients with intercurrent illness), prolonged use of antithyroid drugs may not be

appropriate.

As antithyroid drugs are concentrated within the gland, there is no simple dose/response relationship in man. The effectiveness of drug induced inhibition of iodide organification can be measured using a suitable analysis of iodide binding rate, and from such studies it is apparent that relatively small doses of carbimazole/methimazole are capable of blocking iodide organification (McCruden, Hilditch, Connell, et al., 1986): there appears to be no reason in the majority of patients to use doses in excess of 30 mgs carbimazole daily (Alexander et al. 1981).

After control of thyroid hormone excess has been achieved, continued use of a dose of drug sufficient to inhibit fully iodide organification will lead to iatrogenic hypothyroidism, and consequent goitre enlargement. This may be avoided by using a lower dose of drug or by maintaining circulating thyroid hormone levels with exogenous thyroid hormone. This latter combined treatment will maintain suppression of TSH, and so allow serial measurements of thyroid radioisotope uptake to be made, which may be of value in deciding whether patients have entered remission (Alexander et al. 1970; McLarty, 1973; Wilkin, Isles, Crooks, et al., 1981). There is some evidence to suggest that remission rates after treatment with combined antithyroid drug/thyroid hormone therapy are higher than after antithyroid drug alone: this may reflect the higher dose of drug used in the former regimen (Ratanachaiyavong and McGregor, 1985; Romaldini, Bromberg and Werner, 1983). Although both thyroxine (100-200 µg/day) and tri-iodothyronine (40-80 µg/day) have been used for this purpose, thyroxine is the more effective agent in suppressing TSH secretion (Siwa et al. 1978; Ridgway et al. 1980).

Propylthiouracil is a less potent antithyroid agent than methimazole in man, and doses of 300-600 mg/day are required to control thyroid hormone excess.

It has been customary to give antithyroid drugs to patients with Graves' disease for a fixed course of between 12 and 18 months. However, it would be of value if the likelihood of a patient remaining in remission on withdrawal of drug therapy could be predicted during treatment, so that a rational decision about the need for continued treatment could be made. Alexander and colleagues (1970) suggested that if thyroid iodide trapping, estimated from early radioiodine uptake measurements fell during antithyroid

drug treatment and remained low after 6 months drug treatment, remission was likely and drug treatment could be stopped. However, Wilkins et al. (1981) with a similar design of study noted that a considerable number of patients in this category relapsed shortly after stopping treatment. In contrast, in a large study of Japanese patients, Yamamoto reported that 96% of patients who showed suppression of iodide uptake after a course of antithyroid drugs remained in remission on withdrawal of therapy (Yamamoto, Totsuka, Kojima, et al., 1983). McGregor and colleagues (1980) suggested that a combination of a fall in TSH-receptor directed antibody titre and HLA group DR3 was strongly predictive of sustained remission on withdrawal of treatment. This hypothesis remains to be tested over a long period of follow-up. In studies where short courses (less than 3 months) of antithyroid drugs have been given, it has been shown that the relapse rate on withdrawal of therapy is high (Burr, Fitzgerald and Hoffenberg, 1979; Greer, Kammer and Bouma, 1977; McFarlane, Davies, Longson, et al., 1983). It therefore appears that to achieve a reasonable remission rate, drugs need to be given for a period greater than three months. Indeed it has been suggested that suppression of iodide uptake during antithyroid drug treatment can occur up to three years after the start of therapy. During such long term treatment it has been reported that as many as 65% of patients show suppression of uptake, and that 96% of such patients remain in remission after therapy is stopped (Yamamoto et al. 1983). There is little doubt that where uptake measurements remain high during antithyroid treatment, or where thyroid stimulating antibody measurements do not fall, remission is unlikely and continued treatment indicated.

1.4.5. Toxic effects of antithyroid drugs

Serious side effects with antithyroid drugs are rare. The development of granulocytopaenia can occur at any time during drug ingestion, and presents abruptly (Trotter, 1962). The reported incidence of this potentially serious adverse effect varies from 0.1 to 3% (Amrheinn, Kenney and Ross, 1970). Characteristic manifestations of drug related granulocytopaenia include fever and pharyngitis. Fortunately, the neutrophil count usually rises on drug withdrawal. Although the cause of the selective leucopaenia is unclear, it has been suggested that drug specific leucoagglutinins are responsible for the effect, in a similar manner to the rare leukopaenia noted

with penicillin (Bilezikian, Laleli, Tsan, et al., 1976). Even less common is the occurrence of aplastic anaemia, although this often has a fatal outcome (Aksoy and Erdem, 1968). The incidence of these serious side effects is probably similar for all drugs of this group.

In a few patients a "serum sickness" type reaction has been reported with arthopathy, fever and urticaria (Wiberg and Nuttal, 1972). Much more commonly patients may present with an urticarial or maculopapular rash and pruritis. These effects disappear on drug withdrawal, but may return on rechallenge with the drug (Trotter, 1962; Vasily and Tyler, 1980). Other reactions are uncommon. Hair loss has been reported with carbimazole (Papadopoulos and Harden, 1966), while, with both methimazole and propylthiouracil, hepatotoxicity has been documented with peri-portal inflammatory changes and bile duct stasis (Fedotin and Lefer, 1975; Lunzer, Huang, Ginsberg, et al., 1975). There are isolated case reports of the nephrotic syndrome and peripheral neuropathy occurring in patients taking propylthiouracil (Reynolds and Bhathena, 1979; Roldan and Nigrin, 1972).

SECTION FIVE

AIMS OF THESIS

It will be apparent from the foregoing review that, despite the widespread use of ^{131}I treatment, there are still areas of uncertainty regarding its use in the treatment of thyrotoxicosis. In particular, specific questions relating to the early effects of ^{131}I on thyroid function arise.

1. What is the incidence of worsening of biochemical thyrotoxicosis immediately after ^{131}I, and are such changes clinically important?

2. How do the effects of ^{131}I on thyroid physiological processes such as iodide trapping and organification relate to biochemical and clinical responses in the first year after therapy?

3. Can information about the effect of ^{131}I on thyroid iodide handling be used to reduce the unpredictability of clinical response to therapy over the first few months?

4. How does the use of carbimazole pretreatment affect the response to ^{131}I?

5. Are these potential effects of carbimazole on response to ^{131}I related to the phenomenon of drug accumulation within the thyroid gland?

The present work sets out to address these questions in a series of studies in patients with thyrotoxicosis and in laboratory animals.

The first part of the remainder of the thesis (Chapter 2) deals with techniques used in the study of thyrotoxic patients given therapeutic ^{131}I including uptake of tracer doses of ^{123}I. The principles and potential errors of these measurements are considered and investigated experimentally.

The second part includes the results of studies of thyrotoxic patients treated with ^{131}I. The initial chapter of this section deals with the effect of ^{131}I on thyroid hormone concentrations immediately after treatment, and examines the effect of pretreatment with carbimazole on the potential exacerbation of thyrotoxicosis at this time. The use of early radioiodine uptake measurements to predict initial clinical and biochemical response to ^{131}I therapy is examined in the following chapter, as is the relationship

between the effect of ^{131}I on iodide handling and on biochemical response. In the next chapter the experience of transient hypothyroidism in these studies is presented, and this is followed by data on the effect of ^{131}I therapy on the rate of organification of iodide in a small group of subjects in whom serial measurements have been made. Studies on the effect of carbimazole pretreatment on outcome following ^{131}I treatment in patients with thyrotoxicosis are then presented, and, in the last chapter of this section a study on the effect of antithyroid drug treatment on the uptake and autoradiographic distribution of iodide in rat thyroid is discussed.

The next part deals with the phenomenon of antithyroid drug accumulation by thyroid and other tissue, and the relationship of this with tissue iodide trapping and organification. The effects of inhibition of iodide trapping on accumulation of labelled drug in mouse thyroid is examined; data on accumulation and autoradiographic distribution of labelled drug and iodide by salivary gland are then presented, while in the next section the use of human polymorphonuclears as a model tissue in which to study drug accumulation is discussed.

Finally, an attempt is made to draw together these various strands, and conclusions relevant to clinical use of radioiodine and antithyroid drugs in thyrotoxicosis in relation to the question posed above are discussed.

CHAPTER TWO

METHODS USED IN STUDY OF PATIENTS WITH THYROTOXICOSIS

SECTION ONE
CLINICAL AND BIOCHEMICAL METHODS

2.1.1. Clinical Assessment

All the patients included in this study were assessed, treated and followed-up by the author in the Radioisotope Outpatient Clinic of the Western Infirmary. The patients were seen every 4 weeks in the year following ^{131}I therapy. At each visit, patients were clinically assessed, blood was drawn for measurement of circulating thyroid hormones, and, where part of the study protocol, uptake of ^{123}I was measured either using a simple in vivo scintillation counter or a gamma camera computer system. (2.2.4.) Hypothyroidism was diagnosed in patients with subnormal concentrations of serum thyroxine along with elevated levels of TSH. Thyrotoxicosis was diagnosed in patients with elevated concentrations of T3 and undetectable TSH. Graves' disease was diagnosed if patients had elevated concentrations of T3 and T4, diffuse uptake of tracer radioiodine (^{123}I) over both lobes of the thyroid on scan and an elevated thyroid uptake measurement 20 minutes after intravenous injection of tracer ^{123}I (8% of dose). Toxic nodular goitre was diagnosed in subjects in whom distribution of isotope (^{123}I) on thyroid scan was not uniform over both lobes of the thyroid: in all such patients T3 and T4 concentrations were elevated and initial 20 minute uptake of ^{123}I was $>$ 8% of injected dose. No patients included in studies had been treated previously with thyroid surgery or ^{131}I: patients with known exposure to radiological contrast media within the preceding three years were also excluded. Patients with intercurrent medical problems where rapid control of thyrotoxicosis was desirable were not studied: for this reason no patients in these studies had clinical evidence of cardiac disease. In view of the possible effect of ^{131}I on dysthyroid eye disease, patients with WHO classification grades IV-VI (Werner 1977) were not studied.

The purpose of the studies, which were approved by the local ethical committee, was explained to patients, all of whom gave informed consent

before participation.

Biochemical measurements

Circulating levels of thyroxine, tri-iodothyronine (both measurements of total serum content) and TSH, were measured by radioimmunoassay using commercially available systems. All of these employed single antibody methods, with antibody bound to solid phase. Further details of these assays, including inter- and intra-assay variability are given in Table 2. Apart from the internal quality control of the commercial assay, the reliability of the measurement of T3 and T4 is regularly assessed by participation in the United Kingdom external quality control scheme. Samples from individual subjects were measured serially since these assays have minimal interassay variability.

Thyroglobulin and thyrotrophin receptor directed antibody were also measured using commercially available systems. Details of these assays are given in Table 2. The thyrotrophin receptor antibody (TRAb) is based on the principle of inhibition of binding of labelled TSH to a standard preparation of detergent solubilised TSH receptors (Southgate, Creagh, Teece, et al., 1984). Assays based on this principle have been fully validated in patients with Graves' disease (Rees-Smith et al., 1977; Hardisty et al., 1983). Measurement of both thyroglobulin and TRAb was performed on serum which had been stored frozen at -20^{o}C; samples were assayed from each subject in a single batch. The presence of endogenous antithyroglobulin antibodies in serum can compete for radiolabelled ligand in the assay of thyroglobulin, leading to innaccurate results (Izumi and Larsen, 1978): the presence of anti-thyroglobulin antibodies was screened for before and after ^{131}I therapy using a coated red cell assay for the antibodies (thymune 'T', Wellcome Diagnostics, Table 2) in all samples in which serum thyroglobulin was measured.

TABLE 2

VARIABLE	METHOD	SEPARATED	INTRA-ASSAY CO-EFFICIENT OF VARIATION	INTER-ASSAY CO-EFFICIENT OF VARIATION	NORMAL RANGE OF VALUES
TOTAL T4	SINGLE ANTIBODY DIRECT RADIOIMMUNOASSAY (AMERSHAM UK)	ANTIBODY (ANTI-T4) BOUND TO POLYMER PARTICLES	2.3 - 3.6	5.5 - 7.8	54 - 142 nmol/l
TOTAL T3	SINGLE ANTIBODY DIRECT RADIOIMMUNOASSAY (AMERSHAM UK)	ANTIBODY (ANTI-T3) BOUND TO POLYMER PARTICLES	4.6 - 6.6	6.5 - 9.7	0.8 - 2.46 nmol/l
THYROID STIMULATING HORMONE	DOUBLE ANTIBODY DIRECT RADIOIMMUNOASSAY (AMERSHAM UK)	2nd (ANTI 1gG) ANTIBODY BOUND TO POLYMER PARTICLES	1.9 - 3.2	4.7 - 7.7	1.0 - 5.5 uU/ml
THYROGLOBULIN	DOUBLE ANTIBODY DIRECT RADIOIMMUNOASSAY (NMS PHARMACEUTICALS)	2nd (GOAT ANTI-RABBIT 1gG) ANTIBODY BOUND TO POLYMER PARTICLES	2.9 - 5	4.3 - 5.5	2 - 60 ng/ml
THYROTROPHIN RECEPTOR ANTIBODY	SINGLE ANTIBODY RADIORECEPTOR ASSAY USING ^{125}I - TSH AS COMPETING LIGAND FOR ENDROGENOUS TRAb (RSR LTD)	CENTRIFUGATION TO SEPARATE DETERGENT SOLUBILISED RECEPTORS FROM MEDIUM	-	-	-10% _+10%
ANTI THYROGLOBULIN ANTIBODY	THYROGLOBULIN COATED TANNED TURKEY RED CELLS (WELLCOME LTD)	-	-	-	-

SECTION TWO
RADIOISOTOPE STUDIES

2.2.1. Introduction

Several radioisotopes of iodine emit not only particulate radiation but also gamma rays of suitably high energy for in vivo counting. Concentration of radioiodide by the thyroid gland allows external counting of gamma emmision over the neck to be performed, and, after appropriate calculations are made, permits quantitation of iodide trapping and organification by the thyroid. Throughout the studies described in the following chapters, ^{123}I-iodide was used as tracer. This short lived radioisotope (physical decay half-life = 13 hours, gamma energy, 59 KeV) has favourable emmision characteristics which allow accurate gamma counting and imaging with a gamma camera. As the energy of gamma emmision differs from that of ^{131}I, (365 KeV) tracer studies using ^{123}I can be made shortly after the use of therapeutic ^{131}I (physical half-life 8.05 days). Since the radiation from a tracer dose of ^{123}I is relatively low (1.0 Rad, 80 μCi), it was possible to perform serial radioisotope studies in patients treated with ^{131}I. On average each patient had 8 tracer studies performed, delivering a total of around 8 Rad to the gland: as the therapeutic dose of ^{131}I would expose the gland to between 3000 and 6000 Rad, this additional radiation dose was not considered significant.

^{123}I was obtained from the Atomic Energy Research establishment (Harwell), and ^{131}I from Amersham International, U.K.

2.2.2. Interpretation of early iodide uptake studies

In all studies ^{123}I (80 μCi) was given by rapid intravenous injection. Following injection the isotope is distributed throughout the vascular compartment and equilibrates rapidly with stable (^{127}I) iodide. Thyroidal handling of the radioisotope therefore reflects the behaviour of stable iodide. The time course of typical thyroid accumulation of ^{123}I by normal and thyrotoxic subjects is shown in Figure 5. It will be seen in both instances that initial uptake is rapid, but that in the thyrotoxic subjects a much greater proportion of the dose given is accumulated in the early phase. Uptake within the first 10-20 minutes after radioisotope administration is largely dependent upon trapping of iodide by the thyroid (IAEA report, 1972):

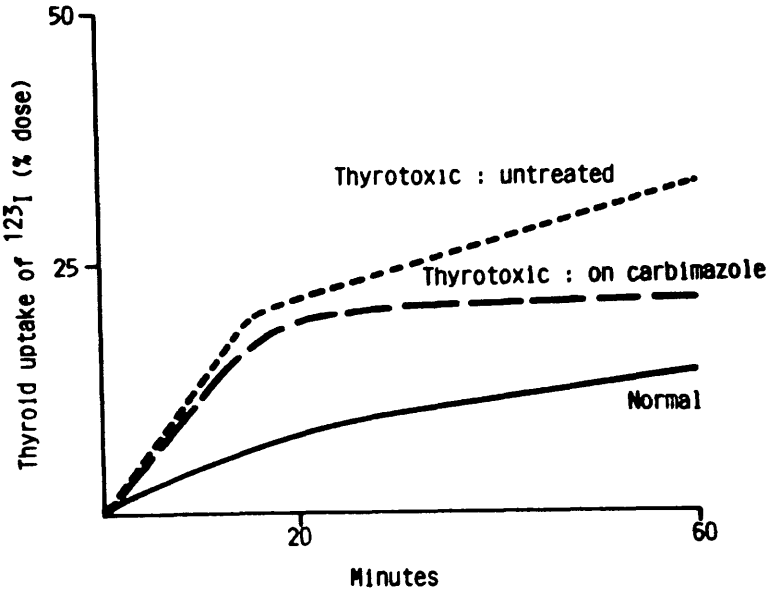


Fig 5

EARLY UPTAKE OF TRACER RADIOIODIDE (^{123}I) BY THYROID GLAND

SCHEMATIC CURVES OF UPTAKE OF A TRACER DOSE (80uCi) OF ^{123}I AFTER INTRAVENOUS INJECTION OF THE ISOTOPE AS MEASURED BY A GAMMA COUNTER. IN THE NORMAL SUBJECT UPTAKE INCREASES OVER THE HOUR OF MEASUREMENT, WITH THE GREATEST INCREASE BEING IN THE FIRST 20 MINUTES. THIS INITIAL PART OF THE UPTAKE CURVE (20 MINUTES) IS LARGELY A REFLECTION OF THYROID IODIDE TRAPPING, WHILE ORGANIFICATION OF TRAPPED IODIDE OCCURS AND ALSO CONTRIBUTES TO THE SECOND PERIOD (20 to 60 MINUTES). IN THE UNTREATED THYROTOXIC SUBJECT IODIDE TRAPPING IS MORE AVID, AND ORGANIFICATION CONTRIBUTES MORE TOTHE INITIAL (FIRST 20 MINUTES) OF UPTAKE THAN IN THE NORMAL. IN THE THYROTOXIC ON CARBIMAZOLE, TRAPPING IS UNAFFECTED BY THE DRUG, WHILE IODIDE ORGANIFICATION IS INHIBITED. WHILE THE INITIAL PORTION OF UPTAKE IS THEREFORE GREATER THAN USUAL, THERE IS LITTLE INCREASE IN UPTAKE BETWEEN 20 AND 60 MINUTES.

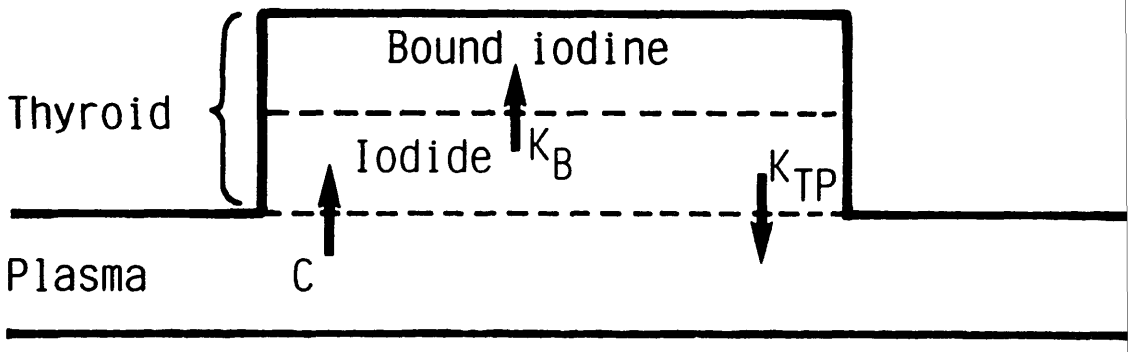


Fig 6

COMPARTMENTAL DISTRIBUTION OF IODIDE

THEORETICAL REPRESENTATION OF THYROIDAL IODIDE HANDLING. CLEARANCE OF IODIDE FROM PLASMA BY THE GLAND CAN BE REPRESENTED BY A CONSTANT (C), WHICH IS PREDOMINANTLY DETERMINED BY IODIDE TRAPPING. ORGANIFICATION OF INTRA-THYROIDAL INORGANIC IODIDE CAN BE REPRESENTED BY ANOTHER RATE CONSTANT (KB). LEAK OF IODIDE FROM THYROID TO PLASMA IS EXPRESSED AS KTP: THIS WILL CONTRIBUTE TO THE OVERALL CLEARANCE FIGURE.

thus, if drugs which inhibit iodide organification are given, uptake over this period should not be affected to any great extent. Subsequent uptake reflects both continued trapping of iodide by the gland and incorporation of iodide into organic form. In subjects where iodide organification is inhibited, there will therefore be no major increase in thyroid uptake after the initial trapping phase (Figure 5). As uptake of ^{123}I at 20 minutes after isotope injection has been shown to be an acceptable measure of thyroid iodide trapping (IAEA report, 1976; McLarty 1973; Wilkins et al., 1981) this has been used as the principal radioisotope measurement in the present work. In some instances, the uptake of radioisotope at 60 minutes was also measured, and the change in uptake between 20 and 60 minutes used to provide an approximate index of capacity for iodide organification (2.3.5.).

2.2.3. Iodide kinetic studies

Distribution of iodide in the thyroid can be described using a simple 3 compartment model (Figure 6) which consists of a plasma and two thyroidal iodine compartments (inorganic and organic). Transfer of iodide from compartment to compartment can be described mathematically, allowing rate constants to be derived for these functions. In Figure 6, C (ml/min) represents clearance of plasma iodide by the gland and is equivalent to thyroid iodide trapping. As discussed above, an early iodine uptake measurement (between 10 and 20 minutes after intravenous injection) gives an estimate of this function. K_B (min^{-1}) represents the incorporation of inorganic iodide to organic form (i.e. the function accomplished by thyroid peroxidase and coupling of iodotyrosines). Leakage of free inorganic iodide back to plasma is described by a exit rate constant (K_{TP}). The amount of iodide remaining within the gland after further uptake is blocked completely by perchlorate and free iodide discharged can be assumed to be in organic form, and this post-perchlorate measurement is directly related to the efficiency of organification (Binding Rate Constant, K_B). Hilditch has shown that analysis of the uptake curve following intravenous injection of tracer ^{123}I allows estimation of K_B to be made (Hilditch, Horton and Alexander, 1980), and has demonstrated that this is closely correlated with the organification rate calculated from residual uptake following administration of perchlorate. This has verified independently the accuracy of measurement of K_B derived from perchlorate discharge studies, and this latter technique has

therefore been used in the remainder of this thesis to measure the effect of ^{131}I treatment on thyroid iodide organification.

2.2.4. Measurement of thyroid uptake

The activity of tracer in the neck region viewed by a standard IAEA collimated neck counter is distributed in thyroidal and extrathyroidal regions. Measurement of thyroid radioisotope uptake depends on accurate estimation of extrathyroidal (i.e. vascular, lymphatic) radioactivity. Correction for extrathyroidal radioactivity is relatively easy when using a gamma camera (described below) (2.2.6.): however, when using a simple uptake counter, this correction is not possible. Extrathyroidal neck radioactivity is a potential source of error in measuring thyroid uptake in patients, especially when iodide trapping is low. In the formula used for calculation of thyroid uptake using a simple uptake counter average values for extrathyroidal activity as found by Hilditch (Hilditch, Gillespie, Shimmins, et al., 1967) and Shimmins (Shimmins, Hilditch, Harden, et al., 1969) have been used. Thus at two minutes extrathyroidal activity within the field of view of the counter was taken to be 7% of the total body radioactivity lying outwith the thyroid gland. At 20 minutes the corresponding figure is 6%. At 60 minutes, extrathyroidal activity is reduced to 84% of the value at 20 minutes.

Following rapid intravenous injection of 80 μCi ^{123}I, neck uptake of isotope was measured at 2 minutes, 20 minutes and, in some instances, 60 minutes using a standard collimated uptake counter, placed 8.5 cms from the patient's neck. An aliquot of the injected solution was placed in an IAEA phantom and also counted under the same conditions. Allowing for radioactive decay the uptakes at 20 (and 60) minutes were calculated using the simple equations shown below which correct for extrathyroidal radioactivity.

IF:-

Total neck uptake (% dose in field of interest)

= true thyroid uptake +

true extra-thyroid counts (% dose)

AND:-

when true uptake tends towards zero,

measured neck activity

$$= \frac{\text{True Extrathyroidal Counts (\% dose)} \times 100}{(100 - \text{True Thyroid Uptake})}$$

Direct measurement in perchlorate blocked glands shows

mean neck activity 7% at 2 mins

6% at 20 mins

THEREFORE:-

At 2 minutes: $7 = \frac{\text{True Extrathyroidal Counts} \times 100}{(100 - \text{True Thyroid Uptake})}$

OR $\text{True Extrathyroidal Counts} = \frac{700 - (7 \times \text{True Thyroid Uptake})}{100}$

THUS:-

Total Neck Uptake

$$= \text{True Uptake} + \frac{700 - (7 \times \text{True Uptake})}{100}$$

$$= 7 + 0.93 \times \text{True Thyroid Uptake}$$

THEREFORE:-

True Uptake = (Neck Uptake - 7) X 0.93

An uptake measurement at 24 hours after intravenous injection of ^{123}I was used for calculation of the dose of ^{131}I. Extrathyroidal activity was considered to be negligible at this time and so total neck counts were taken to represent thyroid uptake. An aliquot of injected solution was counted to allow calculation of percentage uptake.

2.2.5. Statistical variations in thyroid uptake measurements

Statistical variations in count rate resulted in uptakes of greater than 10% having errors of less than 1%; for uptake in the range 1-10% the error was less than 7%.

2.2.6. Thyroid imaging and kinetic studies

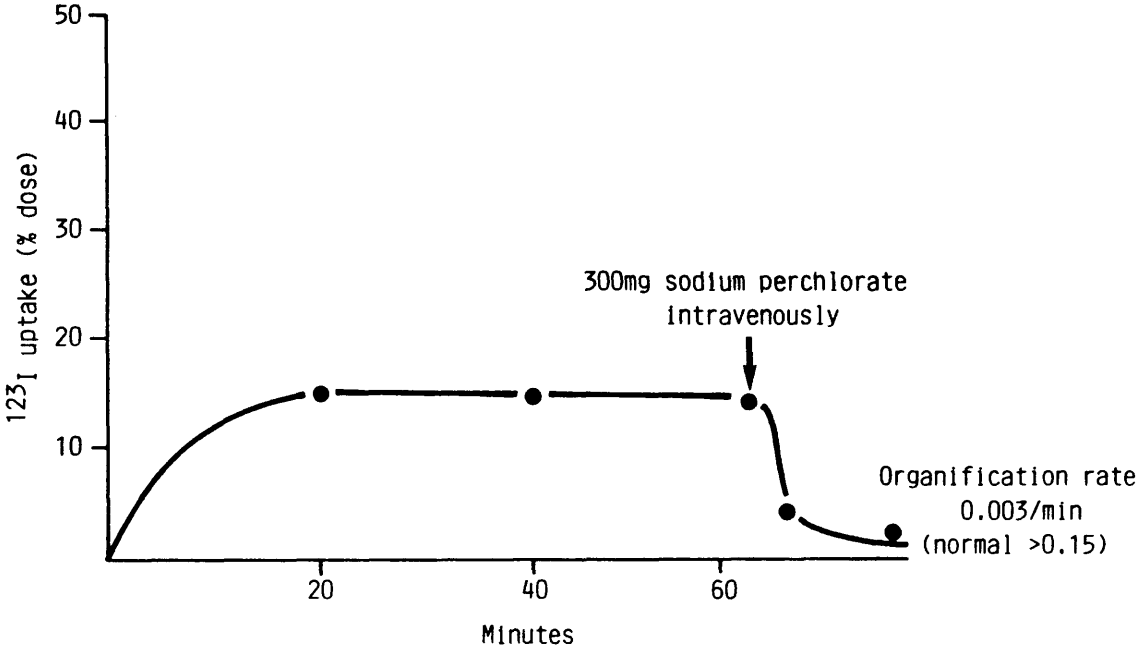


Fig 7

DISCHARGE OF IODIDE BY PERCHLORATE

SCHEMATIC REPRESENTATION OF IODIDE UPTAKE (OF ^{123} I) BY THYROID IN A SUBJECT WITH ORGANIFICATION FAILURE. AS IODIDE TRAPPING IS UNIMPAIRED INITIAL UPTAKE AFTER INTRAVENOUS INJECTION OF ISOTOPE IS NORMAL, BUT THERE IS NO INCREASE IN UPTAKE BETWEEN 20 and 60 MINUTES AS IODIDE TRAPPING IS BALANCED BY EXIT. WHEN SODIUM PERCHLORATE IS GIVEN INTRAVENOUSLY TRAPPING IS BLOCKED, AND IODIDE EXIT FROM THE GLAND IS POSSIBLY INCREASED. THIS RESULTS IN A FALL IN THYROID RADIOISOTOPE CONTENT: THE IODIDE UPTAKE AFTER PERCHLORATE IS A MEASURE OF THE RESIDUAL CAPACITY FOR IODIDE ORGANIFICATION BY THE GLAND.

Scan size To estimate thyroid size, the planar surface area of a gamma camera image of the thyroid was measured. The gamma camera used was an Ohio Nuclear Series 100 camera linked to a Varian V77 mini computer. The image was produced under zoom magnification using a standard parallel hole collimator, 30-60 minutes after intravenous administration of ^{123}I (80 μCi). The image was smoothed using the computer, and an area for the image was derived by circumscribing the image using a cursor. The estimate of size was then made by circumscribing in the same manner a phantom of known surface area. Mass (Grams) was taken to be the area $(cm^2)^3/2 \times 0.36$. This estimate of thyroid mass has been shown previously to correlate reasonably well with thyroid size estimated by ultrasound examination (Brown and Spencer, 1978).

2.2.7. Kinetic studies

^{123}I was given intravenously (150 μCi), and counts were acquired using the gamma camera/computer system. The patient was supine for this measurement, with the standard collimator placed 12 cms above the neck. Extrathyroidal neck radioactivity could be measured directly by counting an area in the field of view outwith but adjacent to the gland. A region to the inferior of the gland displaced away from the arm in which the bolus injection had been given was used in order to eliminate counts derived from the initial injection. A series of 60 dynamic frames each of 60 seconds duration was acquired. For each frame counts were determined for the thyroid area and subtraction of the extrathyroidal activity was made. An aliquot of injected solution was also counted using an IAEA phantom, and thyroid uptake was then derived.

For investigation of organification efficiency, 450 mgs of sodium perchlorate were given by intravenous injection 30 minutes after injection of ^{123}I: uptake of ^{123}I was followed for a further 30 minutes after administration of sodium perchlorate. The effect of sodium perchlorate on the uptake curve in a patient with normal organification and in a patient with an organification defect is shown in Figure 7. The value of the 60 minute uptake measurement was taken as the post-perchlorate uptake and used for derivation of perchlorate discharge data.

2.2.8. Dosimetry

The dose of ^{131}I to be given to patients was calculated using an estimate of thyroid gland mass, and 24 hour uptake of ^{123}I, in an attempt to standardise treatment (100 μCi/g of thyroid tissue giving approximately 5000 Rad/thyroid). The formula for calculation of ^{131}I dose was:
10 x mass in g divided by 24 hour uptake% (mCi).
This dose of ^{131}I was always given orally the day after the tracer dose of ^{123}I used for calculation of 24 hour uptake.

In one group of patients the effective half-life of ^{131}I in the thyroid was measured, and this is discussed more fully later (3.6.1.). This involved counting neck radioactivity at several times (in practice 4 times over 2 weeks) after ^{131}I therapy and calculating the rate of decay of neck counts. For this purpose patients were seen 24 hours after administration of ^{131}I and a 24 hour uptake of the radioisotope calculated using the gamma camera/computer system with a high energy parallel hole collimator. Extrathyroidal activity was assumed to be neglible at this time. As before, an aliquot (1 mCi) of isotope was counted in an IAEA phantom for calibration purposes. Counts at this time were used as a reference point (100%) and subsequent counts expressed as a percentage of this to enable the effective half life of ^{131}I in the gland to be determined.

2.2.9. Analysis of data

Standard statistical methods were used throughout. Mean values are presented with accompanying standard error of the mean; in certain instances, where the data are clearly not distributed in a normal manner, the median point and interquartile ranges are given instead. Comparison of data has been performed using statistical tests where appropriate: where normal distribution of data cannot be assumed non parametric tests have been used. Individual analyses are detailed in the text.

SECTION THREE
VALIDATION OF RADIOISOTOPE TECHNIQUES

2.3.1. Introduction

The human studies presented in this thesis have relied heavily upon radioisotope uptake techniques and in a number of instances data derived in a serial manner from a large number of patients have been used. It was, therefore, of interest to examine some of the problems associated with thyroid radioisotope uptake measurement and to identify how these contributed to the accuracy of measurement.

2.3.2. Extrathyroidal neck radioactivity using thyroid uptake counter

In the previous chapter the use of a standard correction for extrathyroidal neck radioactivity in the measurement of thyroid uptake using an uptake counter was described. This is based on the assumption that extrathyroidal activity within the field of view of the counter is 7% of total body extrathyroidal activity at 2 minutes after the injection and 6% at 20 minutes after injection (Hilditch et al., 1967; Shimmins et al., 1969). These estimates were derived initially using ^{131}I and to ensure that they were appropriate for ^{123}I counting, extrathyroidal neck counts were measured in 15 subjects who had no thyroid uptake of radioiodide. These were 15 patients who were studied after radioiodine treatment. Imaging with the gamma camera showed no identifiable thyroid uptake in all cases and neck counts in these subjects were therefore assumed to represent extrathyroidal radioactivity: counts were expressed as percentage of injected counts measured in a standard thyroid phantom.

At 2 minutes after injection neck counts ranged from 4.4 - 8.4%, with a mean of 6 $\pm$ 1.2 (standard deviation); at 20 minutes the range was 4.2 - 8.3% with a mean of 5.6 $\pm$ 1.3.

Comment

These data confirm that there is decay in extrathyroidal activity between 2 and 20 minutes using ^{123}I. The figures from this small group of patients (6% at 2 minutes and 5.6% at 20 minutes) are not dissimilar to those found with ^{131}I (7 and 6%) and which are used in the routine calculation of uptake by the thyroid. It was assumed that for practical

TABLE 3

COUNT LOSS WITH SHIELDING OF SOURCE OF GAMMA EMISSION

a) UPTAKE COUNTER		SHIELDING (PERSPEX BLOCK)				
		0	0.9cm	2.2cm	3.3cm	4cm
^{123}I STANDARD IN IAEA PHANTOM:	COUNTS/60 SEC	39860	34320	27424	23552	20428
	% LOSS	0	12.8	30.3	40.2	48.0
b) GAMMA CAMERA						
^{123}I STANDARD IN IAEA PHANTOM:	COUNTS/60 SEC	5900	-----	5000	----	----
	% LOSS	0		18%		
^{131}I STANDARD IN IAEA PHANTOM:	COUNTS/60 SEC	1309	1240	1133	1039	1032
	% LOSS	0	5.3	13.4	20.6	21.2

purposes the derivation of thyroid uptake using the standard correction for extrathyroidal activity was satisfactory.

2.3.3. Effect of anatomical variation on accuracy of uptake measurement

In a large series of subjects it was felt possible that inter-individual variation in factors such as anatomical localisation of the thyroid might contribute to differences in uptake measurement. One possible source of error lies in the amount of soft tissue interposing between the neck counter and the thyroid gland. For both uptake counter and gamma camera the collimator is placed at a standard distance from the anterior surface of the neck and no account is taken of soft tissue thickness anterior to the thyroid. In order to estimate the degree of possible error due to this, a series of measurements was made, using the thyroid phantom containing an aliquot of either ^{123}I or ^{131}I. Perspex blocks, which have a density and elemental composition similar to human tissue were then interposed between the phantom and collimator and counts remeasured. The data derived from both uptake counter and gamma camera in this way are shown in Table 3. It will be noted that the thinnest perspex block used (0.9 cms) caused a fall in counts for both ^{123}I (14%) and ^{131}I (5%) using the uptake counter. Thicker blocks caused a progressive fall in count rate, so that at the 2.2. cms thickness a 30% loss of counts was noted for ^{123}I using the gamma counter.

The loss of counts was considerably less using the gamma camera system (20% loss at 2.2 cm thickness with ^{123}I and 13% for ^{131}I) reflecting different collimator geometry. The lower loss of counts with ^{131}I reflects the higher energy of gamma emission from this isotope.

Quantitative radioisotope uptake does not normally take account of inter-individual variability in morphology. The data indicate that interposition of tissue between the collimator and the thyroid does cause loss of counts. However, even in obese individuals it is unlikely that there will be greater than 0.9 cms of soft tissue interposing between the thyroid and collimator, and it is likely that the loss of counts due to this factor will be considerably less than 15%. This clearly should have no impact on serial measurements within an individual subject, and is unlikely to be a major source of error in inter-individual comparisons.

2.3.4. Loss of counting efficiency at high count rates

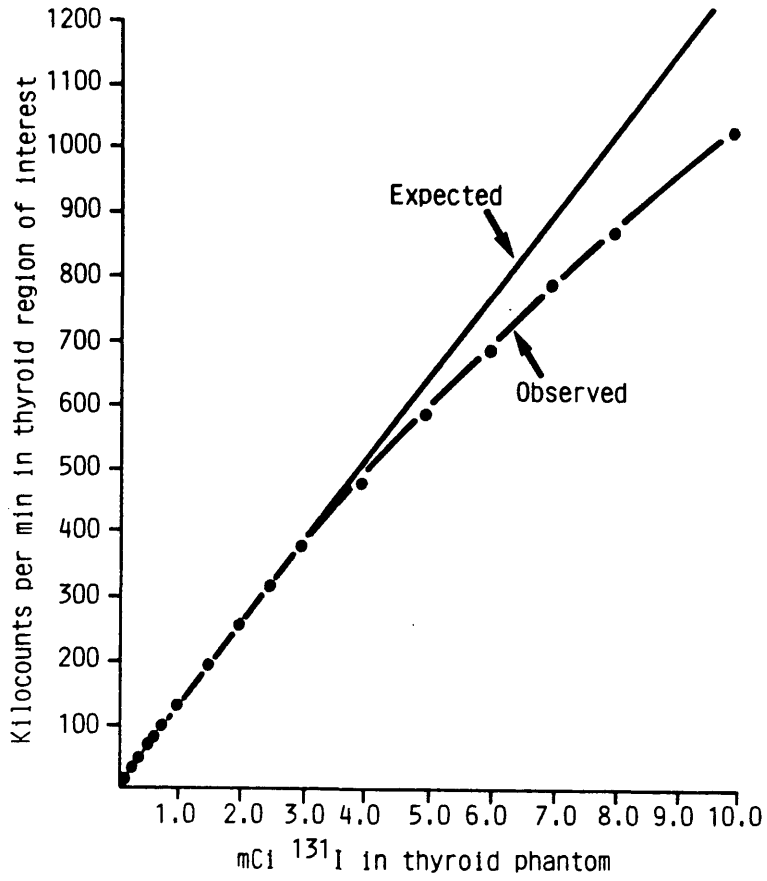


Fig 8

CORRECTION FOR GAMMA CAMERA/COMPUTER DEAD TIME

AT VERY HIGH COUNT RATES (eg AFTER ^{131}THERAPY) THE GAMMA CAMERA/COMPUTER MAY NOT REGISTER ALL COUNTS (DEAD TIME). COUNTS WERE ACQUIRED USING THE GAMMA CAMERA/COMPUTER FROM A SERIES OF THYROID PHANTOMS CONTAINING A RANGE OF ALIQUOTS OF ^{131}I FROM 100 uCi to 10 mCi. ACTUAL COUNTS OBSERVED WERE COMPARED WITH EXPECTED COUNTS FROM THE KNOWN ACTIVITY OF THE SAMPLE. AT LOW COUNT RATES THE ACTUAL AND EXPECTED COUNTS AGREED WELL, BUT THERE WAS LOSS OF COUNTING EFFICIENCY AT RATES OF $>$ 400 KILOCOUNTS/ MINUTE. THIS CURVE WAS USED AS A CALIBRATION CURVE TO CORRECT HIGH COUNTS FOR LOSS OF EFFICIENCY.

Serial measurement of neck counts were made after administration of therapeutic ^{131}I using the gamma camera/computer system. The high doses of radioactivity used (generally around 5 mCi) give rise to very high levels of gamma emission. At such high count rates, the ability of the gamma camera and computer to register and retain counts is crucial for accuracy, and the phenomenon of dead time at high count rates is known to lead to loss of counting efficiency (Adams, Jansen, Grames, et al., 1974). In an effort to quantitate this, and to permit correction of measured counts a calibration curve for loss of counting efficiency at high count rates was constructed.

A series of measurements were made by placing doses of ^{131}I ranging from 0.1 - 10 mCi in the neck phantom. Sixty second counts were acquired for each dose, and are plotted in Figure 8. It will be seen that although the initial increase in counts at low doses is linear (up to 3 mCi) deviation from this straight line becomes progressive as count rate rises above 400 000 counts per minute. This loss of linearity in counts plotted against known phantom content reflects loss of counting effiency of the gamma camera/computer. The calibration curve constructed from this allows correction for this loss of efficiency to be made. For example, a measured count rate of 800 000 counts per minute reflects a true count of 925 000 counts per minute. This correction was routinely applied in calculation of counts following ^{131}I therapy in derivation of effective half-life of ^{131}I.

