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PROBLEMS IN THE TREATMENT AND ASSESSMENT OF PATIENTS WITH THYROTOXICOSIS: A CLINICAL AND LABORATORY STUDY

by

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UNIVERSITY OF GLASGOW, 1976.
THIS THESIS IS DEDICATED TO MY

DEAR WIFE

AND

OUR DAUGHTER
INTRODUCTION

This thesis originated from three years of personal involvement in the clinical assessment of a new radioisotope (iodine-125) therapy for thyrotoxicosis. I was attracted to this study by Dr. W.R. Greig who initiated this therapeutic approach in 1968.

The somewhat heterogeneous nature of the thesis reflects the varied problems that arise in the treatment of thyrotoxic patients. Particular problems dealt with in this thesis include the difficulties involved in assessing such patients after therapy, whether further therapy is required, whether and what adjuvant drug therapy to radioiodine should be used, and what is the optimum mode of using radio-iodine therapy.

In my opinion, the aspects of this thesis which make an original contribution to the therapy of thyrotoxicosis include:

1. The results of the Trial of iodine-125 therapy which show conclusively that iodine-125 is not the answer to the problem of post-radioiodine hypothyroidism.

2. The validation of the TRH-thyrotoxic rat model as a chosen analogue to the human thyrotoxic gland rather than goitrogen-induced hyperplasia.

3. Systolic time intervals and zinc metabolism studies in thyroid disease which offer an objective measure of assessment of a patient's cardiac and metabolic response to hyper- or hypothyroidism and may permit detection of "at risk" patients (requiring urgent therapy).
and assist in deciding whether (further) therapy is required.

4. The laboratory study of beta-adrenoreceptor blocking drugs which contributes to the clarification of the action of such drugs and supports my critique of the current role of such drugs in the therapy of hyperthyroidism.

5. The demonstration of abnormalities of lipid metabolism in "compensated euthyroidism", subclinical hypothyroidism and frank hypothyroidism that underlines the inadequacy of a purely clinical assessment of post-radioiodine patients, emphasises the need for full hormonal assessment and supports my tentative conclusion that the current usage of iodine-131 to produce a euthyroid state is insufficient and potentially dangerous to certain patients.

The techniques adopted in this study include:

1. Measurement of thyroid hormone levels in serum. These assays were performed by the staff of the Regional Radio-immunoassay Centre and the resulting data was marshalled by myself and presented in this thesis. I did not participate in the actual laboratory measurements though I did subsequently spend a period at the Centre to learn something about the techniques involved.

2. Tissue culture techniques. I studied and refined the techniques in a tissue culture laboratory over a one year period and performed the experiments
described here in their entirety. This involved a meticulous aseptic technique, use of a Coulter counter and cell sizing apparatus, microscopy and the handling and counting of radio-isotopes.

3. Systolic time interval measurement. This was a collaborative study performed with Dr. W. S. Hillis who made the measurement in the study of hypo-, hyper-, and euthyroid groups without prior knowledge of their thyroid status. I performed the sequential systolic time interval measurements in patients with "compensated euthyroidism" and "T-3 toxicosis".

4. Laboratory studies of lipid metabolism involved ultracentrifugal separation of plasma lipoprotein fractions, cholesterol and triglyceride assays and spectrophotometric assay of the light-scattering index of sera in the Intralipid Tolerance Test. I performed these studies in their entirety.

5. Autoradiography and staining of thyroid sections. Those preparations were done by Dr. S. Kennedy, Consultant Pathologist. I was responsible for the administration of isotopes and TRH to the rats studied, for the dissection of the thyroid, tissue preparation and preservation, and for blood sampling by cardiac puncture.

This thesis crosses the boundary between the two specialties of endocrinology and cardiology and, in my opinion, is the
richer for this as the two specialties have deepened and 
widened my understanding of the problems of thyroid 
disease.

This thesis is the result of three years of intense 
study, of clinical and of laboratory work, that have led 
to the mastering of new techniques and to a modest 
contribution or two to the current understanding of 
thyroid disease.

In my opinion, the work described in this thesis and 
the techniques acquired in the process of completing 
these studies, satisfy the requirements laid down 
in the University regulations for the degree of doctor 
of philosophy.
ACKNOWLEDGEMENTS

The work described in this thesis was carried out from 1972 to 1975. These studies were commenced in the Department of Nuclear Medicine, Royal Infirmary, under the guidance of Professor E.M. McGirr and Dr. W.R. Craig. I would like to express my gratitude to these individuals for their generous help and guidance.

The financial assistance of the Wellcome Trust is also acknowledged.

I am indebted to Professor T.D.V. Lawrie for the opportunity to complete these studies while holding a registrar appointment in the Department of Medical Cardiology. His helpful advice is also appreciated.

I am grateful for helpful advice from Dr. I.R. McDougall and Dr. H.W. Gray.

My thanks are due to Dr. J.G. Ratcliffe and staff of the Radio-immunoassay Centre, Stobhill Hospital, for undertaking the sequential hormonal assays on these patients.

Dr. Stewart Kennedy, Consultant Pathologist, Royal Infirmary, undertook the preparation of slides of tissue sections, autoradiography and electronmicroscopy for the studies of the effect of chronic TRH administration on rat thyroid and response of this thyroid model to iodine-125 and iodine-131. I am grateful for his help and advice.

The study of systolic time interval measurement was a collaborative one with Dr. W.S. Hillis, Senior Registrar in the Department of Medical Cardiology, and part of the
studies described in this thesis will be incorporated into an M.D. thesis to be submitted by Dr. Hillis in the future. Our fruitful collaboration in this field is continuing.

My grateful thanks are due to Mrs. E. Laurie for undertaking the typing of this manuscript.

THE AUTHOR'S CONTRIBUTION TO THIS THESIS

I was responsible for the clinical assessment, prescription of therapy dose(s) and follow-up of 360 patients participating in the Trial of Iodine-125 therapy. I was responsible for selection of patients of particular interest and for detailed prospective studies of their metabolic and dynamic status. These studies included sequential systolic time interval measurement and lipid studies in selected groups and were performed in their entirety by the author.

The in vitro study of beta-adrenoreceptor blocking drugs necessitated spending over a year in acquiring expertise in tissue culture techniques. During this period, I refined and extended the methodology of a tissue culture laboratory and used this methodology for the studies detailed in this thesis. The stimulus to this study arose from a clinical observation and the in vitro studies were performed in their entirety by myself.

I was responsible for the initiation of the studies of the effect of chronic TRH ingestion on rat thyroid and for subsequent studies of the effect of iodine-131 and iodine-125 on this model for the human thyrotoxic gland.
I saw to the administration of TRH, gave the isotopes to the animals and performed the necessary weighings of animals, extraction and preparation and weighings of thyroids.

The lipid and lipoprotein studies in groups of thyroid patients were performed entirely by myself.
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The following publications concerning material presented in this thesis have appeared or have been submitted to medical journals.

**PUBLICATIONS**


**PRESENTATIONS AND ABSTRACTS**


Hillis, W.S., Bremner, W.F., Murray, R.G., Tweddle, A., Lawrie, T.D.V.
Effect of Beta Adrenergic Blocking Agents on Systolic Time Intervals in Hyperthyroidism.
Presented at the Scottish Society for Experimental Medicine, 1975.

Bremner, W.F., McDougall, I.R., Greig, W.R., Ratcliffe, J.G.
Long-term Results of Iodine-125 Treatment for Thyrotoxicosis.
SUMMARY

This thesis falls logically into 5 sections.

SECTION A outlines the present understanding of the aetiology of thyrotoxicosis and discusses presently available tests of thyroid function regarding their relevance to the Trial of Iodine-125 therapy. This is important as accuracy of assessment of thyroid status has markedly improved since the inception of the Trial in 1968. The currently available treatments for thyrotoxicosis are discussed with particular attention paid to their drawbacks. The problem of post-therapy hypothyroidism is emphasised.

SECTION B outlines the experimental and theoretical basis for the iodine-125 trial and describes, in detail, the design and results of the trial in 360 patients.

Reasons for the failure of this isotope to reduce the incidence of post-therapy hypothyroidism are discussed and further experimental evidence (from the author) is adduced. The validation of a TRH-induced thyrotoxicosis model in the rat as an approach to the human condition is described.

SECTION C describes experimental tissue culture studies of β-adrenoreceptor blocking drugs which have rapidly attained the status of a major adjuvant therapy in treating thyrotoxicosis.

SECTION D This section discusses the need for a test or tests of body response to thyroid hyper- or hypofunction and describes a new test of thyroid status; systolic time interval measurements. Zinc losses in hyperthyroidism are
described and the potential value of such measurements
discussed.

SECTION E concentrates on the cardiac complication of
hypo- and hyperthyroidism with investigations of lipid
abnormalities in hypothyroid patients and electronmicroscopic
studies of hyperthyroid rat hearts.
ABBREVIATIONS

LVET  LEFT VENTRICULAR EJECTION TIME
PB\textsuperscript{127}I  PROTEIN-BOUND IODINE-127
PEP  PRE-EJECTION PERIOD
QS\textsubscript{2}  TOTAL ELECTROMECHANICAL SYSTOLE
STI  SYSTOLIC TIME INTERVAL
T\textsubscript{4}  THYROID STIMULATING HORMONE
T\textsubscript{3}  TRIIODOTHYRONINE

UNITS: ALL UNITS ARE S.I. UNITS UNLESS OTHERWISE STATED
INTRODUCTION

Thyrotoxicosis as a recognisable entity first appears in the literature in the writings of Parry as recently as 1825. Graves subsequently related the ocular complications to the disease (1835) and von Basedow made further contributions to defining the disease entity in 1840. The absence of any mention of the disease from literature prior to 1825 has fostered the belief that thyrotoxicosis is a modern disease but this is pure speculation. Certainly, that most obvious of thyroid disorders, diffuse goitre, was described circa 1215 by Marco Polo in his travels in Yarkand (306).

Thyrotoxicosis (or hyperthyroidism) is due to excessive secretion of thyroid hormones (thyroxine and/or triiodothyronine) with a sustained rise in plasma levels. With this excessive output, the unoccupied binding sites on the thyroid hormone-carrying proteins of plasma decrease in number, and the free or unbound hormone fractions increase, with consequent over-stimulation of tissues responsive to thyroid hormones. In many aspects, the disease is simulated by thyrotoxicosis factitia (excessive ingestion of thyroid hormone).

Graves' disease is characterised by a diffusely enlarged overactive thyroid gland with an onset usually in younger adults (20 - 40 years), frequently with co-existing ocular and skin pathology, whereas toxic nodular goitre presents as an unevenly enlarged, overactive, nodular gland in older
patients, with less prominent ocular and skin involvement. Whether these conditions are distinct entities or an age-
dependent variation of the same disorder is disputed. For the purposes of the iodine-125 trial no distinction was made between these conditions, all such patients being regarded as "thyrotoxic" for the purposes of the Trial.

Toxic adenoma is a term used to distinguish a single hyperactive nodule from the commoner diffusely overactive or multinodular glands. Patients with thyrotoxicosis due to this cause are uncommon (< 5% of patients with thyrotoxicosis) and were not included in the Trial.
Fig 1. PATHOGENESIS of THYROTOXICOSIS
(adapted from R Volpe)

Random Mutation

Lymphocyte capable of producing
Thyroid-Stimulating Immunoglobulins (TSI)

Inherited defect of Immunological Surveillance

T Lymphocyte

B Lymphocyte → IgE

Anaphylactic Pathway → Delayed Hypersensitivity

TSI Formation

Circulating TSI

Thyroid Cell

Hyperthyroidism

$T_4$/$T_3$
PART 1

AETIOLOGY OF THYROTOXICOSIS

"What song the Syrens sang, or what name Achilles assumed when he hid himself among women, though puzzling questions, are not beyond all conjecture" HYDRIGRAPHA V.

There are many pointers to suggest that thyrotoxicosis has an immunological basis. At the clinical level, there is the association of thyrotoxicosis with recognised auto-immune disorders (2, 7, 67, 159, 160, 260-291) and the occasional finding of lymphoid and thymic hyperplasia. Hashimoto's thyroiditis occurs frequently in relatives of thyrotoxic patients and occasionally in the same patient (70, 260, 261, 291). The presence of thyroid antibodies and the discovery of thyroid stimulating immunoglobulins in thyrotoxic sera (2, 69, 153, 211) coupled with abnormalities of cellular immunity (as evidenced by the lymphocyte migration inhibition test (291) and by in vitro stimulation of thyroid cells by lymphocytes from thyrotoxic patients (73)) led to the hypothesis proposed by Volpe et al. (291) and shown diagrammatically opposite. According to Volpe's theory, "forbidden" clones of lymphocytes arise through a defect in immunological surveillance and act directly on thyroid follicle cells (cellular immunity) producing thyrotoxicosis. They also stimulate "bursa dependent" lymphocytes capable of directly affecting follicle cells, through the anaphylactic reaction pathway by way of IgE, or by secretion of thyroid-stimulating immunoglobulins.
One major drawback to this concept is the lack of conclusive evidence for this hypothesis but it serves as a useful working hypothesis.

Studies by Kriss (153) have linked ophthalmopathy to formation of thyroid antigen-antibody complexes on extracocular muscle membranes; the complexes arising by way of the lymph transfer that has been shown to occur from thyroid to orbital tissues.

Such ideas have been a major influence upon the author's approach to thyrotoxicosis.

PART 2: TESTS OF THYROID FUNCTION

(Only tests relevant to the Trial are discussed)

In the absence of simple tests of secretion rates of thyroid hormones, reliance is placed on measurements of plasma hormone levels and of gland avidity for iodine (114). The use of the TRH test is a recent and useful innovation.

1) Protein-bound Iodine (PBI-127)

This is an automated, chemically determined assay which was the only routinely available measure of plasma thyroxine level at the commencement of the Trial. The normal range is 315-630 nmol/L. If circulating iodo-proteins are present in excessive amounts (as in Hashimoto's thyroiditis, or immediately following radio-iodine therapy), the butanol-extractable iodine is a more reliable measure of circulating hormone levels.

Misleadingly high values for the PBI-127 occur if the patient has ingested organic iodine compounds (especially radiographic media.) False values also arise if a sample containing an excessively high PBI-127 is processed before the patient's serum as inter-sample contamination occurs in these circumstances. Studies
of the extent of cryptic iodine contamination(1) have shown this to be a considerable problem (~20% among hospital out-patients(214)), mitigating against the value of PBI-127 as a reliable test of thyroid status. For this reason, routine assay of serum thyroxine was adopted in 1972 as the best test of thyroid status.

2) **Thyroxine assay**

Serum thyroxine, or T\textsubscript{4}, is assayed by non-chemical protein-binding techniques and the assay is readily available as a standard pack (Thyopac-4, Radiochemical Centre, Amersham). The normal range for the Glasgow area is 55-144 nmol/L.

This assay is unaffected by iodine contamination and is valid if the binding capacity of the carrier proteins is normal. Pronounced elevations of thyroid-binding globulins may occur in pregnancy or with ingestion of oestrogens while decreases occur in patients on steroid therapy or with nephrotic syndrome. These alterations may be detected by measuring the unoccupied sites on thyroid hormone binding proteins by means of labelled T\textsubscript{3}. This measure is routinely available as the Thyopac-3 (Radioamersham).

3) The ratio Thyopac-4/Thyopac-3 x 100 is the Free Thyroxine Index and gives a measure that is proportional to the free thyroxine concentration as measured by equilibrium dialysis or gel filtration. This measure is less subject to distortion by iodine contamination or alterations in carrier proteins.
4) **Tri-iodothyronine assay**

This radio-immunoassay method gives a measure of total circulating $T_3$ in serum and became available in the Glasgow region in late 1973. It is of prime value in detecting patients with "$T_3$-toxicosis" (where serum thyroxine and $PBI-127$ measurements are normal). Use of this assay permitted characterisation of a group of patients with biochemical $T_3$ toxicosis following iodine-125 therapy. The clinical course of these patients is described later. The normal range for $T_3$ levels is $0.6 - 2.2$ nmol/L.

To allow continuity in patient assessment over the 7 years of the Trial, $T_3$ assays were not used in reaching a decision regarding further therapy, reliance being placed on thyroxine assays.

5) **Thyroid-stimulating hormone (TSH) assay**

This radio-immunoassay became available in 1971 and permitted detection of patients with biochemical "compensated euthyroidism". ($T_4$ in normal range but elevated TSH). The normal range is 0 to 8 mU/L. This test was not used in assessing patients as hypothyroid to allow continuity of assessment.

6) **TRH Test**

The measurement of the plasma TSH increment after 200 $\mu$g. of intravenously administered thyrotropin-releasing hormone is of value in distinguishing primary from secondary hypothyroidism. The lack of response may be used to confirm hyperthyroidism though euthyroid relatives of patients with Graves' disease and patients
euthyroid after treatment for Graves' disease may show no response (49). This test was used to characterise patients with "compensated euthyroidism".

7) Thyroid gland uptake of radio-iodine

The 24 hour uptake of a tracer dose of iodine-131 offers a measure of gland avidity for iodine. In conjunction with an elevated PBI-127, an uptake in the thyrotoxic range (≥ 50%) was used to confirm the clinical diagnosis of thyrotoxicosis in patients entering the Trial. This procedure was continued to include all 360 patients in the Trial. Routine scintiscans using initially iodine-131 but, more recently, technetium-99, were performed to exclude patients with solitary hyperfunctioning nodules.

PART 3

THERAPIES AVAILABLE FOR THYROTOXICOSIS.

1. NON-INTERVENTION

From a study of hospital records from around 1900 to 1940 when no therapy was available, Wilson (217) deduced that two thirds of thyrotoxic patients failed to recover with, perhaps, up to one half of these dying; the remainder eventually became euthyroid. This, however, was in the days when the only diagnosis was clinical; consequently milder cases may have escaped attention and the mortality estimation may be excessive. Nevertheless, the morbidity and mortality is such that therapy is indicated. Also, a proportion of patients becoming spontaneously euthyroid eventually become hypothyroid (114), possibly due to the presence of Hashimoto's Thyroiditis (109, 186, 317).
2. IMMUNOSUPPRESSIVE THERAPY

Since thyrotoxicosis (excluding toxic solitary adenoma) may be an autoimmune disorder, the use of immunosuppressive therapy is theoretically attractive. The results are difficult to evaluate as the disorder is of variable severity. Large doses of Prednisone were given to 5 patients by Werner et al. (305) with induction of a remission in all, permanent in 2. Steroids, however, have multiple actions and may affect the hypothalmo-pituitary axis directly (309). The main contra-indications to steroid therapy are the frequency and severity of side effects. Other forms of immunosuppression are subject to the severe limitations of toxic side effects.

The three modes of therapy in current use are all directed against the thyroid gland rather than the basic pathology.

3. SURGERY

Prior to the advent of antithyroid drug therapy and radio-iodine therapy, surgery was the only effective therapy. With modern anaesthesia and pre-operative control of thyroid overactivity, surgery is less heroic and much safer. However, there is still a significant morbidity and mortality attendant upon thyroidectomy. Also, the widespread use of radio-iodine and drug therapy has resulted in a marked decline in the number of patients being referred for surgery with potentially deleterious effects on the standards of thyroid surgery. "The occasional thyroidectomist" has been soundly condemned (162).
The results of the surgical approach are perhaps typified by the report of Green and Wilson (107). They reported a rate of 67% for euthyroidism, 5% for hypothyroidism, and 8% for persistence or recurrence of thyrotoxicosis. Subsequent experience from the same group demonstrated an inverse correlation between the incidence of hypothyroidism and recurrence, and extent of the thyroidectomy, with a combined morbidity of some 30% four years post-operatively.

McNeill and Thomson (191) reviewed 123 patients who underwent thyroidectomy more than 5 years earlier and found 81% euthyroid, 12% toxic and 7% hypothyroid. In contrast, a review of 146 patients at a mean period of 9 years post-thyroidectomy by Hedley et al. (133) found 36% hypothyroid. Other reports (114, 195) are in keeping with the last report. The overall incidence of hypothyroidism post-thyroidectomy appears to reach 35% to 49%.

The lower incidences are based on retrospective studies which tend to be incomplete and lack the more recent hormonal assays necessary for accurate assessment.

The risk of hypothyroidism has been correlated with the extent of the thyroidectomy, the presence of thyroid autoantibodies in the circulation and the extent of plasma cell infiltration and number of lymphoid follicles with germinal centres at operation (36, 105).

The various studies of thyroidectomised patients suggest the incidence of hypothyroidism mainly reflects the amount of thyroid tissue resected while, in patients with an active immune process in progress damaging thyroid tissue, the autoimmune process may be contributory.
The other drawbacks to surgery relate mainly to the risks of anaesthesia and operation. The mortality rate is low but probably around 0.2% (191). The major complications include laryngeal palsy (0.6% if nerve identified at operation, 2% if not (235)), phonation disturbances, parathyroid insufficiency, bad cosmetic result, as well as the risks of haemorrhage, embolism and atelectasis consequent upon any operation under anaesthetic (191, 235).

The present indications for surgery are probably limited to patients with pressure symptoms, young women wanting to start a family, patients who relapse after an adequate period of drug therapy and who are under 40 years of age, and perhaps to patients who intend to work as missionaries etc. far from any follow-up centre (114). This last indication is open to argument in the light of the high incidence of post-operative hypothyroidism noted above.

Repeat thyroidectomy is contra-indicated as there is a high risk of recurrent laryngeal nerve palsy and of recurrence of thyrotoxicosis (189).

Treatment of older patients with iodine-131 has improved the peri-operative surgical morbidity figures by removing many at risk patients from the surgical lists.

4. ANTITHYROID DRUGS

Antithyroid drugs, such as carbimazole and propylthiouracil, are usually efficacious in controlling thyrotoxicosis: the exceptions are the occasional very resistant patient and
patients with large goitres where such large doses are required that toxic side effects supervene. Drugs are the treatment of choice in young mildly toxic patients with small goitres, and in such patients where rapid control is essential. Therapy is efficient, safe, and carries none of the potential risks of damage to adjacent structures incurred by surgery.

The main drawback to drug therapy is the high relapse rate which after a course of one to two years is at least 50%. By 10 years at least two-thirds of patients have relapsed which is close to the spontaneous remission rate. Wilson suggests that antithyroid drugs control gland overactivity until normal thyroid function recurs spontaneously (217). It would appear that antithyroid medication does not influence the basic course and pathogenesis of the thyrotoxic process. Beck, however, claims that carbimazole acts not only on thyroid gland organification but also on the thymus gland directly, influencing thymic lymphocytes (personal communication based on thymic biopsy studies in patients undergoing thyroidectomy).

Problems with drug therapy relate to the difficulty of predicting which patients will have a remission and to the optimum duration of therapy. At the clinical level, rapid initial control of symptoms and reduction in goitre size are rough pointers to a favourable outcome, with delayed control or increasing goitre size (despite cutting off TSH stimulation by concomitant T4 thyroxine administration) mitigating against a remission. Alexander et al (34) have used the T3 suppression test to predict which patients are in
remission while on drug therapy on the basis that patients who do suppress are more likely to respond to drug therapy with a prolonged remission. This approach is not generally accepted despite its initial promise—for example, while Harden (129) found the presence of LATS to correlate with non-suppressibility, Sellers et al. (254) did not. Patients have had recurrences after suppression has become normal (126) and some authorities feel the test to have little practical value (121). Also relapse may be obscured by an iodine-deficiency state induced by antithyroid drugs (128).

Side effects of drug therapy, apart from relapse or failure to induce a remission, include a maculo-papular rash and occasional granulocytopenia or frank agranulocytosis. Another risk affects the pregnant patient where placental passage of drug may induce a foetal goitre with dystocia at delivery or frank hypothyroidism.

Relapse after a second course of antithyroid drugs is very common.

5. Radiation Therapy

The use of external irradiation of the thyroid has gained little support because of the very real risk of post-cricoid and nasopharyngeal carcinoma (114). This was reinforced by findings of up to 30% incidence of nodules and 3% of carcinoma in children given radiation therapy for enlarged thymus or tuberculous glands (134). A proposal for combination therapy with x-rays and iodine-131 was put forward and applied by Trotter and Willoughby but, for the above reasons, has not found general acceptance (277). Children's thyroid are particularly
susceptible to neoplastic change induced by radiation.

The thyroid cells treat radioactive iodine as normal iodine and concentrate it in the gland colloid; consequently isotopes of iodine can be used to destroy thyroid tissue with minimal irradiation of contiguous structures. In January 1941, the first thyrotoxic patient was treated with an isotope of iodine-127 (iodine-130, half life 12.5 hours) and two preliminary reports on this therapeutic approach appeared in 1942 (127,136).

Iodine-131 with its longer half-life (8 days) superseded iodine-130 and by 1960 was the therapy of choice for the majority of thyrotoxic patients.

Advantages of Iodine-131 Therapy

The characteristics of this form of therapy that make it the most frequently adopted treatment are its ease of administration - it is taken orally and takes only a few minutes on an out-patient basis - it is inexpensive and it eventually always controls the thyrotoxicosis (115). There is, moreover, only very rarely overt evidence of serious extra-thyroidal damage.

The aim of iodine-131 therapy is the maximum possible cure rate with one therapy dose. 60 per cent of patients respond to one therapy of average size (22,28,218) with some two-thirds of resistant patients responding to each subsequent therapy dose. 3 months minimum have to elapse between doses; if this delay is extended to 6 months, a further 15 per cent of patients became euthyroid without further therapy (45). Conventional doses of iodine-131 leave 50 per cent of patients thyrotoxic 3 months later and a minority require frequent therapies or, in the rare very resistant patient, massive therapy doses.
Disadvantages of Iodine-131 Therapy

1. EARLY COMPLICATIONS.
   
   (a) Radiation Thyroiditis.

   This occurs in a minority of patients, may cause localised discomfort and gland swelling, but subsides spontaneously (46). This potential complication, however, excludes patients with pressure symptoms from radio-iodine therapy.

   (b) Exacerbation of thyrotoxicosis and thyroid crisis.

   Exacerbation of thyrotoxicosis occurs in most reported series of 131-treated patients but thyroid crisis has been reported infrequently (158, 204, 209, 246, 255, 287) and has been absent from some large series (44). Thyrocardiacs are at risk of deterioration or death if they suffer an exacerbation of their hyperthyroidism and should be treated with antithyroid drugs prior to or as a "holding measure" after iodine-131 therapy.

   (c) Radiation Sialitis.

   This may be painful but is uncommon at the levels of dosage used to treat thyrotoxicosis, being more common with cancericidal doses (102). The parotid is most commonly affected because of its high saliva : serum iodine ratio (34, 41). This complication usually subsides spontaneously.

2. LATE COMPLICATIONS.

   (a) Neoplastic change.

1. THYROID.

   Epidemiological surveys have demonstrated an increased
incidence of thyroidal carcinoma in children following external radiation (52,134). Using three data sources, Hempelmann derived a linear relationship between the incidence of thyroid nodules and estimated cumulative radiation dose to the thyroid (134). An association between external irradiation and thyroid neoplasia has also been found in adults (103,313,318). Further epidemiological evidence relating thyroid irradiation to thyroid carcinoma comes from the increased incidence of thyroid carcinoma and benign nodules found among atomic bomb survivors in Hiroshima and Nagasaki (141) and among the Marshallene islanders accidentally exposed to nuclear test fallout (51,52). In the latter group of 67 islanders, four cases of thyroid carcinoma and 16 benign nodules were found. These people received an estimated exposure of 160 Rads from several iodine isotopes (I-131, I-132-I33, I-135) and 175 Rads from gamma rays.