2.3.5. Estimation of organification efficiency using spot uptake measurements

Accurate detection of organification defects for iodide has, in the past, involved the use of perchlorate discharge studies (1.3.5.). An alternative method was described by Hilditch et al. (1980) using sophisticated computer assisted analysis of the uptake curve following intravenous injection of tracer radioiodine. Both of these techniques involve the administration of perchlorate, and normally involve the use of a gamma camera/computer system. Examination of the uptake curve (Figures 5 and 7) indicates that in normal subjects uptake increases between 20 and 60 minutes. It has been noted previously that uptake at 20 minutes is largely a function of iodide trapping (IAEA, 1972) and that subsequent uptake reflects both trapping and organification. Where organification is inhibited, the initial portion of the uptake curve is normal, but little increase is seen between 20 and 60

minutes. The relationship between the 20 and 60 minute uptake is therefore crucially dependent upon thyroid iodide organification, and in an effort to find a simple test for organification efficiency this relationship was examined further in a small group of patients.

Seventeen subjects were studied: 10 were taking Carbimazole as antithyroid therapy for thyrotoxicosis, and 7 were known to be euthyroid on no drug therapy. None of the thyrotoxic subjects had a 20 minute uptake greater than 20%. The thyrotoxic subjects had ingested between 20 and 40 mg of carbimazole 4-6 hours before the study was performed. In all subjects 150 μCi of ^{123}I was injected intravenously and the counts acquired as described in the previous section (2.2.6.) using a gamma camera/computer system over the next 60 minutes. Corrections for background radioactivity was made as described, and uptake value at 20 minutes and 60 minutes calculated. After 60 minutes sodium perchlorate was injected intravenously (300 mg) and uptake followed for the next 30 minutes. The perchlorate discharge was expressed as percentage of 60 minute uptake discharged by perchlorate.

All subjects taking carbimazole had a positive discharge with perchlorate with a range of discharge of 10-63%. No discharge was seen in any normal subject. The change in uptake between 20 and 60 minutes expressed as a percentage of 20 minute uptake is shown in Figure 9. It will be noted that the percentage increase was less than 75% in all subjects who had a positive discharge, and greater than 60% in all subjects who had normal organification. In only one normal subject was the percentage increase less than 75%. The relationship between percentage increase uptake and measured perchlorate discharge is shown in Figure 10. It will be noted that there was a highly significant inverse correlation between the two variables ($r = -0.87$, $p < 0.001$).

These data indicate that the increase in uptake between 20 and 60 minutes gives a crude but fairly reliable measure of organification impairment. In all subjects where the ratio between 60 and 20 minute uptake was less than 1.6 (i.e. a percentage increase from the 20 minute uptake of less than 60%) an organification defect was present, and this was inversely correlated with the more accurately defined perchlorate discharge. A ratio of greater than 1.75 between 60 and 20 minute uptake measurements was invariably associated with normal organification. No subject in this

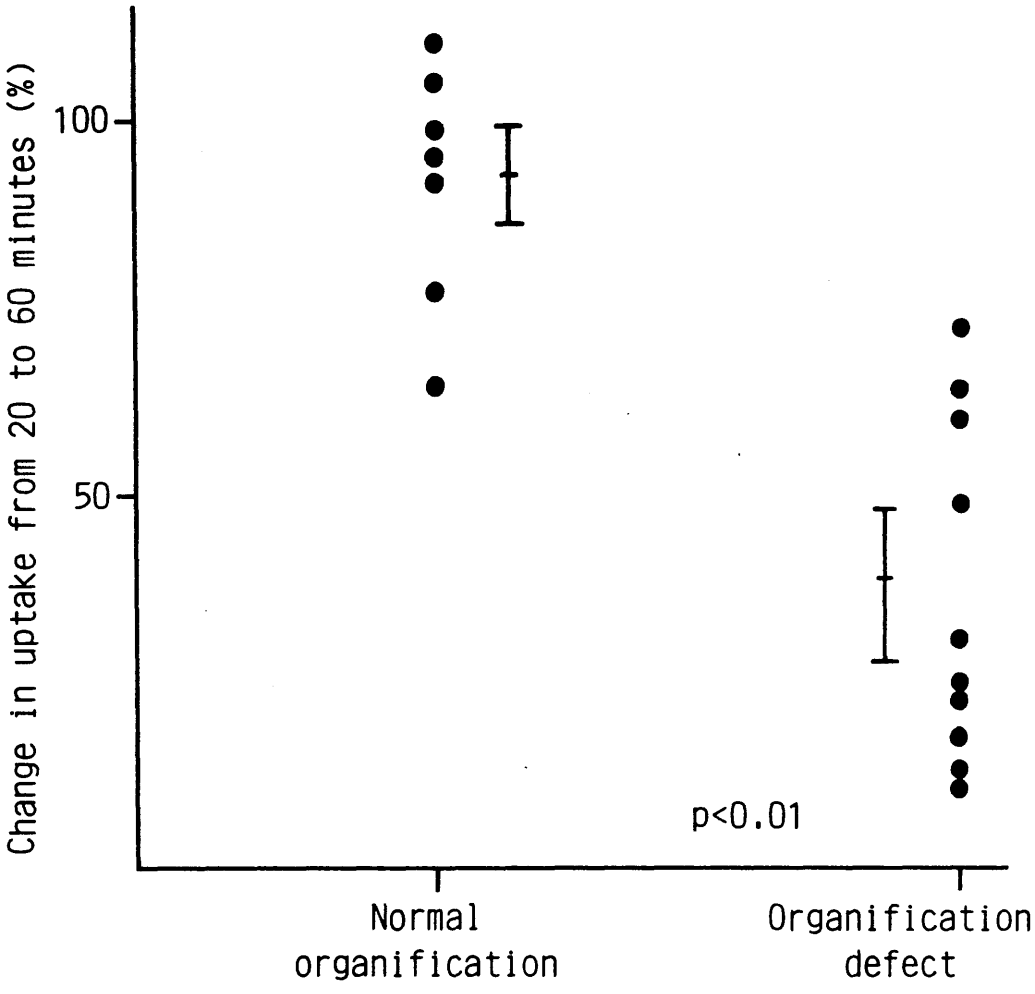


Fig 9

CHANGE IN THYROID UPTAKE OF ^{123}I FROM 20 TO 60 MINUTES

UPTAKE OF ^{123}I BY THE THYROID AFTER INTRAVENOUS INJECTION OF ISOTOPE AT 20 AND 60 MINUTES IN NORMAL SUBJECTS AND THYROTOXIC PATIENTS TAKING A BLOCKING DOSE OF CARBIMAZOLE. THE % INCREASE IN UPTAKE BETWEEN 20 AND 60 MINUTES WAS SIGNIFICANTLY LOWER ($p < 0.01$) IN THE SUBJECTS WITH IATROGENIC ORGANIFICATION BLOCK.

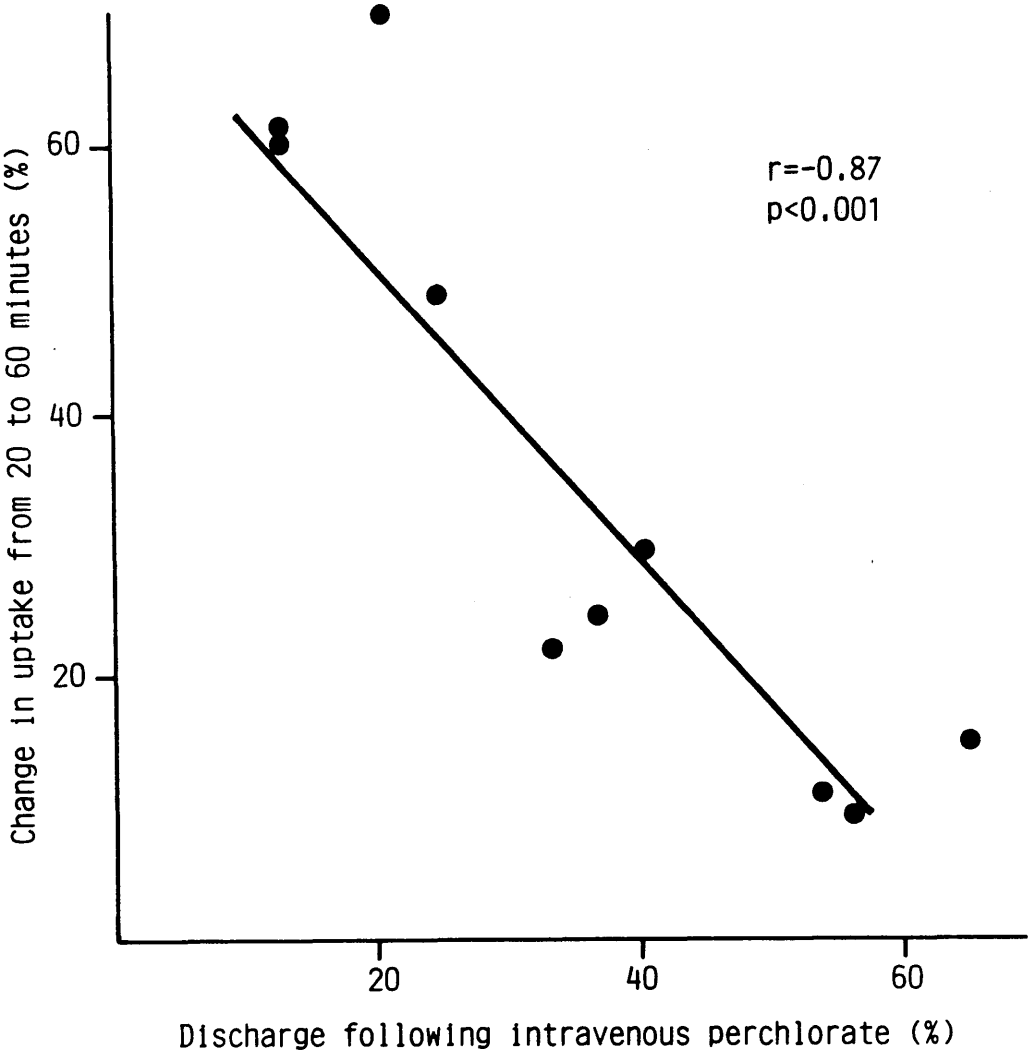


Fig 10

RELATIONSHIP BETWEEN CHANGE IN UPTAKE OF ^{123}I FROM 20 TO 60 MINUTES AND DISCHARGE OF ^{123}I BY PERCHLORATE

THYROIDAL UPTAKE OF ^{123}I WAS MEASURED FOR 60 MINUTES AFTER INTRAVENOUS INJECTION OF ISOTOPE (8CuCi) IN 10 THYROTOXIC SUBJECTS 4–6 HOURS AFTER INGESTION OF 40 mg CARBIMAZOLE. AT 60 MINUTES AFTER ^{123}I INJECTION SODIUM PERCHLORATE 300ug WAS GIVEN INTRAVENOUSLY, AND UPTAKE FOLLOWED FOR A FURTHER 30 MINUTES. THERE WAS A CLOSE INVERSE RELATIONSHIP BETWEEN THE INCREASE IN UPTAKE FROM 20 TO 60 MINUTES AFTER ISOTOPE INJECTION AND THE POST PERCHLORATE DISCHARGE ($r = -0.87$).

study had a 20 minute uptake of greater than 20%: in thyrotoxicosis uptake may often be very rapid with organification making a major contribution to the 20 minute uptake (IAEA, 1972), and in this circumstance the ratio between 20 and 60 minute uptake will be falsely low. This index of organification is therefore not appropriate where initial (i.e. 20 minute) uptake is high. It is therefore suggested that in subjects with normal initial (i.e. 20 minute) uptake measurements, the ratio of 60 to 20 minute uptakes (less than 1.6) may provide a crude index of the presence of an organification defect. This can be performed easily using a thyroid uptake counter without the need to administer perchlorate. This simple measurement is used later in the thesis (3.2.6.) to give an estimate of organification efficiency following ^{131}I therapy in thyrotoxic subjects.

CHAPTER THREE

STUDIES WITH RADIOACTIVE IODINE (^{131}I) IN THE TREATMENT OF THYROTOXICOSIS.

SECTION ONE
IMMEDIATE EFFECTS OF ^{131}I ON THYROID HORMONE CONCENTRATIONS IN THYROTOXIC SUBJECTS.

3.1.1. Introduction

Several authors have reported worsening of clinical features of thyrotoxicosis after ^{131}I therapy, and in a number of instances thyroid hormone levels have been reported to rise in such patients (Creutzig et al. 1976; Shafer and Nuttal, 1975; Tamagna et al. 1979; Viherkoski, 1970). Indeed, Parker and Lawson (1973), in reviewing Scottish mortality statistics, reported that a number of deaths appeared to follow ^{131}I therapy in elderly patients. The potential rise in circulating thyroid hormone concentrations and associated exacerbation of thyrotoxicosis may reflect irradiation thyroiditis, with release of preformed T3 and T4 into the circulation (Einhorn et al. 1967). In that instance it might be expected that the rise in circulating hormone levels might be accompanied by changes in circulating concentrations of thyroglobulin and, at some stage, by immunological changes. Accordingly, the effect of ^{131}I therapy on concentrations of T3 and T4 have been examined in a group of thyrotoxic subjects, and the relationship between these changes and circulating concentrations of thyroglobulin and TSH receptor directed antibodies (TRAb) has been assessed. In addition, the effect of carbimazole treatment before ^{131}I administration on these variables has been examined in a serial manner following ^{131}I treatment.

3.1.2. Patients and methods

Twenty-four patients with thyrotoxicosis were studied. All had diffuse enlargement of the thyroid, and at the time of initial diagnosis had elevation of both T4 and T3. In all subjects the uptake of ^{123}I 20 minutes after intravenous injection of the tracer was elevated.

Patients were assigned randomly to two groups: either immediate

treatment with ^{131}I or initial therapy with carbimazole followed by ^{131}I. In this latter group carbimazole (10-15 mgs twice daily with Thyroxine 0.1 mg per day) was given for a minimum of 3 months. At the time of ^{131}I therapy all patients in this group were clinically and biochemically euthyroid, and none had elevation of TSH. All drugs were discontinued 48 hours before a pre ^{131}I therapy tracer uptake study (using ^{123}I) and therefore 72 hours before ^{131}I administration.

Blood samples were drawn for measurement of T3, T4, thyroglobulin concentration and TRAb. Serum from each subject was frozen and stored at -20°C until subsequent analysis of thyroglobulin and TRAb in a single assay. Derivation and performance of these assays has already been considered. Blood samples were taken immediately before ^{131}I administration and at 2, 6, 9, 16, 23, 42 and 70 days after therapy. T3 and T4 were measured at all of the above time points, whereas thyroglobulin and TRAb were measured only in basal samples, at 2 days and 42 days after ^{131}I treatment. The dose of ^{131}I was calculated using data derived from the ^{123}I tracer study as described (2.2.8.) Comparison of group means was made using the Mann Whitney U Test. Linear correlations were calculated using the sum of least squares method.

3.1.3. Results

Further details of the patients studied are shown in Table 4. The groups were similar with regard to age, goitre size, and dose of ^{131}I given. By definition, in the group given ^{131}I alone all patients were clinically and biochemically thyrotoxic whereas those in the group pretreated with carbimazole were euthyroid at the time of ^{131}I administration.

Serum thyroxine

Individual and mean values for serum thyroxine in both groups of subjects are shown in Table 5 and Figure 11. It will be noted, in the group of subjects given ^{131}I alone that serum thyroxine rose in 5 out of 12 subjects two days after therapy, but the mean change in serum T4 was insignificant. Thereafter, mean serum T4 fell gradually so that by 10 weeks after treatment was within the normal range. In all subjects in whom serum T4 rose after treatment, the maximum change and peak level occurred within the first two days.

TABLE 4

DETAILS OF PATIENTS STUDIED: IMMEDIATE EFFECTS OF ^{131}I ON THYROID HORMONE CONCENTRATIONS

	NO.	SEX	AGE (yr)	^{123}I UPTAKE (20 MIN) BEFORE ^{131}I (%)	ESTIMATED THYROID SIZE (G)	DOSE OF ^{131}I (mCi)
PATIENTS GIVEN ^{131}I ALONE	12	11F : 1M	49.5[x] ± 6.4	24.1 ± 9.6	40 ± 16.7	4.7 ± 1.9
PATIENTS PRETREATED WITH CARBIMAZOLE	12	10F : 2M	51 ± 5.1	28.5 ± 16.1	51.2 ± 22	6.4 ± 3.5
ALL SUBJECTS	24	21F : 3M	50 ± 5.7	26.3 ± 13.1	45.5 ± 21.0	5.6 ± 2.9

[x] MEAN ± SD

TABLE 5

CHANGES IN SERUM THYROXINE AND TRIIODOTHYRONINE IMMEDIATELY AFTER ^{131}I TREATMENT

(A) PATIENTS GIVEN ^{131}I AS SOLE TREATMENT

	THYROXINE (nmol/l)					TRIIODOTHYRONINE (nmol/l)				
PATIENT NO.	BEFORE ^{131}I	DAYS AFTER ^{131}I 2	6	9	16	BEFORE ^{131}I	DAYS AFTER ^{131}I 2	6	9	16
1	170	204	198	175	168	3.79	4.0	2.95	2.98	2.59
2	316	287	175	182	197	7.9	8.25	7.0	5.3	3.66
3	168	140	129	124	109	3.41	2.55	2.6	2.68	1.89
4	270	212	238	220	191	7.3	6.0	5.72	7.03	5.0
5	292	316	230	218	200	11.5	9.87	7.65	6.78	4.05
6	280	298	216	244	220	6.85	8.9	6.12	5.08	5.42
7	228	199	173	180	169	6.0	7.05	5.42	5.05	4.99
8	216	257	227	210	168	4.4	5.85	3.6	4.2	3.2
9	168	206	172	171	161	4.7	4.0	4.45	5.15	3.2
10	246	246	167	150	120	6.58	7.5	4.2	3.34	4.11
11	219	200	151	139	120	4.10	3.8	3.3	2.45	2.02
12	186	182	168	155	136	3.85	3.95	2.62	2.58	2.0

TABLE 5

(B) PATIENTS MADE EUTHYROID WITH CARBIMAZOLE BEFORE ^{131}I

PATIENT NO.	THYROXINE (nmol/l) BEFORE ^{131}I	DAYS AFTER ^{131}I 2	6	9	16	TRIIODOTHYRONINE (nmol/l) BEFORE ^{131}I	DAYS AFTER ^{131}I 2	6	9	16
13	97	109	97	97	93	2.18	2.38	1.48	1.8	1.6
14	126	127	72	67	40	1.8	1.65	1.14	1.11	1.21
15	121	120	112	89	123	1.5	1.5	1.6	1.4	1.26
16	56	56	74	87	103	2.88	2.88	2.69	1.92	2.02
17	117	112	101	103	147	1.7	2.61	2.68	3.1	2.93
18	118	109	122	130	92	3.23	2.2	3.6	2.9	2.2
19	105	90	84	117	111	1.38	0.95	1.1	1.84	1.39
20	148	132	131	127	95	2.15	3.73	2.54	1.85	1.51
21	124	136	130	130	135	2.35	2.96	2.04	2.19	1.58
22	120	112	116	130	141	1.52	1.36	1.32	1.35	1.37
23	115	122	121	141	123	1.75	2.10	2.13	1.9	1.95
24	136	153	137	87	92	3	4.05	2.84	2.27	1.73

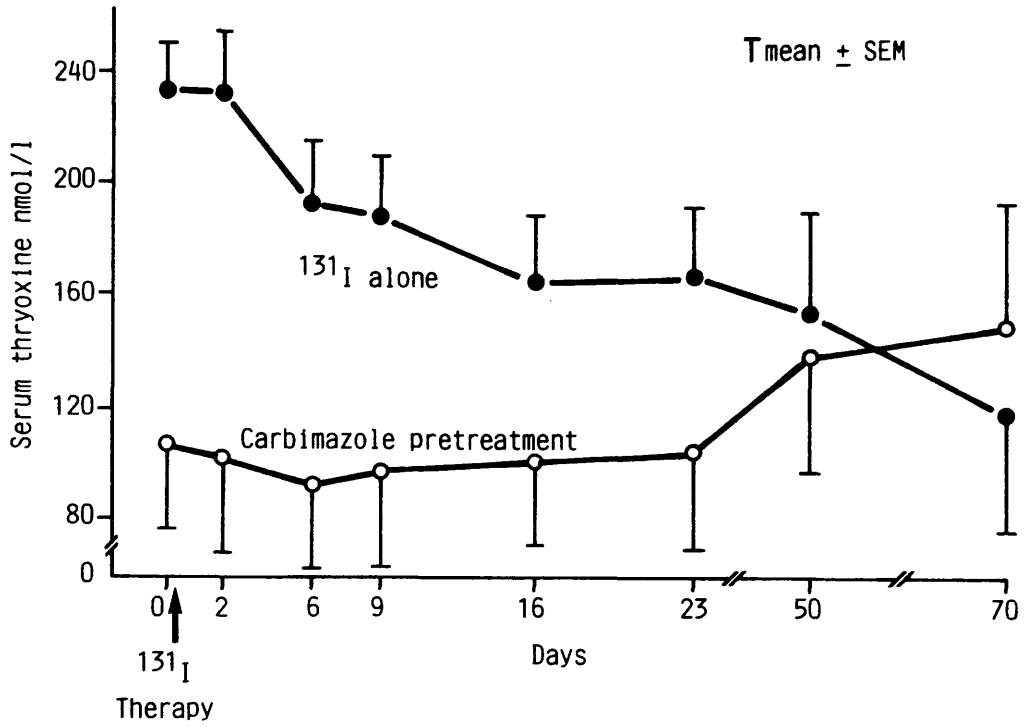


Fig 11

EFFECT OF ^{131}I ON SERUM THYROXINE CONCENTRATIONS

THE EFFECT OF ^{131}I THERAPY ON SERUM THYROXINE CONCENTRATIONS IS SHOWN. 12 SUBJECTS WERE GIVEN ^{131}I AS SOLE TREATMENT, AND 12 WERE MADE EUTHYROID BEFORE ^{131}I WITH CARBIMAZOLE. ^{131}I HAD NO NET EFFECT ON HORMONE LEVELS IMMEDIATELY AFTER ADMINISTRATION, ALTHOUGH IN APPROXIMATELY 50% OF SUBJECTS AN ACUTE INCREASE WAS OBSERVED (SEE TABLE 5).

In the group pretreated with carbimazole, there was again no net change in serum T4 immediately after therapy, although in a small number of patients (4) concentrations rose. Thereafter, mean serum thyroxine levels rose gradually so that by 10 weeks after treatment mean levels were higher than in the group given ^{131}I alone. This reflected a progressive increase in serum thyroxine concentrations to frankly elevated values in 6 of the 12 subjects studied in this group.

Tri-iodothyronine

In the group given ^{131}I alone serum T3 rose in 7 out of 12 subjects and fell in the remaining 5 by 2 days after therapy: mean T3 was higher although this did not reach statistical significance for the group (Table 5, Figure 12). Serum T3 concentrations fell gradually in all subjects thereafter.

Serum T3 concentrations rose within two days of ^{131}I therapy in 6 out of 12 subjects pretreated with carbimazole. As with serum thyroxine concentrations, mean serum T3 concentrations thereafter rose so that by 10 weeks after therapy mean value in this group was significantly higher ($p < 0.05$) than in subjects given ^{131}I alone.

Following the immediate changes in serum T4 and T3 after ^{131}I therapy, patients in the group given ^{131}I alone showed a slow decline in mean thyroid hormone concentrations. In this group 5 subjects remained thyrotoxic by 10 weeks after treatment. Despite this, in all subjects concentrations of both T4 and T3 were lower at this time than before therapy and 4 subjects were hypothyroid (having been euthyroid 9, 16, 23 and 42 days after ^{131}I). Three subjects were euthyroid 10 weeks after therapy and remained so during subsequent follow-up.

In the group pretreated with carbimazole 6 subjects had elevated serum thyroxine levels 10 weeks after treatment, although in only 2 was there clinical evidence of thyrotoxicosis. One subject was hypothyroid and the remainder of this group was euthyroid at this time.

Serum thyroglobulin

Serum thyroglobulin levels were elevated before treatment in only 5 of the subjects given ^{131}I alone and in 3 of the subjects pretreated with Carbimazole. Thyroglobulin levels following ^{131}I therapy are shown in Figure 13. As endogenous antibodies to thyroglobulin can compete for ligand in the

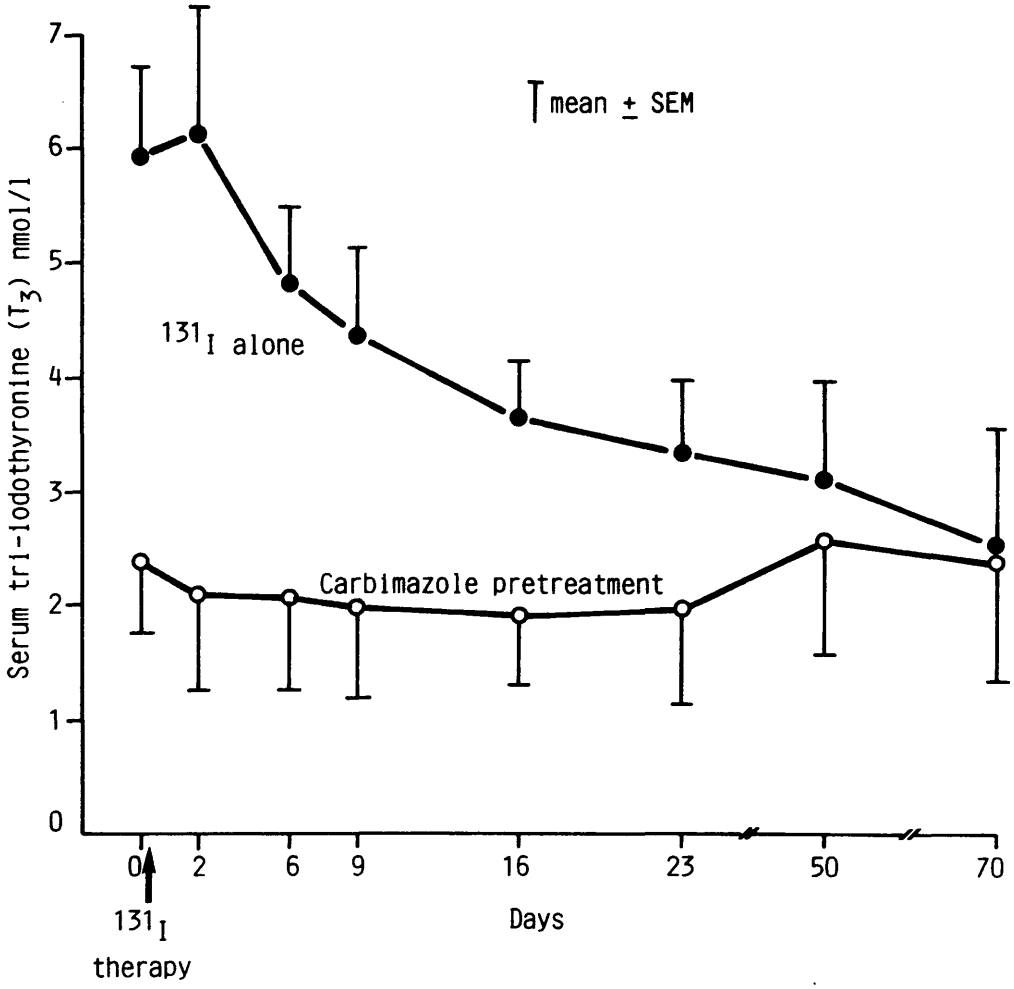


Fig 12

EFFECT OF ^{131}I ON SERUM TRIIODOTHYRONINE

THE EFFECT OF ^{131}I THERAPY ON SERUM TRIIODOTHYRONINE CONCENTRATIONS IS SHOWN. 12 SUBJECTS WERE GIVEN ^{131}I AS SOLE TREATMENT, AND 12 WERE MADE EUTHYROID BEFORE ^{131}I WITH CARBIMAZOLE. IMMEDIATELY AFTER ^{131}I ADMINISTRATION THERE WAS A RISE IN HORMONE CONCENTRATIONS IN APPROXIMATELY 50% OF SUBJECTS, ALTHOUGH NO CHANGE IN MEAN HORMONE LEVELS OCCURRED.

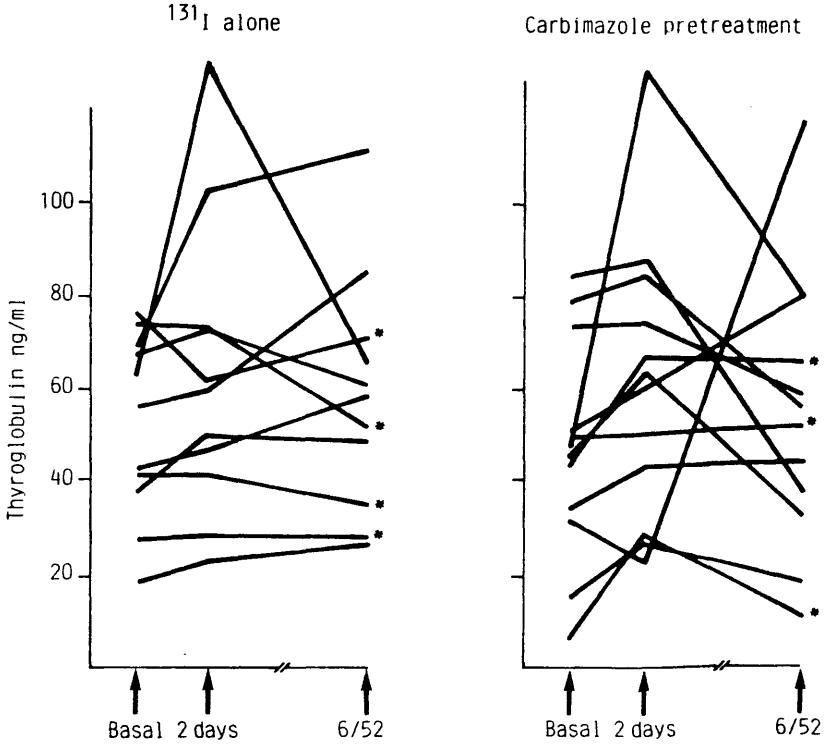


Fig 13

SERUM THYROGLOBULIN CONCENTRATIONS BEFORE AND AFTER ^{131}I TREATMENT

SERUM THYROGLOBULIN CONCENTRATIONS BEFORE, 2 AND 42 DAYS AFTER ^{131}I TREATMENT. 12 SUBJECTS WERE GIVEN ^{131}I AS SOLE THERAPY (LEFT HAND PANEL) AND 12 WERE MADE EUTHYROID WITH CARBIMAZOLE BEFORE ^{131}I. ANTI-THYROGLOBULIN ANTIBODIES WERE DETECTED IN THOSE SUBJECTS MARKED *.

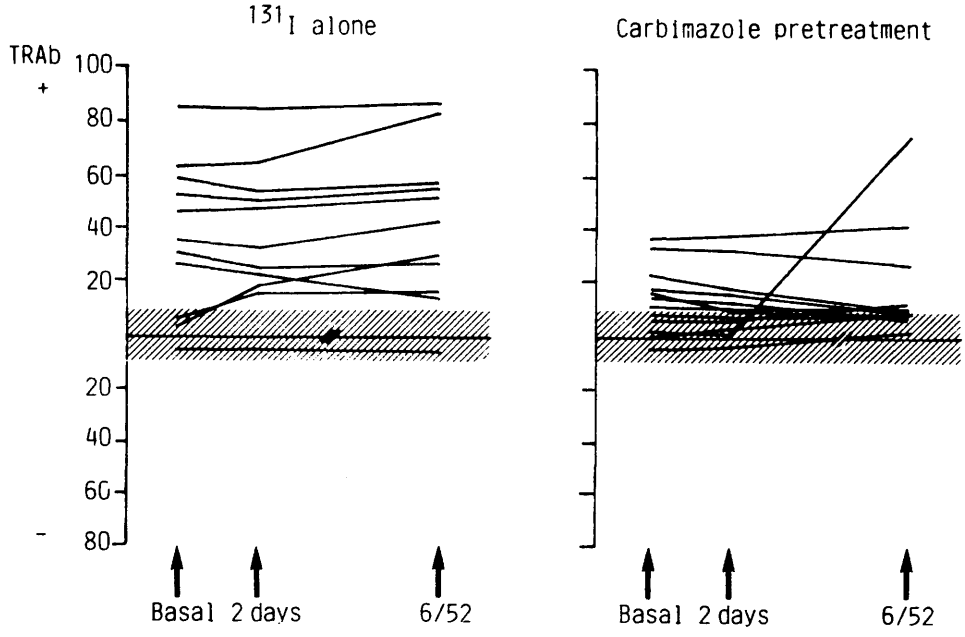


Fig 14

CHANGE IN TSH-RECEPTOR DIRECTED ANTIBODY FOLLOWING ^{131}I THERAPY

SERUM LEVELS OF TSH DISPLACING ANTIBODY (TRAb) BEFORE, 2 AND 42 DAYS AFTER ^{131}I THERAPY. 12 SUBJECTS WERE GIVEN ^{131}I AS SOLE THERAPY (LEFT HAND PANEL) AND 12 WERE GIVEN CARBIMAZOLE BEFORE ^{131}I. THE SHADED AREA REPRESENTS THE RANGE OF VALUES FOUND IN NORMAL SUBJECTS. VALUES IN THE CARBIMAZOLE PRE-TREATED SUBJECTS BEFORE ^{131}I WERE SIGNIFICANTLY LOWER ($p < 0.01$, MANN WHITNEY U TEST) THAN THOSE IN THE ^{131}I-ALONE GROUP.

radioimmunoassay used, antithyroglobulin antibodies were measured in serum before and six weeks after ^{131}I (Izumi and Larsen, 1978): patients who had positive results are identified in Figure 13. In only 3 subjects in the group given ^{131}I alone did serum thyroglobulin rise 48 hours after ^{131}I therapy, and in one of these subjects a further rise was seen during the next 6 weeks. Thyroglobulin levels were higher 6 weeks after ^{131}I therapy than before treatment in 6 subjects.

In the subjects pretreated with carbimazole serum thyroglobulin rose 48 hours after ^{131}I therapy in 6 subjects, and in only one of these subjects did a further rise occur at 6 weeks. Little change was seen in thyroglobulin in the remaining subjects. In 6 out of 12 subjects serum thyroglobulin was higher 6 weeks after therapy than before treatment with ^{131}I. The change in thyroglobulin was not different in this group compared to those patients given ^{131}I alone, and rise which occurred in most subjects in either group was small.

TRAb

Changes in TRAb following ^{131}I therapy are shown in Figure 14. TRAb titres were elevated in 9 out of 12 subjects given ^{131}I alone and in 6 out of 12 of those pretreated with carbimazole (taken immediately before ^{131}I administration): mean concentration was higher ($p < 0.01$) in the former group. After ^{131}I therapy a major change in TRAb occurred in only one subject in the group pretreated with Carbimazole and no change was noted in the group given ^{131}I alone.

3.1.4. Relationship between changes in thyroid hormone levels, thyroglobulin and TRAb after ^{131}I therapy.

^{131}I alone

Changes in T3 and T4 immediately (within 48 hours) after ^{131}I were positively related ($r = 0.44$, $p < 0.05$). There was no relationship between change in serum thyroglobulin and T3 or T4, or between TRAb and T3 or T4 (summarised in Table 6). Similarly, neither size of dose nor pretreatment goitre size was related to subsequent changes in any of the above variables. Subsequent (at 6 weeks) changes in T3 and T4 were highly correlated ($r = 0.88$, $p < 0.01$).

TABLE 6

CORRELATIONS BETWEEN THYROID HORMONES, THYROGLOBULIN AND TRAb FOLLOWING ^{131}I THERAPY (r values shown).

a) TWO DAYS AFTER ^{131}I	PATIENTS GIVEN ^{131}I ALONE	PATIENTS PRETREATED WITH CARBIMAZOLE	ALL SUBJECTS
T_3 v T_4	0.44 ($p<0.05$)	0.34	0.2
THYROGLOBULIN (% CHANGE) v T_3 (% CHANGE)	0.21	0.06	0.18
THYROGLOBULIN (% CHANGE) v T_4 (% CHANGE)	0.12	0.04	0.05
DOSE (mCi) v T_3 (CHANGE)	0.23	0.01	0.11
DOSE (RADS) v T_3 (CHANGE)	0.01	0.08	0.03
b) SIX WEEKS AFTER ^{131}I			
T_3 v T_4	0.88 ($p<0.001$)	0.13	0.33
THYROGLOBULIN (% CHANGE) v TRAB (% CHANGE)	0.46	0.14	0.34
THYROGLOBULIN (% CHANGE AT TWO DAYS) v TRAB (% CHANGE AT 6 WEEKS)	0.14	0.28	0.18

Carbimazole pretreatment

No relationship was seen between change in T3 and T4 either immediately or 6 weeks after ^{131}I therapy (Table 6); further, changes in serum thyroglobulin, TRAb, dose of ^{131}I given, and pretreatment thyroid size were not related to changes in T3 or T4 after ^{131}I therapy.

3.1.5. Discussion

Immediate changes in T4 and T3

Early after ^{131}I therapy both T3 and T4 concentrations rose in approximately 50% of patients studied: the percentage change from basal in both hormones was similar in the two groups of patients (^{131}I alone and carbimazole pretreatment). In no subject was the rise in thyroid hormone concentration associated with adverse clinical consequences. In all subjects in whom T3 or T4 rose as a consequence of ^{131}I treatment this was greatest within 48 hours of therapy. There have been a few reports of worsening of biochemical features of thyrotoxicosis following ^{131}I therapy (Creutzig et al. 1976; Lamberg, 1959; Shafer and Nuttal, 1975; Tamagna et al. 1979; Viherkoski et al. 1970). In one study of 44 patients, serial measurements of T3 and T4 were made during the first week after ^{131}I: only minor changes in T3 were noted, and these were not related to alteration in clinical state (Creutzig et al. 1976). In a study of 13 patients given ^{131}I therapy, Shaffer and Nuttal (1975) reported that T3 rose in a number of patients 5 days after treatment, although measurement of thyroid hormone concentrations was not made at earlier times. In contrast Tamagna and colleagues (1979) reported that in 14 patients given ^{131}I neither mean T3 nor T4 levels changed, although hormone concentrations in 3 subjects showed a small rise at 10 days after therapy. The current study examined the course of thyroid hormone levels after ^{131}I more fully than these previous series and showed a temporary worsening of biochemical thyrotoxicosis in approximately 50% of patients studied. This was shown to be maximal within 48 hours after treatment. The change in T4 was related to that of T3 only in the group of patients given ^{131}I alone. This may reflect the altered relationship between T4 and T3 in the carbimazole pretreated group before ^{131}I therapy. After ^{131}I therapy there was a tendency for T4 to rise and for T3 to fall in this

latter group accounting for the lack of relationship between the two variables.

The cause of the early rise in thyroid hormone concentration after ^{131}I therapy may reflect irradiation thyroiditis with release of preformed thyroid hormone from the colloid of the gland into the circulation. This would be supported by the rapidity of the rise following ^{131}I administration. However, no consistent change in serum thyroglobulin levels following ^{131}I therapy was observed although in 50% of subjects levels were higher after than before ^{131}I: it might be expected that irradiation thyroiditis would lead to release of thyroglobulin from the colloid of the gland. Indeed, in studies of the effect of ^{131}I therapy on serum thyroglobulin levels, other authors (Izumi and Larsen, 1978) have reported that thyroglobulin rose as early as two days after treatment.

The measurement of thyroglobulin in serum using conventional double antibody radioimmunoassay techniques is complicated by the presence of endogenous antithyroglobulin antibodies which compete with the assay antibody for ligand, giving inaccurate results (Izumi and Larsen, 1978). In view of this, most reports of change on serum thyroglobulin after ^{131}I have included only patients who were negative for antithyroglobulin antibody (Feldt-Rasmussen et al. 1982; Gardner et al. 1979; Ullere and Van Herle, 1978). In the present study patients were screened for antithyroglobulin antibodies before and six weeks after ^{131}I therapy: only seven positive results were found (identified in Figure 16). In the majority of patients, therefore, it seems unlikely that the presence of antithyroglobulin antibody in serum interfered with assay performance.

It is possible that the assay used to detect circulating thyroglobulin in the current study was not capable of detecting relatively small changes in concentration. However, the reported sensitivity of the assay in the study of Gardner et al. (1979) was similar to that in the current report, and the cause of the failure to observe a more significant rise in thyroglobulin is therefore uncertain. One possible explanation is that the dose of ^{131}I used in the current study was rather lower than that in the series reported by Gardner: despite this, however, T3 or T4 levels rose in more than 50% of the subjects studied in the current series and a significant proportion of patients subsequently became hypothyroid. Additionally, serum thyroglobulin was measured on only two occasions, and it is possible that a rise in levels

was missed. However, if the rise in T4 and T3 reflects irradiation thyroiditis, serum thyroglobulin concentrations might be expected to rise at the same time as T4 and T3.

Carbimazole pretreatment did not affect the percentage change in either T3 or T4 in subjects in whom levels rose after ^{131}I therapy, although absolute changes in both hormones were less than the group given ^{131}I alone. This smaller absolute rise in T3 and T4 may reflect the depleted intrathyroidal pool of T3 and T4 in subjects pretreated with carbimazole. Thus, in only 4 subjects from this group did T3 concentrations rise outwith the normal reference range immediately after ^{131}I therapy.

In none of the subjects studied either with this protocol, or, indeed, in any of the subjects included in this thesis, did abrupt worsening of clinical condition follow ^{131}I therapy. This contrasts with experience reported from Scandinavia where Lamberg (1959) and Viherkoski (1970) reported two series of patients given ^{131}I therapy in whom there was an appreciable incidence of thyroid storm immediately after treatment. In all of the affected patients there were, however, other complicating medical conditions such as congestive cardiac failure, and the patients were all elderly. In contrast, the majority of patients included in this study were aged less than 60 years and none had significant intercurrent illness. If thyroid storm is consequence of an immediate rise in T3 and T4 levels following ^{131}I administration, pretreatment with carbimazole limited that potential increase by reducing basal hormone levels and provides a rational basis for use of antithyroid drugs as preparatory treatment before ^{131}I administration in subjects at risk of clinical deterioration in the event of a rise in T3 or T4; such patients would include the elderly, subjects with thyrocardiac disease or subjects with other major intercurrent illness. This would be consistent with the observation of Viherkoski et al. (1970) that worsening of clinical features of thyrotoxicosis after ^{131}I was not seen in patients pretreated with carbimazole.

3.1.6. Changes in TRAb status

Basal TRAb measurement was elevated in all but three of the subjects given ^{131}I alone, and in seven of the subjects given carbimazole before ^{131}I treatment. Thus, mean TRAb levels were lower in the group given carbimazole, suggesting that the drug had reduced the level of immunological

stimulation of the gland. This effect of carbimazole was reported initially by McGregor and colleagues (McGregor, Peterson, Capifferi, et al. 1979) and has since been confirmed by other workers (Fenzi et al. 1979; Bech and Madsen, 1980). In the group of subjects reported in the current study ^{131}I treatment had no major effect on TRAb levels. Atkinson and colleagues (Atkinson, McGregor, Kendall-Taylor, et al. 1982) reported that both bioassayable and immunologically detectable thyrotrophin receptor stimulating antibody rose after ^{131}I treatment. Their study showed that the percentage of positive values using both assays increased following therapy, and that at three months after treatment the mean value using both assay systems was higher. Similarly, Bech and Madsen (1980) using a bioassay method to measure thyroid stimulating immunoglobulin showed that there was an initial fall in titre within the first 2 weeks after ^{131}I therapy, but this was followed by a highly significant and sustained rise.

The negative finding in the current study contrasts with these results. The method used to measure TRAb is based on that reported by Atkinson et al. (1982), and was clearly able to detect increased activity in the basal state. The cause of the rise in antibody titre reported by other workers following ^{131}I treatment may reflect release of thyroid antigen leading to stimulation of helper T-lymphocytes and consequent increase in antibody production, and it may be that this would not become evident within 2-42 days of ^{131}I administration. An alternative explanation for the rise in antibody following ^{131}I is that suppressor T-lymphocytes within the thyroid gland are more susceptible than other immunocompetent cells to irradiation damage (McGregor et al. 1979), and that ^{131}I therapy therefore causes further abnormality of the helper-suppressor T-cell ratio leading to a rise in antibody production. However, in only 2 subjects in the current study were TRAb values at six weeks after treatment significantly higher than before ^{131}I administration. For reasons which are not clear this contrasts with experience reported by other workers, but may be a consequence of timing of measurements.

3.1.7. Summary

In summary, serial measurements have shown that in around 50% of patients given ^{131}I treatment there is a temporary rise in both T3 and T4, and that this reaches a maximum within 48 hours of ^{131}I administration.

Carbimazole pretreatment lowered basal levels of both hormones but appeared not to affect significantly the proportional increase following irradiation. No evidence of clinical deterioration in any subject was seen after ^{131}I therapy. There was no relationship between change in thyroid hormone levels and either circulating thyroglobulin or TRAb following ^{131}I administration.

SECTION TWO

RELATIONSHIP BETWEEN THYROID ISOTOPE UPTAKE MEASUREMENTS AND BIOCHEMICAL AND CLINICAL OUTCOME FOLLOWING ^{131}I THERAPY.

3.2.1. Introduction

The clinical and biochemical outcome after treatment of patients with thyrotoxicosis using subablative dose of ^{131}I is uncertain. Thus, patients may either become euthyroid after a variable period of time or remain thyrotoxic; of the former group of patients, some progress to hypothyroidism, some have a relapse of thyrotoxicosis and the remainder remain euthyroid. In addition, immediately after ^{131}I therapy there may be delay of up to several months before the residual capacity for thyroid hormonogenesis is evident. Because of this difficulty in determining the immediate effect of ^{131}I treatment on thyroid function, some efforts have been directed to examining other measures of thyroid status in an attempt to predict outcome early after ^{131}I administration. Myant (1953 a + b) examined the prognostic value of measurement of thyroid uptake of tracer ^{131}I following doses of the same isotope. He reported that at 3 weeks after therapy, thyroid uptake 24 hours after oral tracer administration had fallen in a high proportion of patients, and that subsequent measurements showed partial recovery of this function in the majority of these. He noted that remission from thyrotoxicosis was less likely to develop in those patients who showed no fall in uptake 3 weeks after therapy. Since then, a small number of authors have made similar measurements following ^{131}I therapy (Einhorn and Hastad, 1961; Franco et al. 1970; Larsson, 1955; Sagel et al. 1973). These studies, using a variety of tracer isotopes and techniques of uptake measurement have, in the majority of instances, lacked precise biochemical definition of thyroid hormone levels. In the current study, repeated measurements of early (20 min) thyroid uptake of ^{123}I after intravenous administration of tracer have been made following ^{131}I therapy in a group of patients with thyrotoxicosis. These have been compared with changes in biochemical function over the first year after treatment.

3.2.2. Patients and methods

Fifty-five patients with thyrotoxicosis were treated with ^{131}I. Forty-two had Graves' disease and the remainder had multi-nodular goitre. Twenty-four patients were made euthyroid with carbimazole (30 mg per day) before ^{131}I administration. To prevent iatrogenic hypothyroidism T3 supplements (60 µg)/day were also given to this group of patients. All drugs were discontinued 5 days before ^{131}I therapy. The remaining 31 patients received ^{131}I treatment alone. Further details of the patients studied is given in Table 7.

Patients were seen on the day immediately before ^{131}I treatment, and assessed clinically. Blood was drawn for measurement of thyroid hormone levels, and thyroid uptake of ^{123}I was measured 20 minutes after intravenous administration of the tracer: a further uptake measurement was made at 24 hours. The thyroid was imaged using the gamma camera, and the planar surface area of the scan used to determine thyroid mass. The dose of radioiodine administered was calculated using the method described previously (2.2.8.) Details of thyroid size, uptake and doses of ^{131}I given are shown in Table 8.

Following ^{131}I therapy patients were seen every 4 weeks for a period of one year. On each occasion (unless permanent hypothyroidism had supervened) patients were assessed clinically and blood was drawn for measurement of thyroid hormone levels. Thyroid uptake of ^{123}I was measured 20 and 60 minutes after intravenous administration of the isotope. Patients with permanent hypothyroidism were started on an appropriate dose of thyroxine: permanent hypothyroidism was distinguished from potentially transient hypothyroidism by initially starting all hypothyroid patients on tri-iodothyronine. This was withdrawn after a minimum of 8 weeks, and basal thyroid function reassessed one week later. Subjects with persistently low levels of thyroxine, elevation of TSH and low uptake values were presumed at this stage to have permanent hypothyroidism and started on Thyroxine.

Patients with persistent symptoms and signs of thyrotoxicosis 4 months after ^{131}I treatment were started on Carbimazole with T3 supplements, this was continued for 6 months. At this stage treatment was withdrawn and basal thyroid function reassessed. In all of such instances thyrotoxicosis recurred within 6 weeks.

3.2.3. Results

TABLE 7

DETAILS OF PATIENTS INVESTIGATED WITH SERIAL UPTAKE MEASUREMENTS AFTER ^{131}I TREATMENT

	NO.	SEX	AGE	GRAVES' DISEASE	TOXIC MULTINODULAR GOITRE
PATIENTS GIVEN ^{131}I ALONE	31	29F: 2M	56.9 ± 10.5 *	23	8
PATIENTS PRETREATED WITH CARBIMAZOLE BEFORE ^{131}I	24	22F: 2M	55.5 ± 9.8	19	5
ALL PATIENTS	55	51F: 4M	56.4 ± 9.8	42	13

* MEAN ± SD

TABLE 8

THYROID GLAND SIZE, TRACER ^{123}I UPTAKE AND DOSE OF ^{131}I GIVEN

	GOITRE SIZE (G)	20 MIN UPTAKE (^{123}I) BEFORE ^{131}I (%)	24 HOUR UPTAKE(^{123}I) BEFORE ^{131}I (%)	DOSE OF ^{131}I (mCi)
PATIENTS GIVEN ^{131}I ALONE	33.7 * ± 12.3	20.5 ± 9.1	76 ± 13.2	6.3 ± 1.8
PATIENTS PRETREATED WITH CARBIMAZOLE	40.3 ± 10.6	24.5 ± 20.4	66 ± 16.1	5.4 ± 2.4
ALL PATIENTS	35.2 ± 11.8	22.1 ± 17.2	71 ± 14.1	5.9 ± 2.1

* MEAN ± SD

TABLE 9

OUTCOME AFTER ^{131}I THERAPY

	3 MONTHS AFTER ^{131}I			6 MONTHS AFTER ^{131}I			1 YEAR AFTER ^{131}I		
	T[x]	E[x]	H[x]	T	E	H	T	E	H
PATIENTS GIVEN ^{131}I ALONE	5	13	13	7	10	14	7	10	14
PATIENTS PRETREATED WITH CARBIMAZOLE BEFORE ^{131}I	11	7	6	8	10	6	5	13	6
ALL PATIENTS	16	20	19	15	20	20	12	23	20

x T = THYROTOXIC

E = EUTHYROID

H = HYPOTHYROID

One year after ^{131}I treatment 12 patients were still thyrotoxic, 20 had developed permanent hypothyroidism and the remainder were euthyroid. The distribution of patients into these arbitary biochemical classifications over the first year of follow-up is shown in Table 9 (it is assumed for this purpose that 4 patients given carbimazole because of persistent thyrotoxicosis between 4 and 10 months after treatment were thyrotoxic during this time).

It will be seen that by 4 months after therapy most patients who became euthyroid in the first year of follow-up had already done so, and this is also the case for patients who develop permanent hypothyroidism. The mean time to development of permanent hypothyroidism was 9.6 $\pm$ 1.2 weeks.

The influence of pretreatment with carbimazole on overall clinical and biochemical outcome following ^{131}I is discussed later.

3.2.4. Radioisotope studies and biochemical outcome

Twenty minute uptake measurements before ^{131}I administration are shown in Figure 15: the patients have been subdivided for this purpose according to biochemical outcome one year after treatment. It will be noted that those patients who failed to enter remission had a higher 20 minute uptake of ^{123}I before ^{131}I therapy was given ($p < 0.05$), suggesting that the underlying disease process in these subjects was more severe. Pretreatment goitre size, and dose of ^{131}I given are shown in Table 7. It will be noted that for neither of these variables was there significant difference between the groups classified according to outcome, although there was a tendency for the subjects with the highest uptakes to receive slightly lower doses of ^{131}I (the dose calculation involves the reciprocal of the 24 hour uptake). There was, however, no evidence to suggest that the dose given to these subjects who failed to enter remission was significantly lower than that given to the other 2 groups.

Serial measurements of 20 minute uptake for the 12 months after therapy are shown in Figure 16. In all patients 20 minute uptake of ^{123}I had fallen by 4 weeks after ^{131}I therapy. At this time, uptake in those subjects who were euthyroid one year after treatment was higher than in those who developed permanent hypothyroidism and lower than in those who remained thyrotoxic ($p < 0.05$). Despite this there was considerable overlap between the groups. No recovery of uptake function was seen in those patients becoming hypothyroid, while a rise in uptake between 4-12 weeks

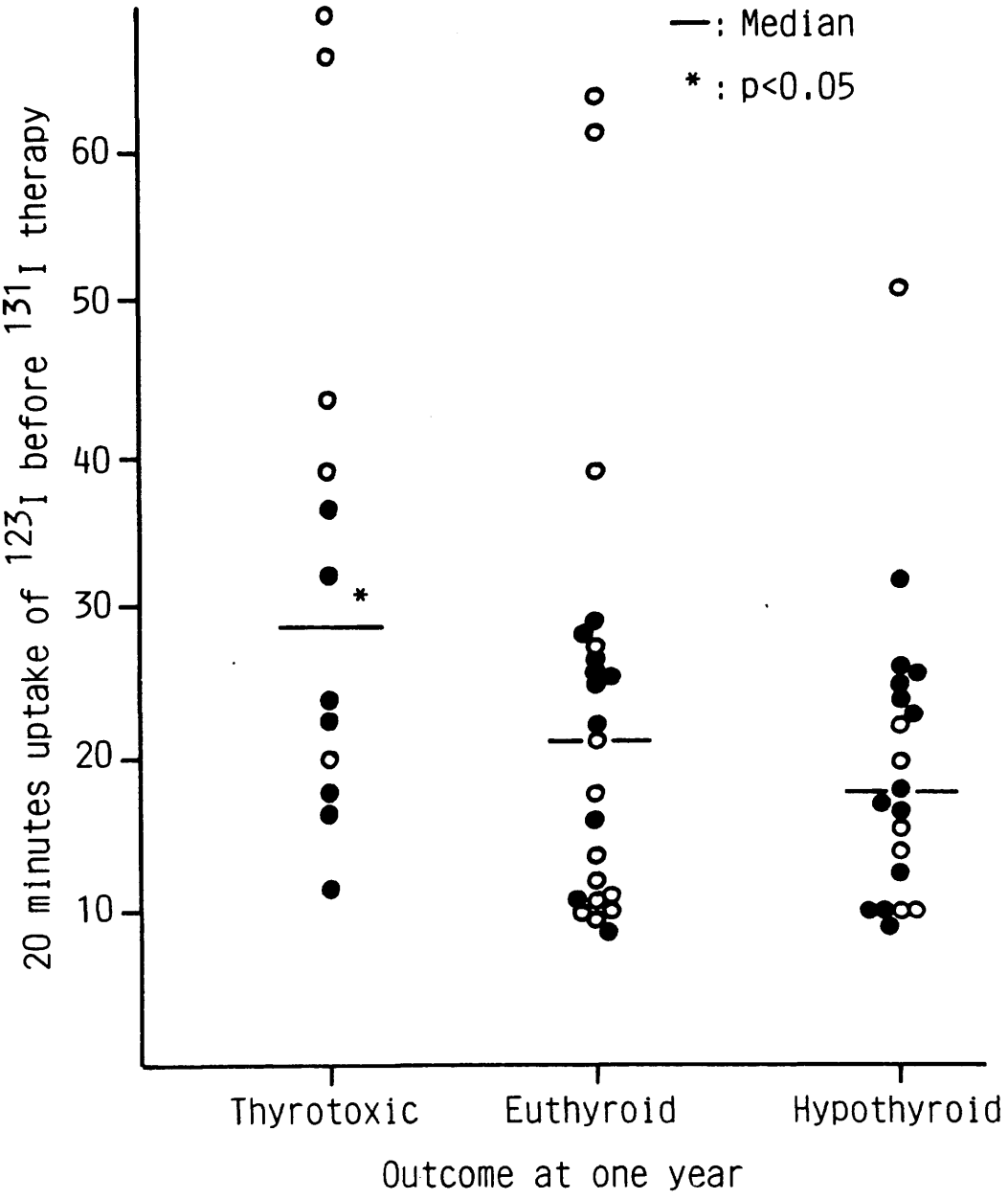


Fig 15

20 MINUTE UPTAKE OF ^{123}I BEFORE ^{131}I TREATMENT

UPTAKE OF ^{123}I 20 MINUTES AFTER INTRAVENOUS INJECTION OF ISOTOPE IN 55 THYROTOXIC SUBJECTS: MEASUREMENT WAS MADE ON THE DAY BEFORE ^{131}I ADMINISTRATION. THE THREE COLUMNS IDENTIFY SUBJECTS ACCORDING TO BIOCHEMICAL STATE 1 YEAR AFTER TREATMENT: MEDIAN UPTAKE WAS HIGHER ($p < 0.05$, MANN WHITNEY U TEST) IN THOSE SUBJECTS WHO REMAINED THYROTOXIC THAN IN THOSE WHO RESPONDED TO ^{131}I. THE OPEN CIRCLES REPRESENT THOSE SUBJECTS WHO WERE MADE EUTHYROID BEFORE ^{131}I TREATMENT WITH CARBIMAZOLE: THE CLOSED CIRCLES THOSE WHO RECEIVED ^{131}I AS SOLE THERAPY.

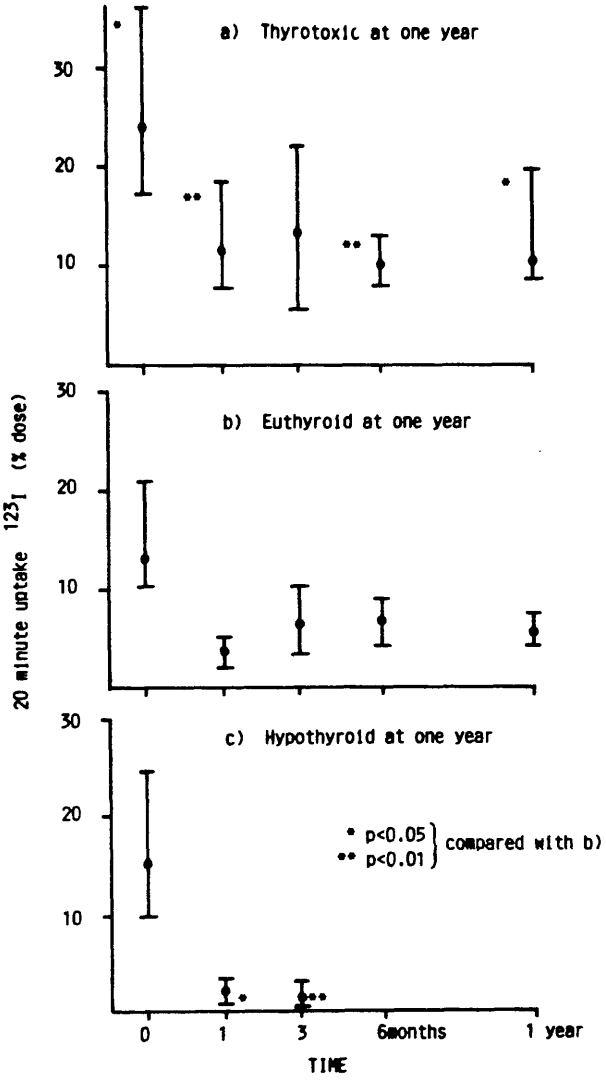


Fig 16

SERIAL CHANGES IN 20 MINUTE UPTAKE OF ^{123}I AFTER ^{131}I TREATMENT

UPTAKE OF ^{123}I WAS MEASURED 20 MINUTES AFTER INTRAVENOUS INJECTION OF ISOTOPE BEFORE AND AT 4-WEEKLY INTERVALS AFTER ^{131}I TREATMENT IN 55 SUBJECTS. SUBJECTS ARE CLASSIFIED ACCORDING TO BIOCHEMICAL OUTCOME ONE YEAR AFTER ^{131}I ADMINISTRATION. VALUES SHOWN ARE MEDIAN AND INTERQUARTILE RANGE. FOUR WEEKS AFTER ^{131}I THERAPY UPTAKE WAS HIGHER IN THOSE SUBJECTS WHO FAILED TO FULLY RESPOND ($p < 0.05$, MANN WHITNEY U TEST) AND LOWER ($p < 0.01$) IN THOSE WHO DEVELOPED PERMANENT HYPOTHYROIDISM THAN IN THOSE WHO WERE EUTHYROID AT ONE YEAR.

occurred in those who were euthyroid one year after ^{131}I. At one year after ^{131}I therapy, uptake in this group remained lower than in those who were still thyrotoxic ($p < 0.01$).

3.2.5. Changes in radioisotope uptake with time

(a)Patients who became permanently hypothyroid

(Table 10) After the initial fall in 20 minute uptake 4 weeks after ^{131}I treatment, no recovery of isotope trapping was seen in the majority (13 out of 20) of patients who developed permanent hypothyroidism, so that mean uptake values of less than 2% of injected dose were found. These subjects all developed biochemical hypothyroidism with 12 weeks of ^{131}I treatment: all subsequent measurements (up to 16 and 24 weeks), when TSH levels were high, were low. In the remaining 7 subjects measurable uptake of ^{123}I was present 8-12 weeks after ^{131}I therapy. This was less than 6% of injected dose in all instances, and by 24 weeks after ^{131}I therapy was less than 4% in all patients. All of these subjects developed biochemical hypothyroidism within 24 weeks of ^{131}I administration. The pattern of uptake changes in those patients pretreated with carbimazole who developed hypothyroidism compared with those given ^{131}I alone was very similar (Figure 17), and the time taken to develop biochemical hypothyroidism was not different in those patients pretreated with Carbimazole compared to those given ^{131}I alone. In the group given ^{131}I alone changes in T4 and T3 were parallel (Figure 17); while hormone levels had fallen by four weeks after ^{131}I, there was a lag period between the major fall in uptake and its full biochemical expression.

(b)Patients euthyroid one year after treatment

In all but one patient, 20 minute uptake showed some recovery after the initial fall noted at 4 weeks after ^{131}I administration, so that mean 20 minute uptake at 12 weeks after ^{131}I treatment was 8.5% (range 1.4-17.1, Table 11). No difference in the pattern of response (degree of fall after therapy or in subsequent measurements) was seen in the group of patients given carbimazole pretreatment compared to those given ^{131}I alone (Figure 18). All but one of the patients given ^{131}I alone had responded to radioiodine with either normal or low thyroid hormone levels (4 patients had an episode of transient hypothyroidism) by 12 weeks after ^{131}I administration. The single patient who did not respond in this time had mild

TABLE 10

SERIAL MEASUREMENTS OF UPTAKE OF ^{123}I IN SUBJECTS WHO DEVELOPED PERMANENT HYPOTHYROIDISM AFTER ^{131}I TREATMENT

		20 MINUTE UPTAKE OF ^{123}I xx WEEKS AFTER ^{131}I				
	NO.	BEFORE ^{131}I	4 WEEKS	8 WEEKS	12 WEEKS	16 WEEKS
PATIENTS GIVEN ^{131}I ALONE	14	18.8 ± 7.5*	2.9 ± 2	2.3 ± 1.9	2.2 ± 1.5	1.9 ± 1.2
		(13.1, 8.2-31.5)+	(1.9, 0-4.9)	(2.8, 0-4.6)	(1.0, 0-4)	(1.8, 0-3.8)
PATIENTS PRETREATED WITH CARBIMAZOLE	6	20.1 ± 5.9	0.85 ± 0.9	1.8 ± 1.2	1.5 ± 1.4	1.4 ± 1.3
		(15.2, 9.1-51)	(1.2, 0-1.9)	(1.20, 0.2-3.2)	(1.4, 0-3.8)	(1.3, 0-2.2)
ALL PATIENTS	20	19.2 ± 10.5	2.3 ± 2.0	2.1 ± 1.8	2 ± 1.5	1.8 ± 1.3
		(15.2, 8.2-51)	(1.5, 0-4.9)	(1.2, 0-4.6)	(1.4, 0-4)	(1.7, 0-3.8)

* MEAN ± SD

\+ MEDIAN, RANGE

xx % OF DOSE

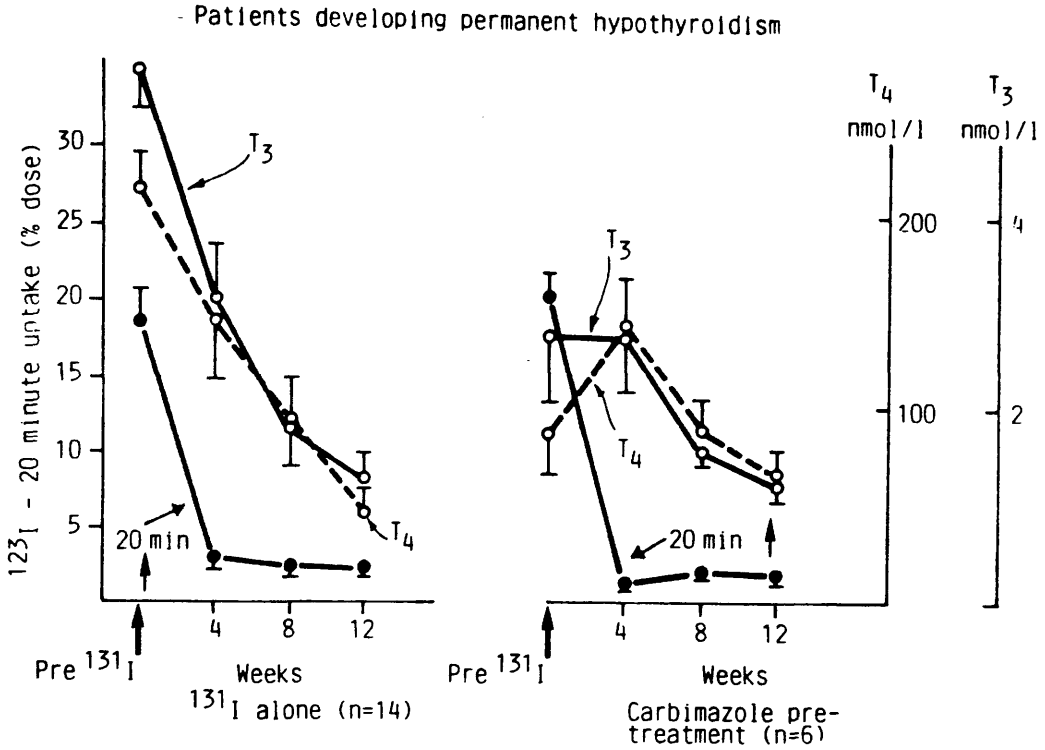


Fig 17

RELATIONSHIP BETWEEN EARLY IODIDE UPTAKE AND THYROID HORMONES IN SUBJECTS HYPOTHYROID AFTER ^{131}I TREATMENT

SERIAL CHANGES IN 20 MINUTE UPTAKE OF ^{123}I (SOLID DOTS), SERUM THYROXINE (BROKEN LINE) AND TRIIODOTHYRONINE (SOLID LINE) BEFORE AND AFTER ^{131}I THERAPY IN PATIENTS WHO WERE HYPOTHYROID 1 YEAR AFTER ^{131}I TREATMENT. PATIENTS GIVEN ^{131}I AS SOLE TREATMENT ARE SHOWN IN THE LEFT HAND PANEL, AND THOSE MADE EUTHYROID WITH CARBIMAZOLE IN THE RIGHT. VALUES SHOWN ARE MEAN AND STANDARD ERROR OF THE MEAN.

TABLE 11 (PART I)

SERIAL MEASUREMENTS OF UPTAKE OF ^{123}I IN SUBJECTS WHO WERE EUTHYROID 1 YEAR AFTER ^{131}I THERAPY

	NO.	20 MINUTE UPTAKE OF $^{123}I^{xx}$ WEEKS AFTER ^{131}I THERAPY			
		BEFORE ^{131}I	4 WEEKS	8 WEEKS	12 WEEKS
PATIENTS GIVEN ^{131}I ALONE	10	19.2 ± 9.1* (24.5, 10-28.6)+	4.4 ± 1.0 (3.8, 0-6.3)	3.7 ± 1.35 (4, 1.2-5)	7.9 ± 5.7 (5.7, 1.4-17.1)
PATIENTS PRETREATED WITH CARBIMAZOLE	13	23.2 ± 18.7 (13, 8.1-63.4)	4.2 ± 6.6 (1.7, 0-6)	4 ± 2.6 (1.7, 0.5-7.4)	8.9 ± 4.6 (8.9, 3.5-14.5)
ALL PATIENTS	23	21.4 + 15.4 (13, 8.1-63.4)	4.3 + 5.3 (3.6, 0-6.3)	3.9 + 2.2 (2.7, 0.5-7.4)	8.5 + 5.1 (7.3, 1.4-17.1)

* MEAN ± SD

\+ MEDIAN, RANGE

xx % OF DOSE.

TABLE 11 (PART II)

SERIAL MEASUREMENTS OF UPTAKE OF ^{123}I IN SUBJECTS WHO WERE EUTHYROID 1 YEAR AFTER ^{131}I THERAPY

		20 MINUTE UPTAKE OF ^{123}I** WEEKS AFTER ^{131}I THERAPY			
	NO.	16 WEEKS	24 WEEKS	32 WEEKS	48 WEEKS
PATIENTS GIVEN ^{131}I ALONE	10	8.3 ± 6.0* (6.1, 3-16.4)+	9.9 ± 8.8 (5.4, 3-25.7)	5.9 ± 3.4 (4.7, 1.8-13.4)	6.1 ± 3.5 (5.6, 2.3-14)
PATIENTS PRETREATED WITH CARBIMAZOLE	13	10 ± 6.1 (8, 3.8-15.1)	11.6 ± 9.1 (8.4, 3.4-18)	9.6 ± 7.3 (4.7, 2.9-13.6)	5.9 ± 2.6 (5.2, 3.8-12.6)
ALL PATIENTS	23	9.3 ± 6.0 (7.5, 3-16.4)	10.9 ± 8.9 (6.5, 3-25.7)	7.8 ± 5.7 (4.7, 18-13.6)	6.0 ± 3.0 (5.1, 2.3-14)

* MEAN ± SD

\+ MEDIAN, RANGE

** % OF DOSE.

T3 toxicosis until 6 months after treatment. In the group given carbimazole pretreatment, 5 subjects remained euthyroid at all times after ^{131}I administration, 2 had an episode of transient hypothyroidism within the first 12 weeks after ^{131}I, and the remainder (6) had a mild episode of biochemical thyrotoxicosis between 4 to 36 weeks after therapy. In none of these patients was the illness severe, and did not warrant other treatment. Uptake values in this small subgroup of patients did not appear to differ from those who remained euthyroid throughout, although numbers are small (Table 12). By one year after therapy all but 2 of this group of patients had uptake measurements within the normal range (Table 11). Again, in the group given ^{131}I alone changes in T4 and T3 levels were very similar after ^{131}I (Figure 18), with a progressive fall to euthyroid values over the first 12 weeks. As with the previous group major effects of ^{131}I on isotope uptake were not immediately expressed in circulating hormone levels, presumably due to preformed stores of hormone within the gland. The partial recovery of trapping function from 4-12 weeks is then responsible for maintaining subsequent hormonogenesis. In the group pretreated with carbimazoleT3 and T4 levels again changed in parallel. However, in this instance there was no overall fall in hormone levels immediately after ^{131}I, and instead a small rise occurred between 0-8 weeks.

(c) Patients who failed to respond

Twelve subjects had persistent biochemical and clinical thyrotoxicosis one year after therapy. In all instances, uptake measurements fell after ^{131}I administration, with the largest decline being within the first 4 weeks (Table 13, Figure 19). Further fall in uptake over the next few months was seen in 4 individuals: in the remainder a rise in uptake then occurred. In no patient, however, was the uptake measurement one year after therapy higher than that before ^{131}I administration, and in 3 patients was just above the normal range. Thyrotoxicosis in all but 4 subjects (3 given pretreatment with carbimazole) was mild, with T3 values between 2.46 and 3.0 nmol/l, and T4 between 150 and 190 nmol/l. In the 4 other subjects there were marked clinical symptoms and signs, and carbimazole was therefore used to control clinical and biochemical thyrotoxicosis between 4 and 10 months after ^{131}I administration. On stopping carbimazole, all patients relapsed within 6 weeks and uptake values remained high. In 2 of these 4 there was very little

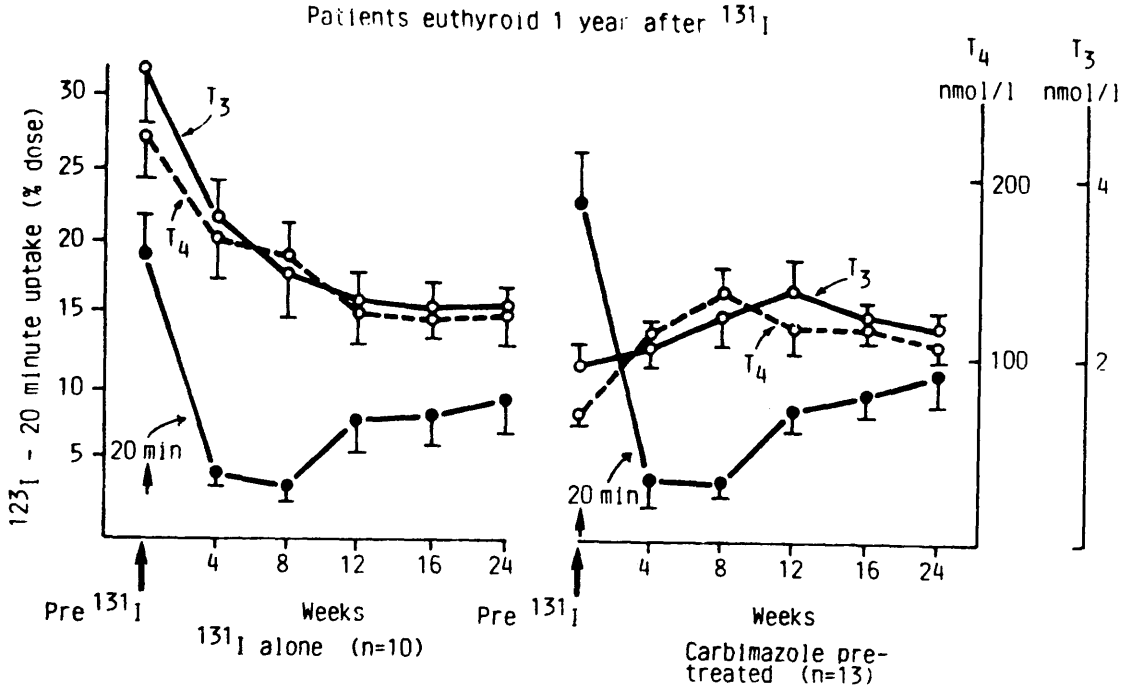


Fig 18

RELATIONSHIP BETWEEN EARLY IODIDE UPTAKE AND THYROID HORMONES IN SUBJECTS EUTHYROID AFTER ^{131}I TREATMENT

SERIAL CHANGES IN 20 MINUTE UPTAKE OF ^{123}I (SOLID DOTS), SERUM THYROXINE (BROKEN LINE) AND TRIIODOTHYRONINE (SOLID LINE) BEFORE AND AFTER ^{131}I THERAPY IN PATIENTS WHO WERE EUTHYROID 1 YEAR AFTER ^{131}I TREATMENT. PATIENTS GIVEN ^{131}I AS SOLE TREATMENT ARE SHOWN IN THE LEFT HAND PANEL, AND THOSE MADE EUTHYROID WITH CARBIMAZOLE IN THE RIGHT. VALUES SHOWN ARE MEAN AND STANDARD ERROR OF THE MEAN.

TABLE 12 (PART I)

PATIENTS PRETREATED WITH CARBIMAZOLE WHO WERE EUTHYROID ONE YEAR AFTER ^{131}I BUT HAD AN EPISODE OF THYROTOXICOSIS WITHIN THAT YEAR.

WEEKS AFTER ^{131}I	PRE-^{131}I			4			8			12		
PATIENT NO.	T4	T3	20	T4	T3	20	T4	T3	20	T4	T3	20
1	84	1.37	17.6	117	1.59	0	212	4.71	8.4	140	4.05	8.9
2	80	3.12	9.1	156	2.72	0.6	189	2.82	0.5	82	1.91	3.4
3	84	2.95	6.12	148	3	23.9	75	1.6	8	169	4.1	10.8
4	73	1.67	9.5	158	2.62	1.7	166	4.15	7.4	200	5.3	14.5
5	51	1.37	8.1	110	1.7	0	197	2.3	6.7	162	5.1	9.5
6	80	2.23	27.4	146	2.15	0	181	2.8	6.6	154	3.08	9.4

T_4 = SERUM THYROXINE; NR = 54-142nmol/l

T_3 = SERUM TRIIODOTHYRONINE; NR = 0.8 - 2.46nmol/l

20 = 20 MINUTE UPTAKE OF ^{123}I; NR = 2 - 8%.

TABLE 12 (PART II)

PATIENTS PRE-TREATED WITH CARBIMAZOLE WHO WERE EUTHYROID ONE YEAR AFTER ^{131}I BUT HAD AN EPISODE OF THYROTOXICOSIS WITHIN THAT YEAR

WEEKS AFTER ^{131}I	24			36			48		
PATIENT NO.	T4	T3	20	T4	T3	20	T4	T3	20
1	77	1.9	12.7	106	2.38	8.8	63	1.28	7.9
2	84	1.4	2.4	88	1.0	2.9	107	1.28	5.1
3	189	5.4	9	134	2.4	8.4	116	1.2	6
4	153	3.33	6.9	172	3.3	5.4	134	2.18	3.8
5	141	3.8	26.8	139	2.9	13.6	119	2.15	7.9
6	152	2.1	8.4	134	2.3	4.7	122	1.68	5.6

T_4 = SERUM THYROXINE; NR = 54 - 142nmol/l

T_3 = SERUM TRIIODOTHYRONINE; NR = 0.8 - 2.46nmol/l

20 = 20 MINUTE UPTAKE OF ^{123}I; NR = 2 - 8%.

TABLE 13 (PART I)

SERIAL MEASUREMENTS OF UPTAKE OF ^{123}I IN SUBJECTS WHO WERE THYROTOXIC ONE YEAR AFTER ^{131}I TREATMENT.

	NO.	20 MINUTE UPTAKE OF $^{123}I^{xx}$ WEEKS AFTER ^{131}I THERAPY			
		BEFORE ^{131}I	4 WEEKS	8 WEEKS	12 WEEKS
PATIENTS GIVEN ^{131}I ALONE	7	22.9 ± 8.4*	10.6 ± 6.7	12.7 ± 6.7	11.1 ± 7.4
		(22.3, 11.2-36.8)+	(8.5, 4.7-24.7)	(8.5, 5.6-26.3)	(8, 3.1-24.6)
PATIENTS PRETREATED WITH CARBIMAZOLE	5	54.4 ± 13.4	26.3 ± 20.8	33.6 ± 27	21.1 ± 8.4
		(43.5, 16-68.8)	(18.4, 8.3-66)	(24, 9.2-71.6)	(24.2, 4.8-22)
ALL PATIENTS	12	34.4 ± 18.4	17.7 ± 16.8	19.7 ± 19.5	15.3 ± 8.9
		(23.9, 11.2-68.8)	(11.5, 4.7-66)	(9.2, 5.6-71.6)	(13.3, 3.1-24.6)

* MEAN ± SD

\+ MEDIAN, RANGE

xx % OF DOSE

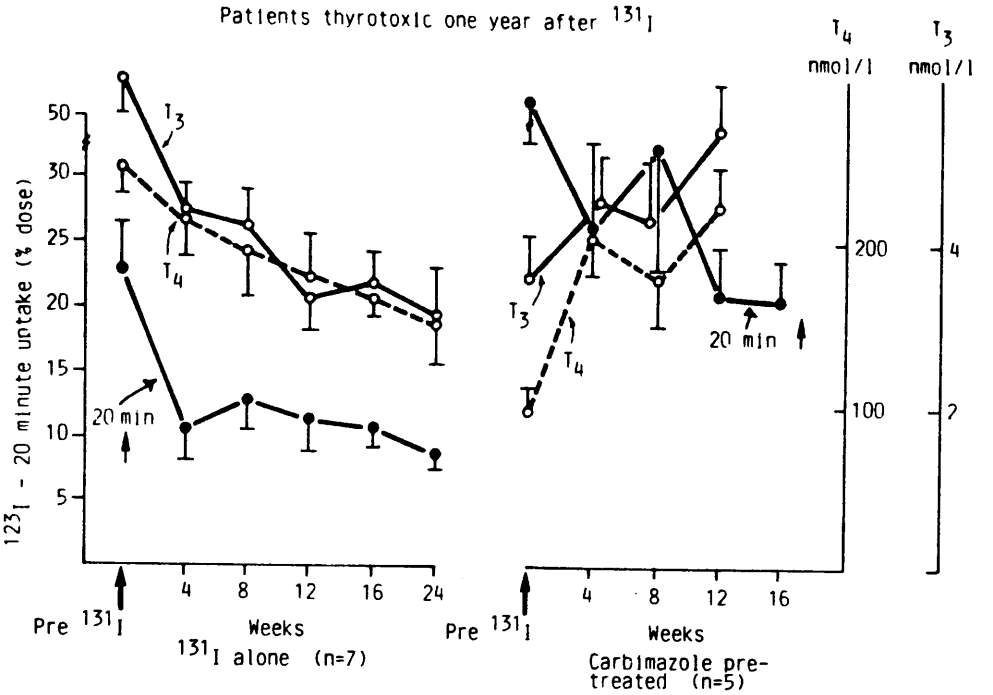


Fig 19

RELATIONSHIP BETWEEN EARLY IODIDE UPTAKE AND THYROID HORMONES IN SUBJECTS THYROTOXIC AFTER ^{131}I TREATMENT

SERIAL CHANGES IN 20 MINUTE UPTAKE OF ^{123}I (SOLID DOTS), SERUM THYROXINE (BROKEN LINE) AND TRIIODOTHYRONINE (SOLID LINE) BEFORE AND AFTER ^{131}I THERAPY IN PATIENTS WHO WERE STILL THYROTOXIC 1 YEAR AFTER ^{131}I TREATMENT. PATIENTS GIVEN ^{131}I AS SOLE TREATMENT ARE SHOWN IN THE LEFT HAND PANEL, AND THOSE MADE EUTHYROID WITH CARBIMAZOLE IN THE RIGHT. VALUES SHOWN ARE MEAN AND STANDARD ERROR OF THE MEAN.

TABLE 13 (PART II)

SERIAL MEASUREMENTS OF UPTAKE OF ^{123}I IN SUBJECTS WHO WERE THYROTOXIC 1 YEAR AFTER ^{131}I TREATMENT

	NO.	20 MINUTE UPTAKE OF $^{123}I^{xx}$ WEEKS AFTER $^{131}I^{xx}$ THERAPY 16 WEEKS	24 WEEKS	32 WEEKS	48 WEEKS
PATIENTS GIVEN ^{131}I ALONE	7	10.6 ± 3.2*	8.7 ± 2.3	9 ± 1.3	11.4 ± 4.2
		(9.2, 3.8-18.1)+	(8.8, 4.9-13)	(9.9, 5.6-16)	(8.5, 6.1-19.6)
PATIENTS PRETREATED WITH CARBIMAZOLE	5	20.6 ± 7.6	18.2 ± 4.9	18.7 ± 6.1	21.5 ± 8
		(20, 6-24.3)	(17.6, 7.9-27)	(21, 6.6-27.4)	(25.1, 8.5-35.8)
ALL PATIENTS	12	14.8 ± 5.6	12.6 ± 4.9	13.1 ± 5.8	15.7 ± 7.6
		(14.1, 3.8-24.3)	(9.9, 4.9-27)	(9.9, 5.6-27.4)	(10.5, 6.1-35.8)

* MEAN ± SD

\+ MEDIAN, RANGE

xx % OF DOSE.