Some of the claims relating to the effects of low levels of radiation of thyroid can be disputed as control populations are often lacking. The frequency of thyroid nodules may seem unduly high because they may go unnoticed in normal populations unless specifically sought. A study of children exposed to fallout in America with control groups found no significant difference in benign nodules nor carcinoma (228) and noted an unexpectedly high incidence of unsuspected thyroid disease in the control groups.

It is still a matter for argument whether iodine-131 therapy for thyrotoxicosis results in thyroid neoplasia. Studies using rats have shown that iodine-131 can produce
thyroid neoplasms particularly in glands rendered hyperplastic with a goitrous challenge (67,101).

Iodine-131 therapy has been administered to some children, particularly in the United States, and occasional reports of neoplasm have limited its use to older patients, either over 25 years of age (some Clinics in the U.S.A.) or over 40 years (more generally) (134,141).

An extensive survey by the Cooperative Thyrotoxicosis Therapy Follow-Up Study (63) offers the most definitive statement on the possible association between iodine-131 therapy and induction of neoplastic.

A 96% follow-up of 36,050 patients treated by iodine-131, thyroidectomy, antithyroid drugs, x-rays or a combination of these therapies between 1946 and 1968, has been achieved. 86 malignant neoplasms of thyroid were found, 9 occurring in 21,714 patients treated with iodine-131 within one year of therapy and 19 occurring one year or more after iodine-131 therapy. 50 malignant lesions were found within one year after therapy in 11,732 patients undergoing thyroidectomy, with 4 further lesions occurring one year or more after thyroidectomy. If it is accepted that the malignant lesions in patients treated by thyroidectomy are incidental, then the risk of malignancy from iodine-131 therapy is not significant. The average follow-up time for patients treated with iodine-131 was 8 years which compares with 12\(\frac{1}{2}\) years for patients treated with thyroidectomy. It must be remembered that the latent interval between irradiation and the detection of carcinoma of thyroid is long -
Fig. 2 - The occurrence of carcinomas and adenomas with respect to the age at the time radioactive iodine was given as therapy. The curved line represents the number of patients in each decade of life when treated with $^{131}$I. The shaded columns represent malignant neoplasms. The open columns represent adenomas.
Faventos and Winship (232) suggested a mean interval of 10.9 years, while Dolphin (65) calculated the mean latent period to be 20 years minimum. In the Marshallese islanders, the first nodule was not found until 9 years after exposure and the first carcinoma not until the 11th year.

Of 438 patients who were given X-ray therapy, 2 were subsequently found to have malignant lesions and 19 benign adenomas.

Few conclusions could be drawn as to frequency of benign adenomas in patients treated with iodine-131 or thyroidectomy due to bias in selection for therapy in the light of the presence of nodular lesions.

A statistically significant increased incidence of adenomata was found in patients under 20 years treated with iodine-131 and it is suggested that young adults treated with iodine-131 for thyrotoxicosis without causing hypothyroidism are more susceptible to the development of adenomata than older adults. The diagram (Fig. 2) opposite relates the occurrence of neoplasia to age at time of therapy. The incidence of malignancy was not statistically significant vis-à-vis mode of therapy but total numbers of patients under 20 years of age were small.

Study of the appearance of malignancy in relation to time since therapy demonstrated that, for iodine-131 treated patients, the number of malignant neoplasms found per year is approximately proportional to the number of patients at risk up to the 10th year. The
Fig. 3 - The occurrence of carcinoma of the thyroid found each year following $^{131}$I therapy and thyroidectomy for hyperthyroidism. The lines represent the number of patients in each group at risk by years. The bars represent the number of patients having a malignant neoplasm removed by years.

Interval After Therapy in years

Number of Patients At Risk

Number of Malignant Neoplasms
latent period for appearance of malignant neoplasm is thought to be several years and so the majority of these tumours were probably present at the time of therapy. After the 10th year, no new malignant lesions were discovered in the iodine-131-treated group even with 22,700 patient-years at risk after the 10th year. 1953 iodine-131-treated patients have been followed for 15 years, and 115 for 20 years. This data suggests the development of lesions with time that occurred and is still occurring in the Marshallese islanders is not developing in patients treated with radio-iodine. The figure (Fig.3) opposite relates occurrence of neoplasm to time following radio-iodine therapy and thyroidectomy.

It may be concluded, with the proviso that a longer period of follow-up is desirable, that iodine-131 therapy carries little increased risk of thyroid neoplasia.

2. LEUKAEMIA.

Radiation increases the incidence of leukaemia as evidenced by the frequency in survivors of atomic bomb explosions (31), in patients treated for ankylosing spondylitis with x-rays (53) and in children of mothers who had diagnostic x-rays during pregnancy (270). It is calculated that 1 mCi of iodine-131 irradiates blood and marrow by 1.7 Rads, so that a thyrotoxic patient receiving an average therapy dose receives between 10 and 20 Rads to the marrow (106).
A personal communication from Professor Dobyns informs me that one of the Marshallese islanders who was operated on for thyroid cancer has subsequently developed and died of leukaemia. A further islander has developed an as yet incompletely characterised haematological neoplasm.

A report from the Cooperative Therapy Follow-Up Study found no significant difference in leukaemia incidence between patients treated with iodine-131 and with thyroidectomy though the age-corrected incidence in all patients was 50 per cent above the expected average for the general population (249). However, this tends to confirm the findings of several earlier studies that radio-iodine therapy does not increase the risk of leukaemogenesis (181,226,304).

3. CHROMOSOMAL ABNORMALITIES.

Chromosomal abnormalities have been demonstrated following iodine-131 therapy. They are most obvious following massive doses for thyroid carcinoma (28,208). Gross, readily detectable abnormalities are not apparently a prodromal phase of leukaemia. Subtler abnormalities produced by small therapy doses may be more significant than those produced by cancericidal doses.

4. DAMAGE TO EXTRATHYROIDAL TISSUES.

The maximum range of $\beta$ rays emitted by iodine-131 is 2000 microns making hypoparathyroidism unlikely unless all the parathyroid glands are intra-thyroidal. Several cases have been recorded (27,74,94,150,273,275)
and none of these patients received an excessive
dose and none was hypothyroid when hypocalcaemic.
A proportion of patients when stressed with D.T.A.
manifest a degree of parathyroid insufficiency (16).

The risk of hypoparathyroidism is therefore present,
but small. The potential hazards of subclinical
hypoparathyroidism are unknown.

5. FERTILITY AND RISKS TO THE FOETUS.

(a) Thyrotoxicosis decreases libido and fertility;
cure in female patients is often followed by pregnancy.
There is evidence of amenorrhea after cancericidal
therapy doses of iodine-131 but not after the amounts
used to treat thyrotoxicosis; the amenorrhea may
have been due to high uptake of neighbouring metastases
though testicular atrophy has been reported after 563 mCi
(165).

(b) Foetal hypothyroidism is rare when radio-iodine
has been given in error to pregnant women, probably
because the maternal thyroid soaks up most of the radio-
iodine; at least 6 cases of foetal hypothyroidism have
been recorded (89,126,220,233,247). Radio-iodine is
contra-indicated during pregnancy.

(c) There is a risk of genetic damage to children
born to mothers after radio-iodine therapy. The doubling
dose (the Rad dose that eventually doubles the number
of gene mutations) is estimated to be between 15 and
30 Rads (169). The number and type of abnormalities
in children born to iodine-131-treated mothers does not
differ from the general population (46).
Fig. 4. Estimated total population with iatrogenic hypothyroidism after treatment of hyperthyroidism by radio-iodine or surgery.
follow up patients

new patients

YEARS

Fig. 5 Cumulative clinical load after radioiodine treatment of hyperthyroidism. Although the number of new patients treated each year may remain constant (open circles), the number of patients at "at risk" of developing hypothyroidism accumulates and becomes part of the clinical load of a follow up programme (shaded circles).

6. HYPOTHYROIDISM FOLLOWING IODINE-131 THERAPY.

Iodine-131 exerts its effect by destroying thyroid follicular cells and from the earliest days of its use, it was expected that a proportion of patients would be rendered hypothyroid. This was soon confirmed with several reports suggesting an incidence of perhaps 10% (15,246,257,264,294).

Much more disquieting was the gradual awareness that euthyroid patients were at risk of eventually becoming hypothyroid(33,72,107,110,121,186,209) and a flurry of reports from the mid-1960's demonstrated a cumulative incidence of post-therapy hypothyroidism, reaching 70% 10 years post-therapy in two reports (19,209) with no evidence of a levelling-off in the annual accrual rate. The figures opposite(figs 4,5) illustrate the problem: the number of patients at risk is cumulative and produces a massive clinical-load in terms of follow-up.

The use of small doses of iodine-131 merely delays the onset of hypothyroidism and at the cost of a prolonged period of toxicosis (45,177).

Several trials of low dosage schedules with concurrent use of inorganic iodine (120), antithyroid drugs (182), or beta-adrenoreceptor blocking drugs (118,171) have failed to reduce the climbing incidence of hypothyroidism.

Hypothyroidism, after radio-iodine therapy, is frequently of insidious onset and patients may not perceive its onset. Delay in diagnosis is serious because of the dangers of accelerated atherosclerosis, cardiac failure, coma and death (224,225,245). This makes routine follow-up essential. The size of the problem has led to computerisation of follow-up (221).
The CausE of Post-Iodine-131 Hypothyroidism.

The therapeutic effect of iodine-131 is exerted by its $\beta$ ray emission which is absorbed over a path length of 400 to 2000 microns. The $\gamma$ emission is largely unabsorbed by the thyroid. The $\beta$ wave path length is sufficient to irradiate follicle cell cytoplasm, cell nucleus and vascular stroma. Hypothyroidism is considered to result primarily from nuclear irradiation with damage to the cell's divisional integrity and subsequent cell death (61,62,109). Vascular damage is thought to be of only minor significance in the development of gland fibrosis and atrophy that ensues (58,121,146) though some authorities consider this view too extreme (55).

There is, of course, the eventual onset of hypothyroidism that will ensue in a proportion of survivors of thyrotoxicosis treated or untreated (see page 7). This will add to the radiation-induced hypothyroidism, the incidence of which is too great to be explained in terms of spontaneous hypothyroidism. High LATS titres persist in the majority of treated thyrotoxics which tends to support a low incidence of spontaneous "cure".

Irradiation-induced auto-immune thyroiditis has been proposed as the cause of hypothyroidism (18) but, despite the transient increase in thyroid antibodies in serum of some patients after radio-iodine therapy, titres do not correlate with eventual status (37) and this hypothesis is not widely held.

**SUMMARY OF SECTION A.**

This section has reviewed the present, partly speculative, knowledge of the aetiology of thyrotoxicosis. Present therapies are directed against thyroid gland rather than against the primary cause.
Tests of thyroid function relevant to the iodine-125 Trial (Section B) have been briefly outlined to emphasise the degree of continuity in defining patients' thyroid status over 7 years.

Therapies available for thyrotoxicosis have been discussed and attention focussed on the advantages of radio-iodine therapy. The risks of neoplasia and the genetic hazards have been reviewed: there is no good evidence of an increased risk of carcinoma of thyroid in adults; risks to younger patients have led to restrictions on the age at which radio-iodine therapy may be given, and pregnant patients are not given radio-iodine.

The major problems appear to be related to the inevitable rise in post-therapy hypothyroidism which necessitates follow-up for patient (until hypothyroid and on replacement therapy, or dead) and places a considerable clinical load on thyroid centres. One way to deal with these problems is deliberately to render patients hypothyroid with large doses (62,125,161) and give them thyroxine. This guarantees control of the thyrotoxicosis but leaves the patient on medication (which he or she may not have required for years with ordinary therapy doses). Also, the risks of large therapy doses may be significant, though Dobyns's data suggest that hypothyroidism protects against thyroid neoplasia (at least in children).

As an alternative to iodine-131 therapy and its associated problems, the use of another isotope, iodine-125, was proposed. Section B deals with the theoretical and experimental basis for this proposal and with a trial of iodine-125 therapy in 360 patients.
Rapid fall-off in dose rate across the boundary of a sphere when all the iodine-125 is within the circumference of the sphere.
Iodine-125, discovered in 1946, is one of the 24 radio-nucleides of iodine. Its decay is complex (112,259) occurring by electron capture, \( \gamma \) emission and internal conversion with production of soft x-rays and low energy \( \beta \) particles. The majority of the low energy x and \( \gamma \) rays pass through the thyroid without dissipating energy. Most of the energy deposited in the thyroid is due to the low energy \( \beta \) particles. It has been calculated (99,112) that few electrons travel further than 15 microns from a point source and the maximum range is 26 microns. From a spherical source of iodine-125 there is a marked fall in dose rate across the sphere boundary. Changing sphere size from 35 to 70 microns (selected for similarity to proportions of colloid in rat follicles, the rat thyroid being the model for the human disorder) makes little difference. The figure opposite illustrates these points (Fig. 6).

Iodine-125 has, of course, to cross the follicle cell to be incorporated into thyroglobulin and it traverses the cell again when the colloid is resorbed and thyroid hormone released and secreted. However, the intracellular content of iodine-125 is less than 10 per cent of the total thyroid gland content and may be much lower than this upper limit (202,321); 90 per cent plus of iodine-125 will, therefore, be in the colloid.

This permits an important comparison to be made between the emissions of iodine-131 and iodine-125 (Fig. 7). Iodine-125 will subject the cell apex - the site of hormonogenesis - to a much higher dose rate than the nucleus - the site of reproductive integrity. The dose rate across the cell does not reach zero by
Rapid fall in radiation dose from the colloid across the apex of the follicular cell towards the base of the cell

A. When 100 per cent of iodine-125 is in the colloid
B. When 90 per cent of iodine-125 is in the colloid and 10 per cent in the cell
C. Mean total gland dose
FIG. 8

COMPARISON OF $^{131}$I and $^{125}$I

$^{131}$I COLLOID

$^{125}$I COLLOID

Nuclear irradiation high
Reproductive capacity reduced.

Apical irradiation high
Functional capacity reduced.
virtue of photon emissions, but the Rad dose at the colloid interface will be 3 to 4 times that at the nucleus 10 microns from the colloid (112,306). To convert Rads to rems a quality factor has to be introduced; the factor is greater than unity for the low energy electrons which travel 0.05 to 5 microns and irradiate the apical segment. The quality factor is unity at nuclear level as the low energy electrons do not travel that distance. This factor raises the colloid interface dose to 5 times the nuclear dose (168).

Iodine-131 emits \( \beta \) and \( \gamma \) rays. The \( \beta \) rays have an average energy of 187 KeV and are absorbed over a path length of 400 to 2000 microns. The \( \gamma \) rays pass through the thyroid largely unabsorbed. The radiation field of iodine-131 is great in comparison to follicle cell height (15 microns) or follicle diameter (300 microns) and so adjacent follicles will irradiate one another, producing a uniform irradiation of cell cytoplasm and nucleus. Only a narrow rim of peripheral follicles will escape this uniform irradiation (10).

By contrast with iodine-125 the path length of the iodine-131 emissions is such as to irradiate cell apex and cell nucleus more or less equally with consequent cell death and eventual hypothyroidism (Fig.8).

The figures 6,7,8 illustrate the actions of iodine-125, its potential benefit residing in its capacity to preferentially reduce hormone production.

Figure 8 illustrates the differential irradiation across the thyroid gland: colloid-cell interface receives proportionately more irradiation than cell nucleus with iodine-125. The question to be answered is the following: is this difference sufficient to permit preferential suppression of hormonogenesis leaving an
FIG. 9: ACTION OF IODINE-125 ON FOLLICLE CELLS.

Follicle Cells — Thyroid Stimulating Immunoglobulins (T.S.I.) — Cell Hypertrophy & Replication
and Lymphocyte Stimulation (L.S.) — (No. of Divisions limited)

125 I

Follicle cells
Destroyed (A)

Cells producing hormones but with damaged Nuclei (B)

?[Life Span

T₄ + T₃

131 I Small
A Eventually includes B+C+D(?Significance of
Stromal Irradiation)

125 I ? Adequacy of C
To provide a Therapeutic advantage vis à vis eventual
Hypothyroidism.

Cells able to divide but no hormone production (C)

Repair (?Significance)

Replication

T₄ + T₃

T₄ + T₃

Follicle cells intact (D)

Division

T₄ + T₃
adequate reserve of cellular replicative capacity to reduce the eventual hypothyroidism that is inevitable with iodine-131. Figure 9 illustrates the effects of iodine-125 and iodine-131 on thyroid cells; the hypothesis on which the therapeutic Trial is based holds that, with iodine-125 therapy, cells grouped as C are increased in number compared with iodine-131 and provide a therapeutic advantage as regards eventual hypothyroidism.

EXPERIMENTAL BASIS FOR THE USE OF IODINE-125.

There is no laboratory animal equivalent of thyrotoxicosis though Seltler (251) describes a thyrotoxic mare that was cured by a subtotal thyroidectomy. There is, however, a useful alternative, for assessing the effects of radiation in the growth response of the rat thyroid to a goitrogenic stimulus (66). Gross et al. (115) found that increasing doses of iodine-131 progressively diminished the growth response whereas equivalent doses of iodine-125 did not interfere with the response. Iodine-125 treated rats grew less well than iodine-131-treated rats and this was attributed to a greater differential destruction of thyroid hormone synthesis in the former group. The pituitary glands from the iodine-125-treated rats were heavier than those of control or 131-irradiated rats; this was taken to indicate increased secretion of TSH in response to the reduced thyroid hormone production. The DNA content of 125-1-treated rat thyroids was found to be increased, indicating the cells still retained the capacity to respond to TSH.

Greig et al. (113) compared rat thyroid survivals after irradiation with x-rays, iodine-131, and iodine-125. Euthyroid glands have a very low rate of cellular replication and a goitrogenic stimulus has to be used to increase replication rate
to permit measurement to be made. The Do values for x-rays, iodine-131, and iodine-125 were 450 rads, 5,500 rads and 9,400 rads. The difference in Do value for the isotopes was explained by the inhomogeneous absorption of radiation across the follicular cells in the iodine-125 treated rats which protected the nucleus. The cell apex, which is concerned in hormonogenesis, receives a greater degree of irradiation but this does not appear to affect cell viability and replication. Irradiation of cell cytoplasm has been shown to have little effect on cell viability (200) and erythrocytes require irradiation doses several orders of magnitude higher than nucleated cells for destruction (253). Vickery and Williams (289) confirm Gross's conclusions. They also demonstrated that the effect of iodine-131 was increased by prior feeding of an iodine-deficient diet to increase uptake of iodine-131. Iodine-125 produced less diminution in goitrogenic response and less thyroid destruction in animals on normal diet. Prior feeding of an iodine-deficient diet produced even less inhibition of goitrogenic response or thyroid destruction. Iodine-deficiency causes follicle cells to elongate and the nuclei are further from the colloid thus resembling thyrotoxic glands. The ratio of destructive efficiency of iodine-131 to iodine-125 was 18 to 1 on normal diets, but this ratio rose to 60 to 1 on iodine-deficient diets. Iodine-125 produced less cell destruction in iodine-deficient glands than in normal glands.

Other reports (177,288) have shown iodine-131 to cause eventual fibrous replacement of rat thyroid whereas large doses of iodine-125 left gland architecture intact.
Lowitus and Shakam (168) noted an increase in thyroid size from a week after administration of iodine-125; this increase persisted for 3 months (without administration of a goitrogen) and, in the light of low serum hormone levels, was thought to be TSH-induced. Konecny (151) reported an increase after iodine-125 but the increase was not detectable until 35 days post-therapy. He confirmed that iodine-125 caused less structural damage than iodine-131.

These studies all tend to confirm the basic premise that the effects of iodine-125 are a reduction in hormonogenesis with less structural damage to the gland.

Not all studies, however, are in accord with this view. Jongejan and von Putten (144) administered iodine-131 and iodine-125 to rats and mice on low-iodine diet and, 4 months later, measured iodine uptake (a function of the basal part of the cell), thyroxine levels in serum as a measure of apical hormonogenesis and cell death and structural alterations as a measure of nuclear damage. They found iodine-131 to be more effective than iodine-125 by a factor of 20 in reducing circulating thyroxine, by a factor of 16 in causing nuclear damage and by a factor of 20 in suppressing iodide uptake. These authors, being unable to demonstrate any gross difference in radiation effects of iodine-125 across the cell, concluded that iodine-125 has no significant extranuclear effect.

On the postulate that iodine-125 might reduce the incidence of hypothyroidism attendant upon iodine-131 therapy for thyrotoxicosis, the Medical Research Council was approached and permission obtained for a therapeutic trial of iodine-125 in humans.
THE TRIAL

The first question to be answered was whether iodine-125 would actually be effective as a therapy for thyrotoxicosis. With iodine-131 therapy, some 50 per cent of patients are no longer thyrotoxic by three months at a dose of 10,000 rads. It was decided to begin with a pilot study of equivalent doses of iodine-125 that would deposit this amount of radiation at a distance of 10 microns from the source (i.e. the nucleus-colloid distance in the elongated thyrotoxic cell). The nuclear dose is reduced by some 80% with iodine-125 and so the doses administered were 4 times the standard iodine-131 doses.

10 patients, all over 60 years, formed the patient material for the pilot study. The average administered dose was 38.2 mCi. Two patients received 56 and 57 mCi, respectively. The mean recovery time was 8.5 weeks and all 10 were euthyroid by 20 weeks (111). This was encouraging as one would have expected at least 3 patients to require re-treatment with iodine-131. The patients receiving the largest doses were clinically euthyroid at 40 weeks. The numbers treated, however, were small.

This initial group has been increased by a further 8 patients (given large doses to control severe thyrotoxicosis). One patient has required a repeat therapy dose and a further patient three therapy doses of this order for control. Mean initial therapy dose for the 18 patients was 27.6 mCi.

One patient has since died (of congestive cardiac failure and atrial fibrillation), three have been lost to follow up (one has emigrated). Of the remaining 14 patients, after a mean follow-up period of 5.4 years, 9 (64%) are hypothyroid. The rest are euthyroid.
This high incidence of hypothyroidism is predictable after such high doses. The study did, however, confirm that iodino-125 is efficacious though the need for further therapy doses at this level suggested that efficacy might be less than ideal.

DESKTOP OF THE TRIAL

1) SELECTION OF PATIENTS

As knowledge regarding the efficacy of iodine-125 at smaller doses was lacking, patients were considered to require frequent review initially. Also, as there was a possibility of side effects and, particularly a potential risk of neoplasm, review had to be life-long. For these reasons, patients were selected if they lived within roughly a 30 mile radius of the Royal Infirmary.

It subsequently proved difficult to recall a proportion of patients living some distance away, particularly as they were also attending for review at their "parent" hospital. Such patients, when euthyroid or hypothyroid for a year, who fell into this category, were then released to the care of the initial referring consultant.

Admission to the Trial was restricted to patients aged around 40 years or older. Only 13 were under 40 years when treated, the youngest was 37 years of age.

2) PRE-THERAPY DIAGNOSIS OF THYROTOXICOSIS

Patients referred for iodine-125 therapy usually had already been diagnosed as thyrotoxic with confirmatory laboratory investigations.
In the early stages of the Trial, each patient required an elevated PBI-127 and a 24 hour thyroidal uptake of a tracer dose (5 uc) of iodine-131 in the thyrotoxic range to confirm the diagnosis. Thyroid scans initially using iodine-131, latterly, technetium-99, were also performed to exclude patients with toxic adenomata.

From the end of 1972, serum thyroxine assays became routinely available and were complimented, from late 1973 onwards, by serum triiodothyronine and thyroid stimulating hormone assays. Thyrotropin releasing hormone (TRH, Roche) was available in 1973 and TRH tests were used to further clarify thyroid status in selected patients.

3) EXPLANATION TO THE PATIENT.

Each patient was seen individually before therapy and at each review. The reasons for using the new isotope were explained and the necessity for frequent review emphasised. Patients were grateful as their thyrotoxicosis became under control and early reviews were well attended. After several years, however, they were less enthusiastic (particularly as they felt "well"), and default became more frequent. There was a particular tendency for patients in the Glasgow city area to move house (as part of an extensive rebuilding programme) and to fail to inform the thyroid clinic of the new address.

All patients still fertile were advised to use contraceptive precautions from the first therapy dose up to one year after the last dose.
4) ADMINISTRATION OF THERAPY DOSES

Individual doses were calculated as described below and were ordered from the Radiochemical Centre Amersham. Doses were administered on a Friday or a Monday orally on an out-patient basis. Patients were advised to avoid contact with children for a fortnight. Patients were advised to be careful with their urine during the first week and to avoid contamination by splashing. The risk of irradiating other individuals is less than with iodine-131. Van Middlesworth has pointed out that contamination of the environment with iodine-125 (half life 60 days) would be prolonged but his radiation levels data shows the environmental hazard to be insignificant (285).

5) REVIEW OF PATIENTS

At the commencement of the Trial, review was at fortnightly intervals but with increasing experience, it was considered safe to reduce the frequency to a first review one month after therapy, then two three-monthly reviews and subsequently six monthly reviews. This basic pattern was altered according to the patient's condition at review.

Patients were assessed clinically and had, initially, routine PBI-127's performed; after 1972, serum thyroxines were performed routinely and other hormonal assays subsequently.

The primary aim of this Trial was to reduce the incidence of late hypothyroidism inevitable with iodine-131 therapy. To this end, patients were assessed clinically
and biochemically. If the serum thyroxine (initially the PBI-127) was in the hypothyroid range, the assay was repeated three weeks later and yet again after a further three weeks. Thus the patient was given a period of six weeks' grace before being considered hypothyroid, and replacement therapy instituted. The point at which hypothyroidism was considered to be present was defined as the presence of one or two clinical indications with a serum thyroxine (initially the PBI-127) persistently in the hypothyroid range.

The reasons for changing to thyroxine assays are detailed in Part 2, 1.

The use of a biochemical standard as part of the diagnosis of hypothyroidism was considered essential in view of the incipient, often unperceived, progression of hypothyroidism after radio-iodine therapy. The serum thyroxine assay was considered the best end point of the titration at which hypothyroidism occurred - both in regard to continuity of patient assessment over 7 years of the Trial and in regard to patient safety.

6) SELECTION OF THERAPY DOSE.