TABLE 14 (PART I)

DETAILS OF PATIENTS WHO REQUIRED CARBIMAZOLE THERAPY FOLLOWING ^{131}I TREATMENT

WEEKS AFTER ^{131}I	PRE-^{131}I			4			8			12		
PATIENT NO.	T4	T3	20	T4	T3	20	T4	T3	20	T4	T3	20
1) ^{131}I ALONE; CMI FROM 16 - 40 WEEKS	184	6.0	32.8	210	4.2	24.7	199	5.13	26.3	174	5.1	24
2) CMI PRETREATED: CMI USED FROM 12 - 40 WEEKS	56	3.1	66.6	221	4.88	66	151	5.6	72	240	5.8	-
3) CMI PRETREATED: CMI USED FROM 8 - 40 WEEKS	100	2.5	13.4	192	3.53	8.3	>270	>7	27	130	2.9	-
4) CMI PRETREATED: CMI USED FROM 12 - 40 WEEKS	113	2.9	68.8	170	4.05	11.8	128	2.66	17	194	3.4	27

CMI = CARBIMAZOLE
T_4 = SERUM THYROXINE; NR = 54-142nmol/l
T_3 = SERUM TRIIODOTHYRONINE; NR = 0.8-2.46nmol/l
20 = 20 MIN. UPTAKE OF ^{123}I; NR = 2-8%

TABLE 14 (PART II)

DETAILS OF PATIENTS WHO REQUIRED CARBIMAZOLE THERAPY FOLLOWING ^{131}I TREATMENT

WEEKS AFTER ^{131}I	24			40			52		
PATIENT NO.	T4	T3	20	T4	T3	20	T4	T3	20
1) ^{131}I ALONE; CMI FROM 16 - 40 WEEKS	120	1.4	13	116	2.38	8.3	168	3.5	19.6
2) CMI PRETREATED: CMI USED FROM 12 - 40 WEEKS	105	113	21	80	1.2	42	130	2.9	25.1
3) CMI PRETREATED: CMI USED FROM 8 - 40 WEEKS	114	1.6	27.4	134	1.98	10	220	5.2	35.8
4) CMI PRETREATED: CMI USED FROM 12 - 40 WEEKS	108	2.8	17.3	62	2.14	15	196	4.4	24.2

CMI = CARBIMAZOLE
T_4 = SERUM THYROXINE; NR = 54-142nmol/l
T_3 = SERUM TRIIODOTHYRONINE; NR = 0.8-2.46nmol/l
20 = 20 MIN. UPTAKE OF ^{123}I; NR = 2-8%

change in uptake after ^{131}I therapy, while in the other 2 uptake measurement 4 weeks after ^{131}I administration were considerably reduced (Table 14).

Patients given carbimazole before ^{131}I administration had all relapsed within 8 weeks of stopping the drug (in all but one patient within the first 4 weeks). Although mean pre-^{131}I therpy uptake was higher in this group, this difference between the groups was not significant using appropriate tests for non parametrically distributed data.

The relationship between changes in ^{123}I uptake and T4 and T3 is shown in Figure 19. It will be noted that in the group given ^{131}I alone, T3 and T4 levels changed to a similar extent following ^{131}I therapy. The maximum fall in uptake values again occurred within the first four weeks after treatment; therefore mean (and median) values changed little. There was again a delay between maximum change in uptake and maximum biochemical response.

In those patients pretreated with carbimazole uptake values were very high, and, owing to small numbers, the changes displayed in Figure 19 are less uniform. However, there was a gradual fall in uptake over the first 12 weeks after ^{131}I therapy. This was not reflected in thyroid hormone levels which progressively rose over this period, presumably due to withdrawal of the iatrogenic organification blockade. As 3 of this group were then restarted on carbimazole therapy, subsequent thyroid homrone changes cannot be analysed.

3.2.6. Assessment of organification impairment using 60 and 20 minute uptake meassurements of ^{123}I

Sixty minute uptake measurements were made in all subjects following ^{131}I therapy. In a preliminary study (2.3.5.) a ratio of 1.6 or less between 60 and 20 minute uptake was shown to be associated with a defect of iodide organification. This may only be applicable where uptake measurement at 20 minutes after intravenous injection of tracer is measurable, preferably greater than 4%. In addition, where uptake is greater than 20% within the first 20 minutes the significance of a ratio of 1.6 or less is uncertain. In 17 patients of the current series a low ratio between 60 and 20 minute uptakes was found within the first 4 months after ^{131}I administration. Further details of these patients are shown in Table 15. No abnormal ratios were

TABLE 15

DETAILS OF ABNORMAL 60 MINUTE TO 20 MINUTE UPTAKE RATIOS AFTER ^{131}I TREATMENT +

	4 WEEKS AFTER ^{131}I			8 WEEKS AFTER ^{131}I			12 WEEKS AFTER ^{131}I			16 WEEKS AFTER ^{131}I		
	20 MIN	60 MIN	RATIO	20 MIN	60 MIN	RATIO	20 MIN	60 MIN	RATIO	20 MIN	60 MIN	RATIO
PATIENTS GIVEN ^{131}I ALONE	3.5*	4.3	1.22	4.4	5.7	1.3	3.1*	4.6	1.5	15.7	23.8	1.5
	4.8	4.7	1	4.6	3.8	0.81						
	5.0*	6.4	1.28				3.5*	4.1	1.2			
	5.1	5.1	1									
	5.2	6.3	1.2				4.6	5.1	1.1			
	5.2	5.9	1.13									
PATIENTS PRETREATED WITH CARBIMAZOLE	6	7.3	1.2	4.4	5.7	1.3	8.4	10.4	1.35			
	6.3	8.2	1.3									
				4.8*	6.5	1.5						

20 MIN = 20 MINUTE UPTAKE OF ^{123}I

60 MIN = 60 MINUTE UPTAKE OF ^{123}I

+ IN PATIENTS WHO DID NOT DEVELOP PERMANENT HYPOTHYROIDISM ABNORMAL RATIOS WERE ONLY SEEN AT ONE TIME POINT: NO PATIENT APPEARS IN THE ABOVE TABLE MORE THAN ONCE.

* PATIENTS WHO SUBSEQUENTLY DEVELOPED PERMANENT HYPOTHYROIDISM.

found later than 4 months after ^{131}I administration. It will noted that the majority of patients had an abnormal ratio within the first 12 weeks after ^{131}I administration, and that 5 of these patients developed permanent hypothyroidism in the following 4 weeks. Five of the 17 patients had been pretreated with carbimazole, the remainder having been given ^{131}I alone. This proportion of carbimazole pretreated patients (20%) developing an abnormal ratio was lower than in patients given ^{131}I alone (40%) ($X = 4.6$, $p < 0.05$).

3.2.7. Dose of ^{131}I: Patients divided by outcome at one year

The doses of ^{131}I administered are summarized in Table 16. Those patients who failed to enter remission were given a slightly higher mean dose of ^{131}I than those who responded, although this did not reach statistical significance. No difference in the dose of ^{131}I given was seen between those pretreated with carbimazole and those given ^{131}I therapy alone.

3.2.8. Gotire size: Patients divided by outcome at one year

Gotire size, estimated from the planar surface area on thyroid isotope scan immediately before ^{131}I therapy is shown in Table 16. It will again be seen that the estimated size was greatest in those patients who subsequently remained thyrotoxic, although this did not reach statistical significance.

3.2.9. Discussion

In this series of 55 patients, 12 remained thyrotoxic one year after radioiodine therapy. Twenty three became euthyroid, of whom 6 had a short episode of transient hypothyroidism, and the remaining 20 developed permanent hypothyroidism within the first year. The incidence of permanent hypothyroidism after ^{131}I is partly a function of dose of radioiodine administered (Glennon et al. 1972; Hagen et al. 1967; Hardisty et al. 1981): it has been suggested from a retrospective analysis of patients treated at the Mayo clinic that the incidence of hypothyroidism is greater currently than in previously years, with as many as 90% of patients becoming hypothyroid one year after treatment (Cunnien et al. 1982): this, however, may reflect a tendency to give higher initial doses of ^{131}I therapy. In the current series, however, those patients who became hypothyroid did not appear to receive higher doses of ^{131}I compared with those who became euthyroid or remained

TABLE 16

GOITRE SIZES AND DOSES OF ^{131}I: PATIENTS DIVIDED BY OUTCOME AT 1 YEAR AFTER TREATMENT

	DOSE OF ^{131}I mCi	ESTIMATED GOITRE SIZE (GRAMS)
OUTCOME AT 1 YEAR AFTER ^{131}I		
HYPOTHYROID (n = 20)	5.4 ± 2.2*	32.1 ± 8.8
EUTHYROID (n = 23)	5.8 ± 1.5	36.3 ± 10.5
THYROTOXIC (n = 12)	7.27+ ± 2.3+	45.4 ± 13.8+

* MEAN ± SD

\+ NOT SIGNIFICANTLY DIFFERENT FROM HYPOTHYROID AND EUTHYROID GROUPS.

thyrotoxic, suggesting the contribution of other factors to eventual outcome.

Of those patients developing permanent hypothyroidism, the majority did so within 12 weeks of ^{131}I administration, many within 8 weeks: no patient became hypothyroid later than 4 months after ^{131}I therapy. This was accompanied in all subjects by a rapid fall in early iodine uptake. The early phase of the uptake curve (within 20 minutes of intravenous admistration of isotope) is an index of iodide trapping (IAEA Report, 1972), while uptake at later times is dependent upon both trapping and organification. Iodide trapping is normally the rate limiting step in thyroid hormonogenesis, and studies of early iodide uptake therefore examine an important physiological variable in control of thyroid function. In all patients, iodide trapping fell to virtually undetectable levels 4 weeks after ^{131}I administration, and showed only minimal recovery in a small number of patients thereafter. This reflects, therefore, severe impairment of the dominant thyroid physiological process, and inevitable progression to hypothyroidism is therefore to be expected. In all such patients this severe loss of iodide trapping was present despite very high level of TSH. Larsson (1955), who made late (24 hours) and indirect measurements of tracer ^{131}I uptake after ^{131}I therapy, showed that in the majority of patients who were hypothyroid after treatment, very low tracer uptake values were recorded.

The response of the thyroid to ^{131}I therapy was rapid (within 4 weeks) in all patients who became hypothyroid. Although it is likely that iodide trapping declines much earlier than this following ^{131}I administration, earlier measurements are not possible because of the very high background levels of radiation in the gland. In all subjects there was a lag between this fall in iodide trapping and the biochemical response (Figure 17): this presumably reflects the long disappearance half-life of thyroxine (7 days) and the store of preformed thyroid hormone within the gland. Thus, although the clinical and biochemical response to ^{131}I administration may be delayed, this does not reflect the rapid effect of radiation on important thyroid physiological processes. This gap between uptake response and biochemical response has been noted by Myant (1953 a + b) and Franco et al. (1970).

Twenty three patients were euthyroid one year after ^{131}I administration. Of those given ^{131}I alone, all but one patient had become euthyroid within 12 weeks of treatment. In contrast, although all patients pretreated with Carbimazole were euthyroid before ^{131}I was given, in 6

subjects an episode of thyrotoxicosis occurred after ^{131}I administration, and these subjects were not euthyroid until 6 months after treatment. Uptake values fell in all patients in this group following ^{131}I therapy, and the median value 4 weeks after treatment was significantly higher than that in those patients who became permanently hypothyroid (Figure 16, Table 11). This suggests that the degree of damage sustained by the thyroid following ^{131}I was less in this euthyroid group of patients. In 10 patients from this group, uptake at this time (4 weeks) was less than 2%, indicating almost complete loss of iodide trapping function. Thereafter, iodide uptake rose in all patients with values remaining within the normal range in the majority of instances. Thus, in this group of patients temporary loss of thyroid function was followed by partial recovery so that normal thyroid hormone levels were maintained. As with permanent hypothyroidism, there was a lag period between the response of iodide trapping to ^{131}I administration (rapid) and the reflection of this in thyroid biochemical change (Figure 18). The pattern of thyroid uptake followed by partial recovery is similar to that noted by Myant (1953 a + b) who noted that uptake measurements fell 3 weeks after ^{131}I administration, and that in a number of patients measurements rose thereafter. He observed that where no further uptake occurred, patients did not become euthyroid. Similarly, Einhorn and Hastad (1961) measured uptake of ^{132} 'lowing ^{131}I therapy and showed that the greatest fall in uptake occurred in those patients who subsequently entered remission. Carbimazole pretreatment had no effect on this pattern of uptake behaviour (Figure 18). In this subgroup mean thyroid hormone levels did not fall after ^{131}I, and indeed, rose slightly. This again demonstrates the delay between effects of ^{131}I on iodide trapping and biochemical response. It is likely that withdrawal of carbimazole for five days before ^{131}I therapy allows an increase in thyroid hormonogenesis, so that following ^{131}I treatment, when iodide trapping temporarily falls, hormone content of the gland is sufficient to maintain circulating T4 and T3 until trapping recovers.

Twelve patients remained thyrotoxic one year after treatment with ^{131}I: of these, 4 remained severely thyrotoxic and required carbimazole therapy to control symptoms between 4 and 10 months after ^{131}I administration. In the remainder, some evidence of biochemical response to treatment was noted and in these patients the clinical signs and symptoms of thyrotoxicosis were mild. All patients showed evidence of some response to

radioiodine in terms of a fall of 20 minute uptake after ^{131}I administration, although this was small in several instances. As with the other categories of patient, the maximum fall in iodide trapping occurred within 4 weeks of ^{131}I, and biochemical response some weeks later (Figure 19).

3.2.10. Implications of altered radioisotope uptake following ^{131}I therapy

The general pattern of 20 minute uptake measurements following ^{131}I therapy was similar in this study to that reported elsewhere (discussed above): in all patients a fall in uptake early after treatment was followed by a variable degree of recovery in the subsequent 4-8 weeks. Uptake measurements thereafter remained fairly stable. Thyroid functional reserve (i.e. the capacity to synthesise thyroid hormone) is determined, after ^{131}I administration, by the remaining ability to trap iodide and the important effects of ^{131}I treatment on this aspect of thyroid function occur early after treatment. As other workers have noted, however, there is a lag between this response and its biochemical expression, presumably because of the store of preformed thyroid hormone within the gland.

The reason for the biphasic response to ^{131}I administration, with an initial fall in uptake followed by partial recovery is unclear. It is possible that there is, at cellular level, temporary disruption of cell function with loss of iodide trapping, and that this gradually recovers over the subsequent few weeks. It seems unlikely that the recovery of iodide trapping function represents replacement of thyroid cells: the dose of radiation administered is one which would prevent further cell replication. In general, thyroid cell function is considered radio-resistant, in contrast to the relative radio-sensitivity of cell replication (Al-Hindawi and Wilson, 1965). This is reflected in studies showing cummulative increases in late hypothyroidism in the years after ^{131}I therapy (Greig, 1973; Dworkin, 1971; Toft et al. 1975).

It is possible that part of the explanation for the change in uptake behaviour lies in the heterogenetity of isotope distribution throughout the gland (Studer, Peter and Gerber, 1985). It is possible that following ^{131}I administration some follicles, which are more active in iodide trapping, receive a higher exposure to radiation than others and it may be that cells from such follicles are permanently damaged: the activity of these follicles will largely determine the preirradiation tracer uptake, and loss of this tissue will account for the initial fall in uptake after ^{131}I administration. Follicles

which are relatively inactive before ^{131}I will trap less isotope and may receive a relatively lower dose of radiation and thus be capable of continued iodide trapping following ^{131}I therapy. However, the steady increase in incidence of hypothyroidism in the years following ^{131}I therapy indicates that cellular damage (at least to the nucleus) is a very generalised phenomenon within the gland.

A spectrum of thyroid damage following ^{131}I therapy ranging from complete loss of function to minimal change in uptake was noted. The reason for this is unclear. Smith and Wilson (1967) showed that the dose of ^{131}I had a major bearing on the incidence of early hypothyroidism, and this has been confirmed in a large number of patients treated with a range of doses of ^{131}I (Cevallos et al. 1974; Glennon et al. 1972; Hagen et al. 1967). However, in the current study an attempt to give the same dose of ^{131}I to all patients was made. The dose of radiation delivered to the thyroid is a function of the administered dose of isotope, gland mass and the effective half-life of ^{131}I. Although some inaccuracy in estimation of gland mass using scintigraphy is inevitable, and although there may be some variability in effective half-life of ^{131}I in patients with thyrotoxicosis, it seems unlikely that these variables would be sufficient to account for the differences in response noted. Thus, the mean estimate of goitre size in the group of patients who failed to become euthyroid after ^{131}I was greater than that in those who responded more fully: this former group therefore received a higher dose of ^{131}I, and the anticipated radiation per unit mass of gland should have been similar. The variability and sensitivity of response cannot be explained by the use of antithyroid drugs, as the pattern of isotope uptake and biochemical response were similiar in those patients given no pretreatment.

Examination of the isotope uptake data suggests that the spectrum of thyroid damage following ^{131}I therapy is continuous. There was, therefore, a range of disruption of function from permanent loss of trapping of iodide to transient loss of trapping with a variable degree of recovery to minimum change in isotope uptake with little change in thyroid hormone production. It is evident from the foregoing data and discussion that the effects of ^{131}I on thyroid cell function occur early after administration of the isotope.

3.2.11. Effects on iodide organification

It was demonstrated in a pilot investigation that the increase in uptake of tracer iodide between 20 and 60 minutes after intravenous injection of tracer was a crude measure of iodide organification (2.3.5.). Of the 55 patients in this study, 30% had an abnormally low ratio of uptake between 20 and 60 minutes within the first 4 months after ^{131}I treatment. This may represent a true defect of iodide organification in such subjects: the figure is remarkably similar to that reported by Gray (1975) who studied a group of patients following ^{131}I therapy using a perchlorate discharge test. Of the patients in the current study shown to have an abnormal ratio, 4 had persistent thyrotoxicosis, 5 developed permanent hypothyroidism and the other 9 became euthyroid over the subsequent few weeks (3 of this group had an episode of transient hypothyroidism). Twenty percent of the patients pretreated with carbimazole had an abnormal ratio within the first the few months after ^{131}I administration, whereas 40% of those not pretreated had an abnormal ratio, this difference was significant using a X^2 test. This may be evidence of a radio-protective effect of carbimazole. It must be stressed that the 60:20 minute uptake ratio is a very indirect means of assessing iodide organification. This is especially so when uptake measurements are low, at a time when statistical errors in counting can significantly affect the result. The data need, therefore, to be interpreted with caution. A more detailed study of iodide organification defects following ^{131}I therapy is presented in chapter 4. From this preliminary data, however, it does appear that such defects are not uncommon after ^{131}I treatment and are most evident in the early months.

3.2.12. Use of radioisotope uptake studies to predict biochemical outcome following ^{131}I therapy

From the above discussion it is clear that the principal determinant of residual thyroid reserve after ^{131}I therapy is the ability to trap iodide. Thus, subjects in whom iodide trapping, as assessed by the 20 minute uptake of ^{123}I, remained elevated after ^{131}I treatment did not enter remission while those subjects in whom iodide trapping became negligible developed permanent hypothyroidism. From Figure 16 it will be noted that, as early as 4 weeks after ^{131}I administration, significant differences between those patients who became euthyroid and those who were either hypothyroid or thyrotoxic one year after treatment can be seen, and that, from 12 weeks

onwards, little change in uptake behaviour occurred in those patients who were not already hypothyroid. Despite this statistical separation, there was some overlap between the groups. However, all patients whose uptake 4 weeks after therapy, was 4% or less responded to ^{131}I (i.e. became either euthyroid or hypothyroid), while no patients whose uptakes were higher than 8% at 4 weeks after treatment responded. An uptake of less than 2% between 8 and 12 weeks after treatment was, in all but one instance, associated with the development of permanent hypothyroidism.

3.2.13. Implications for patient management

The main problem immediately after ^{131}I administration is the uncertainty surrounding the response of an individual patient, and the lag period between treatment and the time for this response to be expressed in clinical or biochemical change. For example, the period between treatment and normalisation of thyroid biochemistry in those patients who did respond (^{131}I group alone) was 12.7 $\pm$ 2.4 weeks. This uncertainty in response may still occur in patients who are given deliberately "ablative" doses of ^{131}I (Kendall-Taylor, Keir and Ross, 1984; Safa and Skillern, 1975) in that hypothyroidism is not inevitable. In elderly subjects, or in patients with thyrocardiac disease this may be unsatisfactory, exposing those patients, who do not respond adequately, to delay before further necessary treatment is arranged. Because of this, antithyroid drugs may sometimes be given before ^{131}I treatment and continued for a variable time thereafter. When the effects of ^{131}I are assumed to have occurred, antithyroid therapy is discontinued. With this policy, residual thyroid function on cessation of drug treatment is unknown and patients may be exposed to risk of relapse of thyrotoxicosis (Viherkoski et al. 1970).

Use of early iodine uptake measurements may be of value in predicting outcome over this period. Where the uptake 4 weeks after ^{131}I therapy is high (i.e. greater than 8%) response to treatment is most unlikely and a second dose of ^{131}I can be given. Where the uptake has fallen to less than 4%, 4 weeks after treatment, further treatment is likely to be unnecessary. Uptake measurements of less than 2% at 8 weeks after treatment are indicative of likely permanent hypothyroidism and such patients should be managed accordingly. While the effects of antithyroid drugs given after ^{131}I therapy on uptake measurements were not examined in this study,

and although antithyroid drug treatment is thought to influence uptake behaviour (Alexander et al. 1967; McLarty, 1973; Wilkin et al. 1981) it is likely that similar assumptions can be made regarding the high or low (i.e. greater than 8% or less than 2%) uptake measurements after ^{131}I therapy in drug treated patients. The use of carbimazole pretreatment alone does not seem to change significantly the pattern of response of uptake, nor its biochemical expression after ^{131}I. The potential radioprotective effect of such therapy is discussed more fully in sections 5-7 of this chapter.

SECTION THREE

TRANSIENT HYPOTHYROIDISM FOLLOWING RADIOIODINE THERAPY FOR THYROTOXICOSIS

3.3.1. Introduction

Recovery of thyroid function following hypothyroidism induced by ^{131}I therapy has been reported in a number of series since the introduction of this form of treatment (Dorfmann et al. 1977; McGirr et al. 1964; Segal et al. 1961). This phenomenon, described as transient hypothyroidism, occurs within 12 months of ^{131}I therapy and adequate thyroid hormone production recovers over the course of a few months. Despite considerable experience gained with ^{131}I therapy over 30 years neither the mechanism underlying the phenomenon nor effective means of predicting recovery of "early hypothyroidism" are clear. The experience of transient hypothyroidism in patients given ^{131}I in the current studies will be presented in this section.

3.3.2. Patients and methods

Fifty five patients with clinical and biochemical evidence of thyrotoxicosis treated with ^{131}I were followed for a minimum of 12 months. During this first year after ^{131}I therapy patients were seen every 4 weeks. The details of these patients have already been given in the preceding section (Tables 7 + 8). Similarly, pretreatment assessment and dosimetry have already been described.

At each visit patients were assessed clinically and blood was taken for measurement of T3, T4 and TSH. Before therapy, and at each visit (unless on thyroid hormone replacement) thyroid uptake of ^{123}I 20 and 60 minutes after intravenous administration of the isotope was measured. Kinetic analysis of ^{123}I uptake was performed in 2 subjects who developed transient hypothyroidism using the gamma camera/computer system.

Hypothyroidism was diagnosed on the basis of a low serum concentration of T4 and elevated serum TSH. Thyroid hormone replacement therapy was given if clinically indicated using tri-iodothyronine 20 µg t.i.d. This was withdrawn after minimum of 8 weeks for at least 7 days, and basal biochemistry and uptake measurements repeated. Permanent hypothyroidism was diagnosed if withdrawal of replacement therapy resulted in a rise of

serum TSH with a persistently subnormal serum T4.

3.3.3. Results

Six patients developed hypothyroidism which was transient during the first 12 months of follow-up, all within 12 weeks of ^{131}I therapy. Further details of these patients are shown in Table 17. It will be noted that 2 patients had received carbimazole before ^{131}I treatment whereas 4 had not. The mean delay before onset of hypothyroidism after ^{131}I was 9 weeks, and the mean duration of hypothyroidism was 8 weeks. Subsequent follow-up of these patients for as long as 5 years has shown that 2 have developed permanent hypothyroidism (both 4 years after ^{131}I therapy, subjects 2 and 6) and 4 have remained euthyroid. Serial uptake data for these 6 patients are also shown in Table 18. In patients 1 and 2 kinetic analysis of radioisotope (^{123}I) uptake showed a major defect of iodide organification with a low derived binding rate for iodide (Kb) at the time of diagnosis of hypothyroidism. The normal thyroid gland incorporates over 15% of the theoretical intrathyroidal inorganic iodide pool into organic form per minute. In both of these patients the value derived for binding rate was less than 0.05 (5%) per minute. With time, serial kinetic studies show a return to normal organification, the improvement coinciding with a rise in serum T4. It is of interest that the ratio between 20 and 60 minute uptakes in these patients at the time of the organification defect was less than 1.6.

In patient 3 an elevated 20 minute uptake at the time of diagnosis of hypothyroidism was noted, with a ratio of 60:20 minute uptake of 1.39. Return of normal thyroid hormone levels was associated with a fall in 20 minute uptake but an increase in the ratio between 60 and 20 minutes (1.8). In patients 4 and 5, 20 minute uptake was not elevated when TSH was high, and in patient 4 the ratio between 60 and 20 minute uptake was low. In patient 6, there was again an abnormally low ratio between 60 and 20 minute uptake at the time of diagnosis of hypothyroidism, with an increase in the ratio associated with return of normal thyroid function.

In contrast, in most subjects in whom hypothyroidism proved to be permanent, early radioiodine uptake measurements were low when TSH was high and did not return to normal within the period of observation.

3.3.4. Discussion

TABLE 17

PATIENTS WHO DEVELOPED TRANSIENT HYPOTHYROIDISM AFTER ^{131}I TREATMENT

NO.	SEX	AGE	DOSE ^{131}I (mCi)	? CARBIMAZOLE PRETREATMENT	PRETREATMENT 20 MIN ^{123}I UPTAKE	TIME TO DEVELOP HYPOTHYROIDISM (WEEKS)	DURATION OF HYPOTHYROIDISM (WEEKS)	EVENTUAL OUTCOME
1	F	55	8.4	YES	63.4	12	4	HYPOTHYROID 4 YRS.
2	F	75	7.6		25.4	8	10	HYPOTHYROID 4 YRS.
3	F	50	4.4		27.6	8	8	EUTHYROID 3 YRS.
4	F	57	3.6		16.9	8	8	EUTHYROID 2 YRS.
5	F	54	3.7	YES	12.4	12	4	EUTHYROID 3 YRS.
6	F	62	8.5		26.9	8	16	EUTHYROID 4 YRS.

TABLE 18 (PART I)

SERIAL THYROID FUNCTION TESTS IN PATIENTS DEVELOPING TRANSIENT HYPOTHYROIDISM

NO	TIME AFTER ^{131}I (WEEKS)	T4 nmol/l	T3 nmol/l	TSH mU/l	20 MIN UPTAKE ^{123}I %	60 MIN UPTAKE ^{123}I %	IODIDE BINDING RATE (KG) FRACTION/MIN
1	0	132	4.32	3	63.4	-	-
	4	186	3.32	-	5.4	5.4 (59.3)*	0.008
	8	124	1.4	-	13.0	13.1 (82)	0.003
	12	40	1.73	16	14.5	18.5 (37.3)	0.017
	16	105	2.52	6.4	13.8	18.1 (25.1)	0.031
	32	107	2.9	6	6.5	10.4 (0)	0.15
2	0	176	5.78	-	25.4	-	-
	4	146	3.2	5.3	5.4	-	0.18
	8	39	1.46	26.7	11.0	13.1 (36)	0.47
	12	78	2.38	9.3	13.5	24.0 (13.3)	0.042
	18	106	2.48	5	6.1	11.0 (15.5)	-
	40	73	2.55	7	4.1	7.8 (0)	0.15
3	0	316	7.54	-	27.6	-	
	4	227	4.22	-	3.4	-	
	8	31	1.37	50	15.1	21.7	
	16	100	2.5	10	5.4	9.7	
	40	122	2.45	4.5	14.0	24.9	

*FIGURES IN PARENTHESES INDICATE % OF IODIDE TAKEN BY THE GLAND WHICH IS DISCHARGED BY PERCHLORATE

TABLE 18 (PART II)

SERIAL THYROID FUNCTION TESTS IN PATIENTS DEVELOPING TRANSIENT HYPOTHYROIDISM

NO	TIME AFTER ^{131}I (WEEKS)	T4 nmol/l	T3 nmol/l	TSH mU/l	20 MIN UPTAKE ^{123}I %	60 MIN UPTAKE ^{123}I %	IODIDE BINDING RATE (KG) FRACTION/MIN
4	0	185	2.8	-	10.5	16.9	
	4	102	1.3	3.6	2.7	3.0	
	8	39	1.02	11.7	2.1	3.3	
	16	45	1.2	12	2.7	5.2	
	32	102	1.88	4.8	6.5	12.4	
5	0	68	3.3	3	12.4	26.6	
	4	112	2.3	6.2	1.7	1.6	
	12	39	1.52	16.6	3.5	7.2	
	16	89	2.45	3.8	5.2	10.7	
	40	92	2.3	1.7	5.6	11.6	
6	0	56	3.0	-	26.9	42	
	4	82	1.6	2	1.1	0.7	
	8	48	0.79	2.5	7.3	6	
	16	18	0.4	72	9.8	-	
	40	100	1.4	1.3	12.9	18.6	

*FIGURES IN PARENTHESES INDICATE % OF IODIDE TAKEN BY THE GLAND WHICH IS DISCHARGED BY PERCHLORATE

The data confirm that transient hypothyroidism occurs in a proportion of thyrotoxic patients treated with ^{131}I. The percentage in this series (11%) is similar to that reported by McGirr et al. (1964) and Dorfman (1977). Until recently, however, precise biochemical definition of thyroid status has not been possible and many earlier reports are, therefore, less well documented. In the most complete series to date, Sawers (Sawers, Toft, Irvine, et al. 1980) suggested that transient hypothyroidism might be due to either a recovery of follicular cell function following irradiation damage or to compensatory hypertrophy/hyperplasia of undamaged cells: no evidence in support of either hypothesis was produced. In 3 patients (1,2 and 3) with transient hypothyroidism in the current series, early ^{123}I uptake was elevated beyond the normal established range. In this situation, it seems likely that hypothyroidism is due to failure of organification of iodide: the elevated 20 minute uptake indicates that the trapping mechanism for iodide was capable of responding in a relatively normal manner to elevated serum concentrations of TSH. Increased iodide trapping at a time of inadequate thyroid hormone production is very suggestive of a defect in iodide organification. We have confirmed that a binding defect for iodide was present at the time of hypothyroidism in 2 patients from this group in whom serial kinetic studies were performed, with recovery of this defect occurring over the following weeks. A similar situation appears to be present in patient 3 in whom the ratio of 60:20 minute uptakes was low at the time of hypothyroidism. In patient 6, 20 minute uptake of ^{123}I was at the upper end of the normal distribution, but again the ratio between 60 and 20 minute uptake was abnormally low, suggesting that organification was severely impaired. A rise in this ratio was associated with recovery of thyroid hormonogenesis.

In patient 4, the 20 minute uptake of ^{123}I was not elevated at the time of diagnosis of hypothyroidism despite an elevated serum concentration of TSH, suggesting radiation damage to the iodide trapping process. Thyroid hormone production increased over the next few weeks without any major change in 20 minute uptake of ^{123}I: there was, however, an increase in the 60 minute uptake over this period. This may again be consistent with initial impairment of organification improving allowing increased thyroid hormone production.

In patient 5 20 minute uptake of ^{123}I was low at the time of

diagnosis of hypothyroidism, again indicating dysfunction of iodide trapping. The increment between 20 and 60 minutes was not abnormal, however, suggesting that organification of iodide was relatively unimpaired. Over the subsequent few weeks thyroid hormone production recovered and associated with this was a small rise in early iodine uptake measurements. This patient received carbimazole treatment prior to ^{131}I therapy and it is possible that this may induce intrathyroidal iodide depletion (Barandes, Hurley and Becker, 1973; Harden, Alexander and Koutras, 1966). With reduction in the amount of iodide trapped by the gland following ^{131}I therapy, insufficient intrathyroidal iodide may have been available to maintain satisfactory thyroid hormone production. Over a period of time, with some recovery of iodide trapping, intrathyroidal stores may have been replenished sufficiently to allow satisfactory thyroid hormone production.

It seems, therefore, that the mechanisms underlying transient hypothyroidism may not be homogeneous. There is evidence, however, in this group of patients that reversible impairment of organification with or without changes in trapping of iodide can lead to transient hypothyroidism. Reversible organification defects following ^{131}I therapy have been noted by Gray (1975), who showed that in some patients treated with ^{131}I, iodide could be discharged from the gland by sodium perchlorate in the early stages after treatment. In all the patients studied by Gray, this defect improved over a period of some weeks. Other authors have claimed that minor degrees of impairment of organification are common following ^{131}I therapy (Kirkland, 1954; Suzuki and Mashimo, 1972), although these abnormalities reported were not associated with any consistent clinical or biochemical changes. In chapter 2 of this section evidence of impairment of organification was seen in around 30% of patients studied within the first 4 months after ^{131}I administration. It is of interest that all cases of transient hypothyroidism in this series also occurred within the first 4 months after therapy, and it seems likely that this reversible impairment of thyroid iodide processing is responsible for the majority of cases of reversible hypothyroidism. In contrast, major damage to the iodide trapping mechanism does not appear to recover and results in permanent hypothyroidism.

Failure to recognise hypothyroidism that is transient following ^{131}I therapy involves placing a significant percentage of patients on unnecessary replacement treatment. If the aim of ^{131}I treatment is to attain

euthyroidism without the need for replacement therapy, it is clearly important that those patients whose hypothyroidism may be transient are recognised and dealt with accordingly. It has been suggested that is reasonable to withhold replacement therapy for a short period in all patients developing hypothyroidism early after ^{131}I, to allow possible recovery to occur (Sawers et al. 1980). Symptoms of hypothyroidism in patients who have been recently thyrotoxic may, however, be uncomfortable and this policy may involve placing an unacceptably high number of patients at risk of such symptoms. An alternative policy might be to use Tri-iodothyronine as replacement therapy in early hypothyroidism if clinically indicated and to withdraw treatment after a period of time to reassess thyroid function. This system would be rather impractical for general application and would also result in abrupt changes in thyroid hormone status in this group of patients. As an alternative, measurement of early ^{123}I uptake at the time of diagnosis of hypothyroidism may be of some predictive value. Where uptake is low (less than 2%) at the time of TSH elevation, hypothyroidism is due to failure of iodide trapping and is unlikely to recover: placement of the patient on permanent replacement therapy would be reasonable. Where early iodine uptake is greater than 2% hypothyroidism may be due, in part, to impairment of intrathyroidal organification of iodide, particularly when uptake is elevated beyond the normal range, and recovery of thyroid function in this situation may be anticipated. In this situation it would be reasonable either to withhold therapy if symptoms are minimal or to use a limited course of replacement therapy before reassessment of thyroid biosynthetic capacity.

SECTION FOUR

IMPAIRMENT OF IODIDE ORGANIFICATION FOLLOWING ^{131}I THERAPY

3.4.1. Introduction

Of the 2 major physiological processes involved in synthesis of thyroid hormones (iodide trapping and subsequent incorporation into organic form) iodide trapping is rate limiting (Ingbar, 1978) in that the majority of trapped iodide is rapidly bound. In certain circumstances, however, impairment of organification of iodide may limit thyroid hormone formation. Thus, in patients treated with the antithyroid drug carbimazole, iodide trapping is not directly affected and thyroid function is reduced by inhibition of organification. Hilditch, using a sensitive perchlorate discharge test validated by kinetic analysis of the uptake curve (Hilditch et al. 1980) studied patients treated with carbimazole and showed that there was dose related inhibition of organification of iodide using the drug, and that clinical and biochemical response mirror the effects of drug on this function (Hilditch, 1978). Earlier in this chapter evidence was presented that organification impairment as assessed by the crude ratio of 60:20 minute uptake of ^{123}I was present in around one third of patients early after ^{131}I treatment, and it was suggested that this might account for transient hypothyroidism.

The effects of ^{131}I on thyroid iodide trapping have been considered earlier in this chapter; in the current section detailed studies of iodide organification capacity following ^{131}I therapy are considered.

3.4.2. Patients and methods

Twenty four patients with thyrotoxicosis were studied: all were clinically thyrotoxic at the time of entry to the study with elevated concentrations of both T3 and T4, elevated uptake measurements of ^{123}I 20 minutes after intravenous injection of tracer and diffuse uptake of isotope on scanning. Twelve of the subjects were given carbimazole with thyroid hormone supplements (20-30 mg carbimazole and 0.1 mg T4 daily) so that subjects in this group was all euthyroid at the time of administration of ^{131}I. The other 12 patients were given ^{131}I alone. Further details of these patients have already been given (3.1.2.). Carbimazole and thyroid hormone

supplements were withdrawn 72 hours before ^{131}I administration. Pre ^{131}I assessment and calculation of dose of ^{131}I were performed as described in chapter 2.

Patients were reviewed every 4 weeks after ^{131}I administration. Measurement of iodide organification was performed at 6, 16, 32 and 48 weeks after ^{131}I administration unless patients were being treated for hypothyroidism (transient hypothyroidism was excluded as described in section 3) or for relapse of thyrotoxicosis. A tracer dose of ^{123}I (150 μCi) was given intravenously and uptake of the isotope measured over the next 30 minutes using a gamma camera/computer system as described earlier. Sodium perchlorate (450 mg) was then given by intravenous injection and the effect on further accumulation and retention of tracer followed for the next 30 minutes. A fall in the uptake measurement after administration of perchlorate was regarded as a positive test, with the difference between the uptake measurement immediately before and 30 minutes after perchlorate being expressed as the post-perchlorate discharge. Hilditch (1978) has shown that there is a close correlation between this measurement and binding rate for iodide (K_B) using kinetic analysis of radioisotope uptake curves. A typical uptake curve in a patient with no evidence of organification impairment, and in one with a positive perchlorate discharge test has been shown in Figure 7.

3.4.3. Results

Patient characteristics and the biochemical outcome following ^{131}I therapy over the subsequent 12 months are summarised in Table 19. The experience of organification defects following ^{131}I administration in this group of patients is summarised in Table 20. It will be seen that of those subjects given ^{131}I therapy alone, 3 (25%) (No. 5,11,12) had an organification defect 6 weeks after ^{131}I administration. Ten weeks later, the defect was still present in those subjects, but a further 3 (in whom uptake measurement was very low at 6 weeks) (No. 2,3,6) had developed positive tests. In all subjects in whom the study could be repeated at 32 weeks (those who had not become hypothyroid) the percentage of ^{123}I discharged by perchlorate fell with time or disappeared. By 48 weeks after treatment a positive test was still present in 2 subjects, although the size of the discharge was very small in both instances. In one subject (number 5) the test had also been

TABLE 19

PATIENTS IN WHOM DETAILED STUDIES OF IODIDE ORGANIFICATION FOLLOWING ^{131}I THERAPY WERE MADE. (Data reproduced in Table 4).

	NO.	SEX	20 MINUTE UPTAKE OF ^{123}I BEFORE ^{131}I (%)	OUTCOME AFTER ^{131}I 3 MONTHS	6 MONTHS	1 YEAR
PATIENTS GIVEN ^{131}I ALONE	12	11F:1M	24.9* ± 9.6	3 EUTHYROID 5 TOXIC 4 HYPOTHYROID	2 EUTHYROID 6 TOXIC 4 HYPOTHYROID	4 EUTHYROID 4 TOXIC 4 HYPOTHYROID
PATIENTS GIVEN PRETREATMENT WITH CARBIMAZOLE	12	10F:2M	28.5 ± 16.1	8 EUTHYROID 3 TOXIC 1 HYPOTHYROID	7 EUTHYROID 4 TOXIC 1 HYPOTHYROID	7 EUTHYROID 4 TOXIC 1 HYPOTHYROID
ALL	24	21F:3M	26.3 ± 13.1	11 EUTHYROID 8 TOXIC 5 HYPOTHYROID	9 EUTHYROID 10 TOXIC 5 HYPOTHYROID	11 EUTHYROID 8 TOXIC 5 HYPOTHYROID

* MEAN ± SD

TABLE 20 (PART I)

SERIAL ORGANIFICATION STUDIES AFTER ^{131}I TREATMENT

A. PATIENTS GIVEN ^{131}I ALONE

NO.	BEFORE ^{131}I 20*	6 WEEKS 20 ; CLO4 DISCHARGE** %	16 WEEKS 20 ; CLO4 DISCHARGE %	32 WEEKS 20 ; CLO4 DISCHARGE %	48 WEEKS 20 ; CLO4 DISCHARGE %
1	19.9	9 ; -	7.2 ; -	5.2 ; -	5.6 ; -
2	29.1	0 ; -	7.8 ; 17.1	9.4 ; 7.1	
3	10.0	0.5 ; -	10.7 ; 40.9		18.5 ; -
4	29.9	12.8 ; -	29.9 ; -	29.5 ; -	18.2 ; 5.3
5	38.8	11.3 ; 31.7	9.5 ; 16	15.5 ; -	5.7 ; 11.3
6	13.7	0 ; -	2.8 ; 24.7	HYPOTHYROID	
7	33.4	12.5 ; -	16.4 ; -	13.7 ; -	11.5 ; -
8	29.2	1.3 ; -	11.6 ; -	11.4 ; -	6.3 ; -
9	19.4	1.6 ; -	1.4 ; -	HYPOTHYROID	
10	8.6	0.5 ; -	HYPOTHYROID		
11	29.2	1.6 ; 63.4	1.9 ; 92.4	HYPOTHYROID	
12	27.6	13.0 ; 12.4	15.4 ; 4.2	16.6 ; 1.8	17.0 ; -

B. PATIENTS PRETREATED WITH CARBIMAZOLE

NO.	BEFORE ^{131}I 20*	6 WEEKS 20 ; CLO4 DISCHARGE** %	16 WEEKS 20 ; CLO4 DISCHARGE %	32 WEEKS 20 ; CLO4 DISCHARGE %	48 WEEKS 20 ; CLO4 DISCHARGE %
13	18.9	3.5 ; 48.9	2.1 ; 42.1	HYPOTHYROID	
14	39.2	17.2 ; 26.0	21.2 ; 7.1	23.5 ; -	22.7 ; -
15	31.1	16.8 ; -	10.6 ; -	47.5 ; -	RETREATED
16	23.3	4.3 ; -	ON CARBIMAZOLE	ON CARBIMAZOLE	14.9 ; -
17	62.9	0 ; -	23.7 ; -	RETREATED	
18	5.1	4.4 ; -	5.2 ; -		
19	6.4		8.4 ; 5.9	6.5 ; -	4.7 ; -
20	22.5		6.5 ; -		4.6 ; -
21	47.3		23.2 ; -	14.3 ; -	
22	29.1		26.0 ; -	17.9 ; -	
23	27.6	10.4 ; -	9.0 ; -	15.1 ; -	8.7 ; -
24	28.1	30.0 ; -	31.2 ; -	RETREATED	

* 20 = UPTAKE OF TRACER ^{123}I AT 20 MINUTES

** CLO4 % = % OF UPTAKE DISCHARGED 30 MINUTES AFTER I.V. PERCHLORATE

positive at 6 and 16 weeks after ^{131}I, while in the other (No. 4) the test had previously been negative. Thus, 50% of subjects in this group had evidence of iodide organification defects within the first 4 months after ^{131}I administration.

Of the subjects given carbimazole before ^{131}I therapy, 2 (No. 13,14) had a positive test 6 weeks after ^{131}I and in one further subject (No. 19) in whom the measurement at 6 weeks could not be made, a positive test was noted at 16 weeks. One of these subjects (number 13) was hypothyroid at 16 weeks and was not studied further. Of the other 2, the defects were not present at subsequent times. Thus, of this group only 25% had a positive perchlorate discharge tests following ^{131}I therapy.

It will be noted that the patients who developed positive perchlorate tests were not identified by any typical pretreatment characteristic or post treatment uptake measurement. Positive tests were present in subjects who had a wide range of pre-perchlorate uptake measurements (1-21%). However, in those subjects in whom the test was positive there was an inverse correlation (Figure 20) between the size of the perchlorate discharge and the pre-perchlorate uptake measurement.

The effect of organification impairment on biochemical function is examined in Table 21. In the subjects with very large defects, uptake of ^{123}I at 30 minutes after injection of the tracer was also very low and those subjects either were or became hypothyroid shortly after the test. In contrast, 2 subjects with persistent thyrotoxicosis had positive tests. In both of these, the perchlorate discharge was small and the rate limiting step in thyroid hormonogensis was still clearly iodide trapping. Of those subjects who were euthyroid at the time of testing, pre-perchlorate uptake was not lower in those who had no discharge (mean = 10.4%) than in those who had a positive test (8.6%).

3.4.4. Discussion

Incorporation of plasma inorganic iodide into thyroid hormone comprises 2 principle biochemical processes (discussed in 1.1.2.): the trapping of iodide across the thyroid cell membrane against a concentration gradient and the subsequent organification of this intrathyroidal inorganic iodide to form iodotyrosine residues. The effect of ^{131}I on iodide trapping has been discussed in section 2 of this chapter.

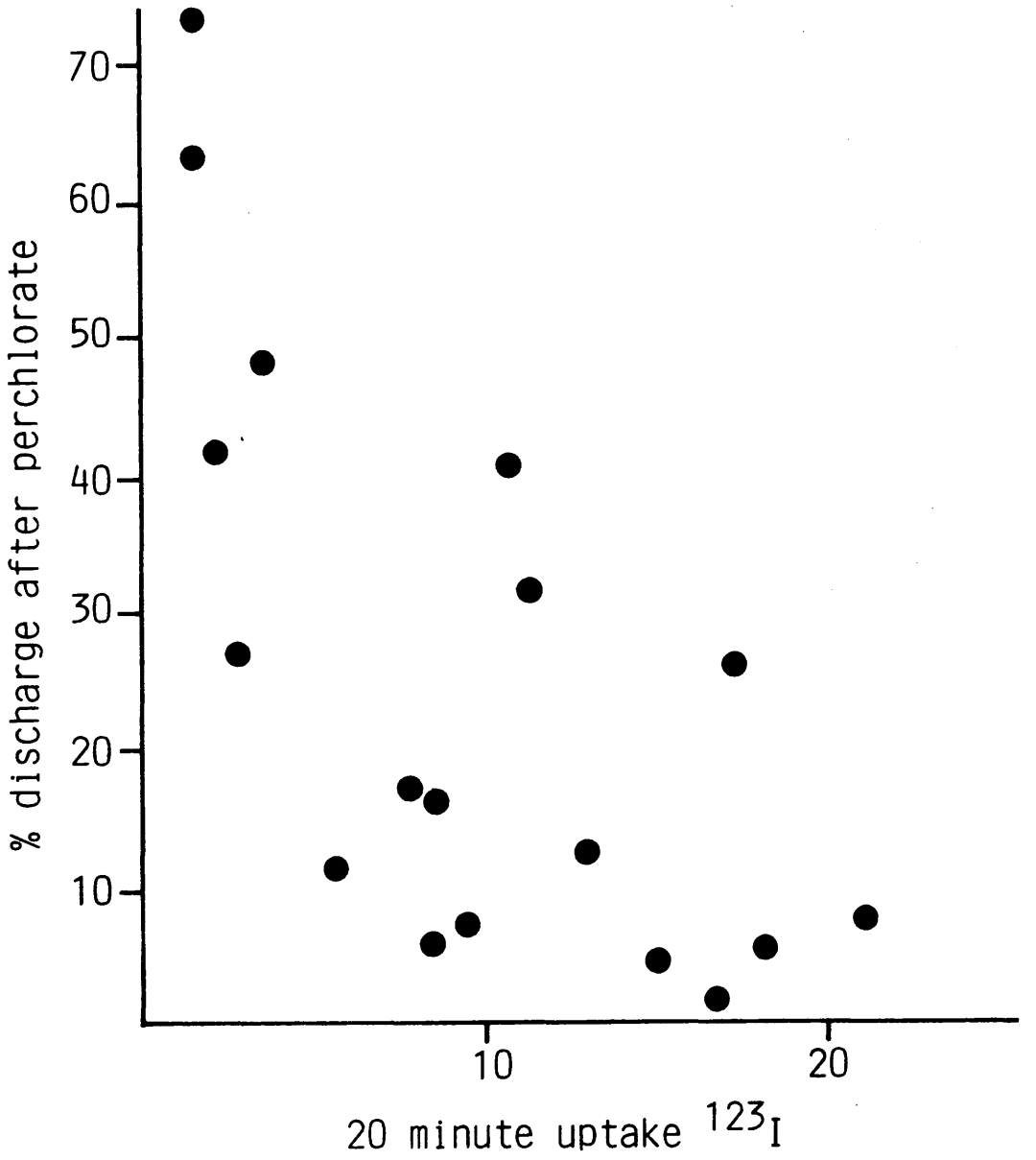


Fig 20

RELATIONSHIP BETWEEN EARLY IODIDE (^{123}I) UPTAKE AND PERCHLORATE DISCHARGE IN SUBJECTS WITH ORGANIFICATION DEFECTS FOLLOWING ^{131}I TREATMENT.

THE UPTAKE OF ^{123}I 20 MINUTES AFTER INTRAVENOUS INJECTION OF ISOTOPE IS PLOTTED AGAINST THE PERCENTAGE OF UPTAKE DISCHARGED BY PERCHLORATE IN THOSE SUBJECTS WHO HAD POSITIVE TESTS FOLLOWING ^{131}I. THERE IS AN INVERSE RELATIONSHIP BETWEEN THE TWO MEASUREMENTS ($r = -0.61$) SUGGESTING THAT WHEN ORGANIFICATION IS IMPAIRED, THE SEVERITY OF DAMAGE TO IODIDE TRAPPING IS REFLECTED IN THE SIZE OF THE ORGANIFICATION DEFECT.

TABLE 21 (PART I)

SERIAL CHANGES IN THYROID FUNCTION IN SUBJECTS WITH ORGANIFICATION DEFECTS

A. ^{131}I ALONE

SUBJECT NO.	BASAL 20↑[1]	%Δ[2]	T4[3]	T3[4]	TSH[5]	SIX WEEKS 20↑	%Δ	T4	T3	TSH	SIXTEEN WEEKS 20↑	%Δ	T4	T3	TSH
2	29.1		216	4.4	<0.1	0	0	192	2.53	<0.5	7.8	17.1	115	3	0.9
3	10		168	3.41	<0.4	0.5	0	82	1.33	1.9	10.7	40.9	72	2.79	1.9
4	29.9		228	6	<0.7	12.8	0	216	5.7	0	29.9	0	206	6.5	C
5	38.8		292	11.5	0	11.3	31.7	119	5.8	<0.6	9.5	16	72	1.34	1.4
11	29.2		246	6.6	<0.2	1.6	63.4	91	1.9	12	1.9	92.4	12	0.3	46
12	27.6		270	7.3	0	13	12.4	211	3.56	0.9	15.1	4.2	167	4.2	<0.2
B.	PRETREATED WITH CARBIMAZOLE														
13	18.9		97	2.18	0.6	3.5	49	111	1.6	1.3	2.1	42.1	37	0.7	31
14	39.2		117	1.7	3	17.2	26	158	2.7	0.1	21.2	7.1	167	4.7	0
19	8.4		105	1.38	2.1			116	1.5	2.4	8.4	5.9	79	1.6	3

1) 20 MINUTE UPTAKE OF ^{123}I: % DOSE.

2) PERCHLORATE DISCHARGE : % OF 20 MINUTE UPTAKE

3) SERUM THYROXINE, nmol/l. nr 54 - 142

4) SERUM TRIIODOTHYRONINE, nmol/l nr 0.8 - 2.46

5) SERUM TSH mU/ml nr < 6

TABLE 21 (PART II)

SERIAL CHANGES IN THYROID FUNCTION IN SUBJECTS WITH ORGANIFICATION DEFECTS

A. ^{131}I ALONE

SUBJECT NO.	THIRTY-TWO WEEKS 20↑ [1]	%Δ [2]	T4 [3]	T3 [4]	TSH [5]	ONE YEAR 20↑	%Δ	T4	T3	TSH
2	9.4	7.1	110	2.1	0.6	8	-	111	1.48	1.1
3			157	4.5	0	18.5	0	140	3.73	< 0.1
4	29.5	0	190	6.9	0	18.5	5.3	134	5.01	0
5	15.5	0	111	3.2	0	5.7	11.3	163	2.9	<0.7
11										
12	16.6	1.8	214	5.24	0	17	0	164	3.09	0
B. PRETREATED WITH CARBIMAZOLE										
13										
14	23.5	0	149	3.8	< 0.6	22.7	0	137	3.3	< 0.6
19	6.5	0	90	1.9	2.1	4.7	0	86	2.1	3.4

1) 20 MINUTE UPTAKE OF ^{123}I: % DOSE

2) PERCHLORATE DISCHARGE : % OF 20 MINUTE UPTAKE

3) SERUM THYROXINE, nmol/l nr 54 - 142

4) SERUM TRIIODOTHYRONINE, nmol/l nr 0.8 - 2.46

5) SERUM TSH mU/l nr < 6

Efficiency of organification of iodide in the current study was assessed by measuring the amount of tracer ^{123}I discharged from the thyroid after intravenous administration of sodium perchlorate. This technique has been validated previously by Hilditch (1978): in a study of patients with thyrotoxicosis it was demonstrated that the test accurately measured the effect of the antithyroid drug carbimazole on iodide organification (Hilditch et al. 1980). Normal subjects and patients with untreated thyrotoxicosis had no iodide discharged by perchlorate indicating normal organification.

In the current study, 3 of the subjects given ^{131}I alone had clear evidence of organification impairment 6 weeks after treatment, and 50% of the subjects in this group had a positive test at 16 weeks. Of these, 2 subjects had very low iodine uptake measurements and were biochemically hypothyroid, 2 were euthyroid and 2 had persistent thyrotoxicosis. Of these last 4 subjects, the organification defects tended to improve with time. In contrast, in the group given carbimazole pretreatment before ^{131}I therapy only 25% of subjects had evidence of organification impairment at any time after ^{131}I therapy. Although small numbers preclude statistical analysis of such data, it seems possible that pretreatment with carbimazole was associated with a lesser degree of radiation damage to the organification process, and may be further evidence of a radioprotective effect of carbimazole. The inverse correlation between the degree of organification impairment (percentage discharged) and the early iodine uptake (Figure 20) may reflect the spectrum of severity of irradiation damage in such patients.

The incidence of organification impairment following ^{131}I has been difficult to assess from previous studies. Kirkland (1954) using a thiocyanate discharge test, reported that almost all patients given ^{131}I showed discharge of tracer iodide and interpreted this as being consistent with organification impairment. It is now recognised, however, that thiocyanate itself impairs iodide organfication and is thus unsuitable for studying the phenomenon. Stewart and Murray (1967) described an alternative means of assessing organification using administration of oral perchlorate along with a range of doses of carrier iodide (100-2000 µg), and measured the discharge of tracer ^{131}I. They showed that a dose of 750 µg of iodide cause the test to become positive in normal subjects, while a smaller dose of iodide induced the test to become positive in subjects with untreated thyrotoxicosis. Similarly, in 7 of 11 patients treated with ^{131}I, administration of greater

than 200 µg of iodide led to a positive test, while perchlorate tests in the absence of iodide were normal. Other workers have used a similar iodide/perchlorate discharge test in patients treated with ^{131}I. Kieffer and colleagues (Kieffer, Medeiros-Neto, Rueta, et al., 1965) showed that all patients who responded to ^{131}I therapy had a positive test within 4 months of ^{131}I, while Suzuki and Mashimo (1972) also found a high incidence of positive tests at a similar time. There is no evidence, however, that combined iodide/perchlorate tests provide an accurate measure of iodide organification rate. While the test may be a sensitive indicator of thyroid dysfunction, it is likely that it basically demonstrates increased sensitivity to the acute effects of iodide administration on iodide organification (Wolff-Chaikoff effect) (Wolff and Chaikoff, 1948). Thus, Koutras and colleagues in 1964 demonstrated that addition of a small amount of iodide to the diet of normal subjects led to an increase in absolute thyroid iodide uptake without changing circulating levels of protein bound iodide (Koutras, Alexander, Harden, et al. 1964). It was deduced from this that the increased dietary intake of iodide caused a fall in iodide organification rate. Although this evidence is indirect, the studies of other workers support this hypothesis. Braverman reported that when patients were given potassium iodide supplements after ^{131}I therapy or subtotal thyroidectomy, more than 50% became hypothyroid (Braverman, Woeber and Ingbar, 1969) (i.e. did not escape as is usual from the Wolff-Chaikoff effect). Before potassium iodide was given only one subject had a positive perchlorate discharge test, while all subjects had a positive test afterwards. The authors suggested that the thyroid gland in Graves' disease is uniquely susceptible to the blocking effect of iodide on organification. Whether this is so is uncertain, but it does suggest that the use of iodide with perchlorate may induce organification impairment rather than demonstrating its presence in the basal state.

More recently Gray (1975) used an intravenous perchlorate test similar to that in the current study. He found that around one third of patients from a group given ^{131}I therapy had a positive test within 3 months of treatment: in contrast, a much higher rate of organification impairment after treatment with ^{125}I was seen. In an earlier section (3.2.6.) indirect evidence of impairment of iodide organification was found in 30% of a larger group of patients given ^{131}I. It is of interest that the peak incidence of positive perchlorate discharge tests in the group of patients described in the current

chapter (16 weeks) is similar to the timing of the abnormal ratio in early uptake measurements (3.2.6.) lending credence to the use of the 60 to 20 minute uptake ratio as a measure of organification impairment. Using the more sensitive test in the current study around 38% of patients had an episode of organification impairment following ^{131}I treatment, and this was more common if patients who were given carbimazole before ^{131}I therapy are excluded.

Iodide organification seems to be less sensitive to the effects of ionising radiation than iodide trapping. Thus, although a marked fall in 20 minute iodide uptake occurred in all subjects in the current study, less than 50% had evidence of impairment of organification at the same time. The reason for this is unclear. Iodide trapping is localised to the basal thyroid cell membrane, whereas organification occurs at the apex of the cell at the cell/colloid interface, near the site of deposition of labelled iodide within the follicle (Ekholm and Wollman, 1975). It might be supposed, therefore, that the biochemical mechanisms involved in organification would be exposed to at least as great an amount of radiation as those involved in iodide trapping. This proximity of the site of organification to the source of radiation was thought to be a possible explanation for the higher incidence of organification defect following ^{125}I, which emits electrons with a very short range of travel within tissue (Gray, 1975; Ertl et al. 1970).

It may be of relevance that the process of iodide organification involves generation of free radicals derived from iodide; there is normally a means of protecting the cell from free radical damage: for example free iodide derived radicals will react with tyrosine residues on the thyroglobulin molecule to form iodotyrosines. The damaging effects of radiation in tissues may also be due to production of free radicals (possibly superoxide or hydroxyl) (Phil and Edjarn, 1958): it may be that the specialised nature of thyroid peroxidase/H_2O_2/iodide/tyrosine interaction can lead to neutralisation of the free radicles produced by radiation and so reduce the extent of radiation damage to the portion of the cell involved in iodide organification. It is known that in other tissues enzymes of the peroxidase class can act, along with superoxide dismutase, to neutralise hydroxyl free radicals.

The impact of organification defects on thyroid hormone production in the current study appears to be small. Thus, persistent biochemical thyrotoxicosis was present in 3 subjects by 16 weeks after ^{131}I therapy at a

time when organification impairment was present. It is clear that there is considerable reserve of thyroid organification capacity within which thyroid hormonogenesis can be regulated by iodide trapping. Hypothyroidism may develop as a consequence of organification impairment only in exceptional circumstances where iodide trapping is critically reduced. Given the tendency for this organification impairment to improve with time in the current study, such hypothyroidism would be expected to be temporary. This phenomenon has been discussed in 3.3.4.

In summary, using a sensitive intravenous perchlorate discharge test less than 50% of patients studied had evidence of organification impairment following ^{131}I therapy: this data is consistent with previous studies; the defects in organification tended to improve with time. No evidence of a major effect of organification impairment on thyroid hormone production was seen. Impairment of organification may be less common in subjects pretreated with Carbimazole, possibly reflecting a radio-protective action of the drug.

SECTION FIVE
EFFECT OF PRETREATMENT WITH CARBIMAZOLE ON OUTCOME AFTER ^{131}I THERAPY

3.5.1. Introduction

The response within one year of treatment to subablative doses of ^{131}I is variable and is influenced by a number of factors which include goitre size, effective half-life of administered dose of ^{131}I and uniformity of radioisotope uptake within the gland (Wilson, 1976). The use of thiourylene antithyroid drugs before ^{131}I therapy may also alter the response to ^{131}I by a "radio-protective" effect. Crooks et al. (1960) showed that pretreatment with methylthiouracil lowered the single dose cure rate following ^{131}I, while a subsequent study showed that potassium perchlorate, which is chemically and pharmacodynamically distinct, did not produce this effect (Buchanan et al. 1965). In a comparison of the effects of pretreatment with carbimazole on the response to ^{131}I therapy with ^{131}I alone Goolden and Fraser (1969) were unable to demonstrate any change in outcome in the combined therapy group. More recent studies, however, have suggested that this drug does lower the incidence of early hypothyroidism following ^{131}I treatment (Aro et al. 1981; Bliddal et al. 1982; Reynolds and Kotchen, 1979; Steinbach et al. 1979). In these latter studies antithyroid drugs were given both before and after ^{131}I therapy, and interpretation of remission rates in this setting may be complicated by the effects of carbimazole on the course of auto-immune thyroid disease (Weetman et al. 1984). The effect of carbimazole pretreatment alone on early outcome following ^{131}I therapy in a group of patients with thyrotoxicosis is examined in the current chapter.

3.5.2. Subjects and methods

Seventy nine patients with clinical and biochemical thyrotoxicosis were studied. Thirty six subjects were treated with carbimazole, 20 to 30 mg per day before ^{131}I therapy. Carbimazole was used for a minimum of 3 months and thyroid hormone supplements (tri-iodothyronine 60 µg per day or thyroxine 0.1 mg per day) given to prevent iatrogenic hypothyroidism. At the time of ^{131}I administration all patients in this group were clinically euthyroid, none having elevation of serum TSH. Carbimazole and thyroid hormone treatment was withdrawn 5 days before ^{131}I therapy in 24 patients

and 72 hours before treatment in the other 12. The other 43 patients were given ^{131}I therapy without carbimazole pretreatment. The age and sex distribution and incidence of thyroid nodularity were similar in the 2 groups. Further details of the treatment groups are given in Table 22. Pretreatment assessment before ^{131}I administration and calculation of dose of ^{131}I was performed as described in chapter 2. The patients were assessed clinically at monthly intervals for one year after ^{131}I therapy, and at each visit blood was taken for measurement of T4, T3 and TSH. Hypothyroidism was diagnosed biochemically on the basis of a low serum T4 and an elevated serum concentration of TSH. Transient hypothyroidism was identified in a small number of patients (6) as described previously: all patients who had an episode of transient hypothyroidism were biochemically euthyroid one year after treatment. A small number of patients had clinically marked thyrotoxicosis following ^{131}I, and carbimazole was given to such subjects between 4 and 10 months after treatment. On withdrawal of treatment with Carbimazole all of these subjects (n = 5) had a relapse of thyrotoxicosis. For the purpose of this study they are regarded as thyrotoxic for the whole of the first year. Similarly, if a second dose of ^{131}I given 4 months after the initial dose such patients (1) were regarded as being thyrotoxic for the whole of the first year.

3.5.3. Results

Outcome following ^{131}I therapy in the 2 groups of patients is shown in Table 23. The incidence of permanent hypothyroidism 3 months after therapy was higher in the patients given ^{131}I alone (40% v 19%). In contrast, patients pretreated with carbimazole had a higher incidence of thyrotoxicosis at this time ($X^2 = 4.5$, $p < 0.05$). One year after therapy the incidence of persistent thyrotoxicosis in the 2 groups was similar (25%). However, more patients in the group given ^{131}I alone were hypothyroid and less were euthyroid than in the combined treatment group ($p < 0.05$). Six patients had an episode of transient hypothyroidism, 2 of these in the group given carbimazole. There was little overall change in thyroid function between 3 months and one year after ^{131}I administration.

TABLE 22

DETAILS OF PATIENTS INCLUDED IN STUDY OF EFFECT OF CARBIMAZOLE ON OUTCOME FOLLOWING ^{131}I TREATMENT

	N	SEX	AGE	GRAVES' DISEASE	TOXIC MULTINODULAR	20 MINUTE UPTAKE ^{123}I%	24 HOUR UPTAKE ^{123}I%	ESTIMATED SIZE (g)	DOSE mCi
^{131}I TREATMENT	43	40F	53.4* ±	35	8	21.2 ±	67.5 ± 15.4	43.3 ±	5.83 ± 3.8
		3M	9.8			21.0		16.1	
CARBIMAZOLE PRETREATMENT	36	32F	55.2 ±	31	5	25.8 ±	75.6 ± 12.6	35.1 ±	6.15 ± 2.5
		4M	10.5			22.2		12.6	

* MEAN ± SD

TABLE 23

OUTCOME AFTER ^{131}I TREATMENT : EFFECT OF CARBIMAZOLE PRETREATMENT

	3 MONTHS			6 MONTHS			9 MONTHS			1 YEAR		
	T[x]	E	H	T	E	H	T	E	H	T	E	H
^{131}I TREATMENT ALONE	10	16	17	13	12	18	12	13	18	11	14	18
	(23)[xx]	(37)	(40)	(30)	(28)	(42)	(28)	(30)	(42)	(25)	(33)	(42)
CARBIMAZOLE PRETREATMENT	14[xxx]	15	7	12	17	7	11	18	7	9	20	7[xxx]
	(39)	(42)	(19)	(33)	(47)	(19)	(31)	(50)	(19)	(25)	(56)	(19)

[x] T = THYROTOXIC
E = EUTHYROID
H = HYPOTHYROID

[xx] FIGURES IN BRACKETS = % OF GROUP

[xxx] $p < 0.05$

3.5.4. Discussion

Pretreatment with carbimazole appeared to result in a lower incidence of hypothyroidism 12 months after ^{131}I.Data were analysed by Chi-squared test using a 2*2 layout(hypothyroid v.non-hypothyroid) without application of Yates' correction.This layout appears to assume that these two alternative outcomes are discrete events.Clearly, thyroid function after ^{131}I forms a continuous spectrum, and the diagnostic classifications adopted are a convenience based on biochemically defined groupings.As these definitions employ more than one hormonal component(e.g.T_4+TSH),it is clearly simpler in this type of analysis to rely on definitions of thyroid status as used herein than to examine other non-independent hormonal variables.This approach also reflects the subdivision of patients used in clinical management of thyroid disease.However,more serious objections are the relatively small sample size and borderline nature of the statistical significance which necessitate caution in interpretion of the apparent radioprotective effect of carbimazole.Although other studies of antithyroid drug/^{131}I treatment have also suggested that drug use is radioprotective(see below),clarification of the significance of such an action of carbimazole requires a larger prospective study.

A further point to be considered concerns study design.It is assumed that the two groups studied were similar in all respects apart from the use of carbimazole(see table 22),and that any difference in outcome reflects a specific interaction of drug with ^{131}I.However, carbimazole made patients in the pretreatment group euthyroid before ^{131}I administration, and it is possible that this difference in thyroid status altered the response to ^{131}I, rather than any specific interaction with drug.The studies of Crooks(1960) and Buchanan(1965) suggest that a radioprotective action of drug pretreatment is specific to thiourylene agents such as methylthiouracil and carbimazole, and is not seen with other drugs which also impair thyroid function(discussed below),and it seems unlikely that a radioprotective effect is a non-specific phenomenon of altered thyroid function.However, the inevitable and major effects of carbimazole on thyroid hormone levels do make assessment of potential interaction of drug and ^{131}I difficult:ideally, the two groups should have similar levels of thyroid hormones at the time of administration of ^{131}I, and not at some earlier period.The nature of the action of carbimazole given for therapeutic reasons excludes this possibility, and this criticism applies to all such studies.Although the current design is appropriate insofar as it reflects clinical practice, a more rigorous

approach, giving carbimazole for a brief time insufficient to alter thyroid hormone levels would be preferred to study radioprotection free of other influences.

Despite these qualifications the results of this study may support the hypothesis that pretreatment alone with carbimazole is radioprotective.Twelve months after ^{131}I this group had a lower incidence of hypothyroidism and contained a higher proportion of euthyroid patients.The mean dose of ^{131}I given to the carbimazole pretreatment group was slightly but not significantly lower than that given to the ^{131}I group and this was accounted for by the higher mean 24 hour ^{123}I uptake in the combined treatment group.As the amount of ^{131}I given was calculated to deliver the same radiation dose to each patient, those with higher 24 hour uptakes received slightly lower doses.It is unlikely that this difference in dose would result in the changes in early outcome observed.

These results are consistent with data reported by Aro and colleagues(1981) and Bliddal et.al.(1982) who independently reported that patients given carbimazole both before and after ^{131}I had a higher relapse rate and a lower incidence of early hypothyroidism compared to those given ^{131}I alone.In a smaller study Steinbach et.al.(1979) examined the effects of methimazole and propylthiouracil on response to ^{131}I.Drugs were not stopped before ^{131}I, and were given for variable times after.While the incidence of hypothyroidism was lower in the combined treatment group,the small numbers(24) and lack of information on treatment regimens detract from the significance of the findings.In the only previous study where carbimazole has been given solely before ^{131}I(drug stopped 48 hours before) there was no major effect on outcome 1 year after treatment(Goolden and Fraser,1969).This study was performed before sensitive assays for T_4 and TSH were available and it is possible that diagnostic classification was less accurate than currently possible.In a major review of ^{131}I therapy Nofal(1966) also noted that antithyroid drug use was associated with a lower incidence of early hypothyroidism.

There are a number of possible mechanisms by which antithyroid drugs might reduce radiation damage following ^{131}I therapy.As tissue damage following ^{131}I may be secondary to generation of superoxide radicals,possibly from H_2O_2, oxidative reduction of peroxidase enzyme in the thyroid cell by carbimazole or related drugs might alter H_2O_2 availability and limit superoxide formation:in view of the rapidity of regeneration of thyroid peroxidase to an oxidised state after antithyroid drug withdrawal *in vitro*(Taurog,1976) it seems unlikely that this

mechanism would be effective 5 days after stopping carbimazole.Drugs of the thiourylene group all contain a -SH moeity, and during intrathyroidal drug metabolism it is possible that disulphide bonds are formed with follicular cell protein(Marchant et.al.1978).Such bonds have been shown to confer radioprotection, possibly by interacting with radiation produced free radicals(Phil and Edjarn,1958).However, antithyroid drugs were withdrawn in the majority of subjects in the current study and in that of Crooks(1960) 5 days before ^{131}I.It is known that the -SH residue of thiourylene drugs is normally oxidised to sulphate and excreted in the urine(Marchant et.al.1978), and it is unclear whether sufficient numbers of sulphydryl groupings in stable covalent bonds with thyroid cell protein would remain to provide radioprotection 5 days after drug withdrawal.

Alteration in thyroid iodide kinetics by antithyroid drugs might influence the response to ^{131}I.In studies where drugs were restarted shortly after ^{131}I therapy, or were continued during treatment, it is likely that drug induced iodide organification block will reduce retention of ^{131}I within the gland.In most studies, however, the dose given to each patient was calculated using tracer iodide uptake data and this should allow for the possibility that drug therapy has altered iodide kinetics, so that the total radiation dose received by each patient is the same.This has generally resulted in patients given combined therapy receiving slightly higher therapeutic doses of ^{131}I than those receiving ^{131}I alone(Bliddal et.al.1982;Crooks et.al.1960;Reynolds and Kotchen, 1979). However, the kinetics of tracer doses of ^{131}I may differ from those of therapeutic doses, especially in patients given medical antithyroid therapy(De Groot and Stanbury,1975) and it is possible that this difference in the handling of therapeutic and tracer quantities of the isotope account for some of the radioprotective effect.

Alternatively, antithyroid drugs might alter the biological and therefore effective half-life of ^{131}I therapy by induction of intrathyroidal iodide depletion.Harden and colleagues(1966) suggested that carbimazole therapy resulted in a contracted intrathyroidal iodide pool:this would lead to a shortened biological and therefore effective half-life of ^{131}I, reducing the radiation dose to the gland.Crooks et.al.(1960), however, found no significant difference in the mean biological half-life of ^{131}I given to patients pretreated with methylthiouracil compared with those given ^{131}I alone.However, carbimazole is a considerably more potent inhibitor of iodide organification than methylthiouracil, and it is possible that more complete blockade of this

process would cause a greater upset in thyroid iodide homeostasis.Data on the effect of carbimazole pretreatment on thyroid iodide turnover are reported in the following section.

A further possibility is that antithyroid drugs might alter the immunological response to ^{131}I.There may be a rise in thyroid autoantibodies after ^{131}I therapy Graves' disease(Atkinson et.al.1982;Bech and Madsen 1980;O'Gorman et.al.1964), and it has been suggested previously that hypothyroidism following ^{131}I is related in part to radiation induced immune thyroiditis, although this mechanism has been thought to be of little importance(Lundell and Holm,1980;O'Gorman et.al.1964).Carbimazole is known to lower autoantibody levels in autoimmune thyroid disease(Weetman et.al.1984):it is possible that pretreatment with carbimazole diminishes the autoantibody rise following ^{131}I therapy and so reduces the incidence of hypothyroidism.In the current study, however, the reduction in early hypothyroidism in patients pretreated with carbimazole was associated a higher incidence of post treatment thyrotoxicosis.If carbimazole was modifying the effect of ^{131}I by inhibiting thyroid autoimmune processes, it might be expected that the incidence of thyrotoxicosis after treatment would also be lower.Further, in section 1 of this chapter no evidence of a rise in TRAb following ^{131}I was noted either in the group given ^{131}I alone or in the group given carbimazole pretreatment, and it seems unlikely that the apparent radioprotective effect of carbimazole is immunologically related.

In summary pretreatment with carbimazole, stopping therapy 2-5 days before ^{131}I, may lead to a reduced incidence of early hypothyroidism.The borderline nature of the significance of the finding by conventional statistical testing demands a degree of caution in interpretation of these data, although they are in keeping with the majority of previous studies of this interaction.As ^{131}I therapy is often given to older patients who are more likely to have intercurrent disease this may lead to a disadvantageous delay in cure.However, data presented in section 1 of this chapter suggested that carbimazole pretreatment prevented major increase in thyroid hormone levels immediately after ^{131}I, so reducing the possibility of clinical deterioration in susceptible patients.This advantage must be set against any reduction in initial response rate;as the early response can be predicted from measurements of ^{123}I uptake shortly after ^{131}I therapy, this may be of no great consequence.The cause of the possible radioprotective action of carbimazole is unclear, and is the subject of further examination in this thesis.

SECTION SIX

INFLUENCE OF CARBIMAZOLE PRETREATMENT ON THYROID IODIDE KINETICS BEFORE AND AFTER ^{131}I THERAPY

3.6.1. Introduction

Evidence that pretreatment with antithyroid drugs reduces the effect of ^{131}I has been presented in the previous chapter. It is possible that this reflects an action of such drugs on thyroidal kinetics of ^{131}I, leading to altered uptake or retention of the isotope within the gland. As the radiation dose to tissue is a consequence of not only the physical properties of the isotope used but also the duration of exposure, such considerations may influence the radiation delivered to the thyroid in subjects given ^{131}I. For example, the amount of radiation delivered is dependent both on the physical decay of the isotope (physical half-life), and the duration of retention of the isotope by the gland (biological half-life). Physical and biological half-life can be combined to give rise to expression of effective half-life, and this is an important determinant of radiation delivered to the thyroid by ^{131}I therapy.

$$\text{THUS: Effective T1/2} = \frac{\text{Physical T1/2} \times \text{Biological T1/2}}{\text{Physical T1/2} + \text{Biological T1/2}}$$

If antithyroid drugs effect thyroid iodide turnover then this might explain the effect of these drugs on response to ^{131}I. Harden et al. (1966) suggested that carbimazole therapy, by blocking incorporation of iodide into organic form, reduced the thyroid iodide content and this is also suggested using more direct measures of thyroid iodide content (Lee, Siegal, Harpen, et al., 1982; Barandes et al., 1973; Hoffer, Bernstein, Gottschalk et al., 1971). This contracted intrathyroidal iodide pool could therefore reduce the biological half-life of ^{131}I within the gland and consequently reduce the effective half-life. Thus, the effect of carbimazole pretreatment may be to lower the radiation delivered to the thyroid from a given dose of isotope. Detailed studies of dosimetry have therefore been made in a small group of patients given ^{131}I. The effect of pretreatment with carbimazole on the relationship between tracer and therapy isotope handling has also been examined.

3.6.2. Patients and methods

Twenty four subjects with thyrotoxicosis (Graves' disease) were studied: these have been described in section one of this chapter. All were clinically and biochemically thyrotoxic at the time of entry to the study. Patients were randomly assigned to receive either ^{131}I directly (Group A) or to have therapy with antithyroid drugs (carbimazole 10-15 mg twice daily with thyroxine 0.1 mg per day) for a minimum of 3 months and until all were clinically and biochemically euthyroid. At that time subjects in this group (B) also received ^{131}I therapy. Drugs were stopped 48 hours before a tracer study, which was performed 24 hours before ^{131}I therapy was given.

3.6.3. Study design

Subjects were studied immediately after lunch on the day of the tracer study. ^{123}I was given (80 μCi) by rapid intravenous bolus injection and uptake of isotope at 20 minutes and 24 hours was measured as described in chapter 2. Thyroid size and the dose of ^{131}I to be administered were also calculated as described. The dose of ^{131}I was given by mouth at between 2 and 3 p.m. on the day after the ^{123}I study. Twenty four hours after the therapeutic dose, uptake of ^{131}I isotope by the thyroid was measured by the gamma camera/computer system using the aliquot of ^{131}I (1-2 mCi) as standard. Correction for camera and computer dead time was made as described earlier (2.3.4.). Thyroid counts were then measured at 5, 15 and 22 days after this initial study and again corrected for loss of efficiency at high count rates. The decay in thyroid counts was then plotted using semi-logarithmic paper to derive an effective half-life for ^{131}I within the thyroid (Figure 21). In all cases a single exponential function was an adequate fit to the data. From these data and from the known physical half-life of the isotope the biological half-life can be derived easily i.e.:

$$\text{RADS} = \frac{740 \times \text{24 hour uptake} \times \text{mCi} \times \text{Effective T1/2} \times \text{Mev}}{\text{Mass (grams)}}$$

3.6.4. Results

Further details of the patients studied are shown in Table 24. It will be seen that the groups had similar distribution of age and sex: estimated

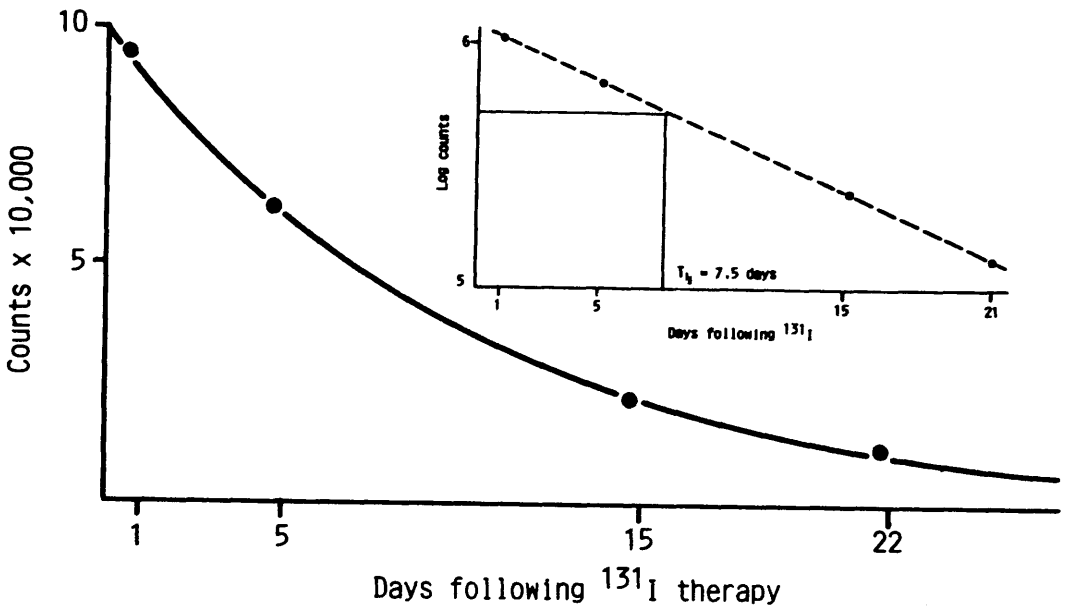


Fig 21

DERIVATION OF EFFECTIVE HALF LIFE OF ^{131}I

THYROID COUNTS FOLLOWING ^{131}I THERAPY WERE ACQUIRED WITH THE GAMMA CAMERA/COMPUTER AT 1, 5, 15 AND 22 DAYS AFTER ^{131}I, WITH APPROPRIATE CORRECTION FOR COMPUTER/CAMERA DEAD TIME. TYPICAL EXPONENTIAL DELAY IN COUNTS IS SHOWN IN THE MAIN GRAPH. THE INSET SHOWS A SEMI-LOGARITHMIC PLOT OF THE DATA, ALLOWING A HALF LIFE FOR THYROID COUNTS (EFFECTIVE HALF LIFE) TO BE PLOTTED.