For iodine-131, gland irradiation is fairly uniform and the mean gland dose (the therapy dose) is an accurate measure of the irradiation of the whole gland. With iodine-125, the major therapeutic emission consists of low energy electrons with ranges less than 1 micron and nuclear dose is only a fraction of the apical dose, even allowing for photon irradiation and up to 10 per cent of the iodine-125 in the cell cytoplasm (see the first part of Section B). From the calculations of
Dose rate (Rads) from 1mCi iodine-125 per day in thyroid glands of masses from 20 grams to 100 grams

A. Dose rate at colloid cell interface

B. Dose rate at 1 micron from apical margin of cell

C. Dose rate at 10 microns from apical margin, the presumed site of the cell nucleus.
of Gillespie et al (99), graphs may be derived giving the
dose rate per mCi iodine-125 per day at different distances
from the colloid in glands of different masses. Figure 10
opposite shows the effect of 1 mCi at colloid-cell interface
and at one and ten microns from the apical membrane assuming
90 per cent of the dose is in the colloid and 10 per cent in
the cell itself, with the colloid fraction being 15\%.
The graph permits calculation of the total rad dose at any point
in the cell for each patient from the formula:

\[
\text{Rad Dose} = R \times \text{Therapy Dose (mCi)} \times \frac{\text{per cent thyroid uptake}}{100}
\]

where \( R \) is the reading from the graph and \( T_e \) the effective half-life of iodine-125 in the colloid. The percentage thyroid uptake
of iodine-125 is difficult to measure and so the 24 hour uptake
of the diagnostic tracer dose of iodine-131 is used as a rough
equivalent. \( T_e \) is subject to great individual variation and
an effective half-life value of 15 days was rather arbitrarily
chosen.

This value was obtained from the relationship:

\[
T_e = \frac{T_B \times T_p}{T_B + T_p}
\]

where \( T_B \) = Biological half-life (on average 20 days in a
thyrotoxic patient) and \( T_p \) is the physical half-life of 60 days.

\( T_B \) is subject to considerable error in adopting the value of
20 days; experimental measurements have ranged in value up to
double this value (183).

Thus calculation of rad dose to various levels along the
follicle cell involves several assumptions which, it is hoped, will be valid when applied to large numbers of patients.

7) **INDICATIONS FOR FURTHER THERAPY**

All patients were left for a full three months before receiving further therapy. A proportion of severely thyrotoxic or frail, elderly "at risk" patients were given adjuvant therapy (carbimazole/propranolol) as a temporary holding measure.

Patients were given further therapy (a repeat of the initial dose) if still severely clinically and biochemically thyrotoxic. Patients mildly thyrotoxic, clinically or biochemically, were not retreated in the expectation that serum hormone levels would fall gradually with passage of time. No patient was retreated without biochemical evidence of thyrotoxicosis. Thus the therapy policy was designed to protect patients from (unnecessary) hypothyroidism.

8) **THE NEED FOR AN OBJECTIVE MEASURE OF HYPOTHYROIDISM**

Following radio-iodine therapy, patients can be placed in one of 6 groups, viz.

1. Clinically and biochemically thyrotoxic.

2. Biochemically toxic (clinical status misleading)

3. Euthyroid (T4, TSH in normal ranges)

4. Compensated euthyroid (TSH elevated but T-4 normal)

5. Biochemically hypothyroid (clinical status misleading)

6. Clinically and biochemically hypothyroid.

The author considers, in agreement with Wenzel et al (303), that these groups merge imperceptibly into one another,
making it essential to have a well-defined concept
of hypothyroidism. Patients with compensated euthyroidism
are not considered hypothyroid by virtue of the adopted
definition.

PATIENTS TREATED WITH IODINE-125

1) SEX DISTRIBUTION

306 are female (85.6%) and 52 male (14.4%): this is
similar to the sex ratio for patients attending for
iodine-131 therapy (185), suggesting no significant bias
in patient selection.

2) AGE DISTRIBUTION

The age range at initial therapy is 37 to 76 years
with only 13 patients under 40 years of age. 78.2% of
patients are aged 40 to 59 years, only 1.7% are 75 years
or older.

3) THYROID GLAND MASS PRIOR TO THERAPY

Thyroid gland mass was estimated by palpation to the
nearest 5 grams by one of three physicians (W.R. Greig,
I.R. McDougall, W.F. Bremner). Impalpable glands were
empirically accorded a weight of 25 grams: this was the
lowest weight. The largest gland size was 80 grams.
76 per cent of the glands weighed ≤ 40 grams.

It is generally realised that assessment of thyroid size
is a major source of inaccuracy in attempting to tailor a
therapy dose to gland size. Soley et al. (264) found that
only 25 per cent of clinical estimates of gland size,
measured by palpation, prior to thyroidectomy fell within
10 per cent of the actual weight. In the remainder, errors
of up to 40 per cent occurred. Similarly, Myhill et al. (201)
found that, for small glands, estimates of size by palpation carried a standard deviation of about 10 grams while, for larger glands, the standard deviation rose to 45 grams. Smaller glands are more accurately estimated but the degree of accuracy in either case is poor.

Myhill compared estimates using radio-isotope scans and showed them to be no more accurate than palpation. Himanka et al. reached similar conclusions (137).

Accordingly, palpation was used in this trial to assess thyroid size and adequate numbers of patients treated to minimise this source of error. Inter-observer variation was limited by restricting assessment to three physicians.

4) Patients were given a preselected therapy dose of iodine-125 per estimated gram of thyroid. The total dose administered was calculated from the formula:

\[
\text{Administered dose (mCi) = Desired dose (\muCi/gram)} \times \frac{\text{Thyroid mass (grams)}}{1,000}
\]

Initially, patients were divided into 7 groups but the author later reclassified patients into 5 groups as it was felt that the greater number of patients per group reduced the possibility of bias due to small numbers per group.

The initial group of patients received large therapy doses, other groups were treated with progressively smaller doses. Most of the patients receiving \( \leq 200 \muCi/gram \) were treated most recently. The initial therapy dose varied from 57 to 5 mCi. 76 per cent of patients received less than 20 mCi as the first therapy dose.
<table>
<thead>
<tr>
<th>CLINICAL STATUS</th>
<th>BIOCHEMICAL STATUS</th>
<th>NO. PATIENTS</th>
<th>SUBGROUP: BIOCHEMICAL STATUS</th>
<th>NO. PATIENTS</th>
<th>CLINICAL/BIOCHEMICAL AGREEMENT %</th>
</tr>
</thead>
<tbody>
<tr>
<td>EUTH</td>
<td>EUTH</td>
<td>96</td>
<td>Normal TSH</td>
<td>83</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T3 + T4 ↑</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T4 ↑</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T3 ↑</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TOXIC</td>
<td>20</td>
<td>T4 + T3 ↓</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T4 ↓ T3 →</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T3 ↓ T4 →</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>EUTH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TOXIC</td>
<td>25</td>
<td>T3 + T4 ↑</td>
<td>18</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T4 ↑</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T3 ↑</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>EUTH</td>
<td></td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MILD TOXIC</td>
<td>TOXIC</td>
<td>11</td>
<td>T3 + T4 ↑</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T3 ↑</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>TOXIC</td>
<td></td>
<td>25</td>
<td>T4 ↓ T3 →</td>
<td>3</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>HYPO</td>
<td>12</td>
<td>T4 ↓ T3 →</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>HYPO</td>
<td>EUTH</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## TABLE 2.

**CLINICAL AND BIOCHEMICAL DISAGREEMENT IN PATIENT CLASSIFICATION**

<table>
<thead>
<tr>
<th>CLINICAL / BIOCHEMICAL STATE</th>
<th>% NOT DETECTABLE CLINICALLY</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOXIC</td>
<td>36  [\frac{20}{56}]</td>
</tr>
<tr>
<td>HYPO</td>
<td>45.5 [\frac{10}{22}]</td>
</tr>
<tr>
<td>EUTHYROID</td>
<td>19.3 [\frac{23}{119}]</td>
</tr>
</tbody>
</table>

*FRACTIONS REFER TO NO. OF PATIENTS NOT DETECTED CLINICALLY AS FRACTION OF TOTAL BIOCHEMICAL GROUP.*
RESULTS

By late 1973, 360 patients had been treated with iodine-125 and sufficient data had accrued for the findings to be documented with a fair degree of reliability.

1. Patient Assessment: Clinical and Biochemical (Hormonal) Correlations.

The two tables opposite detail the degree of agreement between clinical and biochemical status for 197 post-therapy patients assessed by the Wayne indices (56,294) modified in the light of the following remarks.

Correlations for euthyroid and thyrotoxic states is reasonable when one remembers the difficulties inherent in assessing post-therapy patients who may have conflicting signs. As patients treated tend to improve with therapy and to be less obviously thyrotoxic on the full Wayne index, clinical classification tends to concentrate on the amelioration of the more prominent signs and symptoms in each individual patient. Also, in the (relatively) elderly patients who form the subjects of this Trial, the constellation of signs and symptoms that comprise the Wayne index is less frequent and patient assessment by reference to a few stigmata possessed by individual patients is necessary. In the light of these comments, correlations of clinical and biochemical status is reasonable in the euthyroid and thyrotoxic groups.

The correlation as regards hypothyroid patients is less satisfactory: 43 per cent of patients biochemically hypothyroid (T₄ persistently in hypothyroid range) were classified as clinically euthyroid while 57 per cent of patients classified as clinically hypothyroid were, in fact, biochemically euthyroid.
This emphasises the need for adequate hormonal assessment of patients to confirm clinical classification.

2. **Outcome in Whole Patient Group (November 1973 - January 1974).**

   (Follow-up data available for 303 patients)

   Patients who are euthyroid (including those mildly thyrotoxic) form 59.5 per cent of the total. 31.5 per cent are hypothyroid while 9 per cent remain thyrotoxic. The patients who are still thyrotoxic are either among the most recent entrants to the trial or have refused further therapy for irrational reasons.

3. **Relation of Sex to Present Status.**

   62 per cent of the males are euthyroid compared to 60 per cent of the females, while the percentages hypothyroid are 24 and 32 respectively. 8 per cent of the female patients are still thyrotoxic compared to 15 per cent of the males. The follow-up periods are similar and the higher male incidence of persistent thyrotoxicosis may indicate a greater degree of resistance to therapy. The number of treated male patients is, however, small.

4. **Age and Response to Therapy.**

   The highest incidence of hypothyroidism is in the older patients but patients for the pilot study of large doses were all elderly and this produces bias. Omitting this group, the percentages of patients euthyroid and hypothyroid in the age range 45 to 49 years - 61 and 30 respectively - are comparable to the equivalent figures for the age range 60 to 64 years - 63 and 29 respectively. The findings suggest that age at time of therapy has little effect on outcome (for a mean follow-up of 34 months).
<table>
<thead>
<tr>
<th>Status at 3 Months</th>
<th>Hypothyroid by 2 months</th>
<th>Toxic</th>
<th>Toxic</th>
<th>Toxic</th>
<th>Toxic</th>
<th>Toxic but well (T4=154, T3=4.3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 25</td>
<td>30</td>
<td>15</td>
<td>15</td>
<td>30</td>
<td>30</td>
<td>135</td>
</tr>
<tr>
<td>2. 75</td>
<td>55</td>
<td>995</td>
<td>260</td>
<td>995</td>
<td>995</td>
<td>170</td>
</tr>
</tbody>
</table>

**TABLE 3**

VARIATION IN RESPONSE TO RADIOThERAPY
## TABLE 4

FOLLOW-UP DATA ON 303 PATIENTS

<table>
<thead>
<tr>
<th>125\textsuperscript{i} dose group</th>
<th>No. of Patients</th>
<th>Mean Age (yr)</th>
<th>Mean gland size (g)</th>
<th>Mean initial 125\textsuperscript{i} dose (uCi/g thyroid)</th>
<th>Total No. of Additional Doses</th>
<th>% of group requiring further doses (%)</th>
<th>Average duration of follow-up (a)</th>
<th>Average duration of follow-up (months)</th>
<th>Thyroid Status at Follow-Up(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (≤200 uCi/g)</td>
<td>55</td>
<td>51</td>
<td>36</td>
<td>200</td>
<td>53</td>
<td>56</td>
<td>33</td>
<td>7</td>
<td>69</td>
</tr>
<tr>
<td>II (201-350 uCi/g )</td>
<td>87</td>
<td>52</td>
<td>42</td>
<td>330</td>
<td>63</td>
<td>47</td>
<td>30</td>
<td>12</td>
<td>67</td>
</tr>
<tr>
<td>III (351-500 uCi/g)</td>
<td>70</td>
<td>52</td>
<td>41</td>
<td>410</td>
<td>52</td>
<td>44</td>
<td>36</td>
<td>5</td>
<td>77</td>
</tr>
<tr>
<td>IV (501-750 uCi/g)</td>
<td>76</td>
<td>54</td>
<td>37</td>
<td>595</td>
<td>32</td>
<td>26</td>
<td>49</td>
<td>3</td>
<td>41</td>
</tr>
<tr>
<td>V (751-1600 uCi/g)</td>
<td>15</td>
<td>61</td>
<td>30</td>
<td>1100</td>
<td>1</td>
<td>7</td>
<td>54</td>
<td>0</td>
<td>40</td>
</tr>
</tbody>
</table>

Total: 303

31.5%
FIG. 11: CUMULATIVE INCIDENCE OF HYPOTHYROIDISM IN THE FIVE GROUPS.
FIG. 12

POST 125I CLINICAL STATUS RELATED TO DOSE OF 125I

HYPOTHYROID

THYROTOXIC

% Patients

1 201-350μCi/g

2 351-500μCi/g

3 501-750μCi/g

4 >751μCi/g

5

1 2 3 4 5
5. **Gland Size and Response to Therapy.**

Comparison of the outcome at the 1974-75 review between impalpable glands and glands weighing 45 grams and over showed similar results (57 and 28 per cent euthyroid and hypothyroid compared to figures of 62 and 27 respectively). This would appear to indicate that gland size per se has little effect on final outcome though, of course, larger glands will require a proportionately larger therapy dose to give equivalence in terms of μCi per gram of thyroid.

6. **Variation in Thyroid Sensitivity to Irradiation.**

Table 3 contrasts the outcome in two patients and emphasises that there is a marked variation in thyroid sensitivity to iodine-125: this variation is not predictable and confounds attempts to predict individual outcome. Generally, however, cure of toxicosis increases and occurs more rapidly with increasing therapy dose and concurrently the rate of hypothyroidism increases.

7. **Outcome related to initial Therapy Dose.**

Table 4 and figure 11 detail the relevant points about the five therapy groups. This is a general rise in the rate of hypothyroidism with increase in initial therapy dose. Use of small therapy doses (groups 1 and 2) leaves an unacceptably high residue of thyrotoxicosis and repeat therapy (or therapies) is required. Even in the largest initial therapy dose groups, 26 per cent (group 4) and 7 per cent (group 5) required further therapy.

8. **Outcome related to duration of follow-up.**

The figure opposite shows the cumulative incidence of hypothyroidism with the passage of time for the 5 groups.
Irrespective of the administered dose of iodine-125 there is a progressive rise in the number of patients affected: the same rise in hypothyroidism is evident for the total 303 patients.

It should be emphasised that there is no marked decline in the incidence of late hypothyroidism.

9. **Adjuvant Drug Therapy after Iodine-125 Therapy.**

14 per cent (43) of the patients required drug therapy as a holding measure after iodine-125. 60 per cent of those patients were given carbimazole and 40 per cent propranolol. 6 patients were given combination therapy. One was given potassium perchlorate because of a skin rash due to prior carbimazole.

Patients were taken off drugs for at least two months before a final decision as to thyroid status.

10. **Further Therapy Doses.**

79 patients required 2 therapy doses, 24 patients 3 doses, 6 patients 4 doses, 4 patients 5 doses and 3 patients 6 doses, out of the 303 patients reviewed.

11. **Patients lost to Follow-Up.**

57 patients were not available for this review. 9 have died (4 of bronchial carcinoma, 2 of ischaemic heart disease, 1 of systemic lupus erythematosus and 2 of unknown causes). The remainder were either lost to follow-up or were attending other hospitals and information as to thyroid status was not obtained or was incomplete.
21 patients were subsequently traced: 17 were euthyroid, one thyrotoxic and 3 hypothyroid (2 not on therapy). If these ratios hold for the remaining 27 patients not reviewed, then the overall incidence of late hypothyroidism is around 28%, (by "late" is meant at least one year after the last therapy dose).
Table 4 and figure 11 give information about the 303 patients treated the doses of $^{125}$I prescribed, the percentage of patients requiring further therapy/therapies, the length of follow-up and the outcome.

With increasing doses, the percentage of patients requiring further therapy/therapies fell (from 56% for Group 1 to 7% for Group 5) and the opposite trend is noted for hypothyroidism.

In Group 1 where the smallest therapy doses were prescribed ($\leq 200 \mu\text{Ci/g}$) 24% (13 out of 55) are hypothyroid. Of the 22 patients in this group who required only one dose, 7 (32%) became hypothyroid, 3 within 4 months of therapy and one each after 5, 6, 11 and 30 months. Thus, even this small dose is associated with a high incidence of hypothyroidism and the need to retreat 56% of the patients.

Group 3 who were given a mean dose of 410 $\mu\text{Ci/gm.thyroid}$ show the best outcome with only 18% hypothyroid and 77% euthyroid after a mean follow-up of 36 months.

With doses over 500 $\mu\text{Ci/gm.thyroid}$, there is a marked rise in the incidence of hypothyroidism (56% and 60% for Groups 4 and 5 respectively) but persistent hyperthyroidism becomes rare.

The essential conclusion to the Trial resides in figure 11. Irrespective of the administered dose of $^{125}$I, there is a progressive rise in the number of patients being affected by hypothyroidism; the same rise in hypothyroidism is noted for the total of 303 patients, and there is no obvious marked levelling off in accrual rate.
Comparison with Iodine-131

The group of patients showing the best results in terms of the lowest incidence of hypothyroidism was Group 3. This group was selected for comparison with iodine-131. Also, the uniformity of the dose administered makes for a truer indication of the efficacy of iodine-125 which might be obscured by the inhomogeneity of the whole patient group.

A graph of percentage of patients euthyroid after 1, 2, 3, 4 and 5 therapy doses (log scale) was plotted against number of doses (linear scale) and the slope of this line gave the rate constant ($\lambda$).

The fraction of patients remaining thyrotoxic is relatively constant from therapy dose to therapy dose. Consequently, the number of patients not cured is expressed by the exponential equation:

$$N = N_0 e^{-\lambda D}$$

where

- $N$ is the number of patients still thyrotoxic.
- $N_0$ is the original number of patients.
- $D$ is the number of therapy doses.
- $\lambda$ is the rate constant.
Three sets of data are available (180, 209, 259) which give values of \( \lambda \) for iodine-131 for three separate dose regimes. The rate constant for the iodine-125 group selected for comparison was 0.510 which is similar to the rate constant for a dose regime designed to deliver 80 \( \mu \)Ci of iodine-131 to each gram of thyroid tissue (0.676). This indicates that an administered dose of 410 \( \mu \)Ci/iodine-125 gram of thyroid is roughly as effective as 80 \( \mu \)Ci of iodine-131.

A second approach to defining the rate of response to radio-iodine uses the number of doses per patient required to effect a 50 per cent cure. For the iodine-125 group receiving a mean of 410 \( \mu \)Ci/gram this figure is 0.6 which is less than the figure of 1.03 required for an iodine-131 therapy dose of 80 \( \mu \)Ci/gram of thyroid tissue.

Yet a third concept is that of average number of doses prescribed per patient in the entire group. For this particular group, the figure is 1.41 which is similar to the number prescribed by Nofal but less than the number prescribed by Maynard and Silver.

This data suggests that iodine-125 is only about one fifth as effective as iodine-131 on an equivalent dose basis but is more efficient in terms of cure rate.

14. **Side Effects of Iodine-125 Therapy**

No cases of leukaemia nor thyroid carcinoma have been noted. 3 patients have had surgery for carcinoma of breast, subsequent to radio-iodine therapy. 4 women patients have died of bronchial carcinoma - all were heavy smokers. One patient developed systemic lupus erythematosis
and died. None of these conditions appear related to radio-iodine therapy.

Seven years after the start of the Trial there is no evidence that iodine-125 is less safe than iodine-131, but the follow-up in terms of patient-years, is comparatively short.
### TABLE 5.

Classification of Patients Reviewed 1971-1973/74 (n=104)

<table>
<thead>
<tr>
<th>GROUP</th>
<th>No.</th>
<th>Description</th>
<th>1971 TSH Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>6 with normal TSH levels</td>
<td>5 with elevated TSH levels</td>
</tr>
<tr>
<td>II</td>
<td>10</td>
<td>Patients euthyroid in 1971 and becoming hypothyroid prior to 1973.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 with normal TSH levels</td>
<td>4 with elevated TSH levels</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32 with normal TSH levels in 1971 (6 now have elevated TSH levels)</td>
<td>8 with elevated TSH levels in 1971</td>
</tr>
<tr>
<td>IV</td>
<td>15</td>
<td>Patients mildly thyrotoxic in 1971 but well and receiving no further therapy.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>9 patients seeming well and still biochemically mildly thyrotoxic</td>
<td>6 patients now completely euthyroid</td>
</tr>
<tr>
<td>V</td>
<td>28</td>
<td>Thyrotoxic patients in 1971 receiving further therapy</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>13 are now euthyroid (4 with elevated TSH)</td>
<td>9 are hypothyroid</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 are hypothyroid</td>
<td>3 remain thyrotoxic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 are well but biochemically toxic</td>
<td></td>
</tr>
</tbody>
</table>
1971/73/74 Comparison

A review with measurement of all hormonal parameters was performed in selected patients in 1971 by Dr. I. R. McDougall. The opportunity was taken to review these patients again in 1973/74 with a view to discovering if it was possible to predict which patients would become hypothyroid.

Samples from patients reviewed in 1971 were sent by rail to Newcastle and hormone assays performed by arrangement with Professor R. Hall. Measurements in 1973/74 were performed by arrangement with Dr. J. G. Ratcliffe in the Regional Radioimmunoassay Centre, Stobhill Hospital.

Table 5 classifies the patients reviewed according to thyroid status. Patients were classified as euthyroid on the basis of amelioration of symptoms and in the light of their thyroxine assay; these patients were subdivided on the basis of their TSH levels in 1971. Patients were designated hypothyroid on the basis of one or two clinical pointers and a serum thyroxine persistently in the hypothyroid range.

Minor differences in the normal ranges for TSH between Newcastle and Glasgow made valid comparison of changes of TSH over the 2/3 year period rather dubious except in so far as there were marked alterations in TSH levels.

RESULTS AND DISCUSSION

Table 5 illustrates the sub-classes of patients available
<table>
<thead>
<tr>
<th>GROUP</th>
<th>NO. PATIENTS</th>
<th>TIME FROM LAST THERAPY TO REVIEW (MONTHS)</th>
<th>HORMONAL LEVELS IN 1971</th>
<th>T4</th>
<th>T3</th>
<th>TSH (range)</th>
<th>HORMONAL LEVELS IN 1973</th>
<th>T4</th>
<th>T3</th>
<th>TSH (range)</th>
<th>MEAN TIME TO HYPOTHYROIDISM (MONTHS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11-5</td>
<td>EUTHYROID 1971</td>
<td>HYPOTHYROID IN 1973</td>
<td>58 ± 24.5</td>
<td>1.94 ± 0.90</td>
<td>1.0 (0-4)</td>
<td>38.6 ± 12.9</td>
<td>1.12 ± 0.70</td>
<td>30 (15-60)</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
<td></td>
<td>76 ± 23</td>
<td>1.34 ± 0.60</td>
<td>12.6 (6-22)</td>
<td>31 ± 12.9</td>
<td>0.98 ± 0.70</td>
<td>35 (30-60)</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>10-5</td>
<td>EUTHYROID 1571</td>
<td>HYPOTHYROID PRIOR TO 1973 REVIEW</td>
<td>90.0 ± 25.7</td>
<td>1.80 ± 0.60</td>
<td>1.0 (0-4)</td>
<td>27.0 (when hypo)</td>
<td></td>
<td></td>
<td></td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
<td></td>
<td>77.2 ± 25.6</td>
<td>1.20 ± 0.60</td>
<td>12.0 (9-16)</td>
<td>25.7 (when hypo)</td>
<td></td>
<td></td>
<td></td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>40-24</td>
<td>EUTHYROID 1971</td>
<td>EUTHYROID 1973</td>
<td>10.42 ± 25.0</td>
<td>2.00 ± 0.70</td>
<td>0.7 (0-2.2)</td>
<td>86.2 ± 25.7</td>
<td>1.66 ± 0.80</td>
<td>1.9 (0-4.5)</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
<td></td>
<td>79.8 ± 25.1</td>
<td>1.50 ± 0.60</td>
<td>9.8 (6-30)</td>
<td>62.0 ± 25.0</td>
<td>1.11 ± 0.60</td>
<td>15.0 (6-30)</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>15-9</td>
<td>MILDLY TOXIC 1971 AND NOT REQUIRING FURTHER THERAPY</td>
<td>167.3 ± 20.5</td>
<td>3.37 ± 0.62</td>
<td>0.2 (0-2.0)</td>
<td>167 ± 19.3</td>
<td>2.00 ± 0.36</td>
<td>0 (0-4)</td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td></td>
<td>166.6 ± 23.4</td>
<td>3.22 ± 0.65</td>
<td>0.1 (0-1)</td>
<td>126 ± 25.7</td>
<td>1.64 ± 0.50</td>
<td>2.0 (0-5)</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>28</td>
<td>THYROTOXIC 1971 AND GIVEN FURTHER THERAPY/IES</td>
<td>199.5 ± 51</td>
<td>5.24 ± 0.45</td>
<td>0.2 (0-3)</td>
<td>OUTFOME</td>
<td>13 E</td>
<td>9 TSH Normal</td>
<td></td>
<td>9 H, 3T, 3T but well</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: HORMONAL VALUES ARE S.I. UNITS - mean ± S.D.
for re-review in 1973. Of 104 patients 40 were euthyroid in 1971 and remained so in 1973. 24 patients had normal TSH levels in 1971, 16 elevated TSH levels. 30 patients have become hypothyroid and they are classified in 3 groups (1), (2) and part of Group 5. The other sub-classes are as shown.

Table 6 illustrates the hormonal data on these patients. Of 11 patients who were euthyroid in 1971 and who subsequently became hypothyroid in 1973, 6 had originally normal TSH levels while 5 had elevated levels. Two years later these patients were clinically and biochemically hypothyroid, the mean times to hypothyroidism from the initial therapy being 36 and 32 months on average respectively. Whilst these two groups of patients had a similar clinical course, the proportion of patients with elevated TSH levels in 1971 is smaller than those with normal TSH levels suggesting that patients with elevated TSH levels may run a greater risk of hypothyroidism than patients who had normal TSH levels. This compares with the position after I-131 therapy where patients with raised plasma TSH levels develop hypothyroidism at the rate of 5% per annum. As many as 58% of patients euthyroid after I-131 therapy are reputed to have elevated TSH levels (Toft et al. 1974 (274), and Tunbridge et al. 1974 (280) who found 46% of euthyroid patients to have elevated TSH levels.) However, these data are taken from review of patients treated with I-131, at least 6 years after therapy, i.e. they were further on following therapy than the patients reviewed here. It is possible that the 125-I patients will gradually develop a rising titre of TSH levels with time. In fact, the patients with still normal TSH levels in 1973 show a slight but significant rise in TSH levels with a coincidental reduction.
in T4 levels. Also, six patients with normal TSH levels in 1971 have now developed elevated TSH levels though remaining clinically euthyroid with thyroxine level in the low normal range.

From Table 6 it can be seen that the post-therapy patient presents a spectrum of thyroid status ranging from frankly hypothyroid to frankly thyrotoxic. Division of these patients into subgroups on a clinical basis is difficult, if not impossible, and measurement of hormonal parameters is essential, for correct classification of patients following radio-iodine therapy. In late 1974 (September-October) review, a detailed study of clinical and hormonal status was undertaken and the results are detailed as follows.
<table>
<thead>
<tr>
<th>GROUP</th>
<th>CLINICAL STATUS</th>
<th>NO PATIENTS</th>
<th>% of TOTAL GROUP</th>
<th>T₄</th>
<th>T₃</th>
<th>FTI</th>
<th>THYOPAC 3</th>
<th>PB¹²⁵I</th>
<th>TSH</th>
<th>CHOLESTEROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>EUTHYROID</td>
<td>23</td>
<td>66</td>
<td>122.3 ± 37.3</td>
<td>2.82 ± 0.70</td>
<td>120.2 ± 43.8</td>
<td>105 ± 10</td>
<td>496 ± 165</td>
<td>2.05 ± 1.72</td>
<td>6.16 ± 1.04</td>
</tr>
<tr>
<td></td>
<td>TOXIC</td>
<td>6</td>
<td>21</td>
<td>179.0 ± 45.0</td>
<td>3.99 ± 0.54</td>
<td>206.0 ± 56.6</td>
<td>88 ± 9</td>
<td>577 ± 71</td>
<td>1.20 ± 0.49</td>
<td>5.18 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>HYPO</td>
<td>5</td>
<td>15</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>EUTHYROID</td>
<td>34</td>
<td>47</td>
<td>106.8 ± 33.5</td>
<td>2.49 ± 0.68</td>
<td>100.4 ± 36.0</td>
<td>108 ± 8</td>
<td>433 ± 95</td>
<td>6.40 ± 13.46</td>
<td>6.35 ± 0.78</td>
</tr>
<tr>
<td></td>
<td>TOXIC</td>
<td>22</td>
<td>29</td>
<td>168.6 ± 46.3</td>
<td>4.96 ± 1.86</td>
<td>189.2 ± 72.1</td>
<td>93 ± 13</td>
<td>717 ± 181</td>
<td>1.37 ± 1.52</td>
<td>5.02 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>HYPO</td>
<td>19</td>
<td>24</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>EUTHYROID</td>
<td>43</td>
<td>65</td>
<td>99.1 ± 28.3</td>
<td>2.45 ± 0.63</td>
<td>91.4 ± 29.6</td>
<td>108 ± 10</td>
<td>426 ± 110</td>
<td>7.25 ± 11.74</td>
<td>6.09 ± 0.78</td>
</tr>
<tr>
<td></td>
<td>TOXIC</td>
<td>9</td>
<td>14</td>
<td>151.9 ± 32.2</td>
<td>4.13 ± 1.12</td>
<td>150.5 ± 41.2</td>
<td>99 ± 10</td>
<td>583 ± 87</td>
<td>1.66 ± 1.47</td>
<td>5.41 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>HYPO</td>
<td>14</td>
<td>21</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>EUTHYROID</td>
<td>21</td>
<td>31</td>
<td>94.0 ± 27.0</td>
<td>2.37 ± 0.55</td>
<td>87.5 ± 27.0</td>
<td>108 ± 10</td>
<td>449 ± 181</td>
<td>18.00 ± 27.6</td>
<td>36.99 ± 1.30</td>
</tr>
<tr>
<td></td>
<td>TOXIC</td>
<td>5</td>
<td>7</td>
<td>151.9 ± 63.1</td>
<td>4.20 ± 2.27</td>
<td>191.8 ± 104.2</td>
<td>91 ± 21</td>
<td>646 ± 221</td>
<td>2.64 ± 2.55</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>HYPO</td>
<td>42</td>
<td>62</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>EUTHYROID</td>
<td>4</td>
<td>29</td>
<td>84.9 ± 23.2</td>
<td>1.96 ± 0.48</td>
<td>81.1 ± 27.0</td>
<td>108 ± 12</td>
<td>268</td>
<td>35.80 ± 43.70</td>
<td>6.86 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>TOXIC</td>
<td>1</td>
<td>7</td>
<td>114.5</td>
<td>3.42</td>
<td>100.4</td>
<td>114</td>
<td>504</td>
<td>1.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>HYPO</td>
<td>9</td>
<td>64</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Biochemical assessment of the clinically euthyroid and thyrotoxic patient related to dose of Iodine-125. Hypothyroid patients were mainly on thyroxine therapy and their biochemical parameters are not given.

Values are mean ± standard deviation and are in S.I. units.
TABLE 8

BIOCHEMICAL STATUS OF CLINICALLY EUTHYROID PATIENTS

<table>
<thead>
<tr>
<th>Status</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal $T_4$ and $T_3$</td>
<td>96</td>
</tr>
<tr>
<td>(TSH normal)</td>
<td></td>
</tr>
<tr>
<td>(TSH elevated)</td>
<td>13</td>
</tr>
<tr>
<td>Elevated $T_3$, normal $T_4$</td>
<td>11</td>
</tr>
<tr>
<td>Elevated $T_4$, normal $T_3$</td>
<td>4</td>
</tr>
<tr>
<td>Elevated $T_4$ and $T_3$</td>
<td>5</td>
</tr>
<tr>
<td>Low $T_4$, elevated TSH</td>
<td>9</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>125</strong></td>
</tr>
</tbody>
</table>
FIG. 13 MEAN \( T_4 \), PBI, \( T_3 \) AND TSH LEVELS IN THE CLINICALLY EUTHYROID PATIENTS RELATED TO THERAPY GROUP. THE DOSE OF IODINE 125 GIVEN INCREASES FROM THERAPY GROUP 1 TO GROUP 5.
Table 7 details the patients reviewed in each therapy group. Table 8 gives the biochemical status of the 125 patients found to be clinically euthyroid.

Figure 12 relates clinical status to dose. As expected the percentage incidence of clinically apparent hypothyroidism increased and that of persistent thyrotoxicosis decreased with increasing dose.

For the euthyroid patients, all the mean T4 and FTI levels were in the normal range but there was a progressive drop in these means and a concomitant rise in mean TSH with increasing therapy dose (Table 7), Figure 13.

By applying Students' t-test, the mean T4 and FTI of Groups 2 and 4 were shown to be significantly lower than the corresponding Group I values (p < 0.05); also the means of Group 4 were significantly lower than those of Group 2 (p < 0.1); TSH levels remained in the normal range for Groups 1 to 3 but were significantly raised above the normal range in Groups 4 and 5 (p < 0.005).

These findings suggest that, with increasing dose, there is a progressive trend towards suboptimal thyroid hormone concentrations with reduced T4/T3 ratios, leading to elevation of TSH levels.

Only 93 of the clinically euthyroid patients were biochemically euthyroid on the basis of T4 values and 12 of these had elevated TSH levels.

10 patients, who had normal T4 levels, had elevated T3 levels while 4 patients who were clinically euthyroid had elevated T4 levels. A further 6 patients had both
<table>
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<tr>
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<tr>
<td>1</td>
<td>68</td>
<td>62</td>
<td>9.5</td>
<td>E</td>
<td>LFU</td>
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</tr>
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<td>2</td>
<td>51</td>
<td>102</td>
<td>15.4</td>
<td>E</td>
<td>DNA</td>
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<td>59</td>
<td>39.0</td>
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<td>96</td>
<td>Exag.</td>
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<tr>
<td>4</td>
<td>56</td>
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<td>78</td>
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<td>Exag.</td>
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<td>73</td>
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<td>H</td>
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</tr>
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<td>65</td>
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<td>40.0</td>
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<td>117</td>
<td>42.5</td>
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<td>25.0</td>
<td>E</td>
</tr>
<tr>
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<td>58</td>
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<td>15.5</td>
<td>E</td>
<td>117</td>
<td>27.3</td>
<td>Exag.</td>
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<td></td>
<td>95</td>
<td>E</td>
<td></td>
</tr>
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<td>53</td>
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<td>23.1</td>
<td>E</td>
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<td>53</td>
<td>88</td>
<td>28.5</td>
<td>E</td>
<td>111</td>
<td>18.0</td>
<td>Exag.</td>
<td></td>
<td></td>
<td>118</td>
<td>10.6</td>
<td>E</td>
</tr>
<tr>
<td>10</td>
<td>57</td>
<td>53</td>
<td>22.5</td>
<td>E</td>
<td>69</td>
<td>3.0</td>
<td>Exag.</td>
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<td></td>
<td>60</td>
<td>21.7</td>
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<tr>
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<td>70</td>
<td>74</td>
<td>41.9</td>
<td>E</td>
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<td></td>
<td></td>
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<td>65</td>
<td>13.8</td>
<td>E → H</td>
</tr>
<tr>
<td>12</td>
<td>55</td>
<td>134</td>
<td>21.3</td>
<td>E</td>
<td>109</td>
<td>32.0</td>
<td>Exag.</td>
<td></td>
<td></td>
<td>92</td>
<td>41.6</td>
<td>E</td>
</tr>
</tbody>
</table>

**TABLE 9**

REVIEW OF CLINICALLY EUTHYROID PATIENTS WITH ELEVATED TSH LEVELS
**FIG. 14** T.S.H. RESPONSE TO T.R.H. IN PATIENTS WITH "COMPENSATED EUTHYROIDISM".
<table>
<thead>
<tr>
<th>NO.</th>
<th>BASAL TSH μU/L</th>
<th>POST TRH LEVEL μU/L</th>
<th>Δ TSH μU/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>39</td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td>96</td>
<td>170</td>
<td>74</td>
</tr>
<tr>
<td>3</td>
<td>130</td>
<td>210</td>
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<td>75</td>
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<td>6</td>
<td>74</td>
<td>100</td>
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<td>7</td>
<td>115</td>
<td>47.6</td>
<td>364</td>
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<tr>
<td>8</td>
<td>50</td>
<td>108</td>
<td>58</td>
</tr>
</tbody>
</table>
elevated T4 and T3 levels i.e. were biochemically toxic. Another group of 10 patients were clinically euthyroid but were biochemically hypothyroid.

The 20 patients who were biochemically thyrotoxic but classified as clinically euthyroid were, at most, minimally thyrotoxic and did not require further therapy. At a further review one year later in 1975, 11 of these patients were clinically and biochemically euthyroid.

Of the 12 patients with T4 values in the euthyroid range but with elevated levels of TSH (Table 9) 7 were recalled 7 months after initial review and reassessed by the TRH test and 10 of the group were recalled 12 months after the initial review.

As the figure opposite shows, all patients studied had an exaggerated response to TRH, when compared to a control group composed of laboratory personnel and hospitalised patients with no history of thyroid disease. (Fig.14, Table 11)

The 12 month follow-up review showed that 3 of the ten patients were showing clinical signs of hypothyroidism and the T4 level in 8 patients was lower than at the initial review (Table 9).

This condition has been described as "compensated euthyroidism" or "subclinical hypothyroidism" (83).

All patients in this group showed an exaggerated response to TRH. This agrees with the results of Tunbridge et al. 1974 (159), who investigated the thyroid status of 39 I-131 treated patients in this category. The mean T4 level of these patients was shown to be significantly lower than in
patients with normal TSH, in agreement with the results of Toft et al. 1974(156), who found the same result in a group of post-I-131 patients.

Tunbridge et al. thought such patients were no more likely to develop hypothyroidism than euthyroid patients with normal TSH, although Toft et al. thought the subclinically hypothyroid group were most at risk and should be reviewed more regularly.

This study showed that 3 of these patients found to be clinically euthyroid at the initial review had become clinically hypothyroid by the third review one year later and a further patient had developed a hypothyroid T4 level although still judged clinically euthyroid. This represents an annual incidence of hypothyroidism of 25-35 per cent which is significantly higher than the annual incidence in patients with normal TSH levels which is of the order of 5 per cent. It should perhaps be emphasised that this was a prospective study free of the potential bias that may distort retrospective studies.

It should also be remembered that this particular group of patients was treated with iodine-125 and the rapidity of onset of hypothyroidism may possibly be accelerated by use of this particular isotope.

Patients with "compensated euthyroidism" after iodine-125 therapy form an "at risk" group who require close follow-up. However, there is still an incidence of 5 per cent per annum of euthyroid patients who become hypothyroid and who cannot be identified by hormone studies.
<table>
<thead>
<tr>
<th>EUTHYROID GROUP</th>
<th>CONTROL GROUP (TSH normal)</th>
<th>UPPER LIMITS OF NORMAL (FREDRICKSON)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>Age range</td>
<td>40-49</td>
<td>40-51</td>
</tr>
<tr>
<td>Total chol.</td>
<td>7.25 ± 1.17</td>
<td>6.35 ± 1.04</td>
</tr>
<tr>
<td>LDL chol.</td>
<td>4.14 ± 1.04</td>
<td>3.11 ± 0.78</td>
</tr>
<tr>
<td>Trig.</td>
<td>1.58 ± 0.14</td>
<td>1.24 ± 0.17</td>
</tr>
<tr>
<td>VLDL Chol.</td>
<td>0.76 ± 0.26 S.I. units</td>
<td>0.57 ± 0.36</td>
</tr>
</tbody>
</table>

2 values out with normal. *2 values out with normal. (mean ± SD)

Cholesterol values are S.I. units. Triglyceride values are S.I. units.
Doubt exists as to the hormonal status of such patients - hence the two terms, compensated euthyroidism and subclinical hypothyroidism. It is disputed whether such patients should receive thyroxine replacement therapy.

Tunbridge (280) investigated this problem by comparing cholesterol and electrocardiographic appearances before and one month after replacement therapy with 0.2 mg thyroxine per day. No changes were observed.

The author measured cholesterol and triglyceride levels and lipoprotein fractions in 12 patients at each review. The samples were fasting samples and permitted comparison with normal values for individuals of the same ages as the patient (ranges taken from Fredrickson et al. [92]). Groups of patients of the same ages as the compensated euthyroid patients were selected randomly from patients euthyroid with normal TSH levels for further comparison.

Table 10 details the findings. The patients with elevated TSH levels had values in the normal range except for 2 cholesterol and 2 triglyceride values in the frankly hyperlipidaemic range; these latter 4 results persisted in the hyperlipidaemic range when repeated one month later. However, the mean values of both parameters in the compensated euthyroid group were not significantly different from those in the control patients nor from the normal range of values obtained from Fredrickson.

The 3 patients with the abnormal parameters were recalled in the fasting state and full lipoprotein typing carried out by beta quantification and electrophoresis. One patient was
confirmed as having a Type IIa abnormality, one as having a Type IV abnormality and the third patient as having a IIb abnormality (Fredrickson classification). None of these patients had clinical stigmata of a lipoprotein abnormality.

In the light of these findings, these three patients were commenced upon Thyroxine therapy and 3 months later, when they were each on 0.2 mgm. per day, full lipoprotein phenotyping was performed. The patient with the IIb abnormality and the patient with IIa abnormality now showed normal lipoprotein phenotype values in the normal range. The patient with the Type IV abnormality was known to be a heavy drinker and her lipoprotein abnormality persisted. She was taken off thyroxine replacement therapy and her lipoprotein disorder cleared with control of her drinking.

This data suggests, though in view of the small numbers of patients involved, does not prove, that patients with compensated euthyroidism may be at risk of accelerated atherosclerosis and coronary artery disease, as elevated lipoprotein levels in the LDL and VLDL classes are significant risk factors for ischaemic heart disease.

Two of this group of patients have since died of myocardial infarction compared with only one patient in the control group. The patient (who had elevated cholesterol and triglyceride levels suggestive of a Type IIb abnormality) now has an ECG showing inferior ischaemia and frequent sinoatrial block which is causing dizzy spells and will probably necessitate a permanent cardiac pacemaker.

These findings are again, of course, not conclusive but do suggest that replacement therapy should be considered in this group of patients.
TABLE 12
SYSTOLIC TIME INTERVALS IN COMPENSATED EUTHYROIDISM

<table>
<thead>
<tr>
<th>PATIENT NO GROUP</th>
<th>$Q_{2S2I}$</th>
<th>$LUET_I$</th>
<th>$PEP_I$</th>
</tr>
</thead>
</table>
| (1) $\uparrow$ TSH  
   ($m = 8$)        | 539 ± 15 * | 403 ± 8 * | 138 ± 13 * |
| (2) Normal TSH  
   ($m = 10$)       | 535 ± 8    | 404 ± 4  | 128 ± 4  |
| P                | NS         | NS       |

* 3 values outwith normal range.
SYSTOLIC TIME INTERVAL MEASUREMENTS IN COMPENSATED EUTHYROIDISM.

In an attempt to measure the dynamic response of the body to these hormonal alterations, the function of the heart was measured using the non-invasive technique of systolic time interval measurements (see Section D).

Eight patients with compensated euthyroidism were so studied and the results compared with the results from a control group of ten patients with no evidence of thyroid nor of cardiovascular disease. These control patients had normal TSH values, were of the same age and sex as the ten patients studied and none of the patients studied had overt nor ECG evidence of cardiovascular disease. All were normotensive.

RESULTS

The intervals for 5 of the 8 patients studied fell within the normal range of the control group. The other three patients had parameters outwith normal (Table 12). Comparison of the mean values of these 8 patients with the means of the 10 control patients showed no statistically significant difference.

SOME FURTHER LIPID STUDIES IN PATIENTS WITH COMPENSATED EUTHYROIDISM.

1. Lipid Clearing Capacity

Investigation of the clearance rates of triglyceride were measured in six patients with compensated euthyroidism using the intravenous intralipid tolerance test (23, 24, 25).

Patients and Method.

Patients were studied in the fasting state.

0.1 ml/kg body weight of a 10 per cent solution
**TABLE 13**

K2 VALUES IN "COMPENSATED EUTHYROIDISM"

<table>
<thead>
<tr>
<th>PATIENT GROUP</th>
<th>K2 (m⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; EUTHYROID</td>
<td>2.98 ± 0.30</td>
</tr>
<tr>
<td>TSH↑ (n = 6)</td>
<td></td>
</tr>
<tr>
<td>&gt; CONTROL</td>
<td>3.91 ± 0.40</td>
</tr>
<tr>
<td>(TSH normal)</td>
<td></td>
</tr>
<tr>
<td>Clin.euthyroid</td>
<td></td>
</tr>
<tr>
<td>(n = 5)</td>
<td>s p &lt; 0.01</td>
</tr>
</tbody>
</table>

* same age range.
of Intralipid (a soya bean extract available commercially from A.C. Vitrum, Sweden) is administered as an intravenous bolus and venous samples obtained at 10 minute intervals over the subsequent hour from a Vyrunon cannula in the opposite arm, kept patent by a non-heparinised slow saline flush. The samples (taken into EDTA-anticoagulated tubes) are placed in ice and rapidly centrifuged. Plasma aliquots are taken and the degree of light absorption at 360 Å measured on a Beckmann spectrophotometer (to give a measure of the number of remaining fat particles). A one in 2000 dilution of the original Intralipid solution is made and the absorption measured in triplicate. The absorption of fasting plasma is subtracted from that of the samples obtained after the bolus and the subsequent values are expressed as a percentage of the absorption of the injected Intralipid. A semi-log plot of % absorption remaining in plasma against time gives a straight line, the gradient of which, $K_2$ (Å/min), is the required parameter.

The parameter $K_2$ is a measure of the body's capacity to remove chylomicra from plasma, i.e. is a measure of lipid clearing capacity. The $K_2$ parameters are detailed in the table opposite and demonstrate a reduced ability to clear triglycerides from the plasma in these patients compared to normal values obtained from a group of outpatient controls (Table 13).

This again, is suggestive evidence that such individuals have subtle abnormalities of metabolism and may be at risk if "compensated euthyroidism" is prolonged and replacement therapy not instituted.
2. Lipoprotein Lipase Activity

Lipoprotein lipase activity was assayed in 7 patients with "compensated euthyroidism".

Method.

Fasting plasma samples were taken and 1000 units of heparin administered intravenously and further plasma samples obtained exactly 15 minutes after heparin administration. Plasma samples were obtained by immediate centrifugation at 3000 r.p.m. for 3 minutes of EDTA anticoagulated blood. The samples were frozen directly and assayed with control samples from 10 normal lipidemic, 6 Type IV hypertriglyceridaemic and 6 Type II hypercholesterolaemic patients. Substrate was produced by mixing equal volumes of normal serum and 10 per cent Intralipid. 9 ml. of substrate was incubated with 1 ml. of test plasma, after thawing, for 15 minutes at 37°C. (293). The incubation mixtures were immediately assayed in triplicate by extraction with Zol triton X-100 (64) and measurement of total free fatty acid levels by microtitration with 0.02 M sodium hydroxide.

Activity was expressed as microequivalents of free (unesterified) fatty acid released per minute per 100 ml. of plasma.
### Table 14

**Lipoprotein Lipase Activity in Compensated Euthyroidism**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>NO. SUBJECTS</th>
<th>UNESTERIFIED FATTY ACIDS (\mu\text{eq/L plasma})</th>
<th>LIPOPROTEIN LIPASE ACTIVITY (\mu\text{eq/min/100 ml plasma})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PRE-HEP  POST-HEP</td>
<td>PRE-HEP  POST-HEP</td>
</tr>
<tr>
<td>Compensated Euthyroidism</td>
<td>7</td>
<td>0.4 ± 0.4  2.7 ± 0.5</td>
<td>3.0 ± 0.7  12.0 ± 3.2</td>
</tr>
<tr>
<td>Normals</td>
<td>10</td>
<td>0.4 ± 0.3  1.1 ± 0.4</td>
<td>1.2 ± 0.7  12.2 ± 2.1</td>
</tr>
<tr>
<td>Type IV</td>
<td>8</td>
<td>0.5 ± 0.4  3.9 ± 0.5</td>
<td>4.7 ± 0.9  12.1 ± 3.0</td>
</tr>
<tr>
<td>Type II</td>
<td>6</td>
<td>0.4 ± 0.25 1.2 ± 0.5</td>
<td>1.1 ± 0.6  11.9 ± 2.1</td>
</tr>
</tbody>
</table>
RESULTS

Table 14 details the findings.

As expected, there is no significant differences between normals and Type II individuals. Type IV subjects show a high pre-heparin lipase activity that rises to normal levels following heparin. This has been reported before (91, 298). It is postulated that the high pre-heparin level in the Type IV abnormality is insufficient to counterbalance the hypertriglyceridemia, with the result that elevated plasma triglyceride values persist.

Interestingly, the patients with "compensated euthyroidism" are close to the Type IV group in terms of their fasting lipase (pre-heparin lipase) activity.

This is further suggestive evidence that metabolism is subnormal in this patient group.

DISCUSSION

The studies described here suggest that patients with "compensated euthyroidism" may be metabolically "embarrassed" in terms of lipid metabolism and cardiovascular function. This finding suggests that such patients should be given replacement therapy when their TSH levels start to rise.

The mean T3 levels were not significantly different between the "compensated euthyroid" patients and the control group. This finding has been noted by others (274, 280, 303).
**TABLE 15**

**PHYSIOLOGICAL IODIDE IN COMPENSATED EUTHYROIDISM**

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>PRE-IODIDE</th>
<th>6 WEEKS</th>
<th>16 WEEKS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T₄  T₃  TSH</td>
<td>T₄  T₃  TSH</td>
<td>T₄  T₃  TSH</td>
</tr>
<tr>
<td>1</td>
<td>43  1.34 39</td>
<td>70  1.30 17</td>
<td>59  1.32 29</td>
</tr>
<tr>
<td>2</td>
<td>73  1.61 19</td>
<td>84  1.50  4</td>
<td>76  1.49 12</td>
</tr>
<tr>
<td>3</td>
<td>92  1.21 42</td>
<td>100 1.30 11</td>
<td>89  1.29 31</td>
</tr>
<tr>
<td>4</td>
<td>60  1.15 22</td>
<td>65  1.20 13</td>
<td>64  1.18 20</td>
</tr>
<tr>
<td><strong>MEAN n = 4</strong></td>
<td><strong>67  1.33 30.5</strong></td>
<td><strong>80  1.33 11.3</strong></td>
<td><strong>72  1.32 23</strong></td>
</tr>
</tbody>
</table>

HORMONAL PARAMETERS IN S.I. UNITS
There appears to be two possible explanations for this finding. Firstly, it may be that, as the stimulus due to thyroid stimulating immunoglobulins and lymphocytes wanes (as part of the natural course of the disease), hormonal levels fall and TSH is secreted to increase thyroid hormone output. The TSH levels do not rise to the maximum level attainable due to regulation at hypothalamo-pituitary level by T3 levels in the circulation.

Alternatively, the thyroid gland may be failing due to radiation, and TSH production is stimulated to increase hormonal levels of T-4 and T-3. The TSH level does not reach maximal levels necessary to maintain T-4 levels because of a preferential effect at hypothalamo-pituitary level by circulating T-3. The T-3 levels are preferentially sustained by an alteration in the T-3/T-4 secretion ratio by a damaged, failing thyroid gland.

Unfortunately, studies of gland secretion ratios of T-4 and T-3 were not performed to prove or disprove the latter possibility.

In an attempt to evaluate the possible role of relative iodine deficiency in perhaps causing preferential gland production of T-3, 4 patients were given physiological iodide (120 µg/day) orally for 4 weeks and re-assessed at 6 and 16 weeks. The findings are detailed in Table 15.

There was a significant rise in T4 and fall in TSH levels in all patients with a small fall in mean T3 levels in this group at 6 weeks. By 16 weeks, T4 levels had fallen and TSH levels had risen with a small rise in T3 levels.
This small study suggests a role for iodine deficiency in patients with compensated euthyroidism by causing preferential production of T3 in the failing gland. It is possible that patients with elevated TSH levels may become completely euthyroid without hormonal replacement therapy, if given physiological iodine replacement. It is also possible that eventual hypothyroidism may be staved off by this manoeuvre (though not prevented). It should be noted that physiological and not pharmacological doses of iodine were given to these patients. It has been recorded that pharmacological doses of iodine may induce overt hypothyroidism (230).

Patients with Elevated T-3 Levels: "T-3 Toxicosis".

An elevated T-3 level without a concomitant rise in T-4, producing clinical thyrotoxicosis, was first described by Maclagan et al. (175) in 1957. Frequent reports have appeared subsequently, in particular, one from Hollander et al. (140) describing 40 cases. Further studies, by the latter group suggested that elevated T3 levels were frequently an early form of frank thyrotoxicosis where both hormones are elevated (139).

The reasons for selectively high elevation of T-3 levels are unclear; its occurrence in autonomous hyperfunctioning thyroid nodules suggests that L.A.T.S. or TSH stimulation is not the cause. Some cases may result from a relative iodine deficiency (198) with preferential secretion of T-3.