TABLE 24

(SOME DATA REPRODUCED FROM TABLE 4)

DETAILS OF PATIENTS IN WHOM KINETICS OF ^{131}I WERE STUDIED

	No	20 MINUTE UPTAKE OF ^{123}I BEFORE ^{131}I	24 HOUR UPTAKE OF ^{123}I BEFORE ^{131}I	24 HOUR UPTAKE OF ^{131}I	ESTIMATED MASS (g)	DOSE mCi
PATIENTS GIVEN ^{131}I ALONE	12	24.1* ± 9.6	79.9 ± 12.9	61.7 ± 9.5	40 ± 16.7	4.7 ± 1.9
PATIENTS PRETREATED WITH CARBIMAZOLE	12	28.5 ± 16.1	76.9 ± 17.8	63.3 ± 17.8	63.3 ± 15.3	6.4 ± 3.5
ALL SUBJECTS	24	26.3 ± 13.1	78.4 ± 15.3	62.5 ± 12.5	45.5 ± 21.0	5.6 ± 2.9

* MEAN ± SD

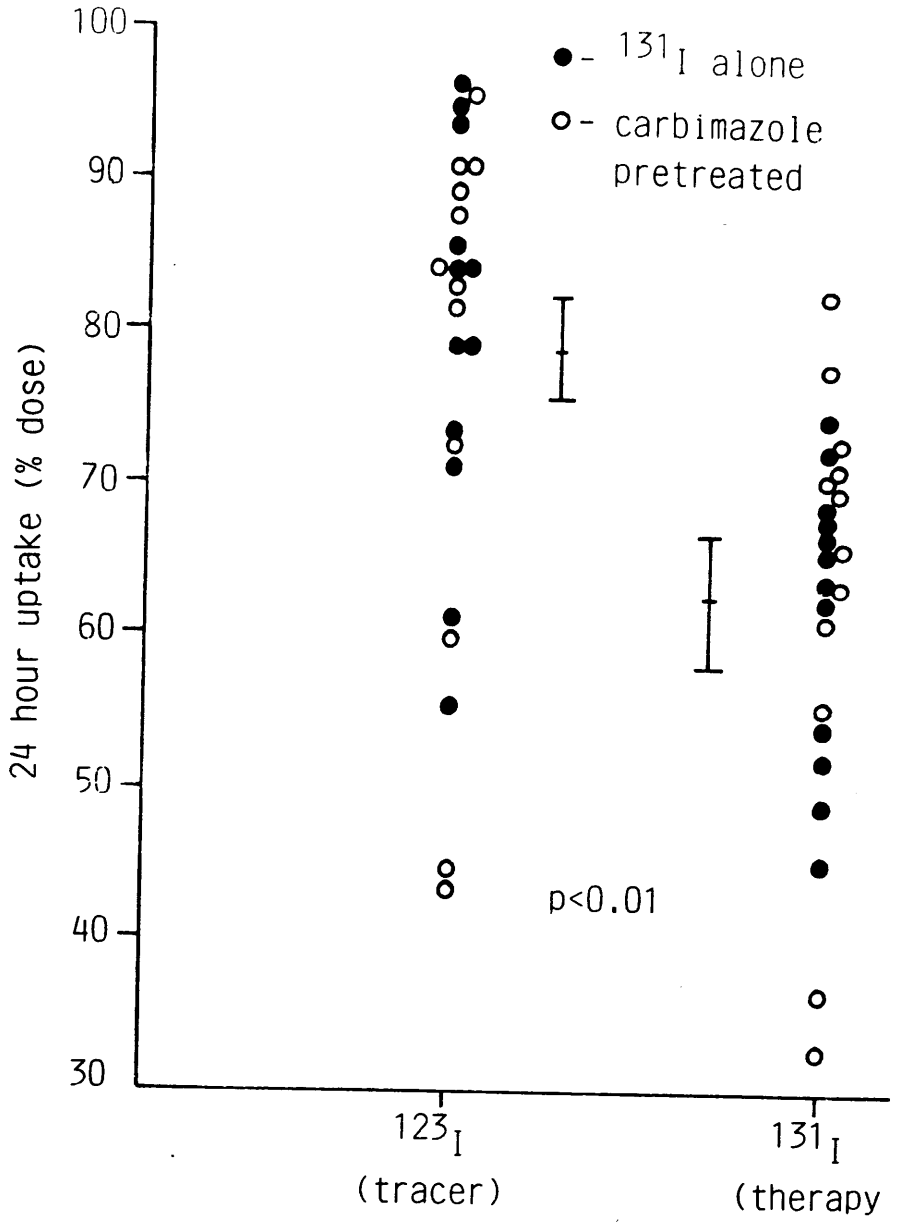


Fig 22

24 HOUR THYROID UPTAKE OF TRACER (^{123}I) AND THERAPY (^{131}I) RADIOIODINE

THYROID UPTAKE OF ^{123}I (80uCi) AND ^{131}I (THERAPY DOSE) IN THYROTOXIC SUBJECTS. ^{123}I WAS GIVEN INTRAVENOUSLY AND ^{131}I ORALLY. SUBJECTS MADE EUTHYROID WITH CARBIMAZOLE BEFORE ^{131}I THERAPY SHOWN AS OPEN CIRCLES; THOSE GIVEN ^{131}I AS SOLE TREATMENT AS CLOSED. MEAN (AND SEM) FOR TRACER AND THERAPY UPTAKES SHOWN: TRACER UPTAKE WAS SIGNIFICANTLY HIGHER THAN THERAPY ($p < 0.01$, MANN, WHITNEY U-TEST).

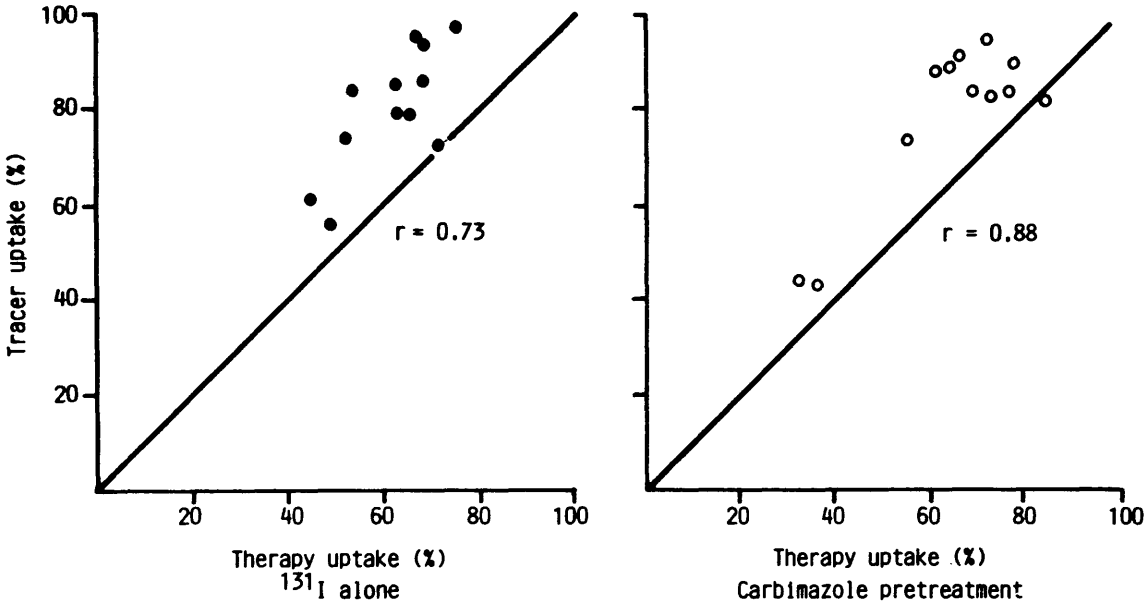


Fig 23

RELATIONSHIP BETWEEN THYROID UPTAKE OF TRACER (^{123}I) AND THERAPY (^{131}I) DOSES OF RADIOIODINE

THE RELATIONSHIP BETWEEN THE 24 HOUR THYROID UPTAKE OF TRACER ^{123}I (80uCi) AND THERAPY ^{131}I IN EACH SUBJECT IS SHOWN. THOSE SUBJECTS GIVEN ^{131}I AS SOLE THERAPY ARE SHOWN IN THE LEFT HAND PANEL, AND THOSE MADE EUTHYROID WITH CARBIMAZOLE BEFORE ^{131}I TREATMENT IN THE RIGHT. THERE WAS A SIGNIFICANT POSITIVE RELATIONSHIP ($p < 0.01$) BETWEEN TRACER AND THERAPY UPTAKE IN BOTH GROUPS OF SUBJECTS, BUT THIS DIFFERED FROM THE LINE OF IDENTITY (SHOWN AS SOLID LINE).

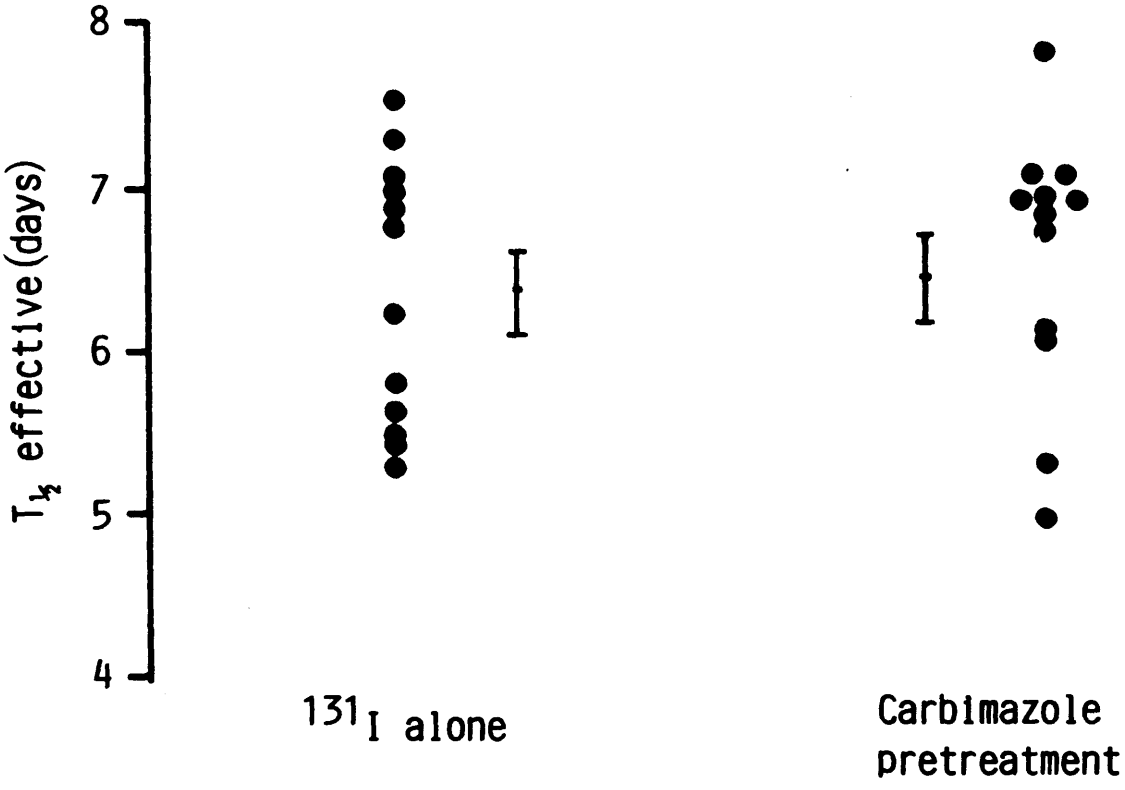


Fig 24

EFFECTIVE HALF LIFE OF RADIOIODINE (^{131}I) FOLLOWING ^{131}I THERAPY IN THYROTOXIC SUBJECTS

THE EFFECTIVE HALF LIFE OF ^{131}I IN THE THYROID IN PATIENTS WITH THYROTOXICOSIS IS SHOWN FOR INDIVIDUAL SUBJECTS. PATIENTS GIVEN ^{131}I AS SOLE THERAPY FOR THYROTOXICOSIS ARE SHOWN IN THE LEFT HAND COLUMN, WHILE THOSE MADE EUTHYROID WITH CARBIMAZOLE BEFORE ^{131}I ARE SHOWN IN THE RIGHT. MEAN AND STANDARD ERROR OF THE MEAN FOR BOTH GROUPS ARE SHOWN.

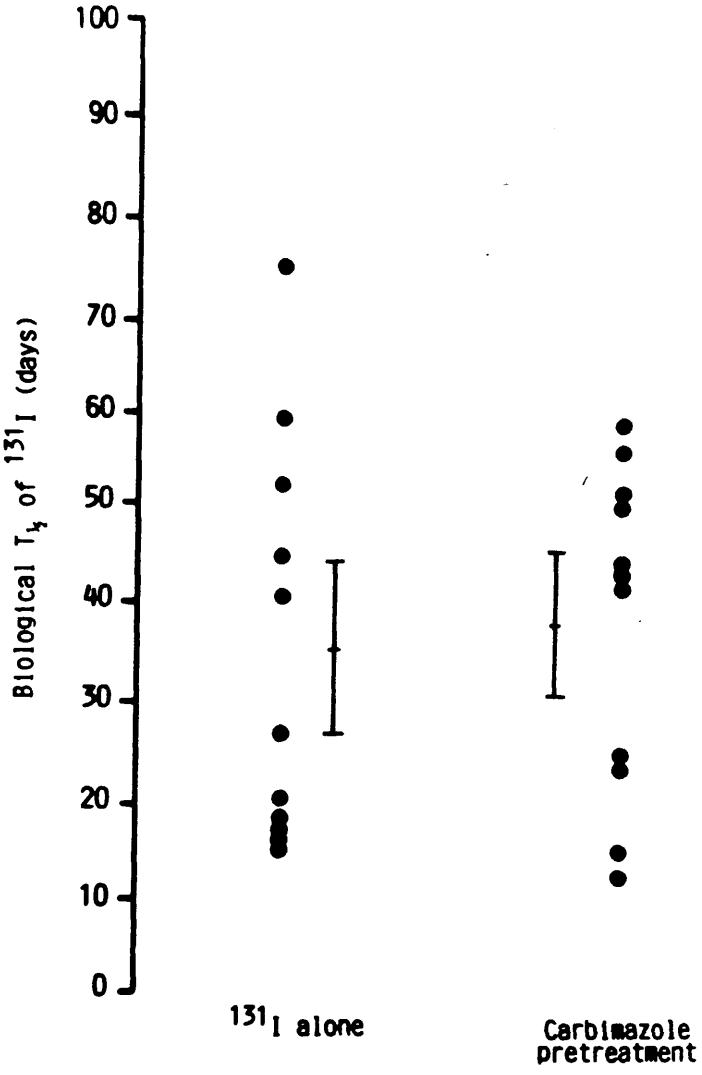


Fig 25

BIOLOGICAL HALF LIFE OF RADIOIODINE (^{131}I) FOLLOWING ^{131}I THERAPY IN THYROTOXIC SUBJECTS

THE BIOLOGICAL HALF LIFE OF ^{131}I IN THE THYROID IN PATIENTS WITH THYROTOXICOSIS IS SHOWN FOR INDIVIDUAL SUBJECTS. PATIENTS GIVEN ^{131}I AS SOLE THERAPY FOR THYROTOXICOSIS ARE SHOWN IN THE LEFT HAND COLUMN WHILE THOSE MADE EUTHYROID WITH CARBIMAZOLE BEFORE ^{131}I ARE SHOWN IN THE RIGHT. MEAN AND STANDARD ERROR OF THE MEAN FOR BOTH GROUPS ARE SHOWN.

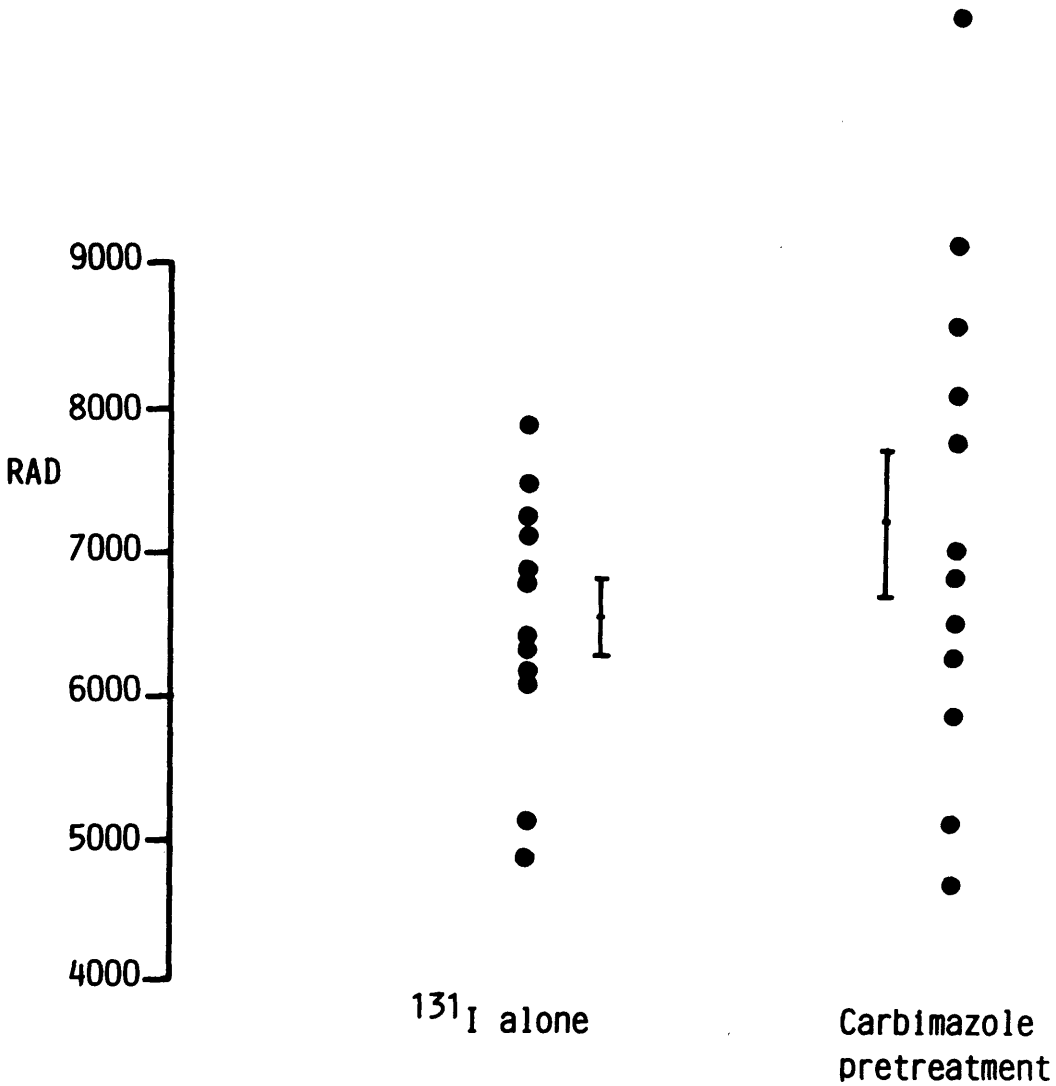


Fig 26

RADIATION DOSE TO THE THYROID IN THYROTOXIC SUBJECTS GIVEN ^{131}I TREATMENT

RADIATION DOSE (EXPRESSED IN RADS) TO THE THYROID IN THYROTOXIC PATIENTS GIVEN ^{131}I. PATIENTS GIVEN ^{131}I AS SOLE THERAPY ARE SHOWN ON THE LEFT, AND THOSE MADE EUTHYROID WITH CARBIMAZOLE BEFORE ^{131}I TREATMENT ON THE RIGHT. MEAN AND STANDARD ERROR OF THE MEAN FOR BOTH GROUPS ARE SHOWN.

thyroid mass, 20 minute uptake, 24 hour uptake of ^{123}I and dose of ^{131}I given to the 2 groups were also not significantly different.

The 24 hour uptake measurements of ^{123}I (tracer) and ^{131}I (therapy) are displayed in Figure 22. It will be noted that measurement of uptake of the latter isotope was consistently lower than that of ^{123}I ($p < 0.001$; Mann-Whitney U test), although a good correlation between the 2 measurements was seen (Figure 23). Thus, the regression line relating the 2 measurements differed significantly from the line of identity ($p < 0.05$). This finding of higher uptake of tracer ^{123}I than therapy ^{131}I was seen both in those subjects pretreated with carbimazole and those given ^{131}I alone and there was no evidence that carbimazole altered this relationship. Effective half-life and derived biological half-life for the 2 groups of patients are shown in Figures 24 and 25; it will be noted that mean effective half-life and biological half-life were not different when the groups were compared. The radiation dose delivered to the thyroid in each patient could be calculated using this data and this derived radiation dose is shown in Figure 26. It will be noted that carbimazole pretreatment had no apparent effect on radiation delivered to the thyroid.

3.6.5. Discussion

Effect of Carbimazole pretreatment on effective half-life of ^{131}I

The measured effective half-life of ^{131}I in this study is similar to that reported by other workers in thyrotoxic subjects (Cullen, Williams and Malone, 1976). There was no difference in effective half-life between the treatment groups: neither the derived value for biological half-life nor the calculated dose of radiation delivered to the gland were affected by carbimazole pretreatment.

It was proposed that carbimazole might affect biological turnover of iodide within the gland by reducing intrathyroidal iodide content. However, carbimazole was used in the current study for between 3 and 5 months, and this may be an insufficiently long time for iodide depletion to develop to a degree that would affect effective half-life of ^{131}I. Further, the patients in this study were given thyroxine supplements along with carbimazole: this represents a significant source of increased dietary iodide availability and may, by increasing plasma inorganic iodide concentration, help maintain thyroidal iodide stores. Both sets of subjects were similar in terms of basal

uptake data and goitre size, and it seems unlikely that there was any reason for the carbimazole pretreated group to have an effective half-life which would otherwise differ from the group of subjects given ^{131}I alone. In an earlier study of the effect of methylthiouracil on biological half-life of ^{131}I, Crooks et al. (1960) reported that the mean biological half-life was 11.7 days. This is rather shorter than the mean biological half-life reported in the current series. However, the measurement of Crooks was made using a simple neck collimator and counter, without computer analysis of data and it is unlikely that the very high count rate following a therapy dose of ^{131}I would be accurately measured with such equipment. In the current study high count rates were corrected for loss of efficiency at high count rates and it is believed that this approach has eliminated a possible source of technical error in counting. Further, changes in dietary iodide availability since the 1950s and 60s may have led to prolongation of biological half-life of iodide in the general population. For these reasons these data and those of Crooks may not be directly comparable.

In section 5 an effect of carbimazole pretreatment on outcome following ^{131}I was described: furthermore, in the major series described by Aro et al. (1981) and Bliddal et al. (1982), in which a radio protective action of carbimazole was noted, doses of carbimazole similar to that used in the current study were given for an equivalent period of time before ^{131}I therapy. It therefore seems unlikely that the effect of carbimazole on early response to ^{131}I is a consequence of altered thyroidal kinetics of ^{131}I.

3.6.6. Discrepancy between therapy and tracer uptake measurements

It was noted in this series of patients that 24 hour uptake of ^{123}I after i.v. injection measured using the gamma counter was lower than the 24 hour uptake of ^{131}I after oral administration in most (23 out of 24) subjects ($p < 0.001$) (Figure 23). The overall correlation between the 2 measurements was good ($r = 0.88$) and this was similar when patients pretreated with carbimazole were examined separately from those given ^{131}I alone.

It is possible that this discrepancy is artefactual: the counts over the neck 24 hours after ^{131}I administration were very high when counting efficiency of the gamma camera system may be less accurate. However, correction for loss of efficiency and computer and camera dead time was made in this study (2.3.4.). It is also unlikely that a non-systematic error

would result in counts using ^{131}I being uniformly lower than those with ^{123}I.

O'Connor and colleagues (1979) and Malone and Cullen (1975) reported that the measurements of oral therapy and tracer uptake by the thyroid gland were very similar, and showed that the regression line relating the data was identical to the line of identity. However, other workers have reported that in some patients the kinetics of therapy doses of ^{131}I may differ from tracer doses (De Groot and Stanbury, 1975; Freedberg et al., 1952). One unexplored possibility is that radiation from the therapy dose has a very early effect on iodide trapping and organification, so that uptake within the first 24 hours is limited by this means.

Although there was a small systematic difference of uptake when therapy and tracer doses of radioiodine were compared in the current study, the good correlation between the measurements implies that tracer uptake studies may still be of value in predicting the general behaviour of therapy doses of ^{131}I, and may still be of use in dosimetry. It has been proposed that antithyroid drug therapy might distort the relationship between tracer and therapy isotope uptake and so make dosimetry less accurate (De Groot and Stanbury, 1975). In the current study no evidence of such an effect was seen: the relationship between tracer and therapy uptake was similar in the group of patients pretreated with carbimazole compared with those given ^{131}I alone.

SECTION SEVEN

EFFECT OF PRETREATMENT WITH CARBIMAZOLE ON IODIDE (^{125}I) UPTAKE, ORGANIFICATION AND DISTRIBUTION IN RAT THYROID

3.7.1. Introduction

Studies in patients with thyrotoxicosis presented earlier in this section indicate that pretreatment with carbimazole, stopping the drug several days before ^{131}I treatment has a radioprotective effect. The mechanism of this is not fully understood, and the possibility that antithyroid drugs might affect the kinetics of ^{131}I within the thyroid has been investigated in the previous chapter. Drug treatment is also known to alter the histological appearance of the thyroid gland (Halmi, 1978), and it is possible that this might affect the intrathyroidal distribution of therapeutic ^{131}I. In the current chapter the effects of antithyroid drug treatment on thyroid histological appearance and the distribution of iodide within the glands of experimental animals are examined.

3.7.2. Methods

Adult male Sprague-Dawley rats were used, of average weight 265 g (± 17, SEM). Animals were housed 2 per cage, and fed diet 41B (Oxoid, Basingstoke) with normal iodine content (iodine content 2.4 µg/g) and received drinking water ad lib. They were divided into 3 groups (6 per group):

(a) no treatment given.

(b) Methimazole 0.5 mg per day, stopping drug 48 hours before end of experiment (Methimazole given for 19 days).

(c) Methimazole 0.5 mg per day, drug continued until end of experiment (21 days).

Methimazole (Sigma) was given by addition to drinking water. Measurement of loss of water from the drinking bottles allowed the average intake of drug to be estimated: this corresponded well to the intended intake.

After 21 days animals were given an intraperitoneal injection of 20 µCi of ^{125}I in 0.1 ml volume (NaCl 0.9%). Ninety minutes later animals

were sacrificed by cardiac puncture under ether anaesthesia and a blood sample was taken for measurement of plasma ^{125}I activity: thyroid glands were rapidly disected and removed. One lobe from each animal was placed immediately in formal saline for histological examination: the other was weighed using a torsion balance and placed in 2 ml ice cold/10% trichloracetic acid (TCA) containing 10^{-3} molar methimazole and 10^{-4} molar potassium iodide to prevent further organification of free iodide. The tissue was immediately dispersed using a ground glass homogeniser.

3.7.3. Calculation of iodide trapping ability

The ability of the thyroid to trap iodide was calculated from the ratio of counts between plasma and tissue (corrected for weight and volume). For this purpose 0.1 ml plasma was added to an appropriate volume (2 ml saline) and counted using an autogamma counter with appropriate background subtraction. Homogenised thyroid in equivalent volume TCA was counted at the same time. Ratio of counts in tissue divided by counts in plasma was then calculated for each animal.

3.7.4. Calculation of organification efficiency

In animals on antithyroid drugs the thyroid can trap iodide but is unable to organify the anion. The organified iodine within the gland will be protein bound whereas inorganic iodide is not. As protein is insoluble in trichloracetic acid, the protein bound iodide will precipitate out in solution and iodide remaining in solution will reflect the inorganic iodide within the gland (Alexander and Wolff, 1966). Thus, the greater the inhibition of organification, the lower will be counts in the precipitate fraction.

After calculation of iodide trapping the TCA suspension was centrifuged at 3000 rpm for 10 minutes: the precipitate at the bottom of the tube was retained and the supernatant discarded. Fresh TCA was then added, homogenisation repeated and the solution again centrifuged. This procedure was repeated on one further occasion: counts in the supernatant at that point were negligibly raised above backgroud and the remaining precipitate counts were assumed to represent protein-bound (i.e. organified) iodide. After counting and appropriate correction for weight, inhibition of organification was then expressed as total thyroid counts minus protein bound thyroid counts divided by total thyroid counts.

3.7.5. Histological studies and autoradiography

Formalin fixed glands were embedded in wax and sections cut. Alternate sections were mounted on glass slides and stained using haematoxylin and eosin for conventional histological examination. The other sections were mounted on glass slides and prepared for autoradiography. Briefly, clean glass slides were coated in Ilford G5 nuclear emulsion (50:50 with distilled water) and allowed to dry. Eight micron thick sections of tissue were cut and attached to the emulsion coated slides by pressure: slides were then stored in a light free container. After exposure the slides were counter stained with haematoxylin and eosin. Control slides wer exposed at weekly intervals until optimum images were produced (best results were achieved at 4 weeks).

3.7.6. Results

The mean weight of rats in each group at the end of the study is shown in Table 25. It will be seen that animals in groups B and C gained significantly less weight than those in group A. Mean thyroid weights (2 lobes) are also shown: despite the lower body weights, animals given Methimazole had substantially larger thyroid glands. Thyroid iodide trapping and organification is also detailed in Table 25. It will be noted that thyroid trapping of iodide as assessed by the tissue/plasma ratio occurred in all groups, but that this was significantly greater in animals in groups B and C than in control animals. Minimal (less than 15%) inhibition of organification as assessed by the proportion of iodide which was TCA insoluble was seen in groups A and B, whereas in animals on methimazole at the time of sacrifice greater than 80% of iodide was in inorganic form (i.e. soluble in TCA).

3.7.7. Histology

Typical thyroid histological specimens and corresponding autoradiographs are displayed in Figure 27 (plates 1-6). Normal rat thyroid histology is represented in plate 1: follicles of normal size with enclosed colloid are seen. The accompanying autoradiograph (plate 2) shows that radiolabelled iodide localises over colloid and at the colloid/cell interface. Uptake by each follicle is not identical highlighting the heterogenous nature

TABLE 25

EFFECTS OF ORAL METHIMAZOLE (0.5mg/DAY) GIVEN FOR 3 WEEKS ON RAT WEIGHT, THYROID WEIGHT AND THYROID IODIDE TRAPPING AND ORGANIFICATION

GROUP	INITIAL WT	FINAL RAT WEIGHT (g)	THYROID WEIGHT (2 LOBES) (mg)	^{125}I TRAPPING (TISSUE:PLASMA RATIO)	% INHIBITION ^{125}I ORGANIFICATION
A: NO TREATMENT	220 ± 16	376 ± 24	17.4 ± 1.8	588 ± 146	8.6 ± 0.6
B: METHIMAZOLE DISCONTINUED 48 HOURS BEFORE SACRIFICE	216 ± 20	246^{x} ± 56	30.8^{xx} ± 2.0	773^{x} ± 79	9.8 ± 1.0
C: METHIMAZOLE CONTINUED UNTIL SACRIFICE	219 ± 23	258^{x} + 52	28.4^{xx} + 4.2	796 + 56	84.6^{xx} + 12.4

VALUES REPRESENT MEANS + SD n = 6

^{x} $p < 0.05$ COMPARED WITH CONTROL

^{xx} $p < 0.01$ COMPARED WITH CONTROL

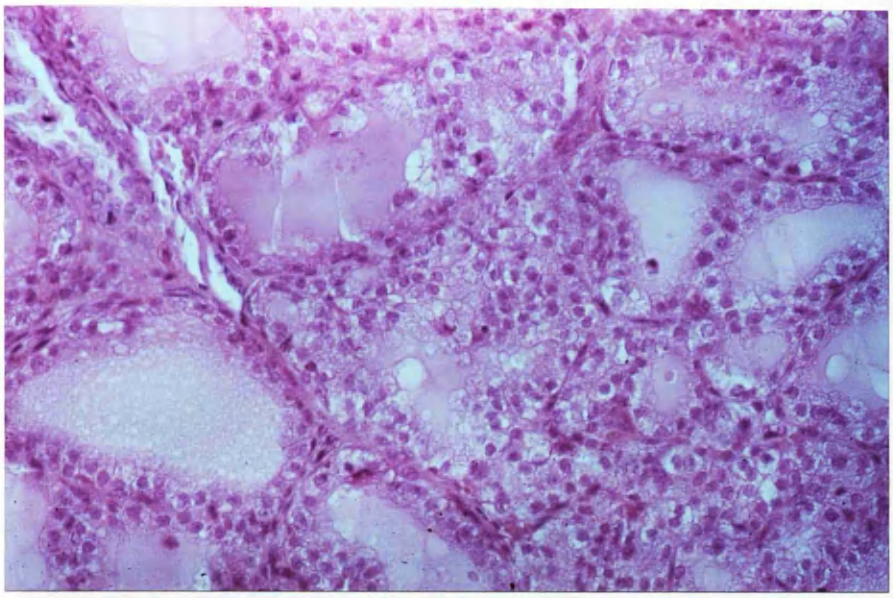


Fig 27

EFFECTS OF ANTITHYROID DRUG TREATMENT ON THYROID HISTOLOGY AND DISTRIBUTION OF TRACER (^{125}I) RADIOIODINE IN THE RAT

PLATE 1 (x300)

NORMAL RAT THYROID HISTOLOGY SHOWING FOLLICLES COMPRISING A BASAL LAYER OF CELLS ENCLOSING COLLOID. (HAEMATOXYLIN AND EOSIN).

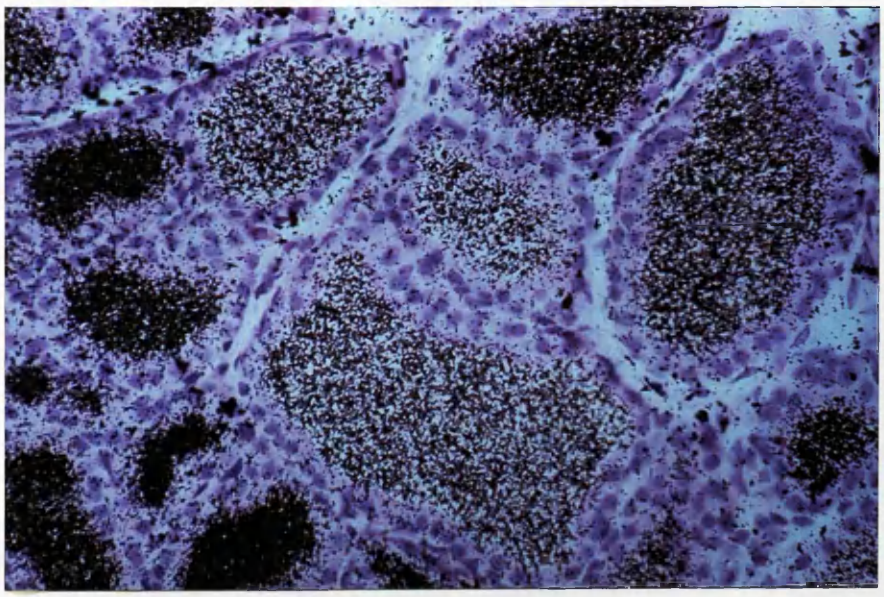


PLATE 2 (x300)

AUTORADIOGRAPH SHOWING DISTRIBUTION OF ^{125}I (ISOTOPE INJECTED 90 MINUTES BEFORE SACRIFICE). NOTE DISTRIBUTION OF IODIDE (SILVER GRAINS) OVER COLLOID AND AT COLLOID/CELL INTERFACE. IODIDE DISTRIBUTION BETWEEN FOLLICLES IS NOT HOMOGENOUS (H + E COUNTERSTAIN).

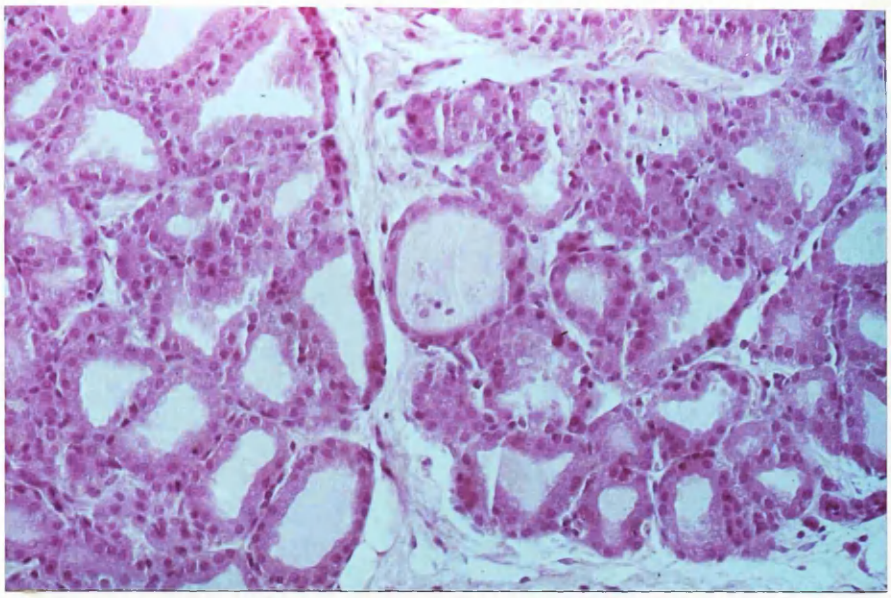


Fig 27

PLATE 3 (x300)

HISTOLOGY OF THYROID: METHIMAZOLE 0.5mg/DAY GIVEN FOR 19 DAYS: DRUG STOPPED 48 HOURS BEFORE SACRIFICE. NOTE NORMAL FOLLICULAR ARCHITECTURE.

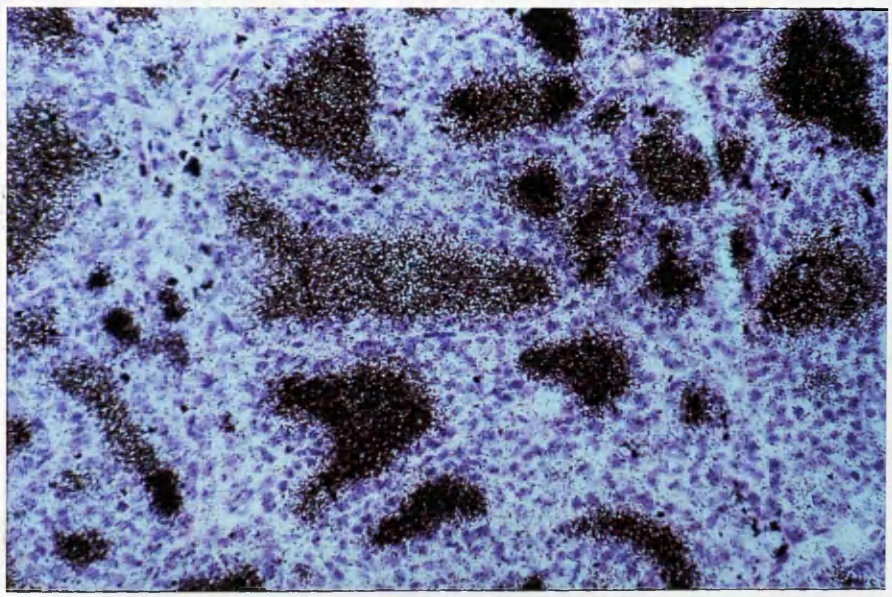


PLATE 4

AUTOGRADIOGRAPH SHOWING DISTRIBUTION OF ^{125}I: METHIMAZOLE 0.5mg/DAY FOR 19 DAYS, STOPPED 48 HOURS BEFORE SACRIFICE. NOTE DISTRIBUTION OF IODIDE SIMILAR TO NORMAL GLAND.

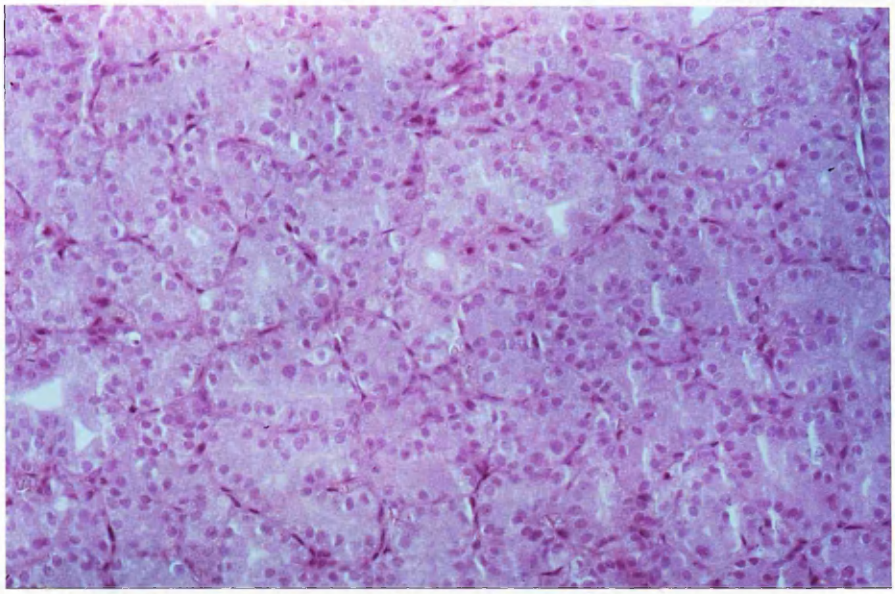


Fig 27

PLATE 5

HISTOLOGY OF THYROID: METHIMAZOLE 0.5mg/DAY GIVEN FOR 21 DAYS: DRUG CONTINUED UNTIL SACRIFICE. NOTE LOSS OF FOLLICULAR ARCHITECTURE, WITH HYPERTROPHY OF CELLS.

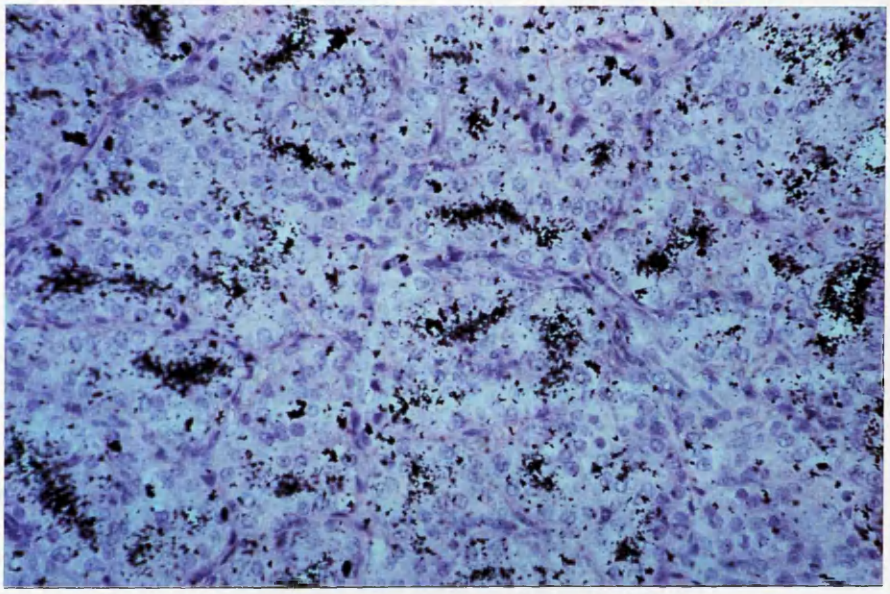


PLATE 6

AUTORADIOGRAPH SHOWING DISTRIBUTION OF ^{125}I: METHIMAZOLE 0.5mg/DAY FOR 21 DAYS, CONTINUED UNTIL SACRIFICE. DISTRIBUTION OF IODIDE IS VERY PATCHY, WITH NO OBVIOUS RELATIONSHIP TO CELLS OR POTENTIAL COLLOID SPACE.

of iodide uptake by follicles (Studer et al. 1985).