During hormonal studies on post-125 patients, 20 patients were noted to have elevated T-3 levels. 10 were selected for prospective review at frequent intervals. These patients were euthyroid or minimally thyrotoxic on clinical assessment.
<table>
<thead>
<tr>
<th>PATIENT NO.</th>
<th>T&lt;sub&gt;4&lt;/sub&gt;</th>
<th>T&lt;sub&gt;3&lt;/sub&gt;</th>
<th>CLINICAL IMPRESSION</th>
<th>T&lt;sub&gt;4&lt;/sub&gt;</th>
<th>T&lt;sub&gt;3&lt;/sub&gt;</th>
<th>CLINICAL IMPRESSION</th>
<th>T&lt;sub&gt;4&lt;/sub&gt;</th>
<th>T&lt;sub&gt;3&lt;/sub&gt;</th>
<th>CLINICAL IMPRESSION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>127</td>
<td>3.42</td>
<td>E</td>
<td>173</td>
<td>5.45</td>
<td>None</td>
<td>113</td>
<td>3.68</td>
<td>E-mild T</td>
</tr>
<tr>
<td>2</td>
<td>126</td>
<td>3.67</td>
<td>E</td>
<td>176</td>
<td>4.78</td>
<td>None</td>
<td>115</td>
<td>3.10</td>
<td>E</td>
</tr>
<tr>
<td>3</td>
<td>106</td>
<td>3.62</td>
<td>E</td>
<td>LFU</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>90</td>
<td>3.39</td>
<td>E</td>
<td>89</td>
<td>2.78</td>
<td>None</td>
<td>74</td>
<td>2.74</td>
<td>E</td>
</tr>
<tr>
<td>5</td>
<td>139</td>
<td>3.47</td>
<td>E</td>
<td>106</td>
<td>3.54</td>
<td>Exag.</td>
<td>96</td>
<td>2.83</td>
<td>E</td>
</tr>
<tr>
<td>6</td>
<td>109</td>
<td>3.20</td>
<td>T</td>
<td>106</td>
<td>3.54</td>
<td>Exag.</td>
<td>94</td>
<td>2.71</td>
<td>E</td>
</tr>
<tr>
<td>7</td>
<td>124</td>
<td>3.85</td>
<td>T</td>
<td>127</td>
<td>4.78</td>
<td>None</td>
<td>106</td>
<td>3.76</td>
<td>E</td>
</tr>
<tr>
<td>8</td>
<td>67</td>
<td>3.60</td>
<td>T</td>
<td>LFU</td>
<td></td>
<td></td>
<td>91</td>
<td>2.16</td>
<td>E</td>
</tr>
<tr>
<td>9</td>
<td>83</td>
<td>3.36</td>
<td>E</td>
<td>112</td>
<td>3.39</td>
<td>None</td>
<td>91</td>
<td>2.62</td>
<td>E</td>
</tr>
<tr>
<td>10</td>
<td>139</td>
<td>3.45</td>
<td>E</td>
<td>120</td>
<td>3.23</td>
<td>None</td>
<td>63</td>
<td>2.32</td>
<td>E</td>
</tr>
</tbody>
</table>

E = EUTHYROID
T = THYROTOXIC
LFU = LOST TO FOLLOW UP
DNA = FAILED TO ATTEND
SI UNITS
The table opposite (Table 16) lists the relevant data for the 10 patients reviewed over one year. At the second review the T-3 levels remained elevated, T-4 levels remained in the normal range except for 2 patients who had elevated T-4 levels.

TRH tests performed at this point showed that these two patients together with six of the rest of the group, had no response to TRH.

Two patients, however, showed an exaggerated response to TRH although both these patients had normal basal TSH levels.

Nine of the group were further reviewed 12 months after the initial review. At this review, the T-3 and T-4 of the two patients who showed classical thyrotoxicosis (biochemical) at the previous review, returned to values similar or slightly below those found initially, although T-3 levels were still elevated (Table 16).

Of the other 7 patients, five now had normal T-3 levels, that is, they had become biochemically euthyroid and the T-4 of all but one of these patients had decreased significantly (an amount greater than could result from inter-assay variation).

The other two patients still had toxic T3 levels, although slightly less than initially, in addition, both patients had significantly reduced T-4 levels.

These findings, indicate that in the context of radio-iodine therapy, (particularly 1-125 therapy), the raised T-3 is probably a transitory state and not necessarily indicative of impending thyrotoxicosis.
### TABLE 17

**PHYSIOLOGICAL IODIDE IN BIOCHEMICAL "T-3 TOXICOSIS"**

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>PRE-IODIDE</th>
<th>6 WEEKS</th>
<th>16 WEEKS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T₄ T₃ TSH</td>
<td>T₄ T₃ TSH</td>
<td>T₄ T₃ TSH</td>
</tr>
<tr>
<td>1</td>
<td>127 3.42  0</td>
<td>180 3.90  0</td>
<td>135 3.91  0</td>
</tr>
<tr>
<td>2</td>
<td>106 3.62  0</td>
<td>176 4.10  0</td>
<td>160 3.70  0</td>
</tr>
<tr>
<td>3</td>
<td>90  3.39  1</td>
<td>114 3.40  0</td>
<td>95  3.36  0</td>
</tr>
<tr>
<td>4</td>
<td>139 3.47  1</td>
<td>173 3.46  1</td>
<td>141 2.01  2</td>
</tr>
<tr>
<td>5</td>
<td>67  3.60  0</td>
<td>96  4.10  0</td>
<td>80  3.70  0</td>
</tr>
<tr>
<td>Mean n = 5</td>
<td>106 3.50 0.4</td>
<td>148 3.79 0.2</td>
<td>122 3.34 0.4</td>
</tr>
</tbody>
</table>

**HORMONAL VALUES IN S.I. UNITS**
In an attempt to evaluate the possible significance of iodide deficiency in the aetiology of T-3 toxicosis, (i.e. biochemical T-3 toxicosis, as 6 of those patients were clinically euthyroid and the rest at most minimally thyrotoxic) five patients with T-3 toxicosis were given physiological iodine (120 μg/day for 4 weeks) and reviewed at 6 and 16 weeks subsequent to the commencement of the oral iodine.

The table opposite (Table 17) details the hormonal changes. Three of the 5 patients developed classical biochemical thyrotoxicosis at the 6-weeks review. There was little clinical change. The other two patients had slight further elevations of T3 levels. By 16 weeks, of the 3 patients who had developed raised T-3 and T-4 levels, one had reverted to T3 toxicosis and the levels in another had declined. The third patient had become biochemically euthyroid. The two patients with T-3 toxicosis and elevation at 6 weeks showed falls at 16 weeks.

This study suggests that relative iodide deficiency may regulate the relative secretions of T-4 and T-3 by the irradiated thyroid gland.

"T-4 Toxicosis"

During the hormonal studies, a small group of patients were noted to have elevated T-4 levels with normal T-3 levels. This finding is termed "T-4 toxicosis".

Doubt has been expressed as to the existence of such an entity (32). Turner et al (291) have suggested this entity may result due to the presence of concurrent severe non-thyroidal illness which is known to lower T-3 levels (198).
LOG-LOG SCATTERGRAM DEMONSTRATING PROBABLE EXISTENCE OF $T_4$-TOXICOSIS AS A CLINICAL ENTITY.
It has also been suggested that T-4 toxicosis is a relatively common finding in elderly patients with thyroid disease \(32,39\) but this is disputed.

The seven patients in this study found to have T-4 toxicosis were free of obvious extra-thyroidal illness. Four were clinically mildly thyrotoxic, three clinically euthyroid. The seven were reviewed regularly and, in 5 cases, hormonal parameters were repeated to confirm the findings two to six weeks later. Hormonal review at 6 months showed 5 patients to be clinically euthyroid; 2 continued to show T-4 toxicosis but three had classical biochemical toxicosis and one was euthyroid. One patient failed to attend. Reviews between 12 and 16 months showed that of the 5 patients attending, all were clinically euthyroid, five were now biochemically euthyroid and one was biochemically thyrotoxic.

T3 and free thyroxine index values for the 20 T-3 toxic and 7 T-4 toxic as well as for 11 compensated euthyroid patients were plotted on a log-log scatter graph \(281\) along with 28 classically thyrotoxic and 48 euthyroid patients' parameters for comparison (Figure 15). The population distributions appear to be discrete and to confirm the existence of T4 toxicosis as an entity following I-125 therapy.
THE T.R.H. TEST IN THE EVALUATION OF TREATED THYROTOXIC PATIENTS

This test has been used to characterise patients with T-3 toxicosis and compensated euthyroidism following 125I-therapy. Its use in the more general situation where post-therapy patients are being assessed is of interest.

The test would appear to be an extremely sensitive measure of thyroid function - Sabri and Utiger (248) have shown that the T.S.H. response is augmented by small decreases in circulating hormone levels whereas Fukuda et al. (95a) have demonstrated inhibition of T.S.H. response to T.R.H. by small increases in circulating hormone levels. Wenzel et al. (303) have demonstrated that both T4 and T3 inhibit the T.S.H. response to T.R.H. and interpret the delay in onset of action of T4 as indicating that the intra-pituitary inhibition is due to T3, time being required for de-iodination of T4 to T3. Biochemical studies, however, indicate high affinity specific T3 receptors in pituitary, but also, in some species, low affinity specific T4 receptors (269,271). In regard to this point, it is of interest that the two T.R.H. tests performed on patients in the Trial with biochemical "T4 - toxicosis" showed no response.

The value of the T.R.H. test following radio-iodine therapy is disputed. Clifton-Bligh et al. (49) found a lack of response in patients biochemically and clinically euthyroid following 131I therapy and a lack of response in some individuals with euthyroid Graves' disease where the subjects had never been (overtly) thyrotoxic.

Twenty-four T.R.H. tests were performed on patients clinically and biochemically euthyroid following 125I therapy.
TABLE 18
TRH TEST POST-125I IN CLINICALLY AND BIOCHEMICALLY EUTHYROID PATIENTS

<table>
<thead>
<tr>
<th>GROUP</th>
<th>NO. PATIENTS</th>
<th>T₄</th>
<th>T₃</th>
<th>TSH</th>
<th>ΔTSH TRH at 20 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 No response</td>
<td>7</td>
<td>114.5 ± 21.9</td>
<td>1.66 ± 0.6</td>
<td>0.1 ± 0.2</td>
<td>0.4 ± 0.6</td>
</tr>
<tr>
<td>2 Response inadequate</td>
<td>10</td>
<td>110.7 ± 27.0</td>
<td>1.61 ± 0.5</td>
<td>0.9 ± 2.0</td>
<td>4.5 ± 2.3</td>
</tr>
<tr>
<td>3 normal response</td>
<td>7</td>
<td>104.2 ± 21.9</td>
<td>1.55 ± 0.4</td>
<td>1.9 ± 2.1</td>
<td>14.0 ± 1.5</td>
</tr>
<tr>
<td>Group I 9 months later</td>
<td>n = 3</td>
<td>108 ± 20.6</td>
<td>1.58 ± 0.7</td>
<td>0.9 ± 1.0</td>
<td>7.1 ± 3.0</td>
</tr>
</tbody>
</table>
Prior to T.R.H. injection (200 μg. intravenously in the morning) full hormonal measurements were performed (T₄, T₃, T.S.H.). Patients were divided into 3 groups according to the change in T.S.H. levels 20 minutes after injection: (1) no response (n = 7), (2) inadequate response (n = 10), (3) normal response (n = 7). The table opposite details the pertinent data.

Grouped in this fashion, the data suggests that the response to T.R.H. depends on circulating hormone levels, even with values in the euthyroid range, as diminution in T.S.H. response occurs with rising T₄ and T₃ levels.

When the responses to T.R.H. (Δ T.S.H.) was graphed against time since last therapy (it was not possible to define accurately the onset of thyrotoxicosis in this group), there was no obvious relationship (for a linear relationship, r = 0.42).

It was not possible to demonstrate that recovery of the capacity to respond to T.R.H. by the pituitary depends on the passage of a period of time sufficient to permit "resetting" of pituitary responsiveness to the altered circulating hormonal levels - i.e. it is possible that prolonged elevation of T₄ and/or T₃ levels may produce pituitary thyrotroph suppression and they may not recover for some time when T₄ / T₃ levels are made normal.

The relationship between circulating thyroid hormonal levels and response to T.R.H. strongly suggests that pituitary responsiveness is related to levels of circulating hormones. Further evidence comes from further T.R.H. test data performed on 3 members of Group 1 some nine months later, when an improvement in response coincided with a fall in T₄ and T₃ levels.
WHY DID THE TRIAL FAIL?

"But facts are chills that winne ding, on' canne be disputed" (Burns).

To date, the Trial has failed in its prime purpose: that is indisputable. The theoretical and experimental basis of the Trial has to be reassessed in the light of this finding.

The author has engaged in studies aimed at improving experimental studies of irradiation therapy for thyrotoxicosis.

A NEW ANIMAL MODEL OF THYROTOXICOSIS

The studies of thyrotoxicosis and of therapies for thyrotoxicosis have been hindered by the lack of a suitable animal model. It has not proved possible to date to produce a thyrotoxic animal. In particular, use of TSH is associated with transitory stimulation but this effect disappears apparently due to antibody formation and annulment of the effects of TSH. Also TSH has to be given by injection.

The closest approach to an animal model has been to use rats or mice subjected to goitrogen stimulus to induce gland hypertrophy. The response of the gland to the goitrogen has been studied subsequent to radio-iodine therapy and indeed was the basic model used for this trial.

Recently, thyrotropin-releasing hormone (TRH) became available from Roche Laboratories. This material, a non-antigenic tri-peptide, is absorbed orally and offered an opportunity of producing an animal equivalent of thyrotoxicosis.
### TABLE 20

**EFFECT OF CHRONIC TRH ADMINISTRATION IN RATS**

<table>
<thead>
<tr>
<th>TIME SACRIFICED</th>
<th>NO. OF ANIMALS</th>
<th>THYROID WEIGHT$^0$ (mg)</th>
<th>THYROID WEIGHT$^0$ (mg/100 mg) body weight</th>
<th>P$^{127}$ I</th>
<th>24 HR. % 131$^I$ UPTAKE</th>
<th>HISTOLOGY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/52</td>
<td>8</td>
<td>22 ($\pm$ 4.0)</td>
<td>10.5 $\pm$ 0.3 (n = 6)</td>
<td>292 $\pm$ 16</td>
<td>-</td>
<td>-ve</td>
</tr>
<tr>
<td>2/52</td>
<td>6</td>
<td>20.5 $\pm$ 4.2</td>
<td>10.3 $\pm$ 0.2</td>
<td>284 $\pm$ 24</td>
<td>-</td>
<td>-ve</td>
</tr>
<tr>
<td>1/12</td>
<td>8</td>
<td>23.9 $\pm$ 4.2</td>
<td>10.6 $\pm$ 0.2 (n = 7)</td>
<td>307 $\pm$ 16</td>
<td>-</td>
<td>-ve</td>
</tr>
<tr>
<td>2/12</td>
<td>8</td>
<td>34.2 $\pm$ 5.1</td>
<td>13.5 $\pm$ 0.3 (n = 6)</td>
<td>394 $\pm$ 17</td>
<td>-</td>
<td>+ve</td>
</tr>
<tr>
<td>3/12</td>
<td>6</td>
<td>36.3 $\pm$ 4.9</td>
<td>13.6 $\pm$ 0.2 (n = 6)</td>
<td>402 $\pm$ 23</td>
<td>-</td>
<td>+ve</td>
</tr>
<tr>
<td>3/12 (Controls)</td>
<td>4</td>
<td>24.0 $\pm$ 3.2</td>
<td>9.7 $\pm$ 0.3</td>
<td>287 $\pm$ 21</td>
<td>-</td>
<td>-ve</td>
</tr>
<tr>
<td>3/12</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>24.2 $\pm$ 3.1</td>
<td>-</td>
</tr>
<tr>
<td>3/12 (Controls)</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6.0 $\pm$ 2.1</td>
<td>-</td>
</tr>
</tbody>
</table>

* +ve indicates evidence of hyperactivity.

** measured on pooled groups of two.
The literature data is rather confusing, there being some reports suggesting that TRH may induce thyrotoxicosis while other studies have been unrewarding.

Sakoda et al. (250) noted elevated $^{127}I$ and TSH levels in the sera of rats given oral TRH (100 or 1000 μg/kg. for 32 days), while Chorea et al. (48) claimed to have induced hyperthyroidism in mice by 2 hourly injections for 10 days of 10 μg TRH—thyroid $^{131}I$ uptake, gland size and serum thyroxine levels were all increased. Researchers in Hoechst, however, found little effect of TRH given to rats and dogs over 14 days, on thyroid weight. There was, however, a slight increase in weight and "some morphological changes indicative of thyroid activation" (personal communication from Dr. J. Garrod, Roche Laboratories). Haigler et al. (122) found that repetitive doses of TRH given to humans did not increase TSH levels, though inappropriate TRH secretion has been found to be a rare cause of hyperthyroidism (76). Rebello et al. (227) found repetitive administration of TRH to humans did elevate TSH levels but the response was limited.

The author studied the effect of chronic oral administration of TRH in drinking water administered to rats over a three month period. 43 male Wistar rats were given 2 mg. crystalline TRH per day for up to three months and sacrificed by ether anaesthesia in batches at one, two, four eight and twelve weeks. Diet consisted of Purina chow of normal iodide content. Thyroid status was assessed by increase in thyroid weight, by histological evidence of increased colloid resorption in tissue sections of thyroid stained with haematoxylin and eosin, and by changes in serum $^{127}I$. The results are detailed in Table 20.
FIGURE 17
HISTOLOGY OF THYROID IN RATS GIVEN TRH FOR THREE MONTHS.

(A) GENERAL VIEW OF HYPERACTIVE THYROID (H. and E. x 220).
ACTIVE COLLOID RESORPTION IS EVIDENT.

(B) VIEW OF CONTROL GLAND. (H. and E. x 560).
(c) ANOTHER VIEW OF HYPERACTIVE RAT THYROID.
(\textit{H. and E. \texttimes560}).
Findings of glandular hyperactivity were detected at two months and persisted until month three by which time gland weight was 30% more than control values. 4 control rats sacrificed at three months showed no change in the thyroid parameters studied. The histological appearances in treated rats consisted of increased follicular resorption suggestive of a hyperthyroidism. The $^{131}$I uptake in 5 treated rats at three months was $24.2\% \pm 3.1$ (mean $\pm$ 1SD) compared to an uptake of $60\% \pm 2.0$ in 3 control rats (over 24 hours). Figure 17 illustrates the histological appearance at three months.

This evidence suggests fairly decisively that TRH given chronically at this level for 12 weeks produces a hyperactive gland. This may serve as a model for the thyrotoxic human gland.

**RADIATION STUDIES OF THE EFFECTS OF IODINE-125 USING THE TRH-TOXIC RAT MODEL: COMPARISON WITH IODINE-131.**

The author felt that the "TRH-toxic" rat model offered a more accurate model of the human thyrotoxic gland and its response to radiation therapy. The goitrogen-induced hyperplasia model has usually involved administration of radio-iodine to a normal thyroid followed by studies of goitrogen induced hyperplasia. Preliminary use of goitrogen to render the gland hyperplastic and more akin to a toxic gland requires cessation of goitrogen therapy to permit uptake of radio-iodine with the possibility that cells may return to a resting state in the interim and so be protected against taking up much radio-iodine (unlike a continuously stimulated toxic cell).
With TRH administration, it is possible to continue gland stimulation before, during radio-iodine administration, and afterwards - an experimental situation much more analogous to human thyrotoxicosis. Also, the relatively mild thyrotoxicosis induced may be closer to the average patient of today, who is detected relatively early in the course of the disease and whose thyroid is less hypertrophied than the 2-3 fold enlargement usually induced in animals by goitrogen administration.

METHODS

40 male white rats were fed oral TRH (2mg./day) in their drinking water and maintained on normal Purina Chow diet for ten weeks when graded doses of iodine-125 or iodine-131 were administered intraperitoneally on the same day to groups of the animals.

The doses administered were 2.5, 10, 40 and 160 μCi iodine-125 and 10, 40, 100 μCi iodine-131. Animals from each group were sacrificed 24 hours after administration and on days five, nine and sixteen after therapy. The animals were killed by ether anaesthesia, the thyroids rapidly dissected out, weighed and transferred to formal saline preservative. Microtome sections of thyroid were taken for histology (haematoxylin and eosin preparations) and autoradiography (using Ilford coarse and fine grain nuclear emulsion).

Slides were studied for evidence of histological damage and scored in terms of the presence or absence of frank follicular damage, Askenazy change (cell cytoplasmic structural change) and extravasation of follicular colloid into the intravascular space.
## Table 21

**Radio biological actions of $^{125}$I and $^{131}$I on TRH-stimulated rat thyroid**

<table>
<thead>
<tr>
<th>ISOTOPES</th>
<th>DOSE $\mu$Ci</th>
<th>TIME AFTER ADMINISTRATION (DAYS)</th>
<th>NO. RATS STUDIED</th>
<th>MEAN THYROID WEIGHT (mg)</th>
<th>PST $\mu$g/100ml</th>
<th>AUTORADIOGRAPHIC PATTERN</th>
<th>ASHANAZY CHANGE</th>
<th>HISTOLOGY</th>
<th>EXTRA-FOSSICULAR COLLOID</th>
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</thead>
<tbody>
<tr>
<td>$^{125}$I</td>
<td>10</td>
<td>1</td>
<td>2</td>
<td>35</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>1</td>
<td>5</td>
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<td>+</td>
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<td>-</td>
<td>+ (n=1)</td>
</tr>
<tr>
<td></td>
<td>160</td>
<td>1</td>
<td>2</td>
<td>29</td>
<td>5.5</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+ n=4</td>
</tr>
<tr>
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<td>1</td>
<td>2</td>
<td>34</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>1</td>
<td>2</td>
<td>32</td>
<td>5.1</td>
<td>+</td>
<td>-</td>
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</tr>
<tr>
<td></td>
<td>100</td>
<td>1</td>
<td>2</td>
<td>32</td>
<td>5.3</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>$^{125}$I</td>
<td>2.5</td>
<td>5</td>
<td>1</td>
<td>31</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td>10</td>
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<td>3</td>
<td>32</td>
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<td>40</td>
<td>5</td>
<td>2</td>
<td>31</td>
<td>5.3</td>
<td>+</td>
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<td></td>
<td>160</td>
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<td>1</td>
<td>27</td>
<td>6.8</td>
<td>+</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>$^{131}$I</td>
<td>10</td>
<td>5</td>
<td>1</td>
<td>31</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td>40</td>
<td>5</td>
<td>1</td>
<td>30</td>
<td>5.4</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td></td>
<td>100</td>
<td>5</td>
<td>2</td>
<td>31</td>
<td>6.7</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+ n=1</td>
</tr>
<tr>
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<td>3</td>
<td>25</td>
<td>7.2</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>$^{131}$I</td>
<td>10</td>
<td>9</td>
<td>1</td>
<td>31</td>
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<td>40</td>
<td>9</td>
<td>2</td>
<td>30</td>
<td>5.9</td>
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<td>+</td>
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<tr>
<td></td>
<td>100</td>
<td>9</td>
<td>2</td>
<td>28</td>
<td>6.9</td>
<td>+</td>
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<td>+</td>
<td>-</td>
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<tr>
<td>$^{125}$I</td>
<td>40</td>
<td>16</td>
<td>3</td>
<td>30</td>
<td>5.0</td>
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<td>+</td>
</tr>
<tr>
<td></td>
<td>160</td>
<td>16</td>
<td>1</td>
<td>22</td>
<td>7.0</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>$^{131}$I</td>
<td>10</td>
<td>16</td>
<td>1</td>
<td>31</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td></td>
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<td>16</td>
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<td>30</td>
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<td>100</td>
<td>16</td>
<td>1</td>
<td>22</td>
<td>6.8</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>
Autoradiographic patterns were classified as radial with evidence of colloid leakage between follicular cells or annular when colloid leakage produced an annular image around the follicle externally.

Particular attention was paid to these early autoradiographic appearances of follicular damage with extravasation of colloid.

RESULTS

Comparison of effects of radio-iodine nuclides.

No significant change in thyroid weight was noted until Day 5 when a small fall was noted in rats given 160 μCi $^{125}$I and this fall was greater by Day 16. Rats given $^{131}$I showed a small fall by Day 9 with 100 μCi $^{131}$I and a more dramatic fall by Day 14.

PBI$^{127}$ measurement on pooled sera from cardiac puncture (all of each group pooled) showed no significant changes except at the higher doses of both nuclides with a rise by Day 5 in rats receiving 160 μCi $^{125}$I or 100 μCi $^{131}$I.

Table 21 lists the relevant findings with particular regard to the histological and autoradiographic appearances. A general pattern is apparent. 24 hours after isotope administration, changes are minimal though there is clear evidence of extra-follicular colloid in the $^{125}$I-treated group at a dose of 160 μCi (annular autoradiographic pattern). There is no unequivocal evidence of radiation damage in terms of Askanazy change nor disruption of follicular architecture at this time.

By Day 5, the evidence of colloid leakage and frank follicular breakdown is apparent at 100 μCi $^{131}$I with no frank structural or autoradiographic changes at 40 μCi $^{131}$I. The $^{125}$I-treated group
(A) Frank follicular damage (upper half of field)
Day 16 dose - 160 μc 125I
(H. and E. x 225)
(B) ASKANAZY CHANGE. THE THYROID EPITHELIAL CELLS ARE LARGER THAN NORMAL, CUBICAL, HAVE A GRANULAR CYTOPLASM WHICH IS EOSINOPHILIC.
DAY 16 DOSE 160 μC 125I
H. and E. x 560.

(C) AUTORADIOPHAPH SHOWING CONSIDERABLE LOSS OF LABELLED COLLOID FROM FOLLICLES INTO PERIFOLLICULAR TISSUE.
DAY 9 DOSE 160 μC 125I.
Ilford 45 x 225.
Autoradiograph showing radial and annular patterns of labelled colloid labels.

Ilford 44 x 560.

Day 9  Dose 160 μc I.

H. and E.  -  Haematoxylin and Eosin Stain.
show little change; autoradiographic appearances are equivocal but probably show an early radial pattern of labelled colloid.

By Day 9, frank follicular breakdown and continuing colloid leakage is quite unequivocal at 160 μCi\(^{125}\)I and autoradiographic follicular change is more obvious at 40 μCi\(^{131}\)I.

By Day 16, radiation change is unequivocal at 160 μCi\(^{125}\)I and 40 μCi\(^{131}\)I with follicular disruption and colloid loss clearly seen.

No frank histological changes are seen at dose levels of up to 40 μCi\(^{125}\)I (borderline changes only at Day 16) or 10 μCi\(^{131}\)I.

Fig. 18 illustrates typical appearances of irradiated glands.

DISCUSSION

The model of TRH-induced thyrotoxicosis was used to compare the irradiation effects of \(^{131}\)I and \(^{125}\)I on rat thyroid. Up to 10 μCi\(^{125}\)I and 10 μCi\(^{131}\)I gave no frank follicular damage, whereas at 40 μCi\(^{125}\)I and, more so, at 160 μCi\(^{125}\)I, evidence of follicular damage was apparent. The changes due to 160 μCi\(^{125}\)I appeared as early as Day 1 and persisted for the 16 days of study. The 40 μCi\(^{125}\)I dose produced less clearcut evidence of damage with no Askanazy change nor frank follicular disruption but with some autoradiographic evidence of colloid extravasation. By comparison, 40 and 100 μCi\(^{131}\)I produced frank follicular damage. There was a tendency for \(^{125}\)I-induced damage to occur earlier at a dose level of 160 μCi in comparison to 40 μCi\(^{131}\)I.

The results suggest a threshold level for observable radiation damage over 16 days, of a minimum of 10 μCi\(^{131}\)I and 10-40 μCi\(^{125}\)I,
with higher doses of $^{125}\text{I}$ producing an effect earlier than $^{131}\text{I}$. The dose ratio for similar effect would appear to be of the order of $4:1 \, ^{125}\text{I} : ^{131}\text{I}$. There is no demonstrable sparing effect of $^{125}\text{I}$ in terms of preservation of follicular architecture or increase in thyroid weight due to retention of cellular capacity to proliferate.

This finding is in accord with the results of the clinical trial of $^{125}\text{I}$ in 360 thyrotoxic patients, where there is no demonstrable reduction in the rate of hypothyroidism at three years after $^{125}\text{I}$ therapy and where the dose ratio of $^{125}\text{I}$ to $^{131}\text{I}$ for optimum control of symptoms with minimal evidence of hypothyroidism was approximately 4 to 1.

The present study has to be considered in regard to other relevant experiments. Greig et al. (113) studied the response of rat thyroid irradiated with $^{125}\text{I}$ or $^{131}\text{I}$ to goitrogen, found a difference in DNA values of roughly 2 to 1 and considered this to indicate a relative sparing of nuclear replicative capacity for the same reduction in hormonogenesis by the shorter radiations of iodine-125 - i.e. there was held to be a differential effect across the follicle cell with the apical cytoplasm (the site of hormonogenesis) being more highly irradiated by iodine-125 emissions (coming largely from the colloid store), than the more distant nucleus.

Vickery & Williams (289) also found a qualitative difference in effect of iodine-125 compared to iodine-131 with greater damage to hormone production than to growth (goitrogen-induced). This effect was exaggerated by feeding a low iodide diet prior to isotope administration with consequent elongation of the cells.
Three criticisms may be raised regarding these studies. Firstly, the glands produced by goitrogen administration in these studies are large (> 2 x normal size) with elongated cells and bear less structural similarity to the relatively mild cases of thyrotoxicosis treated nowadays (due to early detection). Secondly, administration to resting thyroid of radio-iodine may produce spurious differences with subsequent goitrogen administration. The therapeutic irradiation from $^{125}_{1}$ is of low energy and so its effect will be confined to follicles where it is taken up - i.e. non-stimulated follicles will be protected. $^{131}_{1}$, on the other hand, has a much longer path-length in time and so will irradiate adjacent follicles with more uniform effect. Prior administration of a low iodide diet will elongate cells and so cross-irradiation by $^{125}_{1}$ (or extravasated colloid) will also be reduced. Thirdly, the differential radiation concept is dependent on follicular integrity. There is evidence in the present work of early colloid leakage between the cells of apparently intact follicles.

The hypothyroidism that appears to be an inevitable accompaniment of $^{131}_{1}$ therapy has been shown to be due to a damaged capacity of follicular cells to replicate (3).

Konecny (151) observed a striking increase in gland weight in rats after $^{125}_{1}$ treatment (detected after Day 30 and peaking at Day 60) and interpreted this effect as due to a nuclear sparing action of $^{125}_{1}$ and retained capacity to respond to TSH: this, however, is explicable in terms of the lewish dose of $^{125}_{1}$ administered (50 µCi). Damage was not apparent on histological examination until Day 15 - in accord with the findings demonstrated here for 40 µCi $^{125}_{1}$. 
The present findings agree with those of Jongejan and von Putten (144) who fed rats a low iodine diet for 2-3 weeks and gave graded doses of both nuclides. Iodide uptake (a basal function of the cell), serum T4 levels (an apical function) and histological evidence of cell death 100 days after isotope administration show no evidence for a differential action of 125I on hormonogenesis. Also, in mice, where the nucleus is not basally sited, comparable results were obtained. These data refer to the late results in irradiated thyroids but accord with these more acute findings described here.

Figure 9 outlines the potential actions of 125I and 131I. The evidence for a therapeutic advantage of 125I over 131I will depend on C being large enough in comparison to 131I action to produce a differential effect.

Neither the data presented here, nor the results of the clinical trial of 125I in humans support the claims for a differential effect with 125I.
## Table 19

**RESULTS OF PREVIOUS TRIALS OF IODINE 125 THERAPY FOR THYROTOXICOSIS**

<table>
<thead>
<tr>
<th>REF.</th>
<th>NUMBER OF PATIENTS TREATED</th>
<th>DOSE (mC)</th>
<th>THYROID STATUS (% OF GROUP)</th>
<th>DURATION OF FOLLOW-UP (MONTHS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mean</td>
<td>range</td>
<td>EUTHYROID</td>
</tr>
<tr>
<td>1</td>
<td>45</td>
<td>2.5</td>
<td>-</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>5.0</td>
<td>(4-6.5)</td>
<td>54</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>4-9.5*</td>
<td></td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>10-19.5*</td>
<td></td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>20-36*</td>
<td></td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4-36*</td>
<td></td>
<td>60</td>
</tr>
<tr>
<td>3</td>
<td>42</td>
<td>*22</td>
<td>(6-60)*</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>*10</td>
<td>(2.8-32)*</td>
<td>43</td>
</tr>
</tbody>
</table>

**REFERENCES:**

1. Lewitus et al. (1971) (168)
2. Weidinger et al. (1974) (297)

* indicates total dose prescribed.
CONCLUSION TO THE TRIAL

Briefly, this Trial has failed to show any therapeutic superiority of iodine-125 in comparison to iodine-131. In particular, the same unacceptably high incidence of hypothyroidism is found and, 7 years after the commencement of the Trial, there is no evidence of a decline in the accrual rate. Data from other trials of iodine-125 is detailed opposite (Table 19); the slightly better results are explained in terms of small numbers of patients treated and the short period of follow-up. The patient's variability in response to therapy is obvious on looking at the two groups B and C treated by Weidinger where doubling the dose is associated with a decreased incidence of hypothyroidism.

Review and prospective studies of patients using hormonal measurements has defined 3 groups of particular interest and they have been dealt with in some detail. Patients with "compensated euthyroidism" are probably hypothyroid in their intermediary metabolism and do not have "normal" biochemical status and should have replacement therapy; T3 toxicosis post-radio-iodine therapy is a transitory state, and T-4 toxicosis does appear to exist as a biochemical entity.
**SECTION C**

*β*-ADRENOCEPTOR BLOCKING DRUGS: A CRITIQUE OF THEIR USE IN THYROTOXICOSIS AND A LABORATORY STUDY OF THEIR MODE OF ACTION ON THE HEART.

**INTRODUCTION**

Many of the features of thyrotoxicosis - the tachycardia, tremor, stare, sweating - bear a striking similarity to the features of sympathetic activity and several reports have claimed that sympathetic blockers such as reserpine or guanethidine have an ameliorating effect in thyrotoxicosis (30, 43). Schafer (252) considered that tissues were rendered more sensitive to the actions of adrenaline in thyrotoxicosis (this, in 1916) and this belief forms the basis for using sympathetic antagonists in thyrotoxicosis. The specific *β*-adrenoceptor blocking agents introduced recently have gained wide acceptance as adjuvant therapy for thyrotoxicosis.

However, Levey et al (80, 164) have criticised the concept of increased sensitivity to catecholamines in hyperthyroidism on the grounds that the investigations were unscientific and biased and, in particular, did not look at dose-response relationship and placebo effect. Levey's studies suggest that there is no increased sensitivity of heart to catecholamines and he found that *β*-adrenoceptor blocking drugs are incapable of reducing the resting heart rate in the thyrotoxic state to normal. The relationship between thyrotoxicosis and palpitation first recorded in 1825 by Parry (216) is possibly explained by the finding of an increase in free adrenaline in the heart of rats rendered thyrotoxic (322). Levey's group have also demonstrated the existence of two discrete adenyl cyclase systems in heart, one stimulated by thyroid hormones (and glucagon),
the other by catecholamines. Also the similarity to sympathetic stimulation is only partial - for example, vasoconstriction is conspicuously absent.

The experiments described in this section arose from the above considerations and from the following clinical incident: a male patient aged 65 years with no history of ischaemic heart disease presented with thyrotoxicosis and a large goitre. He was averse to surgery and ablation therapy with radio-iodine (30 mCi$^{131}$I) was selected as an alternative. His resting heart was 96/min. prior to therapy. He was commenced upon Propranolol 40 mg. t.i.d. orally as a holding measure to protect him from the anticipated sudden release of thyroid hormones from the goitre into the blood stream. Six days later he returned in gross cardiac failure with a weight gain of ten pounds. His cardiac rate was 58/minute. His failure cleared with diuretic therapy and withdrawal of propranolol. He is now euthyroid (on thyroxine replacement therapy) and has no failure.

This observation suggested that the potency of propranolol was more than sufficient to outweigh the positive inotropic effects of thyroid hormones. It emphasised the need for caution in administration of $\beta$-blocking drugs to thyrotoxic patients and suggested that a more critical evaluation of the action of $\beta$-adrenoreceptor blocking agents was required. Obviously, if there is an element of adrenergic overactivity in hyperthyroidism then a $\beta$-blocker would be of potential value in alleviating symptomatology but, if the myocardial depressant effect (quinidine-like action) of the drug is such as to induce failure in a proportion of patients then selection of another $\beta$-blocker with less cardio-depressant action might be safer and yet as beneficial in controlling symptoms.
The action of catecholamines upon the cardiac action potential (in Purkinje and ventricular muscle fibres) has been shown by Carmeliet et al (43a) to be exerted upon the inward calcium current which is largely responsible for the action potential plateau. It has been demonstrated that, in sodium-free Tyrode solution (containing choline chloride as substitute for sodium chloride), the rapid inward sodium current disappears, leaving an action potential due solely to inward calcium and outward potassium currents. This has the appearance shown in the figure opposite. Addition of adrenaline augments the plateau height. The increased intracellular calcium concentration enhances myocardial contractility by promoting actin–myosin interaction.

It has not been possible to demonstrate an effect of \( \beta \)-adrenoreceptor blocking drugs on the inward calcium current that is independent of their action in blocking catecholamine action. This suggests that any postulated negative inotropic action of such drugs on cardiac tissue in a basal state (i.e., no sympathomimetic stimulation) is not exerted at this site but at some other locus, if indeed such an action is produced by such drugs.

This study was designed to study the action of several such drugs on sodium and potassium fluxes as these functions of the cardiac cell membrane would seem an obvious site for a postulated direct depressant effect of \( \beta \)-adrenoreceptor drugs.

Moreover, cardiac glycosides are known to inhibit the Na, K-ATPase dependent ionic pump with changes in cellular concentrations and membrane fluxes of these ions (17a).

It is generally held, in the light of a large body of experimental
FIG. 3. ACTION POTENTIAL RECORDED IN CALF PURKINJE FIBRES IN PRESENCE OF ADRENALINE (5.5 X 10^{-5} M). THE NA CURRENT WAS PROGRESSIVELY BLOCKED WITH INCREASING DOSES OF LORODOTIN: (a) CONTROL; (b) 2 X 10^{-2} M; (c) 5 X 10^{-7} M; (d) AND (e) 2 X 10^{-6} M. NOTE COMPLETE ABSENCE OF INITIAL SPOKE.
evidence (17a) that the positive inotropic effect of
glycosides on cardiac tissue is due to the increased
intracellular sodium levels displacing sarcolemmal
calcium, increasing intracellular free calcium levels, and
so enhancing myocardial contractility. The efficacy of
\( \beta \)-adrenoreceptor blocking drugs in opposing the toxic,
arrhythmogenic actions of glycosides (276) could be
due to an action on glycoside-mediated Na, K, ATP-ase
dependent pump inhibition and, if such an effect was
demonstrated, would support the contention that such
drugs exert a direct depressant effect at cardiac cell
membrane level.

Failure to demonstrate such an effect would suggest
another locus for the anti-arrhythmogenic action of
\( \beta \)-blocking drugs and, indeed, there is evidence that
sympathetic blockade reduces the incidence and threshold
of glycoside-induced dysrhythmias.

A NOTE ON CATECHOLAMINE ACTION ON CARDIAC
TISSUE

Present understanding of the action of catecholamines on
cardiac tissue is in a state of flux (207a) but a unified
analysis may be attempted as follows. Such an analysis
is obviously relevant to an understanding of the function
of \( \beta \)-adrenoreceptor blocking drugs.

Firstly, the inward calcium current is increased (by
adrenaline) and this effect is responsible for the increased
height of the action potential, for (in part, at least) the
positive inotropic action, and for the increased pacemaker
rate in sino-atrial and atrial tissue.

Secondly, the activation curve for the outward pacemaker
An increase in intra-cellular calcium augments myocardial contraction by stimulating sarcolemmal calcium uptake and release. Intra-cellular calcium is increased in turn by direct inflow of calcium (slow calcium current) shown here as part of the Na-Ca exchange, which in turn is stimulated by cyclic AMP produced by catecholamine action at the β-adrenoceptor site. Intra-cellular calcium is increased indirectly by a rise in intra-cellular sodium which displaces bound calcium. Cardiac glycosides have this effect by inhibiting the sodium pump.

The increased intra-cellular calcium increases the background outward potassium current (shown here as a function of membrane potential and time), thereby shortening the action potential duration, reducing the inflow of calcium ions and thus preventing decoupling of cells.
potassium current in Purkinje fibres is shifted in a depolarizing direction, so accelerating Purkinje pacemaker activity (207a). The outward potassium current is increased in amplitude and, in some studies, its activation threshold is lowered, so shortening the action potential and, in sino-atrial and atrial tissue increasing the maximum negative potential in diastole.

Thirdly, adrenaline increases the activity of Na⁺-K⁺ exchange pump and this may be the cause of the increased maximum negative potential in diastole in Purkinje fibres (207a).

Studies by McGuigan (10a) and others on mammalian ventricular fibres suggest that the inward calcium current regulates the outward potassium current and that this regulation controls the action potential duration. Moreover, it is suggested that the calcium current in turn is regulated by cyclic AMP levels which in turn are regulated by catecholamine levels. The overall postulated scheme is diagrammed opposite. Such a synthesis of currently available data may apply to cardiac tissues in general.

Thus it may be perceived that β-adrenoreceptor blocking drugs may have many actions which are, however, indirect and exerted via the adrenoreceptor site.
To evaluate the apparent cardiu-depressant action of β-blocker
drugs, a cultured cardiac cell preparation was used and the
effects of 4 β-blocker drugs on transmembrane ion fluxes were
measured. The drugs chosen for study were propranolol, which
is now widely used in thyrotoxicosis (26,104), practolol which
is a cardio-selective beta-blocker, has some intrinsic
sympathetic activity and which has some benefit clinically
in toxic patients (30,205); oxprenolol which has some intrinsic
sympathetic activity and is of little benefit clinically in
hypothyroidism (222, 282) and a new drug, tolamolol which has
no intrinsic sympathomimetic activity. This latter drug has
recently been reported to cause tumours in animals and its
general release has not been permitted.

In this study, particular attention was paid to the drug
concentrations used as some of the actions of β-blockers are
demonstrable only at levels far beyond clinically attainable
concentrations.

The study was extended to the action of propranolol on
the effect of a cardiac glycoside (ouabain) on ionic fluxes
as it has been suggested that the action of β-blocking drugs
in protecting against glycoside-induced dysrhythmias is exerted
at cell membrane level (173, 174,265). Also β-blockers is of benefit
in thyrocardiac patients in potentiating the slowing effect of
digoxin on impulse conduction through the A-V node in atrial
fibrillation, may slow sinus tachycardia and it is of interest
to study the interaction of these drugs at cellular level (282,312).

IMPROVEMENT TO A TISSUE CULTURE LABORATORY

The laboratory used for the tissue culture experiment was
located in a small portakabin which presented various structural
difficulties to adequate organisation and sterility considerations.
The following improvements were made:

1. The laboratory was emptied of all non-tissue culture (e.g., flame photometer for ion assays, bench centrifuge) to reduce the risks of contamination and to give adequate room.

2. Techniques for transferring solutions were re-designed. Long steel needles with wide bore were used to suck up cells to break up clumps with minimal damage to the cells. A wide bore glass filter funnel was fashioned to fit the necks of bottles of media and the rubber tube of a Cornwall syringe weighted with a perforated steel hollow ball that slipped down the funnel into the bottle and permitted transfer of media aseptically.

3. Cell counting procedures were automated using a Coulter counter. The instrument was calibrated using latex beads of accurately defined diameter and set to the diameter of Girardi cells in suspension. A PZ64 cell size plotter was added to the counter to permit cell sizing and detection of any volume changes (which would alter intracellular ionic concentration significantly). Automation of cell counting (in place of the use of a haemocytometer) permitted processing of large numbers of plates (up to 40 per experiment) with increased accuracy and reliability of results.

4. By trial and error, the constant clogging of the Coulter counter orifice by serum (used to stop trypsinisation of cell layers) was circumvented by the use of a specific trypsin inhibitor, methylphenylsulphonylfluoride.

5. Rigid standardisation of experimental protocol was adopted so that cell numbers per plate lay within a narrow range and the same correction factor for
persisting clumping of cells could be used.

(5) Emphasis on meticulous aseptic procedure which obviated the use of antibodies. This included repeated checking of media, soak solutions and plates for contaminating bacteria (bacteriological culture on agar plates) and/or the presence of pyrogens (animal studies).
INTRODUCTION

Exchanges of ions between cells and their surroundings are best expressed in absolute units, without prejudice as to the mechanism involved. The common unit used is moles/cm$^2$ sec. In practice, the result is expressed in units of $\text{p-mole/cm}^2 \text{sec}$.

Under steady state conditions, the quantity of the ion going into the cell (the influx) is equal to that leaving the cell (the efflux) in unit time, so that it is only necessary to measure one of these and the internal concentration if the ion does not change with time. Generally, however, most isolated tissues are not in a steady state and will eventually reach a similar composition to that of the environment. This means that the influx and the efflux are not the same and so both must be measured and also that the internal concentration is changing with time.

The Flux Equations

The model chosen for deriving these equations is that of a cell consisting of a bag of fluid separated from the surrounding medium by a thin membrane of high resistance to the movement of the ion, but with a low capacity for the storage of the ion.

Let such a cell of area $A$ and volume $V$, and with an internal concentration of $C_1$ be suspended in a solution containing the same ion at a concentration $C_0$. For experimental purposes some of the external ions are replaced by the labelled ions $C_0^x$. The specific activity of the bathing fluid is then $C_0^x/C_0$. After a sufficiently long time the internal
ions will be labelled in the same ratio. Let the fluxes of this ion across the membrane be \( M_{\text{in}} \) and \( M_{\text{out}} \) for the influx and the efflux respectively. The labelled fluxes of the ion are then \( M_{\text{in}}^x \) and \( M_{\text{out}}^x \) respectively.

In unit time the amount of ion in the cell changes by

\[
M_{\text{in}}^x - M_{\text{out}}^x \quad \text{therefore the concentration changes by}
\]

\[
\frac{M_{\text{in}}^x}{V} - \frac{M_{\text{out}}^x}{V} \quad \text{in unit time,}
\]

i.e.

\[
d\left(\frac{C}{V}\right) = \frac{M_{\text{in}}^x}{V} - \frac{M_{\text{out}}^x}{V} \quad \text{equation (1)}
\]

Using this basic equation both the influx and the efflux can be measured by suitable adjustment of the experimental conditions.

**INFLUX**

If the cell is placed in a solution containing the labelled ion then for a short time there is an influx of the labelled ion, with only a small efflux of the labelled ion (for there are only very few of the labelled ions in the cell at this time). Therefore, equation (1) becomes:

\[
d\left(\frac{C_{1}^x}{V}\right) = \frac{M_{\text{in}}^x}{V} \quad \text{equation (2)}
\]

Rearranging \( M_{\text{in}}^x = d\left(\frac{C_{1}^x}{V}\right) / A \quad \text{equation (3)} \)

\( M_{\text{in}}^x \) is then obtained by multiplying \( M_{\text{in}}^x \) by \( C_{0}/C_{0}^x \).

**EFFLUX**

If the cell is allowed to accumulate some radioactivity, and is then washed in a solution containing no radioactive ions, then the influx term \( M_{\text{in}}^x A/V \) in equation (1) becomes very small (theoretically zero), and so

\[
d\left(\frac{C_{1}^x}{V}\right) / A = -\frac{M_{\text{out}}^x}{V} \quad \text{equation (4)}
\]

At this stage it is necessary to assume some relationship
between the flux and the internal concentration of the ion. That usually assumed is to suppose that the flux is proportional to the concentration (a physical model of this would be that of Brownian movement causing the ions to impinge in a random way on one side of a thin membrane with holes in it).

Thus \( M_{\text{out}} \) is proportional to \( (C_1) \)

or \( M_{\text{out}} = K_2 C_1 \) ..........................................................(5)

where \( K_2 \) is a constant.

Therefore from equation 4:-

\[
\frac{d(C_1^X)}{dt} = -\frac{A}{V}K_2(C_1^X) \quad \text{.................}(6)
\]

\( \frac{A}{V}K_2 \) can conveniently be replaced by \( k \) giving,

\[
\frac{d(C_1^X)}{dt} = -k(C_1^X) \quad \text{.................}(7)
\]

or, \( (C_1^X) = (C_1^X) \exp(-kt), \text{.................}(8) \)

t=0

where \( (C_1^X) \) is the activity left in the cell after time \( t \),

where \( (C_1^X) \) was the original activity at time zero.

A more convenient form of this equation is obtained by taking the log. when \( \ln(C_1^X) = \ln(C_1^X) - kt \) ...........(9)

or \( \log_{10}(C_1^X) = \log_{10}(C_1^X) - kt \log_{10} \text{.........}(9a) \)

t=0

Experimentally one obtains \( (C_1^X) \) at various times and so a plot of \( \log(C_1^X) \) against time gives a straight line of intercept \( \log(C_1^X) \) and slope proportional to \( k \).

\( k \) in those equations is a rate constant (or an exchange constant in this particular case), and is the reciprocal of a time constant, so that it has the dimensions of \( 1/\text{time} \) (i.e. min. \(^{-1}\) etc.)
FIGURE 20.
Flow Diagram For Measurement of Intracellular Ion Concentrations and Influxes.

**Concentration**
- 10^6 cells/plate
  - 3 plates in soak sol^n
  - ± Isotope ± drug

Wash x 4 in Na⁺,K⁺-free, Isotonic KREBS at 0°C

TRYSINIZE 0.5 ml of 0.25% w/v + inhibitor + isoton

Make up to 25 ml

Count cell No° (Coulter Counter) Count Isotope (Tricarb)

**Influx**
- 3 plates in soak sol^n
- ± drug (no isotope)

Replace Soak Sol^n with Medium containing Isotope ± Drug.

- 3 ml of sol^n
- 22Na
  - 30 Secs Later
- 86Rb
  - 10 mins Later

Wash x 4 in Na⁺,K⁺-free Isotonic KREBS at 0°C

Assay Na,K (Photometer)

TRYSINIZE + inhibitor + isoton

PIPETTE

Make up to 25 ml

Count Cell No° Count Isotope

30 second ²²Na Influx
Flow Diagram for Efflux Measurements.

**PLATES OF CELLS EQUILIBRATED IN $^{22}\text{Na}/^{36}\text{Rb}$ MEDIUM $\pm$ DRUG.**

- Wash x4 in Non-Radioactive Soak Sol. $\pm$ Drug for 30 Seconds
- Leave in Soak Solution
  - 1 min ($^{22}\text{Na}$)
  - 9.5 min ($^{86}\text{Rb}$)
  - 2 min ($^{22}\text{Na}$)
  - 29.5 min ($^{86}\text{Rb}$)
  - 5 min ($^{22}\text{Na}$)
  - 59.5 min ($^{86}\text{Rb}$)

Wash x4 at 0°C in Isotonic Na$^+$, K$^+$-free Krebs. for 30 seconds.

- Residual $^{22}\text{Na}$ Content measured at $t=0$ or $t=1.5$ min
- Residual $^{86}\text{Rb}$ Content measured at $t=10$ min, $t=30$ min, $t=60$ min

(10 Plates per Assay)

(3 Plates per Assay)
The value of k thus found from the experimental results is equally applicable to the total ion in the cell. From the above it is also equal to $k_2\cdot A/V$, so from equation 5

$$k = \frac{M_{out}}{C_1\cdot A/V} \quad \text{(10)}$$

Rearranging $M_{out} = k \cdot V/A \cdot (C_1)$ \quad \text{(11)}

POINTS

1) This equation can be used in non-steady state conditions, provided that the rate of change of the total ion in the cell is small compared with the rates at which the tracers are being exchanged. If not then some expression for the change of the total ion must be incorporated in equation 11.

2) Since $k \cdot T_2 = \ln 2$ (i.e. 0.693), where $T_2$ is the half time of the exponential process, equation 11 becomes:

$$M_{out} = \frac{\ln 2}{T_2} \cdot V/A \cdot (C_1) \quad \text{(11a)}$$

The halftime is found by noting the time that the initial activity in the cell takes to fall to half its value.

3) In equations 11 and 11a the flux is in moles/cm$^2 \cdot$ sec. if the halftime is in seconds, the internal concentration is in moles/cm$^3$, and the volume to surface ratio is in cm.

EXPERIMENTAL PROTOCOL

Figures 20 and 21 illustrate the flow plan for measurement of intracellular concentration influx and efflux of sodium and potassium in the presence of the beta-adrenoceptor blocking drugs studied. Measurements were made over a range of drug concentration varying from levels therapeutically obtainable to levels well outwith the therapeutic range.
Measurements were made in the presence of d-1 Propranolol, Dxprenolol, Prcitolol and Tolmolol, in concentrations from 0.1 µg/ml to 100 µg/ml. The effect of Propranolol on Quabaine-induced ionic changes were measured in the presence of Quabaine with or without 5 µg/ml, Propranolol.

METHODS

Preparation of Culture Plates.

Girardi cells, an established cell line derived from human right atrium, were supplied as a trypsinised suspension of $10^5$ cells/ml. (Gibco-Biocult Ltd.). The cells were thoroughly pipetted to dissociate clumps and were transferred to growth medium composed of basal medium Earle (containing 2 gm. sodium bicarbonate/litre), 10% foetal calf serum and 1% Glutamine to give a cell concentration of about $2.5 \times 10^4$ cells/ml.

Four ml. aliquots of cells and medium were plated out in 35 mm. vented Petri dishes and the dishes stacked in a sealed container, which was gassed with 95% CO$_2$ : 5%CO$_2$ to maintain pH.

In the studies of the effect of beta-adrenoceptor blocking drugs on ionic parameters, the medium was poured off plates four days later and replaced with fresh medium containing *isotope and drug at the appropriate concentration. Four hours later, this growth medium was replaced with Earle's buffer salt solution containing isotope and drug (soak solution). After four hours in this solution, when the cells were in a non-dividing state, they were used for the experiment. Final cell concentration was a confluent layer of about $10^6$ cells/plate.

In the experiment to study the effect of Propranolol on Quabaine-induced ionic changes, plates were incubated for four days and then the medium was replaced with growth medium * where appropriate.