Tissue from group B (methimazole stopped 48 hours before sacrifice) is shown in plates 3 and 4. It will be seen that average follicular size is less than in plate 1, but that the overall histological appearance is similar. There appears to be some enlargement of follicular cell size, suggested by increased nuclear/cytoplasmic ratio. Autoradiography again shows localisation of iodide over colloid, and at the cell/colloid interface. The heterogeneity in uptake is more evident in these sections than in group A.

Typical slides prepared from group C animals (methimazole continued until sacrifice) are shown in plates 5 and 6. The thyroid histological appearance in these sections is abnormal with marked loss of colloid from follicles: apparent hypertrophy of follicle cells is again seen. Autoradiography shows less accumulation of iodide over the internal aspects of follicles with rather patchy distribution of iodide throughout the section.

3.7.8. Discussion

Antithyroid drug therapy for 3 weeks impaired normal thyroid function resulting in reduced weight gain in the animals in groups B and C. Increased thyroid gland weight in these animals confirmed that hypothyroidism had developed, resulting in TSH stimulation of thyroid growth. In rats from group B (methimazole stopped 48 hours before sacrifice) increased TSH stimulation in the gland was also reflected in increased thyroid iodide trapping. However, iodide organification was normal and did not differ from untreated rats. The data for thyroid trapping and organification of iodide in untreated animals is very similar to that described by Marchant (1971) using the same technique. It seems, therefore, that 48 hours after stopping methimazole there is no detectable effect on thyroid iodide organification. In contrast, in rats in whom the drug was continued until the time of sacrifice, thyroid iodide organification was severely inhibited (greater than 80%): trapping of iodide and thyroid weight in this group of animals was very similar to animals in group B.

The effect of antithyroid drugs on thyroid histological appearance has been reported previously (Halmi, 1978). In rats still receiving methimazole in the current study there was almost complete absence of colloid with hypertrophied follicular cells. In contrast, where drug had been stopped for as little as 48 hours previously, histological appearance was little different

from untreated animals with reappearance of colloid material. Average follicular diameter did, however, appear a little less than in untreated rats, and there was apparent hypertrophy of follicular cells. The effects of antithyroid drug treatment on the distribution of labelled iodide appeared to be transient. Thus, in the animals still receiving drug, iodide distribution was patchy and much less clearly identified with the lumen of the follicle than in animals in groups A and B, reflecting the inability of animals in group C to organify iodide. In both group A and B animals, follicular distribution of iodide did not appear homogeneous. This seems unlikely to be due to artefact related to section of tissues, as follicles of very similar size had considerable differences in apparent density of silver granules. This variability in follicular accumulation of iodide has been noted in other autoradiographic studies and may reflect genetically determined differences in iodide trapping by 'parent' follicle cells (Studer et al. 1985). However, there was no evidence that distribution of iodide within the gland was abnormal in animals where methimazole had been given until 48 hours before sacrifice.

In summary, normal thyroid avidly trapped iodide and organified greater than 90% of the anion taken up. Stopping methimazole (which is the active compound in plasma derived from carbimazole) 48 hours before the study allowed normal organification to occur and had no major effect on thyroid histological appearance or on intrathyroidal iodide distribution. In contrast, where drug was continued until the time of study, profound effects on iodide organification, histological appearance and thyroidal iodide distribution were noted.

When antithyroid drugs had been given before ^{131}I therapy in thyrotoxic patients they have generally been discontinued at least 48 hours beforehand (see previous section). Drug metabolism in man appears similar to that in rat (Marchant, 19719; Marchant et al. 1978), and it would therefore be expected that in man no effect on organification of iodide would be present at this time: indeed, studies of the duration of action of methimazole in man suggest that inhibition of organification persists for less than 24 hours (McCruden, Hilditch, Connell, et al. 1985). Similarly, the effects of methimazole on histological appearances and on intrathyroidal iodide distribution were much less evident when the drug had been stopped for 48 hours, so that labelled iodide appeared in a normal relationship with colloid and follicular cell borders. It therefore seems unlikely that the

effect of antithyroid drugs following ^{131}I treatment on early outcome is a function of altered organification (and thus retention) or distribution of ^{131}I. However, where antithyroid drugs continue to be given when ^{131}I is administered profound effects on the retention and distribution of iodide within the gland will occur and this is likely to affect the response of the thyroid to treatment. In contrast, the practice of discontinuing drugs for at least 48 hours before ^{131}I administration appears to restore thyroid histology and function to relative normality.

CHAPTER FOUR
TISSUE ACCUMULATION OF ANTITHYROID DRUGS: INTERACTIONS WITH IODIDE

SECTION ONE
EFFECT OF INHIBITION OF IODIDE TRAPPING ON UPTAKE OF RADIOLABELLED PROPYLTHIOURACIL AND METHIMAZOLE BY MOUSE THYROID GLAND

4.1.1. Introduction

In the preceding section it was shown that carbimazole influences the reponse of patients with thyrotoxicosis to ^{131}I therapy: evidence has been presented that this effect is not associated with alteration in the retention or distribution of iodide within the gland, and it seems likely to be associated with some chemical property of the drug. Antithyroid drugs of the thiourylene group are unusual in that they are concentrated within their target tissue in both man and experimental animal (Marchant et al. 1978), and factors which modify the accumulation of drug by the thyroid gland may, therefore, significantly influence antithyroid drug action, which may include radioprotection.

Marchant investigated the phenomenon of antithyroid drug accumulation in the rat, and showed that accumulation was positively correlated with dietary iodide intake and increased by factors which acutely stimulated iodide uptake by the thyroid (Marchant et al. 1978). This suggested that drug accumulation might be in some way associated with the anion trap, although the mechanism remained obscure. The effect of the opposite manoeuvre, namely acute inhibition of thyroid iodide trapping on antithyroid drug accumulation by the gland is examined here.

4.1.2. Animals and drugs

Adult male mice of TO strain (Animal Suppliers, Welwyn Garden City) were used. They were maintained on commercial diet 41B (oxoid, iodine content 2.4 µg/g) for at least 7 days before the experiment. Tap water was available ad lib.

^{35}S-Methimazole (28 µCi/mmol) and ^{35}S-Propylthiouracil (20 µCi/mmol) were obtained from Amersham International Ltd., Bucks. Both

drugs had been shown to be more than 98% pure by thin layer chromatography (Lang, 1983 a + b). Carrier free sodium ^{125}I was obtained from Amersham International.

4.1.3. Methods

Radiolabelled drugs were dissolved immediately before use in alkaline solution (pH 8) to give a range of concentrations from 0.1 µg - 1 mg in 0.1 ml volume. Sodium perchlorate (10 mg) dissolved in 0.9% sodium chloride solution (0.1 ml volume) was injected intraperitoneally 30 minutes before intraperitoneal injection of ^{35}S-labelled drug. Control animals received normal saline instead of perchlorate. Sixty minutes after the administration of ^{35}S-labelled drug, animals were killed by exsanguination, blood being withdrawn into a heparinized syringe by cardiac puncture under ether anaesthesia. Thyroid glands were dissected out, weighed on a torsion balance and digested in soluene (Packard). Samples of 0.1 ml plasma were digested in hyamine hydroxide (10% volume in 50% methanol). Plasma samples were counted in NE250 scintillator (Nuclear Enterprises, Edinburgh) and tissue in toluene scintillator using a liquid scintillation counter with an internal standard/channels-ratio method of quench correction. For calcuation of ^{125}I uptake, separate animals were injected intraperitoneally with 0.5 µCi sodium ^{125}I in 0.1 ml volume 30 mintues after administration of sodium perchlorate or saline. Tissue was removed as before and all samples counted in an automatic gamma counter.

In all experiments 6 animals were used in each group.

4.1.4. Results

Effect of sodium perchlorate on accumulation of iodide

In the control animals avid trapping of iodide (carrier free ^{125}I) was seen with a mean tissue/plasma ratio of 307 $\pm$ 92 (SD). In animals pretreated with perchlorate, trapping was inhibited with a mean tissue/plasma ratio of 1.37 $\pm$ 0.4 ($p < 0.001$).

4.1.5. Effect of sodium perchlorate on accumulation of ^{35}S-Methimazole

These results are shown in Table 26. At doses of 0.1 and 10 µg/animal intrathyroidal accumulation of methimazole was seen with thyroid/plasma ratios in excess of 2. Higher doses (100 and 1000 µg)

TABLE 26

EFFECT OF SODIUM PERCHLORATE ON THYROIDAL ACCUMULATION OF ^{35}S-METHIMAZOLE (MMI) IN MICE

DOSE OF ^{35}S-MMI	THYROID ^{35}S-MMI CONCENTRATION nmol/gm	PLASMA ^{35}S-MMI CONCENTRATION umol/l	THYROID/PLASMA RATIO
0.1ug	8.0 ± 2.2	0.3 ± 0.06	36.1 ± 16.0
0.1ug + PERCHLORATE	4.1 ± 1.2*	0.3 ± 0.02	15.5 ± 12.2*
10ug	51.0 ± 14.0	25.7 ± 7.2	3.29 ± 1.6
10ug + PERCHLORATE	24 ± 4.4*	29.3 ± 17.8	1.04 ± 0.2**
100ug	280 ± 10	206 ± 36	1.19 ± 0.2
100ug + PERCHLORATE	180 ± 42	215 ± 74	0.82 ± 0.18
1000ug	2250 ± 418	1980 ± 208	1.07 ± 0.14
1000ug + PERCHLORATE	2740 ± 860	2840 ± 64	0.93 ± 0.16

VALUES REPRESENT MEAN ± SD (n = 6)

* $p < 0.05$ (MANN WHITNEY U TEST) COMPARED WITH ^{35}S-MMI ALONE

** $p < 0.005$

TABLE 27

EFFECT OF SODIUM PERCHLORATE ON THYROID ACCUMULATION OF ^{35}S-PROPYLTHIOURACIL (PTU) IN MICE

DOSE OF ^{35}S-PTU	THYROID ^{35}S-PTU CONCENTRATION nmol/gm	PLASMA ^{35}S-PTU CONCENTRATION umol/l	TISSUE/ PLASMA RATIO
0.1ug	9.9 ± 6.2	0.5 ± 0.02	8.2 ± 0.7
0.1ug + PERCHLORATE (10mg)	3.6* ± 2.4	0.6 ± 0.04	3.6* ± 1.80
10ug	20? ± 28.2	25.3 ± 2.4	9.6 ± 1.6
10ug + PERCHLORATE (10mg)	28.7* ± 1.8	31.3 ± 3.2	1.07* + 0.2

ALL VALUES REPRESENT MEAN ± SD (n = 6)

* $p < 0.005$ COMPARED WITH ^{35}S-PTU ALONE (MANN-WHITNEY U-TEST)

resulted in thyroid/plasma ratios near unity.

For the 0.1 and 10 µg doses, pretreatment with sodium perchlorate was associated with a significant fall in the absolute amount of labelled drug accumulated within the gland and also a significant fall in tissue/plasma ratio for the drug. No significant difference was seen in plasma concentrations of ^{35}S-Methimazole in the animals treated with sodium perchlorate. At the higher dose studied, perchlorate had no significant effect on drug accumulation.

4.1.7. Effect of sodium perchlorate on accumulation of ^{35}S-Propylthiouracil

These results are shown in Table 27. At both doses studied (0.1 and 10 µg) intrathyroidal accumulation of ^{35}S-Propylthiouracil was noted, although a fall in tissue/plasma ratio for the drug was noted at the higher dose studied. In both instances pretreatment with sodium perchlorate significantly diminished the tissue/plasma ratio for ^{35}S-labelled drug. A significant fall in the absolute amount of drug accumulated within the thyroid was also noted at the 10 µg dose. No effect on plasma concentration of ^{35}S-Propylthiouracil was noted in the animals given sodium perchlorate compared with control.

4.1.8. Discussion

Accumulation of ^{35}S-Methimazole and ^{35}S-Propylthiouracil within mouse thyroid gland has been demonstrated: maximal tissue/plasma ratios occurred at the lowest drug concentrations studied. While it is accepted that some metabolic degradation of ^{35}S labelled drug would occur during the time course of the experiment, ^{35}S uptake is taken to represent all ^{35}S labelled compound and referred to as parent compound. Acute administration of sodium perchlorate caused a significant fall in the absolute amount of ^{35}S-Methimazole accumulated at the two lower (0.1 and 10 µg) dose levels and consequently a fall in the tissue/plasma ratios. A similar effect was seen for ^{35}S-Propylthiouracil. Perchlorate could not be shown to alter the amount of drug accumulated at higher dose levels of ^{35}S-Methimazole studied, and did not significantly influence plasma concentrations of either ^{35}S-Methimazole or ^{35}S-Propylthiouracil.

These data suggest that it is possible that two separate processes influence intrathyroidal drug content. A saturable mechanism which is

influenced by acute inhibition of the anion trap may operate at low plasma concentrations of drug. At higher drug dosage, passage of drug to within the thyroid cells may be wholly dependent on the plasma concentration, and not affected by acute inhibition of the anion trap.

The cause of this partial inhibition by perchlorate of antithyroid drug accumulation is not immediately clear. Although there is considerable information available on factors which modify antithyroid drug accumulation within the gland, the mechanism of this is not known (Marchant, 1971; Lang, 1982; Lees, 1976). Factors such as TSH and LATS which stimulate iodide trapping have been shown to increase drug uptake and metabolism by rat thyroid (Lees and Alexander, 1978). It seems unlikely, however, that this energy-dependent, membrane associated anion trap is directly responsible for drug accumulation. Although it has been demonstrated in the current study that perchlorate administration was associated with a fall in drug accumulation within the gland, drug accumulation still persisted at a time when iodide trapping was almost completely inhibited. Using an in vitro system, Lang (1982) showed that ouabain and dinitrophenol, both inhibitors of active transport, did not abolish thyroid drug accumulation. There is, therefore, evidence from both in vivo and in vitro studies that antithyroid drug accumulation within the gland does not depend solely on a functioning iodide trap.

Perchlorate competitively blocks iodide trapping by the gland. As iodide exit from the follicular cell is also increased by perchlorate administrtion (Hilditch, 1978) this will have the effect of causing an acute fall in intrathyroidal inorganic iodide. Previous studies have shown that antithyroid drug accumulation is enhanced by increasing iodide availability: less drug is accumulated by animals on a low iodine intake than by animals on a normal diet (Lang et al. 1983 a + b). Similarly, acute administration of potassium iodide in experimental animals causes increased drug accumulation although at very high doses of iodide administration an unexplained paradoxical decrease in drug accumulation may be seen (Lang et al. 1983 a + b). The results of the current study are consistent with these previous reports and suggest that acute intrathyroidal iodide depletion is associated with a decrease in drug accumulation. Other factors which have been shown to increase drug accumulation, such as thyroid stimulating hormone may do so indirectly by increasing intrathyroidal iodide content (Lees and Alexander,

1978).

The reason why drug accumulation is related in this way to thyroid iodide content is not known. Previous studies in rat have shown that radiolabelled antithyroid drug is accumulated within the thyroid gland and that the autoradiographic localisation of drug within the gland is identical to that of iodide, being within the colloid and at the colloid/cell interface (Ferguson et al. 1971). After trapping of iodide by the follicular cell, the anion is rapidly organified by the thyroid peroxidase enzyme system, and this is probably also responsible for oxidation of drugs such as methimazole and propylthiouracil. It therefore appears that iodide and antithyroid drugs compete for a common oxidative enzyme after initial uptake by the gland, and subsequent localisation within the gland may reflect this.

Antithyroid drug oxidation is also increased in animals on a high iodine intake, and reduced in animals on a low intake (Marchant et al. 1978): enhanced drug metabolism in the presence of a competing substrate appears paradoxical. Taurog (1976), who has reported a similar phenomenon in vitro, has suggested that with a high intrathyroidal drug/iodide ratio, drug:enzyme interaction causes irreversible inactivation of thyroid peroxidase and subsequent drug oxidation is therefore prevented. With a lower drug/iodide ratio, an enzyme:iodide complex may be responsible for oxidising the drug and reducing iodine to iodide. This accounts for drug inhibition of iodide organification and it is suggested that such a process may spare the enzyme from irreversible inactivation and allow continued drug oxidation to occur (Davidson et al. 1978). If, therefore, drug accumulation is dependent on subsequent metabolic stops, a fall in intrathyroidal iodide could inhibit accumulation by primarily retarding drug oxidation. This concept is further considered at the end of this section.

SECTION TWO
ACCUMULATION OF THIOURYLENE ANTITHYROID DRUGS IN MOUSE SALIVARY GLAND

4.2.1. Introduction

In the preceding section it was suggested that antithyroid drug accumulation was related to the ability of thyroid tissue to organify iodide, and not specifically related to iodide trapping. If this were so, it might be expected that antithyroid drug accumulation might not occur in tissues where dissociation of iodide trapping and organification exists. Salivary gland tissue has an anion trap which is very similar to that on the thyroid, but lacks the ability to organify iodide (Fletcher et al. 1956; Lazarus, 1972): the potential uptake of antithyroid drugs by mouse salivary (submandibular) gland has therefore been examined. As initial findings indicated that the gland did, in fact, accumulate antithyroid drug this was further investigated using histochemical methods and autoradiography in an attempt to localise the site of drug and iodide deposition within salivary gland. In view of the close relationship in thyroid gland between the site of antithyroid drug and iodide deposition and of thyroid peroxidase, this was investigated in salivary gland by enzyme histochemical techniques which localise peroxidase activity and was further assessed by identifying the site of glucose-6-phosphate dehydrogenase activity, which is essential for generation of hydrogen peroxide in tissues (Bancroft, 1977).

4.2.2. Methods

Adult male mice of the TO strain (Animal Suppliers) were used. They were maintained on commercial diet 41B (Oxoid) for at least 7 days before the experiment; tap water was available ad lib.

^{35}S-labelled Methimazole (28 µCi/mmol) and ^{35}S-Propylthiouracil (20 µCi/mmol) were obtained from Amersham International Ltd. ^{125}I sodium iodide (carrier free) was also obtained from Amersham International.

Drugs labelled with ^{35}S were dissolved immediately before use in alkaline solution (pH 8) to give a series of concentrations ranging from 0.1 µg to 1 mg drug in 0.1 ml volume. Drug was injected intraperitoneally at varying times from 2.5 to 320 minutes before death. Animals were killed by exsanguination under ether anaesthesia. Submandibular and thyroid glands

were dissected out and digested in soluene (Packard). Samples of 0.1 ml plasma were digested in hyamine hydroxide (10% by volume in 50% methanol). The samples were counted in appropriate scintillators (plasma in a dioxin based scintillator, NE 250, Nuclear Enterprises; tissue in a toluene based scintillator, 5g PPO and 0.1g POPOP/litre toluene) in a Packard Tricarb liquid scintillation counter using an internal standard, channels ratio method of quench correction. For calculation of ^{125}I uptake, separate animals were injected intraperitoneally with 1 µCi ^{125}I sodium iodide in 0.1 ml volume: tissues were removed as before and all samples counted in an automatic gamma counter.

To assess the effect of competitive inhibition of the anion trap on uptake of iodide and drugs, a separate group of animals was injected intraperitoneally with 10 mgs sodium perchlorate dissolved in 0.1 ml saline solution 30 minute before administration of drug or iodide.

Six animals were used in each group.

4.2.3. Autoradiography

Aliquots (0.1 ml) of solution containing 100 µCi ^{35}S-Methimazole, ^{35}S-Propylthiouracil, or ^{125}I sodium iodide were injected intraperitoneally. Animals were killed after 30 minutes, submandibular glands were dissected out, and a portion was immediately frozen in isopentane cooled with liquid nitrogen. Clean glass slides subbed in gelatin were dipped in Ilford G5 Nuclear emulsion which had been diluted with an equal volume of distilled water. These were allowed to dry and then stored at -20°C. The frozen salivary glands were mounted on a chuck and 8 µM sections cut in a cryostat maintained at -20°C. Tissue sections were attached to the emulsion-coated slides by pressure and were then left in a light-free box for 6 weeks. Control (i.e. non radioactive tissue) sections were included for chemography and latent image fading. At exposure the slides were thawed and the tissues fixed in phosphate buffered 10% formalin before developing. Counter staining was with haematoxylin and eosin.

4.2.4. Localisation of peroxidase activity

Mouse submandibular glands were frozen and 10 µM sections cut in a cryostat. These were incubated in Tris-HCl buffer (pH 7.2) with 3,3-diaminobenzadine (2×10^{-3} mol/l) and hydrogen peroxide (1%). After

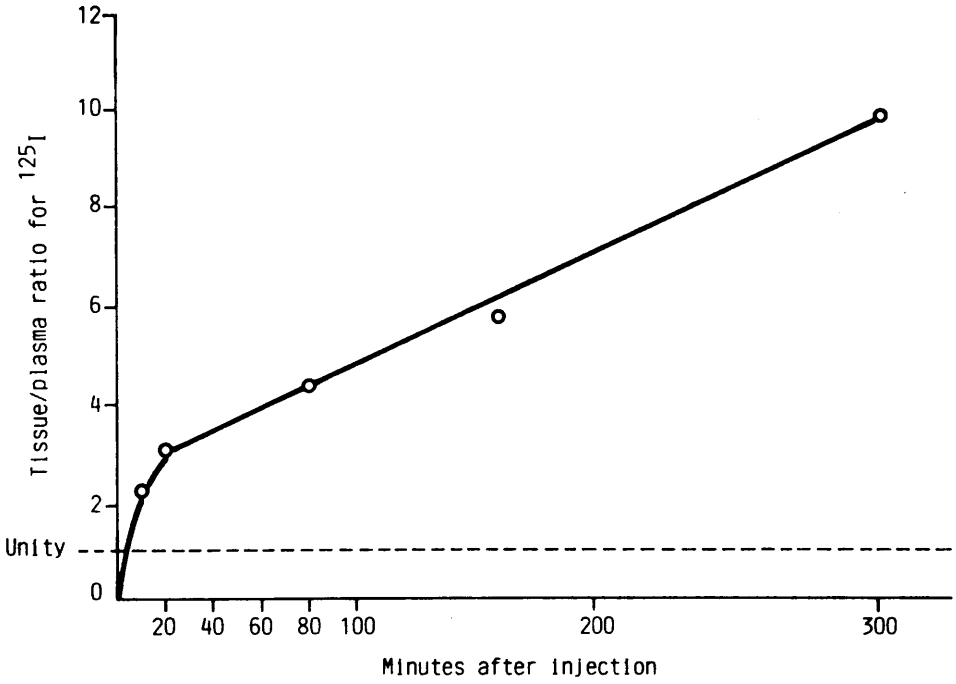


Fig 28

ACCUMULATION OF IODIDE (^{125}I) BY MOUSE SUBMANDIBULAR GLAND

THE ACCUMULATION OF ^{125}I BY MOUSE SUBMANDIBULAR GLAND IS EXPRESSED AS TISSUE/PLASMA RATIO OF THE ISOTOPE. 1.0uCi OF ^{125}I WAS INJECTED INTRAPERITONEALLY, AND SUBMANDIBULAR GLANDS REMOVED AT VARYING TIMES UP TO 320 MINUTES AFTER INJECTION. IT WILL BE SEEN THAT UPTAKE OF IODIDE BY SUBMANDIBULAR GLAND RAPIDLY INCREASED OVER THE FIRST HOUR AFTER INJECTION, AND MORE SLOWLY THEREAFTER. EACH POINT REPRESENTS THE MEAN OF 3 ANIMALS.

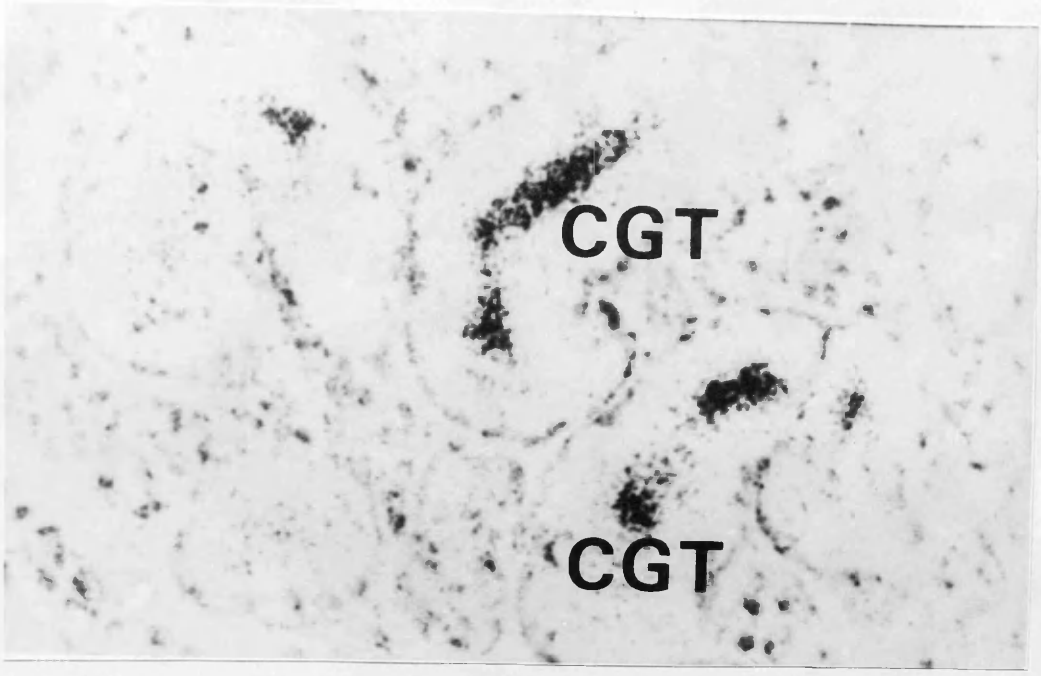


Fig 29

PLATE 1

AUTORADIOGRAPH OF ^{35}S-METHIMAZOLE ACTIVITY IN MOUSE SUBMANDIBULAR GLAND. DARK GRANULES (SILVER GRAINS) ARE SEEN IN THE LUMEN OR THE CONVOLUTED GRANULAR TUBULE (CGT) AND SURROUNDING LINING CELLS (x300).

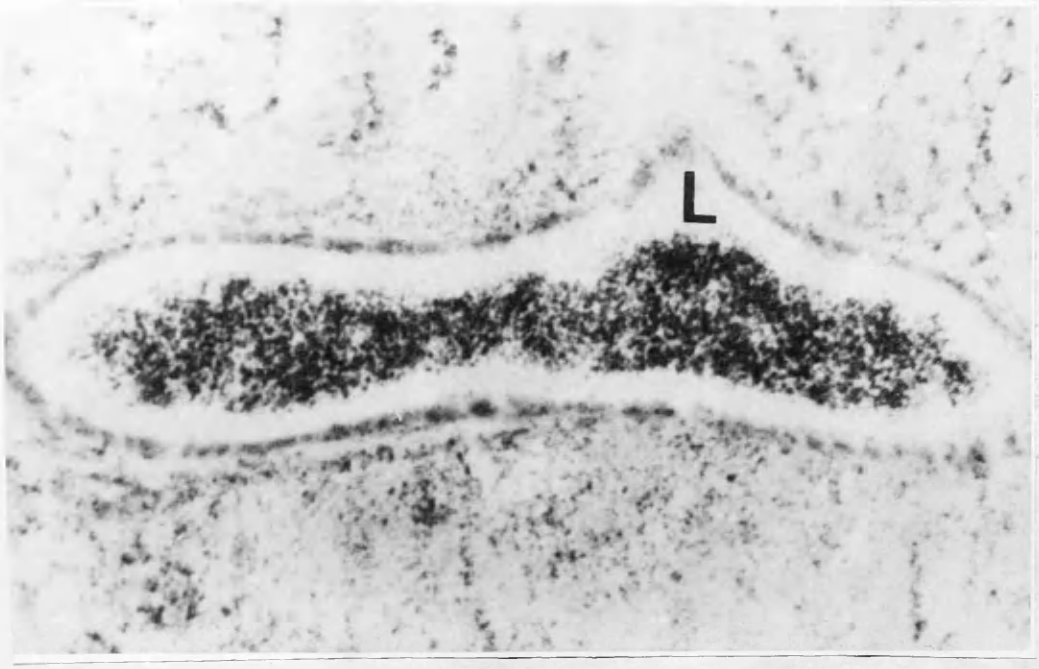


Fig 29

PLATE 2

AUTORADIOGRAPH OF ^{35}S-PROPYLTHIOURACIL ACTIVITY IN MOUSE SUBMANDIBULAR GLAND. DARK GRANULES (SILVER GRAINS) SEEN WITHIN LUMEN (L) INTRALOBULAR DUCT. (x250).

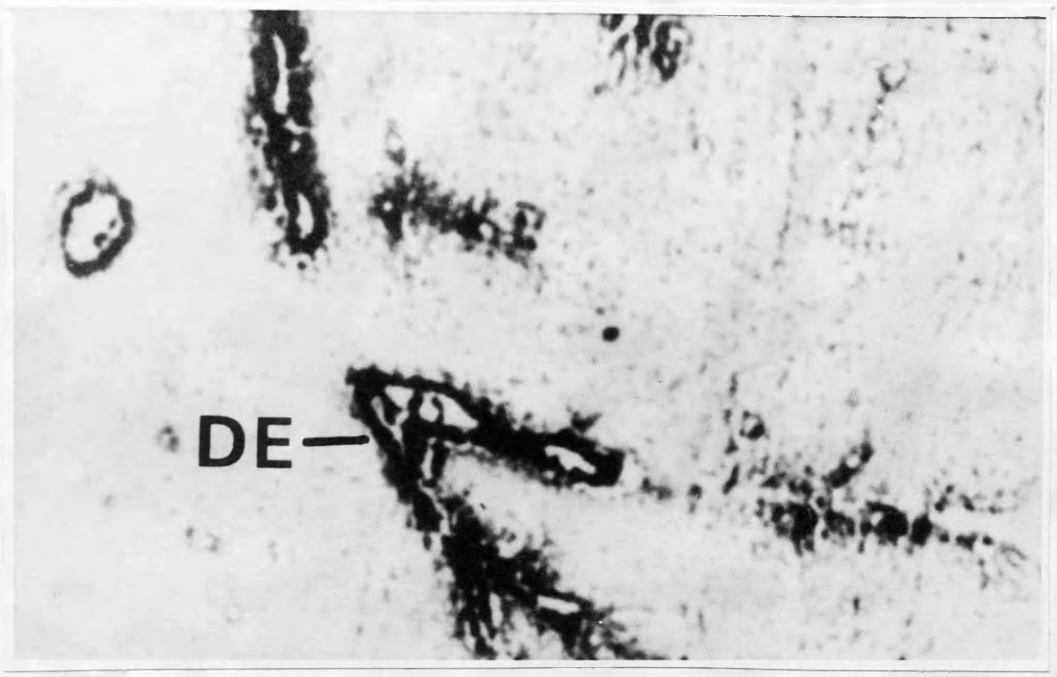


Fig 29

PLATE 3

AUTORADIOGRAPH OF ^{125}I IN MOUSE SUBMANDIBULAR GLAND. DARK GRANULES (SILVER GRAINS) SEEN IN DUCTAL EPITHELIUM. (x200). (DE).

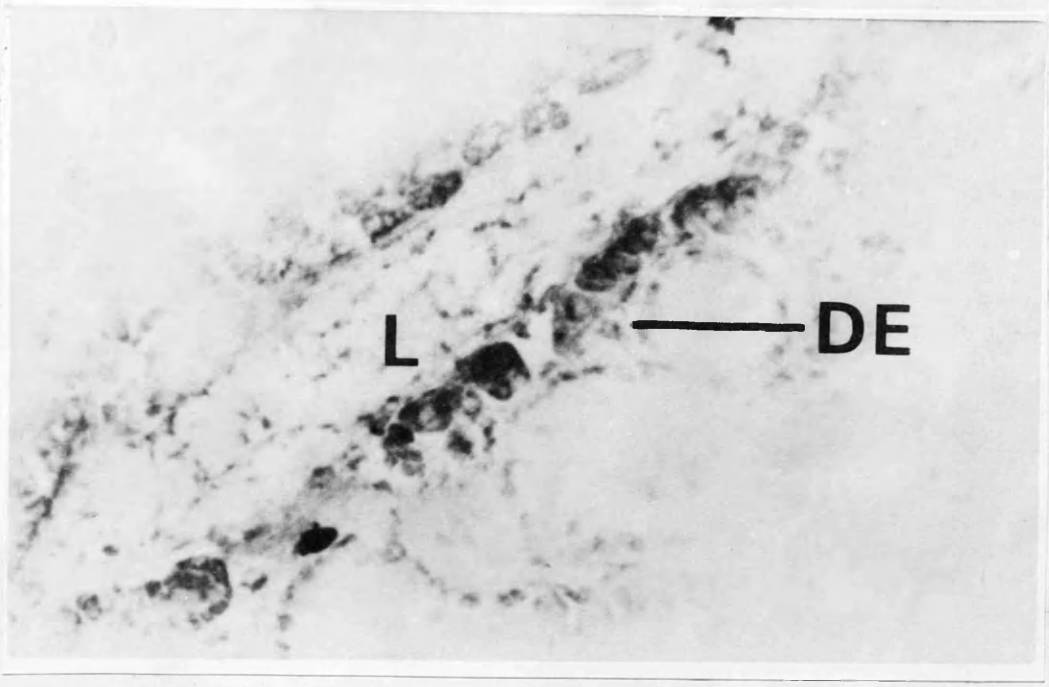


Fig 30

LOCALISATION OF PEROXIDASE ACTIVITY IN SALIVARY GLAND OF MOUSE

DARK STAINING (OXIDISED DIAMINO BENZADINE) SEEN IN CELLS LINING INTRALOBULAR DUCT LUMEN (L) (INTRALOBULAR DUCTAL EPITHELIUM, (DE) (x350).

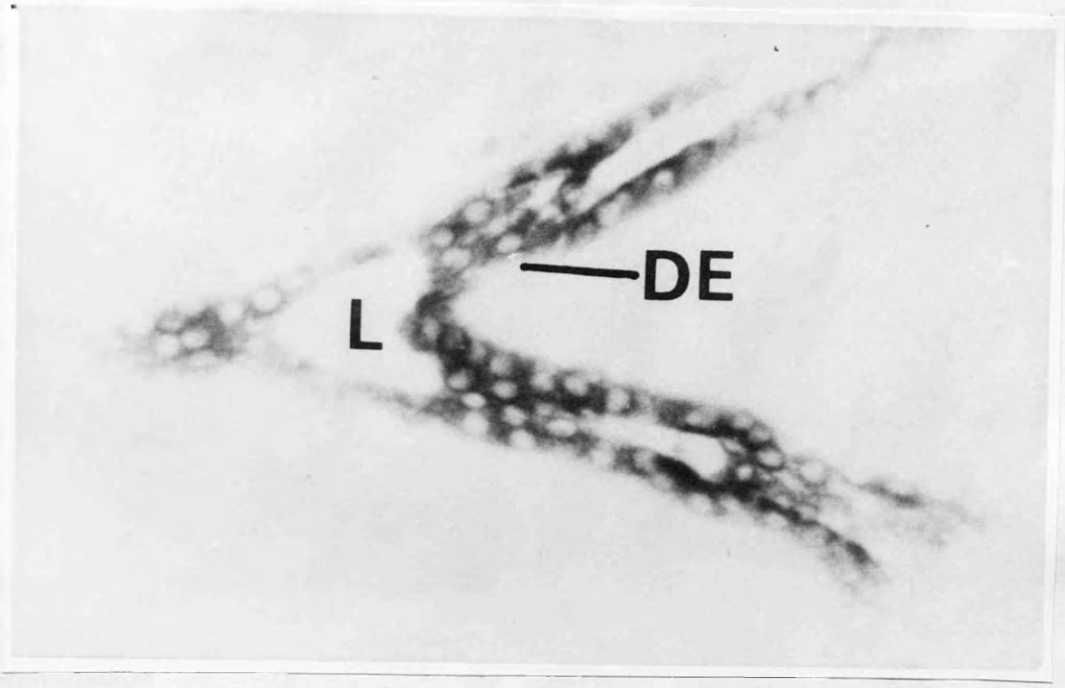


Fig 31

LOCALISATION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY IN SALIVARY GLAND OF MOUSE

Dark staining (precipitated nitroblue tetrazolium) in intralobular ductal epithelium (DE) (x350).

incubation at 25°C for 15 minutes, section were dehydrated through graded alcohols and mounted. Peroxidase activity was marked by a brown deposit (due to oxidised diaminobenzadine) (Bancroft, 1977).

4.2.5. Localisation of glucose-6-phosphate dehydrogenase activity

Frozen sections (8 µM thick) of mouse submandibular gland were incubated at 37°C in phosphate-buffered medium (pH 7.4) containing disodium-glucose-6-phosphate (10^{-2} mol/l), NADP (10^{-2} mol/l) and nitroblue tetrazolium (10^{-3} mol/l). At the end of 30 minute incubation the sections were mounted in glycerol jelly. Glucose-6-phosphate dehydrogenase activity was indicated by purple precipitate (Bancroft, 1977).

4.2.6. Results

(a) Accumulation of iodide and ^{35}S-Methimazole by submandibular gland

Data for iodide are shown graphically in Figure 28. Accumulation of iodide by salivary gland was confirmed with increasing tissue/plasma ratios occurring over a 5 hour period. Table 28 shows the results obtained for accumulation of ^{35}S-Methimazole. It will be noted that the highest tissue/plasma ratio was achieved with the lowest dose of methimazole, and with higher doses of drug, tissue/plasma ratios fell. Accumulation was seen to be a rapidly occurring process with a maximal tissue/plasma ratio being observed very shortly after intraperitoneal injection of drug.

(b) Effect of sodium perchlorate on accumulation of ^{35}S-Propylthiouracil, ^{35}S-Methimazole and ^{125}I Sodium iodide by submandibular gland

These results are summarised in Table 29. Animals were given either saline or 10 mgs sodium perchlorate 30 minute before drug or iodide administration, and were killed one hour after this. Sodium perchlorate completely inhibited accumulation of iodide by the submandibular gland. Antithyroid drug accumulation, however, continued although tissue/plasma ratios and absolute amount of drug accumulated within the gland appeared lower in animals given perchlorate (not significant).

(c) Localisation of ^{35}S-Methimazole, ^{35}S-Propylthiouracil and ^{125}I Sodium iodide in mouse salivary gland

Typical autoradiographs of ^{35}S-Methimazole (Plate 1) and ^{35}S-

TABLE 28

ACCUMULATION OF ^{35}S-METHIMAZOLE BY MOUSE SUBMANDIBULAR GLAND (EXPRESSED AS TISSUE PLASMA RATIO. VALUES ARE MEAN + SD OF 3 ANIMALS)

TIME AFTER	DOSE OF ^{35}S-MMI (ug) GIVEN INTRAPERITONEALLY			
INJECTION	1	10	100	1000
2.5	2.3 ± 0.2	2.1 ± 0.3	0.8 ± 0.2	0.7 ± 0.2
10	2.3 ± 0.2	1.8 ± 0.3	0.8 ± 0.2	0.8 ± 0.1
20	2.6 ± 0.3	1.6 ± 0.1	1.0 ± 0.2	0.9 ± 0.3
40	2.4 ± 0.3	1.4 ± 0.2	1.1 ± 0.2	0.9 ± 0.1
160	2.0 ± 0.4	1.2 ± 0.3	1.0 ± 0.2	1.0 ± 0.1
320	1.8 ± 0.5	1.1 ± 0.2	0.9 ± 0.1	0.8 ± 0.1

TABLE 29

EFFECT OF PERCHLORATE (CLO4, 10mg) ON ACCUMULATION OF ^{35}S-PROPYLTHIOURACIL (PTU), ^{35}S-METHIMAZOLE (MMI) AND SODIUM IODIDE (^{125}I) BY MOUSE SUBMANDIBULAR GLAND.

	^{125}I	^{125}I + CLO4	^{35}S-PTU (0.1ug)	^{35}S-PTU + CLO4	^{35}S-MMI (0.1ug)	^{35}S-MMI + CLO4
PLASMA CONCENTRATION (umol/l)			0.050 ± 0.004	0.05 ± 0.002	0.03 ± 0.006	0.03 ± 0.002
TISSUE/PLASMA	3.54 + 2.06	0.36* ± 0.02	6.73 ± 4.8	4.4 ± 3.0	4.49 ± 1.28	2.48 ± 0.54

*$p < 0.001$ COMPARED WITH ^{125}I ALONE

ALL VALUES REPRESENT MEAN + SD, n = 6.

Propylthiouracil (Plate 2) are shown in Figure 29. For both drugs the site of localisation was the same as radiolabelled iodide being within the lumen of the convoluted granular tubule and intralobular ductal epithelium of the gland. No accumulation of silver granules, which would indicate accumulation of radiolabel was seen elsewhere in the tissue. Figure 29 also shows an autoradiograph of ^{125}I sodium iodide showing distribution of radiolabel in intralobular ductal epithelium.

(d) Localisation of peroxidase

A typical histochemically stained section is shown in Figure 30. It was apparent that peroxidase activity (dark staining) was localised to intralobular ductal epithelium.

(e) Localisation of glucose-6-phosphate dehydrogenase activity

Glucose-6-phosphate dehydrogenase activity (dark staining) was again localised to the intralobular ductal epithelium (Figure 31). These findings indicate that peroxidase activity and the energy pathways required for peroxide generation were localised to the same area of the gland as iodide and radiolabelled antithyroid drug accumulation.