<table>
<thead>
<tr>
<th>No.</th>
<th>Drug Combinations</th>
<th>Times of Exposure to Drug (Hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Quabaine alone</td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td>Quabaine and Propranolol</td>
<td>24, last 12</td>
</tr>
<tr>
<td>3</td>
<td>Quabaine and Propranolol</td>
<td>24, last 1/2</td>
</tr>
<tr>
<td>4</td>
<td>Propranolol and Quabaine</td>
<td>24, last 12</td>
</tr>
</tbody>
</table>
containing glycoside and propranolol in the combinations and for the times shown in Table 22.

**Counting of Cells.**

Medium was aspirated from the cell layer which was washed with ice-cold isotonic sodium-and-potassium-free Kreb's solution to remove external isotope. The cell layer was then covered with 0.5 ml. of 0.25% W/V Trypsin solution and the plates viewed under an inverted phase microscope until, with repeated shaking, the cell layer became detached. The trypsinisation procedure was then stopped immediately using the trypsin inhibitor, methylphenylsulphonyl fluoride (85), and 1 ml. Isoton added subsequently. An inhibitor concentration of $10^{-4}$ molar was sufficient to stop the process of digestion. The cells were pipetted up and down six times to break up clumps, using a wide bore steel needle and syringe. The cells were transferred to a 25 ml. measuring cylinder using an Isoton wash, which was used to make up the final volume to the 25 ml. mark. Cells were counted and also sized using a Coulter counter (model Z with P64 size plotter), calibrated in the usual fashion using latex beads. Six plates were dealt with at one time and every plate was counted in duplicate at least.

**Counting of Isotope**

Immediately after cell counting, 5 ml. of the final solution of cells and isoton were taken into counting vials. Counting was performed on the Packard Tricarb instrument, No.3375. The isotopes studied were $^{22}$Na and $^{86}$Rb (which behaves as $^{42}$K, but is more convenient to handle by virtue of its longer half-life (17C)). Concentrations of isotopes in the growth and soak media were, for $^{86}$Rb 1 uc/ml. and for $^{22}$Na 5 uc/ml. Activities were sufficient to allow counting without addition of scintillant.
Measurement of ion concentrations.

Concentration of sodium and potassium ions in the soak solutions were measured in an EEL flame photometer, each assay being performed in duplicate.

Calculations


The diameters of 50 single cells were measured and averaged using a micrometer eye-piece, calibrated against a slide engraved with lines $10^{-3}$ cm ($10 \mu$) apart. Since trypsinised cells are spherical in shape, the volume may be calculated from cell volume, $V = \frac{4}{3}\pi r^3$ where $r$ is the measured cell radius (cm.). This is an approximate measure of cell volume, but Burrows & Lamb (40) and Mackinnon (188) have demonstrated that, whereas surface area changes markedly with cell shape (e.g. spherical to flat and elongate) there is no significant change in cell volume and that this is a satisfactory estimate of cell volume.


Direct measurement of cell surface area was performed as described by Mackinnon, 1969 (168), from measurements of cell depth, perimeter, and surface area facing the solution. The latter two measurements were obtained using a drawing tube attachment to the microscope, tracing the cell outlines on graph paper and measuring the area and perimeter against a standard obtained from a calibrated slide. The surface area facing into the solution was doubled to a count for the area facing the bottom of Petri dish (Burrows & Lamb (40)).
CALCULATED AND OBSERVED MEAN CELL VOLUME AS A FUNCTION OF CELL NUMBER IN MONOLAYER CULTURES OF GIRARDI CELLS.

**Fig. 22** Showing the mean cell volume, calculated regarding the cells as oblate spheroids, compared to measured mean cell volume over a range of cell populations.
A total of 50 cells from a thickly populated monolayer were so measured, 50 cells from a thinly populated monolayer were similarly measured and a figure for the near cell surface area for monolayers of different population densities was obtained, knowing the percentages of rounded and flattened cells in the monolayers.

Volume to surface area ($V/A$) ratios were calculated and graphed as shown opposite (Fig. 22). The required value for each plate was taken from this graph. No allowance was made for possible small surface projections and therefore the $V/A$ ratio is probably an over-estimate.

3. Determination of intracellular water.

The mean diameter of $10^6$ cells equilibrated as a suspension in Kreb's solution for one hour was calculated from measured diameters of 40 cells. 200mM excess KCl was then added and cell volume shrank. After one hour, the mean diameter was calculated from measured diameters of 40 cells.

Calculation.

The dry weight, $D$, of the cells stays constant. If the cell behaves as an osmometer, then $0_p x W_1 = 0_{p2} x W_2$ (equ. 1) where $0_p$ and $W$ are the cell water and osmotic pressures in Kreb (suffix 1) and hyperosmolar Krebs (suffix 2). On transfer, the cells lose water ($W_1 - W_2$).

\[
D + W_1 = V_1 \\
D + W_2 = V_2
\]

\[
W_1 - W_2 = V_1 - V_2
\]

and from this, $W_2$ is substituted in equ.1 and the volume of cell occupied by water is given by $W_1 = \frac{V_1 - V_2}{1 - \frac{0_{p1}}{0_{p2}}}$
TABLE 23.
IONIC PARAMETERS IN THE PRESENCE OF B-ADRENERGIC RECEPTOR BLOCKING DRUGS

<table>
<thead>
<tr>
<th>Drug</th>
<th>Drug Conc. (µg/ml)</th>
<th>Intracellular Ion Conc. a (µg/1 cell H₂O)</th>
<th>Ion Influx b (p-mole/cm² sec.)</th>
<th>Ion Efflux c (p-mole/cm² sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>K</td>
<td>Na</td>
<td>K</td>
</tr>
<tr>
<td>Propranolol</td>
<td>0.1</td>
<td>163±4</td>
<td>26±8</td>
<td>11±3</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>167±4</td>
<td>30±8</td>
<td>13±3</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>159±5</td>
<td>27±8</td>
<td>13±3</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>160±4</td>
<td>27±8</td>
<td>11±3</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>159±4</td>
<td>26±8</td>
<td>12±3</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>161±4</td>
<td>27±8</td>
<td>14±3</td>
</tr>
<tr>
<td></td>
<td>50.0</td>
<td>120±8</td>
<td>50±10</td>
<td>20±5</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>100±8</td>
<td>62±10</td>
<td>36±8</td>
</tr>
<tr>
<td>Practolol</td>
<td>0.5</td>
<td>159±4</td>
<td>26±8</td>
<td>13±3</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>163±4</td>
<td>30±8</td>
<td>13±4</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>165±4</td>
<td>26±8</td>
<td>12±3</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>90±8</td>
<td>70±11</td>
<td>40±7</td>
</tr>
<tr>
<td>Oxprenolol</td>
<td>0.5</td>
<td>155±4</td>
<td>27±8</td>
<td>13±3</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>159±4</td>
<td>22±8</td>
<td>11±3</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>160±4</td>
<td>26±8</td>
<td>14±3</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>105±6</td>
<td>60±10</td>
<td>35±8</td>
</tr>
<tr>
<td>Tolamolol</td>
<td>1</td>
<td>158±5</td>
<td>27±8</td>
<td>12±3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>160±4</td>
<td>22±8</td>
<td>11±3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>160±4</td>
<td>23±8</td>
<td>10±3</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>159±3</td>
<td>25±8</td>
<td>13±3</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>110±6</td>
<td>62±10</td>
<td>2±0</td>
</tr>
<tr>
<td>DRUG</td>
<td>OUABAINE CONC. (M)</td>
<td>INTRACELLULAR ION CONC. (meq/1 cell H₂O)</td>
<td>ION INFLUX (p-mole/cm² sec.)</td>
<td>ION EFFLUX (p-mole/cm² sec.)</td>
</tr>
<tr>
<td>----------------------</td>
<td>--------------------</td>
<td>------------------------------------------</td>
<td>-----------------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>K</td>
<td>Na</td>
<td>K</td>
</tr>
<tr>
<td>OUABAINE</td>
<td>0</td>
<td>160 ± 4</td>
<td>24 ± 8</td>
<td>11 ± 3</td>
</tr>
<tr>
<td>for 24 hours</td>
<td>10⁻⁸</td>
<td>150 ± 4</td>
<td>46 ± 8</td>
<td>10.5 ± 3</td>
</tr>
<tr>
<td></td>
<td>5.10⁻⁸</td>
<td>40 ± 6</td>
<td>130 ± 10</td>
<td>90 ± 3</td>
</tr>
<tr>
<td>OUABAINE + 5 ug/ml</td>
<td>10⁻⁸</td>
<td>148 ± 4</td>
<td>45 ± 8</td>
<td>10 ± 3</td>
</tr>
<tr>
<td>PROPRANOLOL</td>
<td>5.10⁻⁸</td>
<td>42 ± 6</td>
<td>126 ± 10</td>
<td>65 ± 3</td>
</tr>
<tr>
<td>OUABAINE (24 hrs) + 5 ug/ml PROPRANOLOL for 30 minutes</td>
<td>10⁻⁸</td>
<td>151 ± 4</td>
<td>46 ± 7</td>
<td>105 ± 3</td>
</tr>
<tr>
<td>for 30 minutes</td>
<td>5.10⁻⁸</td>
<td>35 ± 6</td>
<td>125 ± 9</td>
<td>90 ± 3</td>
</tr>
<tr>
<td>5 ug/ml PROPRANOLOL (24 hrs.) + OUABAINE for 12 hrs.</td>
<td>10⁻⁸</td>
<td>150 ± 4</td>
<td>45 ± 8</td>
<td>10 ± 3</td>
</tr>
<tr>
<td></td>
<td>5.10⁻⁸</td>
<td>45 ± 6</td>
<td>120 ± 10</td>
<td>8.5 ± 3</td>
</tr>
</tbody>
</table>
RESULTS

Table 23 lists the results obtained for the action of the beta-adrenoreceptor blocking drugs, Propranolol, Practolol, Oxprenolol and Tolamolol on sodium and potassium concentrations and fluxes. Over the therapeutically obtainable levels of these four drugs, intracellular potassium concentration and sodium concentration remained unchanged at around 160 and 26 meq/litre of cell water. Similarly, there is no change in ion influx and ion efflux parameters.

At drug concentration levels of 50 and 100 ug/ml, an apparent effect on these parameters is seen. This, however, is associated with reduction in cell numbers (to less than 75% of control plate values), with marked staining of cells with trypan blue, indicating loss of viability or membrane leakage, with increase in cell size when the cells are sized and with the presence of cell debris.

Table 24 shows that the ionic changes produced by two concentrations of Ouabaine (10^-8 and 5 x 10^-8 molar) in contact with the cells for 24 hours were unaffected by the addition of 5 ug/ml of Propranolol for the last 12 hours of the incubation. Nor was there any effect over a shorter period of incubation with Propranolol (30 minutes). Similarly, when the cells were incubated with Propranolol first and then with Ouabaine plus Propranolol, giving an exposure time to Propranolol of 24 hours and to Ouabaine of 12 hours, there was yet again no effect.

DISCUSSION

Studies designed to demonstrate a depressant effect of myocardial cells by beta-adrenoreceptor blocking drugs have largely utilised isolated cardiac papillary muscle preparations.
Using such a preparation, Naylar et al. (203) and Levy et al. (163) have claimed that Propranolol apparently depresses the twitch response of isolated cardiac muscle preparations. However, these results have been criticised on the grounds that the diameter of the muscle strips used was such that central hypoxia must have occurred and the apparent decline in twitch response with time in the presence of Propranolol was, in fact, the result of increasing hypoxia, as the tissue was repeatedly stimulated (Harry et al. 1974, (131), Blinks et al. 1963, (20)). Several workers - Harry et al. (131), Blinks (21), and Meier (193) have, in fact, produced data from this self-same experimental approach to support the thesis that beta-adrenoreceptor blocking drugs do not depress isolated cardiac muscle function when used in clinically attainable concentrations.

Recently, in a series of carefully controlled experiments using thin papillary muscle preparations, and skeletal muscle preparations as a control, Harry et al.(131) have shown no depression of twitch response by d-l Propranolol, Oxprenolol and Practolol at therapeutic concentrations.

The experiments described here have failed to show any effect of the four drugs studied, Propranolol, Practolol, Oxprenolol and Tolamolol, on intracellular ionic concentrations, ionic effluxes and influxes at therapeutic levels of drugs. At very high levels, an apparent effect is seen with a fall in intracellular potassium and a rise in intracellular sodium and increases in influx and efflux rates. However, this is almost certainly a toxic effect as evidenced by the points mentioned in the Results Section.
It is possible that many of the experimental claims for a direct depressant effect of beta-blockers on papillary muscle preparations are due to the presence of catecholamines in these preparations and apparent depressant effects are the results of beta-adrenergceptor blockade. Indeed, Choi et al. (47) have demonstrated that pre-treatment of a papillary muscle preparation by reserpine (which does not per se alter transmembrane sodium and potassium exchanges, but depletes adrenergic terminals of catecholamines) markedly reduces the ability of Propranolol to reduce potassium efflux in these preparations, suggesting that this drug may be functioning as a pure beta-blocker.

The experiments described here demonstrate that beta-adrenergceptor blocking drugs do not affect transmembrane sodium and potassium ion exchanges in this cell preparation, which is entirely free of catecholamine influence. Though these cells are an established cell line (i.e. are dedifferentiated dividing cells) they are still useful for studying transmembrane ion exchanges. In particular, they are sensitive to the action of cardiac glycosides with changes in intracellular ionic concentrations and fluxes that bear a proportional relationship to increasing glycoside concentrations Lamb et al. (156, 157).

The studies described here demonstrate that addition of Propranolol does not affect the ionic changes produced by two different concentrations of Ouabaine in contact with Girardi cells. No effect has been demonstrated despite incubation with Propranolol over 12 hours nor has any effect been demonstrated with a shorter incubation of 30 minutes, tending to suggest that Propranolol does not have a transitory action on Ouabaine-induced ionic alterations. When cells were incubated
with Propranolol first and then with Ouabaine plus Propranolol, there was yet again no effect, excluding the possibility that Ouabaine might become membrane-bound and so resistant to the action of Propranolol.

The lack of effect of Propranolol on this effect of cardiac glycosides indicates that the protective action in safe-guarding against glycoside-induced dysrhythmias is not exerted at the Na,K-ATPase level. Tse and Han (276) have demonstrated that the effect of Ouabaine on increasing Purkinje fibre automaticity is greatly augmented by the presence of adrenaline while Roberts et al (238) have shown that reserpine reduces the capacity of glycosides to induce extrasystoles in canine hearts. The experimental results detailed here would also tend to suggest that the action of digoxin in producing dysrythmia may be in large measure due to coincidental presence of catecholamines, and the protective action of beta-adrenoreceptor blocking drugs is, in fact, being exerted at this single site. Against this suggestion must be set the finding that d-Propranolol is less effective against catecholamine-induced tachyarrhythmias compared to l-Propranolol while d-Propranolol is as effective as d-l Propranolol in preventing Ouabaine-induced dysrhythmia in experimental animals; this appears to indicate a double site of action of d-l Propranolol (Parmley et al. (215)).

The lack of effect of Propranolol on sodium and potassium flexes contrasts sharply with the marked effect of this drug on human erythrocytes where major changes in fluxes occur with, as the drug concentration is raised, eventual rupture of the cells (Ekman et al. (75)). This might suggest that beta-adrenoreceptor blocking drugs exhibit a degree of tissue specificity and that their action, for example, on Purkinje
fibres may be quite different from their action on cardiac muscle fibres. However, El Shehawy et al. (76,77) have measured intracardiac conduction using His bundle electrograms in acutely and chronically hyperthyroid rats and demonstrated no effect of large doses of propranolol on the atrial–His time interval nor on the His-ventricular activation time. There was some reduction in heart rate in chronically hyperthyroid animals but the rate did not attain the euthyroid resting value; which is in keeping with a direct action of thyroid hormone on a thyroid-hormone specific adenyl cyclase.

The above studies, in conjunction with the clinical anecdote of induction of cardiac failure by $\beta$-blockade in a thyrotoxic patient, are evidence for a single cardiac site of action of $\beta$-blockers — the sympathetic receptor site and a lack of sympathetic hyperactivity in thyrotoxicosis. This would suggest that the effect of $\beta$-blockade on heart is a reduction in rate due to removal of sympathetic tone but not a normalising of rate — due to the direct cardiac actions of thyroid hormone. It raises the possibility that heart rate in hyperthyroidism, after $\beta$-blockade, may give a measure of response of a target tissue — the heart — to the severity of the thyrotoxicosis.

This data indicates that cardiac protection (important in elderly and cardiac patients) by $\beta$-blockade will be at best only partial and, therefore, treatment with $\beta$-blockade alone (as has been advocated prior to surgery (195) is potentially hazardous.

It is pertinent to observe that while $\beta$-blockade reduces pulse rate, tremor, stare and skin conductance (117,178,261), the effects of such therapy in one trial, were not sufficient to allow patient preference for $\beta$-blocker (propranolol, practolol) over placebo to reach statistical significance (204). Similarly,
patients showed no preference for oxprenolol (98). Another study failed to show any reduction in anxiety levels with β-blockade (229). Propranolol does not reduce the metabolic rate in hyperthyroidism (223) which further emphasises the secondary role of β-blockade in thyrotoxicosis - namely to alleviate some of the peripheral manifestations of the disorder while definitive therapy is introduced.
<table>
<thead>
<tr>
<th>PATIENT</th>
<th>SEX</th>
<th>AGE (YEARS)</th>
<th>CLINICAL STATUS</th>
<th>THERAPY</th>
<th>TIME SINCE THERAPY (MONTHS)</th>
<th>HORMONAL LEVELS</th>
<th>TRH TEST ΔTSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>W.L.</td>
<td>F</td>
<td>52</td>
<td>E</td>
<td>E</td>
<td>12</td>
<td>T4: 273</td>
<td>T3: 5.60</td>
</tr>
</tbody>
</table>

HORMONAL VALUES IN S.I. UNITS
PHYSIOLOGICAL AND METABOLIC RESPONSES TO THYROID HORMONES

I. SYSTOLIC TIME INTERVAL MEASUREMENTS: A NEW TEST OF THYROID STATUS

INTRODUCTION

The details opposite (Table 25) describe a patient one year after a therapy dose of radio-iodine for thyrotoxicosis. She is biochemically very toxic but clinically euthyroid.

At the other extreme are found patients with modest biochemical alterations but a severely thyrotoxic clinical presentation.

Discrepancies such as these point to the need for a means of measuring the body's response to thyroid hormones, in particular, a measure of cardiovascular response would be particularly valuable as this system is the most frequently compromised and most often at risk, particularly, in the elderly thyroid patients.

Measurement of basal metabolic rate is a measure of whole body response to thyroid hormones; it is, however, a time consuming, unwieldy investigation necessitating preparation of the patient (who has to be in the basal state), and it is disturbed by many factors unrelated to thyroid status (14). These drawbacks have relegated this test to a position of relative unimportance.

Likewise, measurement of the Achilles tendon reflex time has found a place in assessment of hypothyroidism but its value in hyperthyroidism is disputed (237,255).
In collaboration with Dr. M.S. Hilles, Senior Registrar, Department of Medical Cardiology, the author embarked on an assessment of systolic time interval measurements as there was suggestive evidence in published work that such measurement might offer a test of sufficient sensitivity to permit assessment of cardiovascular response to circulating thyroid hormone levels.

**Significance and Origin of the term: Systolic Time Intervals (STI's)**

Systolic time interval refers to a period within the systolic part of the cardiac cycle. Emphasis on the significance of the duration of systole dates back to Parrot (1874)(97) but it was with the detailed studies of Wiggers in 1921 (311,312) that the foundations were laid for the current widespread use of STI's in clinical cardiological practice. Most of the present-day use of STI's stems from the work of Weissler's group (299-302).

The heart may be regarded as two pumps in series, both of variable rate, stroke and pressure production. They both share the same rate. Each pump has two cylinders. The atrium is equivalent to a receptor for returning low pressure fluid and as a pump for pumping this fluid into the second cylinder, the ventricle, which is the high pressure pump.

The pressure that this pump is capable of exerting on the fluid depends on the diastolic or resting muscle fibre length (Frank-Starling Law) and up to a limit the pressure exerted increases with increasing fibre length. Thus, the greater the volume of the ventricle prior to contraction (end diastolic volume), the greater the force that can be produced by the fibres. The velocity of fibre contraction is related to the resistance that the blood meets as it is ejected (the...
afterload), increasing afterload reducing velocity of contraction. This is the physiological basis of STI's. The pre-ejection period, or P.E.P., is subdivided into the electro-mechanical delay (E.M.D.) and the isovolumetric contraction time (I.V.C.T.). These two intervals are not divisible accurately as some contraction of the ventricular muscle will have been initiated before the excitation wave spreads throughout the entire ventricle, but contraction is generally taken as that point at which intraventricular pressure (measured invasively) begins to rise.

The E.M.D. reflects the intraventricular conduction pattern and is lengthened, for example, by bundle branch block or altered by biochemical delay or acceleration of cell to cell excitation.

The I.V.C.T. is related to the rate of rise of left ventricular pressure (LV dp/dt) which is an index of myocardial contractility.

The contractile state of the muscle is affected by positive inotropic agents such as thyroid hormone, an increase in metabolism and by cardiac glycosides and sympathetic venous stimulation: all these factors shorten P.E.P. An increase in end-diastolic volume will shorten P.E.P. Negative inotropic agents depress contractility and cause P.E.P. to lengthen.

I.V.C.T. is affected by the diastolic pressure in the arteries: if the pressure is low, the aortic valve opens sooner and thus a low diastolic pressure tends to shorten I.V.C.T. and hence P.E.P.

An increased velocity of muscle fibre contraction raises the systolic blood pressure (and mean aortic pressure). An increased aortic pressure lengthens P.E.P. as the intra-ventricular pressure has to be raised to open the aortic valve, prolonging
Increases in stroke volume and heart rate increase cardiac output which has the same effect of raising mean aortic pressure.

Heart rate is related to P.E.P. Increased sympathetic tone increases rate and this also tends to increase the contractility; this increase tends to reduce the I.V.C.T. by increasing the velocity of contraction.

Four factors, therefore, primarily effect I.V.C.T.

1) Preload which is the diastolic muscle fibre length, or the end-diastolic volume.

2) Afterload or the resistance to outflow which is determined by the aortic diastolic pressure.

3) Myocardial contractility.

4) Ventricular dyssnergy.

L.V.E.T. is affected by the same factors. Heart rate, however, appears to be more significant and L.V.E.T. is directly related to stroke volume.

An increase in preload means muscle fibres have further to contract which tends to take longer and so increase L.V.E.T. and stroke volume.

The clinical uses of systolic time interval measurement have been thoroughly reviewed by Weissler (302).

This study was intended to assess the sensitivity of systolic time interval measurement to thyroid status to determine whether such a measure would afford an accurate and sensitive measure of cardiovascular response to thyroid hormones.
Systolic Ejection Times

QS₂ Total Electromechanical Systole
LVET Left Ventricular Ejection Time
PEP Pre Ejection Period

FIG. 23 MEASUREMENT OF SYSTOLIC TIME INTERVALS.
METHODS:

Systolic time intervals were measured from simultaneous recordings of an electrocardiogram, phonocardiogram and carotid arterial pulse tracing (Fig. 23). The phonocardiogram was taken from a crystal microphone (Elma Schonander EMT 25C) placed over the aortic valve area. An electrocardiogram lead was used which gave a clear QRS complex representing ventricular depolarisation. The carotid pulse wave was recorded using a plastic tambour connected by an air-filled conducting system to a high speed ink jet recorder (Kongograf 34). The recording speed was 100 millimetres per second, the measured intervals were obtained by averaging values from six beats to the nearest five milliseconds.

The frequency response of the Elma Schonander EMT 510 C transducer and its amplifier is greater than 80 Herz.

The intervals measured were (1) the total electromechanical systole (QS2) measured from the commencement of ventricular depolarisation to the first high frequency vibrations of the aortic component of the second heart sounds, representing aortic valve closure; (2) the left ventricular ejection time (LVET) measured from the upstroke of the arterial pulse to the trough of the incisural notch; (3) the pre-ejection period (PEP) is obtained by subtracting the left ventricular ejection time from the electro-mechanical systole and represents the interval from ventricular depolarisation delay and the isovolumic contraction time of the left ventricle; (4) the ratio of pre-ejection period to left ventricular ejection time (PEP/LVET) was also calculated. This ratio encompasses deviations in both basic intervals and may reflect abnormality when neither is clearly abnormal.
<table>
<thead>
<tr>
<th>Systolic Interval</th>
<th>Sex</th>
<th>Regression Equation</th>
<th>S.D. Index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$QS_2$</td>
<td>M</td>
<td>$QS_2 = -2.1 \times HR + 53.6$</td>
<td>5.6 ± 11</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>$QS_2 = -2 \times HR + 53.9$</td>
<td>5.9 ± 11</td>
</tr>
<tr>
<td>$FEP$</td>
<td>M</td>
<td>$FEP = -0.4 \times HR + 131$</td>
<td>131 ± 13</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>$FEP = -0.4 \times HR + 133$</td>
<td>133 ± 11</td>
</tr>
<tr>
<td>$LVEF$</td>
<td>M</td>
<td>$LVEF = -1.7 \times HR + 413$</td>
<td>413 ± 10</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>$LVEF = -1.6 \times HR + 418$</td>
<td>418 ± 10</td>
</tr>
</tbody>
</table>

$FEP/LVEF$ ratio = 0.333 ± 0.044
The duration of the QRS, LVET and PEP are linearly related to the heart rate and regression equations have been derived from observations on normal individuals (Weissler, 301) (Fig. 24). Results from the present studies were expressed as "measured" intervals, measured in absolute terms (milliseconds) and were compared with corresponding normal values ("calculated" values) calculated from the appropriate regression equations. The results were also expressed and shown as the systolic time interval indices (suffix I) calculated as the sum of the measured interval and product of the heart rate and normal regression slope. This offers a convenient expression for studying serial changes and facilitates comparisons among patients by normalising the results for heart rate. The measured values of each of the systolic time intervals expressed as the systolic time interval indices were compared to the calculated values ("calculated" parameters) obtained from the regression equations using the Student's T-test. The results are expressed as the mean and standard deviation of the mean for each group.

Systolic time intervals were measured in 20 hyperthyroid patients (Table 2b) and 15 hypothyroid subjects (Table 27). The diagnosis of hyperthyroidism was made on clinical examination and confirmed by PB$^{127}$I estimation (values > 670 nmol/l) and $^{131}$I uptake studies (24 hour thyroidal uptake > 50%), PB$^{131}$I at 48 hours > 0.3% dose/l plasma). The diagnosis of hypothyroidism was suggested by clinical examination and confirmed by PB$^{127}$I estimation (values < 230 nmol/l) and serum thyroxine assay (values < 60 nmol/l).

No patients had signs nor symptoms of concomitant cardiac disease, none had conduction defects on routine electrocardiography, all were in sinus rhythm and none was receiving
therapy which might affect the systolic time intervals.

Five patients from each group (hyper- and hypothyroid) had measurements performed after appropriate therapy. These patients appeared euthyroid on clinical examination and this was confirmed by normal $PB^I_{127}$ and serum thyroxine estimations (patients number 1 to 5 in Tables 1 and 2) (Table 28).