4.2.7. Discussion

Accumulation of ^{35}S-Propylthiouracil and ^{35}S-Methimazole has been shown in mouse submandibular gland. Autoradiography indicated that this occurred in the intralobular ductal epithelium: radiolabel was also seen within the lumen of the convoluted granular tubule. (The anatomy of the submandibular gland of mouse is shown schematically in Figure 32). This was identical to the localisation of iodide within the gland. Peroxidase activity, which was demonstrated using the technique of histochemistry with diaminobenzadine as substrate, was similarly seen in these areas, and glucose-6-phosphate dehydrogenase, which is responsible for generation of hydrogen peroxide through the hexose-monophosphate shunt, was found in the cells lining the intralobular duct, adding support to the concept that this is the locus of peroxidase enzyme complex activity. It is of interest that autoradiographic studies of the localisation of ^{35}S-Propylthiouracil in rat thyroid gland indicate that the histological localisation of the drug again resembles that of iodide, in that both are concentrated within the colloid

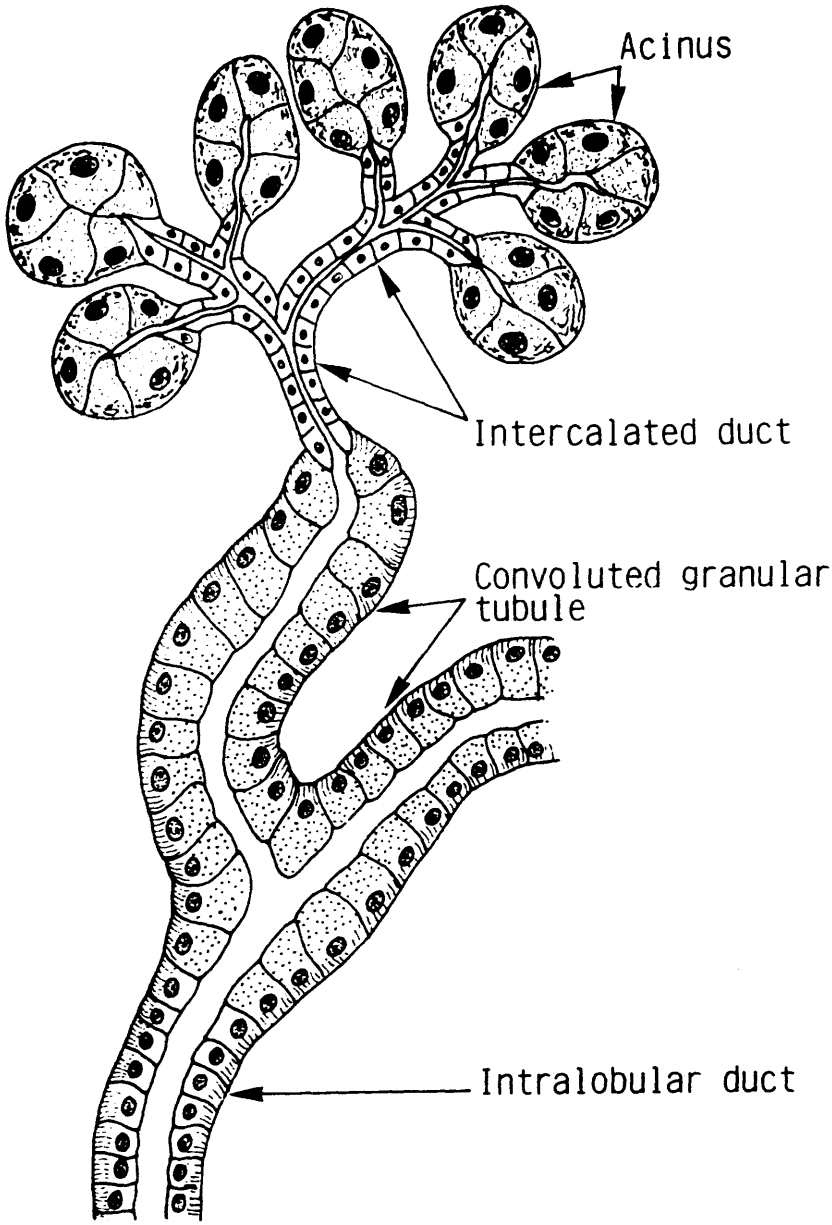


Fig 32

ANATOMY OF MOUSE SALIVARY GLAND

A SCHEMATIC REPRESENTATION OF SALIVARY GLAND ANATOMY IS SHOWN: THIS ILLUSTRATES THE RELATIONSHIP BETWEEN CONVOLUTED GRANULAR TUBULE AND INTRALOBULAR DUCT.

(Ferguson et al. 1971).

Accumulation of drug by the submandibular gland appeared to be a saturable process, with maximal tissue/plasma ratios being achieved with the lowest concentration of drug used. This would suggest that the accumulation of the drug is not merely a product of passive transmembrane passage. Furthermore, the highest tissue/plasma ratio for drug was found very shortly after injection, again suggesting that initial accumulation was specific and active. This evidence, and the similarity in anatomical localisation between radiolabelled drug and iodide, raises the possibility that the drug shares with iodide a similar mechanism of accumulation. However, when iodide trapping by salivary gland was completely inhibited by the perchlorate anion, accumulation of both ^{35}S-Propylthiouracil and ^{35}S-Methimazole by the gland was not abolished: this is similar to data presented in the previous chapter on the effect of perchlorate on thyroidal drug accumulation. This suggests that for both drugs some other mechanism is responsible for drug accumulation.

Both thyroid and salivary gland tissue possess a peroxidase enzyme system (Alexander, 1959), although these may not be chemically identical. Using histochemical techniques, peroxidase activity was localised to areas in which accumulation of both iodide and ^{35}S-labelled drug occurred. The function of a salivary peroxidase/iodide system is unclear. If the anion trap, as these results suggest, is not directly implicated in antithyroid drug accumulation by the submandibular gland, it is possible that the peroxidase enzyme system which is responsible for antithyroid drug oxidation might also be involved in drug accumulation by both thyroid and salivary gland tissue. It is of interest that accumulation of propylthiouracil and methimazole has been reported in polymorphonuclear leucocytes (Lam and Lindsay, 1979): these cells also contain a peroxidase (myeloperoxidase) system and also accumulate iodide, but lack an anion trapping mechanism. It may be that it is the possession of a peroxidase enzyme system which determines the ability of tissues to oxidise and accumulate thiourylene antithyroid drugs, and not primarily the ability to accumulate iodide. In contrast, lymphocytes have been shown not to concentrate 35S-labelled methimazole (Shewring and Lazarus, 1983) and these cells lack peroxidase activity (Weetman, 1984 c)

In conclusion, therefore, ^{35}S-Propylthiouracil and ^{35}S-Methimazole accumulated in the mouse submandibular gland. Although both the thyroid

and salivary gland in the mouse contain an active anion trap, and although the site of localisation for iodide and drug in both tissues is the same, it seems very likely that the method of accumulation for iodide and drug differ. The presence of peroxidase activity in all tissues which have been shown to accumulate thiourylene antithyroid drugs raises the possibility that this may be important in the mechanism of accumulation, and it is suggested (see previous section) that this is related to subsequent drug metabolism.

SECTION THREE
ANTITHYROID DRUG ACCUMULATION IN HUMAN POLYMORPHONUCLEAR CELLS

4.3.1. Introduction

In the preceding sections the accumulation of antithyroid drugs in tissues which possess an energy dependent, membrane associated anion trap has been investigated, and evidence presented that drug accumulation is a function not of this, but of peroxidase enzyme capable of oxidising antithyroid drug. There have been reports that human white blood cells (polymorphs) accumulate antithyroid drugs (Lam and Lindsay, 1979): these cells do not, as far as is known, have a specific anion trap and therefore present a useful model in which to examine this phenomenon further. Polymorphonuclears are metabolically very much more active when phagocytosing than when resting, and for this reason cell uptake of drug was studied during phagocytosis stimulated by zymosan particles opsonised with plasma components (including gammaglobulin and fibronectin).

4.3.2. Methods

Isotopes and chemicals

^{35}S-Propylthiouracil (46 µCi/mmol) and carrier free ^{125}I sodium iodide and ^{99M}Tc-Pertechnetate (50 µCi/mmol) were obtained from the Radiochemical Centre, Amersham. Sodium iodide, potassium perchlorate, methimazole, zymosan and ouabain were obtained from the Sigma Corporation.

Cell preparation

Venous blood (50 ml) was drawn from healthy volunteers and mixed immediately with preservative free heparin. No subject was taking any drug treatment at the time of the study. The total leucocytes were obtained by selectively sedimenting the erythrocytes with plasmagel (Laboratoire Roger Bellon) and the mononuclear cells were then separated from the polymorphs by density centrifugation on Ficoll-Paque (Pharmacia). The residual erythrocytes were lysed in hypertonic saline and the polymorphs finally resuspended in Gey's solution (Gibco, Biocult) with the pH adjusted to 7.4. The average yield from 50 ml blood was 4 x 10^7 cells. These were shown

to be at least 95% viable by exclusion of trypan blue at the start of incubations. All isotopes and drugs were dissolved in Gey's solution: drugs were in a range of concentrations from 10^{-3}-10^{-4} molar.

Phagocytosis

Zymosan particles were opsonised by incubation at 37°C for 30 minutes with fresh autologous plasma, and then suspended in Gey's solution. A final zymosan concentration of 1 mg/ml was used to stimulate phagocytosis by polymorphs: phagocytosis was initiated 10 minutes after test drugs and radioisotopes were placed in the cell suspension.

Radioisotope uptake by cells

In order to measure cell/supernatant ratios, 30 x 10^6 cells in 1 ml were incubated at 37°C for one hour after adding opsonised zymosan. The activities of the radioisotopes in the cell suspension and in the supernatant were then counted. Cells were separated by centrifugation at 400 g for 10 minutes. The polymorph volume of the suspension was calculated based on cell count and assumption of a uniform cell volume (1 x 10^6 cells = 0.7 µl).

Inhibition of cell peroxidase activity

Effect of drugs on cell peroxidase activity was examined by measuring chemiluminescence of phagocytosing polymorphs. This is assayed using photon emission from interaction between a test chemical (luminol) and peroxidase. A light sensitive instrument (luminometer) is used for this purpose. Following dark adaptation of the cells for 30 minutes, chemiluminescence was measured by incubating 5 x 10^6 cells in 175 µl phosphate buffered saline at 37°C together with 25 µl luminol (10^{-5}). Test chemicals were added (25 µl) to give a final concentration of 10^{-4} molar, and phagocytosis initiated by addition of 25 µl of a 10 µg/ml solution of opsonised zymosan. Chemiluminescence was measured for 100 seconds using a Packard Picolite luminometer (Stock, Coderre and Levine, 1982). The results are expressed as a percentage of the control in which saline was substituted for test drug.

All incubations were performed in duplicates of six.

4.3.3. Results

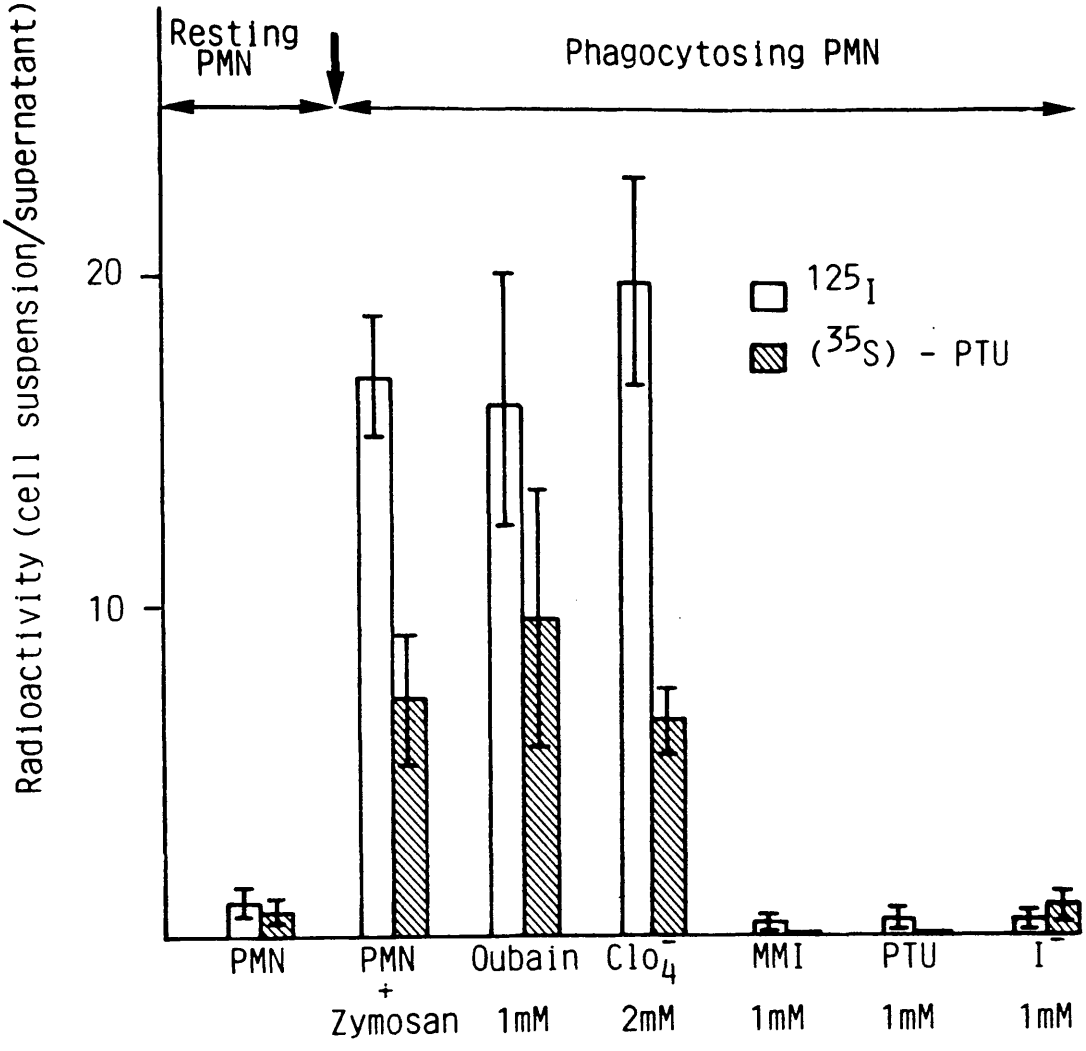


Fig 33

UPTAKE OF IODIDE (^{125}I) AND ^{35}S-PROPYLTHIOURACIL BY POLYMORPHONUCLEAR CELLS

THE UPTAKE OF IODIDE (^{125}I) (OPEN COLUMNS) AND ^{35}S-PROPYLTHIOURACIL (HATCHED COLUMNS) BY RESTING (EXTREME LEFT) AND PHAGOCYTOSING POLYMORPHONUCLEAR CELLS (STIMULATED BY ZYMOSAN). OUABAIN (1mM) AND PERCHLORATE (2mM) HAD NO EFFECT ON UPTAKE OF ^{125}I OR ^{35}S-PROPYLTHIOURACIL; METHIMAZOLE (1mM), PROPYLTHIOURACIL (1mM) AND IODIDE (1mM) ALL INHIBITED UPTAKE OF BOTH ^{125}I AND ^{35}S-PROPYLTHIOURACIL (ALL $p<0.01$). ALL INCUBATIONS WERE PERFORMED IN GROUPS OF 6. MEAN AND STANDARD ERROR OF MEAN ARE SHOWN.

TABLE 30

INHIBITION OF MYELOPEROXIDASE ACTIVITY* IN HUMAN POLYMORPHONUCLEAR CELLS

	CHEMILUMINESCENCE (% OF CONTROL) POLYMORPHS
OUABAIN	98.2
PERCHLORATE	97.8
METHIMAZOLE	61.2
PROPYLTHIOURACIL	78.5
IODIDE	100.9

*MEASURED USING CHEMILUMINESCENCE WITH LUMINOL.

VALUES EXPRESSED ARE A PERCENTAGE OF CONTROL MEASURED IN THE ABSENCE OF ADDED COMPOUNDS. EACH VALUE REPRESENTS MEAN OF 3 MEASUREMENTS. ALL DRUGS ADDED AT A CONCENTRATION OF 10^{-4}M.

Neither resting nor phagocytosing cells accumulated ^{99m}Tc-Pertechnetate after 60 minutes incubation while clear uptake of both iodide (^{125}I) and ^{35}S-Propylthiouracil occurred with cell/supernatant ratios of 16.8 and 7.4 respectively (Figure 33). The effects of various drugs on polymorph accumulation of iodide and propylthiouracil by phagocytosing polymorphs are shown in Figure 33. These agents were added to the cell suspension 10 minutes before zymosan. Neither ouabain nor perchlorate affected uptake of ^{125}I or ^{35}S-Propylthiouracil. Unlabelled methimazole, however, inhibited uptake of both agents. Both unlabelled propylthiouracil and unlabelled iodide at high concentration also inhibited uptake of labelled material, although this is difficult to evaluate as these unlabelled drugs would greatly diminish the specific activity of the corresponding radioisotopic chemical. It was evident, however, that iodide inhibited propylthiouracil accumulation and vice versa.

The effect of these chemicals on myeloperoxidase activity is shown in Table 30: this was measured by chemiluminescence, and values are expressed as a percentage of control. Methimazole and propylthiouracil (0.1 mmol) both decreased myeloperoxidase activity whereas iodide, ouabain and perchlorate had no discernible effect.

4.3.4. Discussion

The cell model used in this study was the human polymorph in which myeloperoxidase activity had been stimulated by phagocytosis of opsonized zymosan particles. Polymorphonuclear myeloperoxidase is necessary for the generation of oxygen singlet free radicals (Belch, J. Personal Communication), possibly using chloride ion as an enzyme co-factor. These very reactive particles are responsible for the bactericidal properties of the cells. Neither methimazole or propylthiouracil affect ingestion of zymosan by polymorphs and this has been confirmed using a labelled zymosan system (Ferguson, Alexander, Connell, et al. 1983; Lam and Lindsay, 1979).

Uptake of iodide by tissue such as thyroid, is dependent upon an energy dependent anion trap, which can be competitively inhibited by other anions such as perchlorate, and is abolished by ouabain which inhibits sodium/potassium ATPase activity. In the polymorph, neither ouabain nor perchlorate affected iodide accumulation during phagocytosis, and this suggests that uptake of the anion is therefore independent of the membrane associated anion trap. It is unlikely that iodide accumulation is a non

specific effect of phagocytosis as uptake of iodide was inhibited by methimazole and propylthiouracil which do not affect accumulation of opsonized zymosan particles (Ferguson et al. 1983). It seems, therefore, that the uptake of iodide is a consequence of myeloperoxidase activity; this enzyme has a similar oxidation potential to thyroid peroxidase, and may be capable of oxidising iodide. This would account for the inhibition of iodide uptake by the thiourylene drugs, which were shown in the current study to inhibit also myeloperoxidase activity as measured by chemiluminesce.

This system, therefore, allowed cell accumulation of propylthiouracil to be studied independently from an anion trapping mechanism associated with cell membranes. The drug was accumulated by polymorphs, confirming an initial report by Lam and Lindsay (1979).

It is of interest that antithyroid drugs appear not to be concentrated in lymphocytes (Shewring and Lazarus, 1983), cells which lack the high concentrations of myeloperoxidase present in polymorphs. Although initial work on the immunosuppressive actions of antithyroid drugs in Graves' disease suggested that this might be due to an effect on lymphocyte function (McGregor et al. 1980), it now appears more likely that this effect is a consequence of impairment of monocyte processing and presentation of antigen (Ratanachaiyavong and McGregor, 1985). Potential accumulation of antithyroid drug by monocytes has not been studied, but it is known that such cells contain an active H_2O_2/peroxidase system: it may be that concentration of antithyroid drugs by monocytes as a consequence of this enzyme complex is a prerequisite for any effect on immune functions (discussed in chapter 5).

Perchlorate, which is a specific competitive blocker of the anion trap in the thyroid and salivary gland, did not affect drug uptake, and ouabain, an inhibitor of membrane associated sodium/potassium ATPase activity also had no effect: these data therefore supplement findings presented in the previous two chapters derived from tissues which have an active anion trapping mechanism. Addition of unlabelled propylthiouracil and methimazole to polymorphs blocked labelled propylthiouracil accumulation, and similar concentrations of both drugs also inhibited myeloperoxidase activity. Iodide also blocked accumulation of drug, although did not significantly inhibit myeloperoxidase. This may reflect differences in the interaction between thyroid peroxidase,iodide and drugs discussed by Taurog (1978) and Davidson

et al. (1978). It is possible that the accumulation of iodide and propylthiouracil by human polymorphonuclear cells is a function of interaction with myeloperoxidase, activated during phagocytosis. Resting cells did not accumulate either iodide or drug and this may reflect inactivity of myeloperoxidase in such cells.

The implication of the above data to the mechanism of thyroidal accumulation of antithyroid drugs is discussed in the concluding portion of this chapter.

Antithyroid drugs are not reported to affect mature white cell function in man during clinical use, although are occasionally a cause of leucopenia (Trotter, 1962). Accumulation of drug only occurred when cells were actively phagocytosing: most polymorphs in the peripheral circulation are not activated and it seems unlikely that drug accumulation would occur to a great extent in vivo; although absence of peroxidase in polymorphs in man is a cause of chronic granulomatous disease, the effect on myeloperoxidase of methimazole and propylthiouracil at high concentration in vivo was one of partial inhibition only. It seems unlikely that antithyroid drug therapy would have an important effect on the efficiency of polymorph function in man.

SECTION FOUR
SUMMARY OF STUDIES ON ACCUMULATION OF ANTITHYROID DRUGS

4.4.1.

The data presented in this section, derived from animal thyroid and salivary tissue and from human white blood cells show that tissue concentration of thiourylene antithyroid drugs is a more generalised phenomenon than hitherto suspected. It seemed initially that drug accumulation might be related to iodide trapping (Marchant et al. 1978): acute blockade of the iodide trap, however, reduced, but did not abolish, drug accumulation in mouse thyroid gland and had little effect on accumulation in mouse submandibular gland. Further, drug accumulation was found in human polymorphs which lack a specific membrane associated iodide trap: iodide uptake by these cells was not inhibited by a competitive anion (perchlorate) or by ouabain. It is clear, therefore, that the presence of an active iodide trap is not a prerequisite for antithyroid drug accumulation.

Subsequent evidence suggested that the presence of a peroxidase enzyme system is a feature of tissues which accumulate antithyroid drugs. Indeed, in mouse salivary gland the localisation of labelled antithyroid drug was very similar to that of the site of peroxidase generation and activity. Previous studies have suggested that this is also the case for rat thyroid tissue (Ferguson et al. 1971). Additionally, in human polymorphs, antithyroid drugs were shown to inhibit peroxidase activity at doses which also reduced uptake of labelled propylthiouracil. Conversely, lymphocytes, which lack a peroxidase enzyme complex, do not concentrate these drugs (Shewring and Lazarus, 1983).

It is possible that drug accumulation within the thyroid is dependent on subsequent drug oxidation: this might explain the relationship between iodide intake and drug concentration by the gland. The process of accumulation may, in part, be one of passive transmembrane diffusion which reaches an equilibrium between extracellular and intracellular drug (Figure 34). In the suggested scheme, outlined in Figure 34, the intracellular concentration of free, non oxidised drug is governed by the rate of oxidation of drug by peroxidase. Where drug oxidation is inhibited, as during restricted iodine intake or by other agents which inhibit peroxidase (such as other

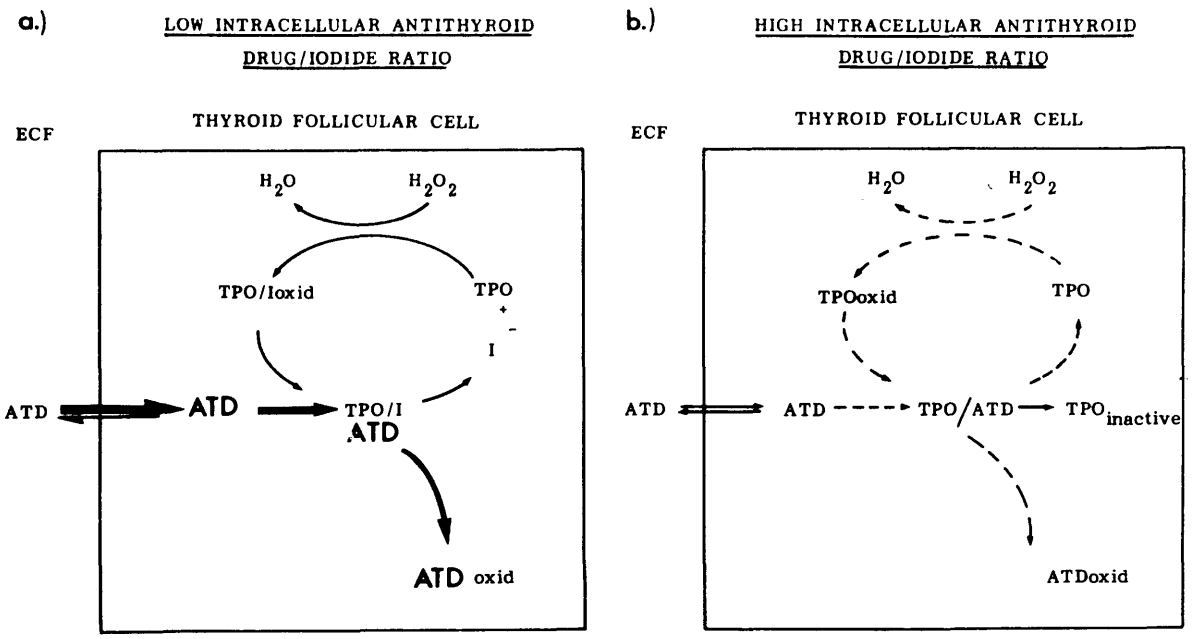


Fig 34

HYPOTHETICAL SCHEME FOR UPTAKE OF ANTITHYROID DRUGS (ATD) BY THYROID TISSUE

SUGGESTED INTERACTION BETWEEN INTRATHYROIDAL IODIDE AND ANTITHYROID DRUG TO REGULATE INTRACELLULAR ACCUMULATION OF LATTER.

(a) (LEFT) NORMAL IODIDE AVAILABILITY: LOW INTRACELLULAR ANTITHYROID DRUG/IODIDE RATIO. THYROID PEROXIDASE (TPO) AND IODIDE (I) FORM AN OXIDISED COMPLEX WITH HYDROGEN PEROXIDE (H_2O_2) ACTING AS AN ELECTRON ACCEPTOR. THIS WOULD NORMALLY BE AVAILABLE FOR IODINATION OF TYROSINE RESIDUES ON THYROGLOBULIN, BUT ATD ACTS AS AN ALTERNATIVE ELECTRON DONOR RESULTING IN DRUG OXIDATION (ATDOXID). TPO AND I^- ARE THEN AVAILABLE TO FORM AN OXIDISED COMPLEX WHICH CAN IN TURN OXIDISE MORE ATD.

(b) (RIGHT) IODIDE DEPLETION: HIGH INTRACELLULAR ANTI-THYROID DRUG/IODIDE RATIO. TPO IS OXIDISED NORMALLY BY H_2O_2, BUT AS I^- IS NOT AVAILABLE IT DIRECTLY OXIDISES ATD. IT IS SUGGESTED (DAVIDSON ET AL 1978) THAT ATDOXID INACTIVATES TPO SO THAT IT CANNOT BE REOXIDISED BY H_2O_2: THUS FURTHER ATD OXIDATION CANNOT PROCEED. IF ENTRY OF ATD TO FOLLICULAR CELLS IS GOVERNED BY SUBSEQUENT ATD METABOLISM, MORE ATD SHOULD BE ACCUMULATED IN SITUATION (a) ABOVE THAN (b).

OTHER PROCESSES WHICH ALTER ATD OXIDATION (INCREASE: TSH, DECREASE: OTHER INHIBITORS OF TPO) WILL SIMILARLY AFFECT INTRACELLULAR DRUG ACCUMULATION.

antithyroid drugs) intracellular levels of free, non oxidised drug may rise and so inhibit further accumulation; the opposite will occur when drug oxidation is enhanced (e.g. by high iodide intake or by TSH). Such a scheme might explain the close relationship in animal studies between events which enhance antithyroid drug oxidation and those which increase accumulation such as iodide availability, TSH and LATS stimulation of the thyroid. It is known that oxidative metabolism of methimazole and propylthiouracil is directly related to intra-thyroidal iodide content (Marchant et al, 1978; Lang et al. 1983 a + b). Tissue/plasma ratios in excess of 40 have been reported for thyroid accumulation of ^{35}S-methimazole (Marchant, 1971; Lees, 1978), and the hypothesis outlined above is unlikely to account alone for such high gradients. However, other physio-chemical factors, such as pK of drugs, are also likely to influence distribution of non-metabolised drug across a semi-permeable membrane (e.g. plasma membrane).

It is possible that this has implications for clinical use of antithyroid drugs. It may be that interaction between thyroid peroxidase and drugs and iodide regulates intrathyroidal accumulation of drug: this interaction may act to maintain relatively constant intrathyroidal drug levels over a range of plasma concentrations of drug. This may partly explain the much longer intrathyroidal action of such agents when compared with the relatively short plasma half-life of the drugs (McCruden et al. 1985). It might also suggest that high doses of antithyroid drugs offer little advantage over lower doses, as both might be expected to generate similar intrathyroidal drug levels. This concept is partly borne out by clinical experience, with similar extent and duration of inhibition of iodide organification occurring with a range of doses of carbimazole (McCruden et al. 1985; McCruden, Low, Connell, et al. 1981; Low, Hilditch and Alexander 1979).

CHAPTER FIVE

FINAL CONCLUSIONS

5.1.1. Introduction

In considering the aims of this thesis a number of questions relating to the early effects of ^{131}I treatment on thyroid function, and the interactions between ^{131}I, antithyroid drugs and iodide were considered. In the subsequent chapters containing experimental data (3 and 4) studies relavent to these questions have been presented. The principal conclusions from these studies are summarised below, with reiteration of the original questions.

5.1.2. What is the incidence of worsening of biochemical thyrotoxicois after ^{131}I, and are such changes clinically important?

Data on the immediate effects of ^{131}I therapy on circulating thyroid hormone levels was presented in chapter 3, section 1. These showed that in around 50% of patients a rise in both T3 and T4 occurred, and that pretreatment with the antithyroid drug carbimazole did not prevent an increase in hormone levels. As most patients on carbimazole had lower initial concentrations of T3 and T4, however, the peak levels achieved were much reduced and such therapy should, in theory, eliminate the risk of exacerbation of thyrotoxicosis after ^{131}I. In no patient in either group was the rise in thyroid hormone levels associated with clinical deterioration. The maximum rise in hormone levels occurred within 48 hours of ^{131}I therapy, and was shortlived (less than 1 week). This seems likely to be a consequence of radiation thyroiditis leading to disruption of thyroid follicular integrity, with leakage of preformed T4 and T3 from the gland. This was, surprisingly, not associated with a consistent change in circulating concentrations of thyroglobulin, and the cause of this is not clear. It is possible that the frequency and timing of sampling for thyroglobulin was not appropriate. However, assays for serum thyroglobulin concentrations are prone to interference from endogenous autoantibody, and this may also have obfuscated small changes in serum thyroglobulin levels. Although the majority of patients in this series were negative for antithyroglobulin antibody using a red cell agglutination technique, there is some evidence that

this may be a relatively insensitive means of measuring antibody levels (Isumi and Larsen, 1978). Additionally, no change in levels of TSH receptor directed immunoglobulin (thyrotrophin receptor binding displacing antibody) was detected following ^{131}I: this contrasts with some previous studies which have shown a rise in thyroid stimulating immunoglobulin and TRAb following ^{131}I therapy (Atkinson et al. 1982; Bech and Madsen, 1980). Although in this instance sample timing was similar to that of other studies, it is possible that the relatively small number of subjects (24) in whom the measurement was made in this thesis may account for the negative finding. Previous studies have, however, failed to show any relationship between immunological changes following ^{131}I and clinical or biochemical events.

5.1.3. How do the effects of ^{131}I on thyroid physiological processes such as iodide trapping and organification relate to biochemical and clinical responses in the first year after therapy, and can such information reduce the uncertainty about the early response to ^{131}I?

The effects of ^{131}I on thyroid iodide handling and the biochemical consequences of these changes were presented in sections 2 to 6 of chapter 3. In all patients ^{131}I caused a fall in 20 minute uptake of ^{123}I, which reflects iodide trapping. This fall was maximal in most patients by 4 weeks after ^{131}I administration. Where major inhibition of iodide trapping at this time was not followed by at least partial recovery, permanent hypothyroidism ensued within 2 months. In contrast, those patients whose trapping function was not severely impaired at this time (in practice a 20 minute uptake of greater than 8%) tended to remain thyrotoxic. Although there was a degree of overlap between patient groups, it was demonstrated that the outcome within the first year after ^{131}I therapy was predicted by this measure of iodide trapping 4 weeks after ^{131}I administration. For the majority of patients the important consideration following ^{131}I therapy is whether cure of thyrotoxicosis results: if not further treatment is required. When the 20 minute uptake of ^{123}I at 4 weeks after treatment was 4% or less, patients invariably became either euthyroid or hypothyroid within the next few months. As mentioned above, an uptake of greater than 8% indicated failure of treatment. This may have some value in the planning of future therapy for patients, as at this time most patients have not shown a consistent biochemical response. Uptake measurements longer than 4 weeks from ^{131}I

administration tended to mirror the biochemical and clinical status of the patients at that time. These measurements may be of especial use in patients whose true thyroid functional reserve following ^{131}I treatment is obscured by continued antithyroid drug treatment; withdrawal of drugs in such instances may be associated with relapse of thyrotoxicosis. Uptake measurements may identify patients who have failed to respond to an initial dose of ^{131}I, allowing more rational planning of future management.

These data indicate that the principal effects of ^{131}I on thyroid cell function occur early after treatment, and that these determine clinical outcome. In those patients who do respond to ^{131}I, the effects of treatment are maximal early after ^{131} administration, and apparent delay in response is due to the lag between changes in thyroid iodide handling and corresponding biochemical change. Although patients with more severe thyrotoxicosis (as measured by the 24 hour uptake of ^{123}I) tended to respond less well to therapy with a greater incidence of persistant thyrotoxicosis, this was a poor predictor of outcome. Similarly, there was little difference in pretherapy goitre size in those patients who responded and those who did not.

The effects of ^{131}I therapy on iodide organification were also considered using both indirect measurement (the ratio of 60:20 minute uptake of ^{123}I) and a quantitative direct perchlorate discharge test. These studies suggested that impairment of organification occurred in up to 50% of patients following ^{131}I treatment: this was most frequently seen between 2 and 4 months after therapy and tended to recover spontaneously. These findings are in general agreement with previous studies of organification impairment following ^{131}I therapy (Gray, 1975).

Evidence of organification impairment was seen in 5 out of 6 patients who developed transient hypothyroidism, and recovery of hypothyroidism appeared to coincide with improvement in organification efficiency. These studies suggest that transient hypothyroidism following ^{131}I therapy is a consequence of organification failure in a gland with a critical fall in thyroid iodide trapping. As permanent hypothyroidism was invariably associated with severe loss of trapping function it is suggested that hypothyroidism occurring in the early months after ^{131}I administration in patients with measurable iodide trapping function (20 minute uptake of ^{123}I greater than 4%) is potentially temporary in nature.

In summary, therefore, ^{131}I therapy causes an early fall of variable extent in trapping function for iodide in all patients. This seems to be important in determining subsequent biochemical and clinical course over the next 12 months. Impairment of organification of iodide seems to be a less frequent occurrence, and has a lesser impact on overall thyroid hormogenetic capacity. Unlike impairment of iodide trapping, organification defects appear fully reversible over a period of several weeks.

If the theory that the early (first year) effects of ^{131}I on thyroid function reflect irradiation damage to cell metabolic processes, and that late effects (i.e. after the first year) reflect cell sterilisation is correct (Dworkin, 1971) then the early response to ^{131}I is determined within four weeks of administration of treatment. This is borne out by the prediction of first year outcome from uptake data at four weeks (discussed above). It is said that the initial response to ^{131}I treatment can be slow and unpredictable: by measuring early iodide uptake four weeks after ^{131}I administration, patient response can be easily assessed and future management thereby facilitated. This may be especially so where patients are taking antithyroid drug treatment after ^{131}I administration, thus obscuring biochemical response to irradiation. Indeed, as the response to ^{131}I (as measured by iodide uptake) occurs within four weeks of therapy, and as patients who do not respond adequately at this time will have persistant thyrotoxicosis, and thus require a second dose of ^{131}I, there appears to be no great justification for using antithyroid drugs after ^{131}I therapy.

5.1.4. How does the use of carbimazole pretreatment affect the response to ^{131}I?

A major portion of the studies in this thesis have examined the effect of carbimazole pretreatment on response to ^{131}I therapy. Of 79 patients studied, 36 had been pretreated with carbimazole before ^{131}I and the remainder were given ^{131}I as initial therapy. Carbimazole pretreatment was associated with a reduced incidence of hypothyroidism during the first year after ^{131}I, and a correspondingly higher proportion of persistently thyrotoxic patients. This finding is in agreement with the majority of studies which have prospectively examined this effect of carbimazole (Aro et al, 1979; Bliddal et al, 1982; Steinbach et al, 1979). Carbimazole also appeared to reduce the occurrence of organification defects following ^{131}I administration,

but had no major effect on the pattern of change in trapping function following ^{131}I therapy. Carbimazole pretreatment did not prevent a rise in T4 and T3 concentrations following ^{131}I administration, although this rise was not associated with thyroid hormone levels above the upper end of the normal range. Carbimazole pretreatment before ^{131}I may, therefore, reduce the possibility of clinical deterioration due to a rise in T3 and T4 following irradiation.

The possible mechanism of the apparent radioprotective effect of carbimazole in reducing response to ^{131}I is of interest: that this might be due to a change in the kinetics, or distribution, of ^{131}I was examined in the final 2 sections of chapter 3. Carbimazole pretreatment did not alter the effective or biological half-life of ^{131}I within the thyroid and, therefore, had no major effect on the radiation dose delivered to the gland. In addition, carbimazole stopped 48 hours before iodide administration had no major effect on the organification or distribution of ^{131}I in rat thyroid. It is unlikely, therefore, that the radioprotective effect of carbimazole is due to the above effects. The other possible effects of carbimazole in reducing the radiation damage from ^{131}I are considered in section 5 of chapter 3. The most likely explanation may be that carbimazole, or a breakdown product of the drug (perhaps sulphate coupled to thyroid cell protein) reacts with free radicals produced from irradiation of thyroid tissue. The effect of sulphydryl groups in reducing free radical damage has been known for many years (Phil and Edjarn, 1958), and it is also known that antithyroid drugs are metabolised within thyroid cells to give rise to sulphate (Marchant, 1971). It is perhaps of relevance that it has recently been suggested that antithyroid drugs may affect mononuclear cell function by scavenging free radicals in monocytes (Taylor et al, 1984; Weetman et al, 1984), thus inhibiting antigen processing and presentation to lymphocytes. It is suggested that this may account for the apparent immunosuppressive effect of carbimazole in Graves' disease (Ratanchaiyavong and McGregor 1985). The potential interaction between antithyroid drugs of this class and free radicals appears to be a widespread property in tissues and may, indeed, underlie the basis of antithyroid action: the organification of iodide may involve the generation of iodine free radicals which can then interact with antithyroid drug or tyrosine residues on thyroglobulin. As free radical generation may be the mechanism by which tissue damage in a number of disease states may occur, such as ischaemic

damage to myocardial cells, joint inflammation in rheumatoid arthritis and alcohol induced hepatitis (Anon, 1985b; Halliwell and Gutteridge, 1984; Rowley, Gutteridge and Blake, 1984; Ryle, 1984) the potential protection of tissue by drugs such as methimazole is interesting and of potential therapeutic importance.

5.1.5. Are the effects of carbimazole on response to ^{131}I related to the phenomenon of drug accumulation within the thyroid?

In the final section of this thesis the phenomenon of accumulation of antithyroid drugs by both human (polymorphs) and animal (thyroid and submandibular gland) tissue was investigated. Accumulation of drug was seen to be independent of the presence of a functional anion trapping mechanism, but to be reduced by blockade of the trap by perchlorate. Drug accumulation was enhanced in polymorphs by stimulation of myeloperoxidase activity by phagocytosis, and reduced by inhibitors of peroxidase. In mouse submandibular gland drug localisation was shown to be the same as that of the peroxidase enzyme complex and also of the energy pathway necessary for generation of H_2O_2 (glucose-6-phosphate dehydrogenase), which can act as substrate for peroxidase. It therefore seems possible that the phenomenon of drug accumulation is critically related to drug interaction with tissue peroxidase, and a suggested scheme based on this interaction is proposed in section 4 of chapter 4. If this is correct, then concentration of thiourylene antithyroid drugs may occur in any cell type where oxidation of antithyroid drugs by a suitable peroxidase/oxidase/catalase enzyme occurs. This appears to be the case for the tissues studied in this thesis, and also appears to so for macrophage/monocyte cells (Weetman et al, 1984c). In monocytes one consequence of intracellular drug concentration is that antithyroid drug can act as a scavenger of free oxygen derived radicals (Taylor et al. 1984), and this may account for the immunosuppressive effect of the drugs. ^{131}I induced damage to thyroid cells is probably a consequence of free radical generation; it seems reasonable to suppose that the free radical scavenging property of antithyroid drug, or of drug metabolites including sulphate/cell protein complex, can account for the radioprotective effect of the drug. Thus, the process of drug accumulation and of radioprotection/immunosuppression appear to be linked through drug/peroxidase interaction. The interaction between drug and peroxidase appears to be a

consequence of the ability of the enzyme to use drug as an alternative substrate, and reflects the appropriateness of the redox potential of drug. Peroxidase can also act as a scavenger of free radicals within tissue, and the properties of antithyroid drugs in this role probably reflect the similarity of redox potentials for drug and enzyme.

In summary, therefore, the apparently unique properties of thiourylene antithyroid drugs discussed above appear to share a common link through drug interaction with cellular peroxidase enzymes. Thus, drug concentration within the cell, acute inhibition of iodide organification, impairment of antigen presentation and consequent immunosuppression and radioprotection are all facets of drug activity which may be direct consequences of the chemical properties of the compounds which allow oxidative interaction with tissue peroxidase and oxygen free radicals.

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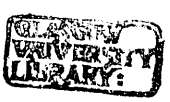
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