In the third study, 74 thyroid patients were assessed without knowledge of their clinical details. Their thyroid status was predicted from the changes in systolic time intervals, when compared to normal, as determined in the first study. Their thyroid status was also determined independently by the referring physician from clinical and routine thyroid investigation. This group included previously undiagnosed patients, but also others being reviewed following $^{125}$I radio-iodine therapy for thyrotoxicosis. The predicted thyroid state was then compared with the independent prediction inferred from systolic time interval measurements. The diagnoses established after full investigations were: 36 patients hyperthyroid, 12 of whom had had previous therapy; 11 patients were hypothyroid, eight had been previously thyrotoxic; 27 patients were euthyroid, 21 had had therapy for hyperthyroidism and six presented with signs and symptoms of anxiety and were referred to exclude thyrotoxicosis (29,30,31,32,33).

RESULTS

The hyperthyroid patients showed shortening of the total electro-mechanical systole 517± 21 (calculated value 549±, 1/p < 0.001) resulting from a normal left ventricular ejection time, 418± 20 (calculated value 417± 1, and
<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (Years)</th>
<th>Sex</th>
<th>Hours Full Time/Week</th>
<th>CGS</th>
<th>CGS 1 Mean, Cals.</th>
<th>LNS</th>
<th>LNS 1 Mean, Cals.</th>
<th>ESP</th>
<th>ESP 1 Mean, Cals.</th>
<th>ESP/ESP 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24</td>
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Total: 418±20 417±21

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**Note:**
- **QRS 1** and **QRS 2** are measured and calculated heart rates.
- **LVEF 1** and **LVEF 2** are measured and calculated left ventricular ejection fractions.
- **PEP** and **PEP 1** are measured and calculated parameters related to the left ventricular ejection fraction.
- **PEP/LVEF** is the ratio of **PEP** to **LVEF**.

**Statistical Significance:**
- **P < 0.01** indicates statistical significance at the 0.01 level.


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Values before treatment:

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- Hyper Group B: 555±10 153±7 405±12 0.424±0.034
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<td>41.0</td>
<td>123</td>
<td>133</td>
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<td>Patient No.</td>
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<td>Heart Rate/Minute</td>
<td>( \text{S}_2 ) L</td>
<td>DEI 1 Meas. Prod.</td>
<td>DEI 1 Pred.</td>
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<td>4</td>
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<td>F</td>
<td>65</td>
<td>570</td>
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shortening of the pre-ejection period $100^{+\pm} 8$ (calculated value $132^{+\pm} 1$, $p<0.001$). The PEP/LVET ratio was reduced $0.231^{+\pm} 0.04$ (calculated ratio $0.340^{+\pm} 0.40$, $p<0.001$). (Table 26).

The hypothyroid subjects showed prolongation of the total electro-mechanical systole $563^{+\pm} 14$ (calculated value $548^{+\pm} 1$, $p>0.01$) which resulted from shortening of the left ventricular ejection time $402^{+\pm} 17$ (calculated value $416^{+\pm} 2$, $p<0.01$) and marked prolongation of the pre-ejection period $161^{+\pm} 10$ (calculated value $132^{+\pm} 2$, $p<0.01$). The PEP/LVET was increased $0.459^{+\pm} 0.056$ (calculated value $0.350^{+\pm} 0.04$, $p<0.001$). (Table 27).

In each of the patients studied after therapy, the pre-ejection period and PEP/LVET ratio were within the normal range (mean plus one standard deviation of calculated values). Some variance was found in the duration of the QS and LVET (Table 28).

The systolic time interval values for each patients in the third study are shown (Tables 29, 30, 31, 33). From the first two studies the pre-ejection period and PEP/LVET showed the most consistent changes and these intervals were used to predict the status of the patients. The diagnosis of hyperthyroidism was predicted from the association of a shortened pre-ejection period and reduced PEP/LVET (the normal comparative range was taken as the calculated mean $\pm$ one standard deviation). In two patients the PEP was shortened, but the PEP/LVET was borderline, these patients were assessed as hyperthyroid (numbers 1 + 10, Table 29).

Subjects were designated hypothyroid from lengthening of the pre-ejection period and an increased PEP/LVET.
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<th>Patient No.</th>
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<th>LVED 1</th>
<th>FDP 1</th>
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<td>82</td>
<td>52.2</td>
<td>413</td>
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</tbody>
</table>

Note: X/LVED 17/1 suggests results in normal ranges.
Two patients with a prolonged PEP, but borderline PEP/LVET, were also designated hypothyroid (numbers 3 and 10, Table 30).

All patients, euthyroid following radio-iodine therapy, had normal pre-ejection periods and PEP/LVET ratios. Three patients designated as "anxious" had a slightly increased PEP/LVET, but all had normal pre-ejection periods (Table 31).

The predicted diagnoses made, as indicated above, agreed with that made by the referring physician in 70 of 74 cases, (95%). Discordant diagnosis occurred in four patients (Table 32). No obvious cardiological explanation is available to explain the difference in these patients. They were of similar age, duration of symptoms and therapy as the other patient groups.
DISCUSSION

Systolic time interval measurements afford a non-invasive, repeatable and rapid assessment of cardiac function and have been reviewed by Kumar and Spodick, 1970 (154), Weissler, Harris and Schoenfield 1965 (301) and in a textbook by Weissler (302).

This study falls into two parts: in the first, it is demonstrated that consistent changes in the pre-ejection period and PEP/LVET ratios occur in unequivocally hypo- and hyperthyroid patients. The return to normal of these values in ten patients rendered euthyroid supports the belief that measurement of such values may be of value in determining the thyroid status of an individual.

To confirm this hypothesis, the thyroid status was predicted in a mixed group of thyroid clinic patients providing a difficult clinical spectrum; which resulted in hyper-, hypo- and euthyroid subgroups. Some patients were minimally hypo- or hyperthyroid and retained conflicting clinical signs but, even in this group, there was a high degree of correlation between predicted diagnosis and that determined independently after full investigation of thyroid function. This appears to confirm systolic time interval measurements as a sensitive indication of the cardiovascular changes induced by thyroid disease.

Six patients presented with tremor, weight loss and/or tachycardia and were found to have interval and thyroid studies within the normal ranges; a diagnosis of anxiety state was eventually made in these patients by psychiatric assessment and exclusion of other possible organic pathologies. Tri-iodothyronine levels were normal in this group, excluding $T_3$ - toxicosis.
Harris et al (139) reported shortening of the pre-ejection period in subjects with increased endogenous sympathetic activity under emotional tension, anxiety, and with psychosis. Such patients may be differentiated from thyrotoxic patients by administration of a beta-adrenoreceptor blocking agent which reverses the changes in anxiety, but does not affect the pre-ejection period in thyrotoxic patients (117).

The diagnosis of thyroid disease depends in the first instance on suspicion followed by adequate clinical examination; this is adequate to detect severe examples of thyrotoxicosis or myxœdème and diagnosis is confirmed by subsequent investigation.

Problems arise when patients have minimal stigmata of thyroid disorder; this is particularly so in the minimally hypothyroid patient who may remain undetected until presenting with angina or congestive cardiac failure. Clinical assessment may frequently be misleading (65a, 279). Assessment after therapy also poses obvious difficulties. The use of biochemical tests as screening procedures is also open to error (132); in particular, a large number of the general population have spuriously elevated PBI's, and the contraceptive pill elevates thyroxine levels. The diagnosis of T3 toxicosis requires the radio immunoassay of tri-iodothyronine, an expensive, time-consuming technique not generally available. In addition, there is the group of patients with "compensated" euthyroidism, i.e. with normal T4 and T3 levels, but raised TSH levels, indicative of thyroid dysfunction compensated for by pituitary hyper-stimulation. At present, no single ideal thyroid test is available and a compromise battery of tests may be required covering many aspects of thyroid function in the individual patient. In the presence
of conflicting tests the clinical assessment of the functional status of the patient by the physician often decides the management of the patient.

It should be noted that the patients studied had no overt cardiac disease on clinical and ECG examination and fall into discrete "hemodynamic" groups according to their thyroid status, so that alterations in systolic time intervals carry a higher degree of diagnostic specificity than is the case in myocardial infarction or cardiac failure where many variables influence cardiac function.

It would seem that measurement of systolic time intervals may have a place in the assessment of this group of patients and may be of more general value where a fully comprehensive set of thyroid function tests is not readily available. These studies have shown such measurements to carry a high degree of specificity, much more so than the now outmoded measurement of basal metabolic rate. Systolic time interval measurements offer an accurate measure of the "peripheral" or target-organ effect in thyroid disease and may well be useful in assessing the elderly who are most sensitive to the cardiac effects of thyroid hormones.

CONCLUSION

The studies described here suggest the following potential benefits of STI measurement in thyroid disease.

1) A useful measure of cardiac response to thyroid hormonal status that may assist in deciding, for example, whether to treat or re-treat apparently mildly thyrotoxic patients.
2) A useful measure for sequential studies of individual thyroid patients.

3) A potential means of detecting "at risk" patients, if it can be shown that shortening or prolongation of P.E.P. in hyper- and hypothyroidism is a measure of severity of thyroid hormone effect on heart and indicative of impending cardiac decompensation, dysrhythmias etc.

THE RELATIONSHIP BETWEEN SERUM THYROID HORMONE LEVELS AND SYSTOLIC TIME INTERVAL MEASUREMENTS.

In an attempt to define a simple relationship between the levels of circulating thyroid hormone levels and systolic time interval measurement, data from 20 patients was analysed for simple mathematical correlations. The data covered euthyroid, thyrotoxic and hypothyroid patients and the scatter seemed sufficiently wide to obviate selection bias. Table 36 lists the raw data.

Using a Wang desk top electronic calculator, with a programme for deriving linear regressions, the values of \( T_4 \) and \( T_3 \) were separately correlated with each of the systolic time interval parameters in turn and a linear relation sought. No significant correlation was obtained.

Next, the \( T_4 \) and \( T_3 \) values were run against the log values of each of the corresponding STI measurements. Again, no significant linear correlation was obtained. This approach was extended, using a Philip's computer and a "Polfit" programme for fitting of variables to a polynomial function - i.e., a relationship of the form
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<th>II</th>
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<td>70</td>
<td>107</td>
<td>2413</td>
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</tbody>
</table>

**NOTE:** Hormonal data was processed when thyroxine was assayed as $\mu g/100$ ml. and triiodothyronine as mg/ml.
<table>
<thead>
<tr>
<th>S.T.I.</th>
<th>RELATIONSHIP</th>
<th>ACCURACY OF FIT</th>
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</tr>
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<td>P.E.P.</td>
<td>$PEP = 100.9 - 3.43 T_4 + 6.69 T_3$</td>
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</table>
\[ Y = aZ + bZ^2 + cZ^3 + \text{etc.} \] where \( Z \) is the STI measurement under study \( Y \) the \( T_4 \) or \( T_3 \) value and \( a, b, c \) etc. are constants.

No significant relationship up to \( Z^4 \) appeared and repeat fitting, this time using the log of \( Z \), was similarly unproductive.

Finally, as neither hormone gave a significant relationship when compared repeatedly to the STI value (as estimated by the standard error of the estimate for \( Y \)), a relationship of the form \( Z = aX + bY + c \), was sought, where \( Z \) is the STI parameter, \( X \) and \( Y \) are the \( T_4 \) and \( T_3 \) values and \( a, b, c \) are contrasts. The relationship \( \log Z = aX + bY + c \) was also evaluated. This approach was performed using a computer programme for multiple regression.

Accuracy of fit was assessed by the root mean square as a fraction of the mean value of \( Z \).

Table 37 opposite lists the significant relationships. Serum hormone levels relate well to heart rate and pre-ejection period, less well to the other parameters.

Such derivations do not, of course, prove that such postulated relationships between cardiovascular and hormonal parameters actually exist.
II. ZINC HANDLING AND THYROID STATUS

A MEASURE OF SEVERITY OF THYROTOXICOSIS

INTRODUCTION

Certain aspects of the thyrotoxic process such as the exophthalmos and pre-tibial myxoedema, may not necessarily reflect the metabolic severity of the disease process, but rather relate to the underlying pathological process. This may render indices such as the Wayne index misleading in individual cases as a measure of the severity of thyrotoxicosis.

What is required is a measure of the metabolic impact of the disorder. It seemed that degree of weight loss might afford a measure of the severity of the condition and a metabolic correlate of weight loss was sought. In the light of reports of increased zinc losses in malnutrition in kwashiorkor (135), the metabolism of this metal was studied in patients with thyroid disease.

Plasma zinc levels and 24 hour urinary zinc outputs were measured in 24 thyrotoxic and ten hypothyroid patients. The findings were related to thyroid status, hormonal parameters, weight changes and to a scale assessing acuteness and severity of the metabolic response to hyper- or hypothyroidism.

PATIENTS & METHODS

Thirty-four patients with unequivocal clinical evidence and biochemical confirmation of hyper- or hypothyroidism were studied. Hyperthyroid patients were selected on the basis of fairly severe symptomatology, particularly of rapid weight loss.
<table>
<thead>
<tr>
<th>Thyroid Status</th>
<th>Thyrotoxic</th>
<th>Euthyroid</th>
<th>Hypothyroid</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Patients</td>
<td>24</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Heart rate</td>
<td>100 (12)  <em>P</em> &lt; 0.001</td>
<td>70 (8)  P NS</td>
<td>65 (15)</td>
</tr>
<tr>
<td>mean (SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting serum</td>
<td>5.44 (1.04)  P &lt; 0.05</td>
<td>6.22 (0.78)  P &lt; 0.005</td>
<td>7.51 (0.91)</td>
</tr>
<tr>
<td>cholesterol</td>
<td>mean (SD) mmol/l.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum thyroxine</td>
<td>194 (23)  <em>P</em> &lt; 0.001</td>
<td>105.5 (17)  P &lt; 0.001</td>
<td>27 (13)</td>
</tr>
<tr>
<td>mean (SD) mmol/l.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum tri-iodothyronine</td>
<td>3.0 (1.2)  <em>P</em> &lt; 0.01</td>
<td>1.7 (0.2)  P &lt; 0.001</td>
<td>0.58 (0.21)</td>
</tr>
<tr>
<td>mean (SD) mmol/l.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma zinc</td>
<td>14.74 (2.23)  P NS</td>
<td>14.24 (2.50)  P NS</td>
<td>14.12 (2.39)</td>
</tr>
<tr>
<td>mean (SD) umol/l.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc clearance</td>
<td>0.70 (0.33)  <em>P</em> &lt; 0.001</td>
<td>0.34 (0.12)  P NS</td>
<td>0.32 (0.05)</td>
</tr>
<tr>
<td>mean (SD) ml/min.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc clearance x 100</td>
<td>0.636 (0.31)  <em>P</em> &lt; 0.001</td>
<td>0.309 (0.110)  P NS</td>
<td>0.32 (0.041)</td>
</tr>
<tr>
<td>Creatinine clearance</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* *P* values calculated by paired t-testing.
Patients were scored on the Wayne Index (17,56) as a first approximation of the clinical severity of thyroid malfunction. They were also assessed on a scale designed to give a measure of acuteness and severity of the metabolic response to thyroid over- or underactivity (fig. 25). This rating was composed of rate of weight change (amount of weight gain or loss; duration of symptoms), basal (resting) heart rate, fasting plasma cholesterol, serum thyroxine level and serum tri-iodothyronine level.

Plasma levels of zinc and 24 hour urinary zinc excretion were measured by the method of Peaston (217).

Zinc clearances (ml/min) were calculated as rate of urinary excretion over 24 hours (ml/min) x urinary zinc concentration + plasma zinc concentration.

Serum thyroxine levels were measured by the Thyopac 4 (Amersham) method and serum tri-iodothyronine ($T_3$) levels by a radio-immunoassay procedure.

Serum cholesterol was measured on samples obtained after a 12 hour fast by the automated method of Annan and Isherwood (9).

Twenty-two patients were studied as outpatients and 12 as inpatients. All the patients were female, except for four thyrotoxic ones. Ten healthy subjects, clinically and biochemically euthyroid, served as controls. The mean age and ranges for hyper-, hypo- and euthyroid control groups were similar. All subjects studied had normal renal function as judged by plasma urea levels and creatinine clearances performed at the time of the zinc clearance studies.

RESULTS

Table 34 lists relevant data for subjects grouped according to thyroid status. Zinc clearance, but not plasma zinc levels
### TABLE 35

CORRELATIONS BETWEEN ZINC CLEARANCE AND RELEVANT PARAMETERS IN 24 THYROTOXIC PATIENTS

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>CORRELATION COEFFICIENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (resting)</td>
<td>0.87</td>
</tr>
<tr>
<td>Rate of weight loss</td>
<td>0.92</td>
</tr>
<tr>
<td>Serum thyroxine</td>
<td>0.71</td>
</tr>
<tr>
<td>Serum tri-iodothyronine</td>
<td>0.64</td>
</tr>
<tr>
<td>Serum cholesterol</td>
<td>0.60</td>
</tr>
<tr>
<td>Wayne Index</td>
<td>0.61</td>
</tr>
<tr>
<td>Severity/acuteness score</td>
<td>0.86</td>
</tr>
<tr>
<td>Plasma zinc</td>
<td>0.42</td>
</tr>
</tbody>
</table>
### Figure 25

**Rating Scale to Assess Acuteness and Severity of Metabolic Response to Thyrotoxicosis**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Score</th>
<th>Parameter</th>
<th>Score</th>
<th>Parameter</th>
<th>Score</th>
<th>Parameter</th>
<th>Score</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Rate</td>
<td></td>
<td>Serum Cholesterol</td>
<td></td>
<td>Serum Thyroxine</td>
<td></td>
<td>Serum Tri-iodothyronine</td>
<td></td>
<td>Rate of weight loss</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mmol/l</td>
<td></td>
<td>mmol/l</td>
<td></td>
<td>mmol/l</td>
<td></td>
<td>Kg/week</td>
</tr>
<tr>
<td>70 - 80</td>
<td>0</td>
<td>2.59</td>
<td>5</td>
<td>154</td>
<td>0</td>
<td>2.2 - 2.5</td>
<td>0</td>
<td>0.27</td>
</tr>
<tr>
<td>91 - 90</td>
<td>1</td>
<td>2.59 - 3.63</td>
<td>4</td>
<td>154 - 180</td>
<td>1</td>
<td>2.6 - 3.0</td>
<td>1</td>
<td>0.28 - 0.32</td>
</tr>
<tr>
<td>91 - 100</td>
<td>2</td>
<td>3.64 - 4.66</td>
<td>3</td>
<td>181 - 206</td>
<td>2</td>
<td>3.1 - 3.5</td>
<td>2</td>
<td>0.33 - 0.36</td>
</tr>
<tr>
<td>101 - 110</td>
<td>3</td>
<td>4.67 - 5.70</td>
<td>2</td>
<td>207 - 232</td>
<td>3</td>
<td>3.6 - 4.0</td>
<td>3</td>
<td>0.37 - 0.41</td>
</tr>
<tr>
<td>111 - 120</td>
<td>4</td>
<td>5.71 - 6.73</td>
<td>1</td>
<td>233 - 257</td>
<td>4</td>
<td>4.1 - 4.5</td>
<td>4</td>
<td>0.42 - 0.45</td>
</tr>
<tr>
<td>121</td>
<td>5*</td>
<td>6.74</td>
<td>0</td>
<td>258</td>
<td>5</td>
<td>4.6 - 5.0</td>
<td>5</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>75.0</td>
</tr>
</tbody>
</table>

* including 2 patients in atrial fibrillation.
are significantly higher in thyrotoxic patients compared with euthyroid or hypothyroid subjects (p < 0.001). The measured parameters of zinc metabolism do not differ significantly in euthyroid and hypothyroid groups.

Table 35 details the degree of correlation between zinc clearance and other measures of thyroid status in thyrotoxic patients. Rate of weight loss shows the best correlation (r = 0.92). The Wayne Index score correlates rather poorly (r = 0.61) whereas there is a highly significant correlation (r = 0.86) between the score for the index of severity and acuteness of metabolic response shown in Fig. 25.

Correlations for hypothyroid patients are not significant. It proved impossible to assess accurately the duration of symptoms as onset was usually insidious and so rate of weight gain could not be estimated with any accuracy.

**DISCUSSION**

It is perhaps surprising that, despite widespread interest in zincuria, as an indicator of catabolism (135), few studies have been performed in thyrotoxic patients who would seem to offer a good model for tissue catabolism. One study did report a fall in red cell zinc concentrations in thyrotoxics and considered this a possible specific action of thyroxine on red cell zinc metalloenzymes (218).

This present investigation has demonstrated a significantly higher zinc output in urine in thyrotoxic patients compared to euthyroid or hypothyroid patients. The correlation between clearance and Wayne Index score was poor, possibly because the Index contains items (e.g. ocular signs) that relate more to the underlying pathogenesis of the disorder than to the severity of the metabolic changes induced by elevated hormone levels. The best correlation was with rate of weight
No significant correlations were found in the ten hypothyroid patients, though five of them had marked weight gain (> 12.5 Kg.). The onset of the disorder was insidious and the duration of the disorder uncertain, and this may explain the lack of positive findings. It may be that a study of zinc metabolism in "acutely" hypothyroid patients would show evidence of zinc retention.

Plasma zinc levels were maintained in thyrotoxic patients (no significant difference from levels in euthyroid controls) despite the increased urinary losses. Dividing zinc clearance by creatinine clearance did not affect the significance of the correlations, and suggests that the increased urinary zinc loss is not due to a renal effect of thyroid hormones.

The data presented here suggests that urinary zinc losses in thyrotoxicosis are correlated with catabolic status and not a specific effect of thyroid hormones on, say, zinc metalloenzymes.

It has been suggested that tissue catabolism releases amino acids and other constituents into the circulation; these compounds form stable co-ordination compounds with zinc. Normally, some 10% of total plasma zinc exists in this form in equilibrium with a further 60-70% of plasma zinc loosely attached to plasma albumin. The remaining plasma zinc is largely present in chemical combination as part of the metalloprotein 2 2 μ2-macroglobulin (87,100). The main excretory pathway for zinc is via the gut. Renal losses are in the form of low molecular weight complexes as these alone normally pass the glomerulus. The excessive losses described in severe thyrotoxicosis in this study indicate an
increased loss of tissue breakdown products or an alteration in the equilibrium with albumin-bound zinc. This point appears worthy of further study.

No clinically significant consequence of increased zincuria was noted in the thyrotoxic patients, but it seems likely that the occurrence of thyrotoxicosis in patients with malabsorption of zinc, phenylketonuria, prolonged parenteral nutrition, or other conditions where zinc is deficient (87,100) will exacerbate the latter disorders.

This investigation suggests that measurement of zinc losses in thyrotoxic patients may offer a measure of the "metabolic stress" of the disease process and permit grading of thyrotoxic patients in terms of severity of the impact of the disease on the body.

CONCLUSION

These two studies, which form Section D of this thesis, offer methods of assessment of patients' response to thyroid malfunction and may permit selection of "at risk" patients, sequential measurement of therapy in terms of change in severity of the condition and may, particularly, offer objective evidence for the physician's assessment of his patient.
SECTION E

THYROID AND HEART

INTRODUCTION

This section consists of 2 parts. It is partly speculative but important in that it examines some aspects of the relationship between thyroid and heart.

Whereas the typical appearance of the young woman with Graves' disease presents little diagnostic difficulty, older patients - and such patients formed the bulk of those participating in the Trial - often have occult thyrotoxicosis until a cardiac problem is precipitated. The cardiac complications of thyrotoxicosis are a major source of the morbidity and mortality that still attends this disease.

Part I examines the evidence for the clinical entity of thyrotoxic cardiomyopathy and describes an animal study pertinent to the concept.

In part II, the relationship between lipid and lipoprotein metabolism and thyroid status is explored with particular emphasis on the acceleration of coronary atherosclerosis. Studies on patients with compensated euthyroidism and subclinical hypothyroidism (see page 73) are adduced and the possible risks of delaying thyroxine replacement therapy argued.

SECTION E

PART I. THYROID AND HEART: THYROTOXIC CARDIOMYOPATHY

Thyrotoxicosis is an important factor in the production of atrial fibrillation - 10% of the patients in the Trial were in atrial fibrillation - in the production of cardiac
enlargement - 8% of 165 x-rayed had cardiomegaly -, and in the production of congestive cardiac failure - 6% were in failure at diagnosis. There is still, however, considerable uncertainty whether thyrotoxicosis per se is responsible for these manifestations, that is, it is still a matter of dispute as to whether there is a distinct entity of heart disease caused by thyrotoxicosis, a "thyrotoxic cardiomyopathy". Symptoms such as dyspnoea or palpitation are common in uncomplicated thyrotoxicosis and so the term thyroid heart disease is generally restricted to patients with thyrotoxicosis and atrial fibrillation or congestive cardiac failure.

The vast majority of patients with thyroid heart disease have evidence of associated coronary, hypertensive, rheumatic or syphilitic heart disease. Kepler and Barnes (147) found evidence of organic heart disease in 67 per cent of 27 cases of fatal thyrotoxicosis where there had been congestive cardiac failure. Maher and Sittler (176) found organic heart disease in 75% of 180 cases of thyrotoxicosis, including all 30 patients with congestive failure and 41 out of 42 patients with atrial fibrillation. Despite the lack of definite evidence in support of a unique thyrotoxic cardiomyopathy, some workers believe in the entity and have adduced the occasional case as such an entity (60, 132).

However, definite proof of such an entity would require vigorous exclusion of other possibilities and the author is unaware of any patient with presumed thyrotoxic cardiomyopathy who has, for instance, had normal coronary arteries
demonstrated by angiography.

The increasing incidence of thyroid heart disease with age (it is exceptional under the age of 40 years) and the reduction in the usual predominance of females in Graves' disease to around 2-3 to 1 in thyroid heart disease may be explained in the terms of the rising incidence of significant coronary artery disease with age and the greater predilection for males to be so affected.

Thyrotoxicosis increases body metabolism and lays an increased demand on the heart which has to increase output and deal with an increased venous return. If the demands are outwith the heart's capacity to meet them rhythm upsets or failure will develop; however, the cardiac reserve is such that these events are likely to supervene in a heart already diseased.

It is, however, established that thyroid hormones have direct action on cardiac muscle with augmentation of contractility (35) and on the conduction system with accelerated A-V nodal conduction (76,117). The possibility, therefore, exists that atrial fibrillation, cardiomegaly and failure may be the results of a true thyrotoxic cardiomyopathy.

The possibility was further examined in two ways:

1. CLINICALLY.

Over three years, forty thyrotoxic (including 24 previously thyrotoxic) patients under the age of 35 were seen and examined for possible thyrotoxic cardiomyopathy. Nine presented with atrial fibrillation (2 had cardiomegaly on chest x-ray) 2 with cardiac failure and 7 were noted to have incidental cardiomegaly on chest x-ray. 12 patients had evidence of
pre-existing cardiac disease, particularly, rheumatic fever (8 cases), atrial septal defect (1 case) hypertension (1 case essential, 2 renal aetiology), 7 patients had a history of respiratory or urinary infections shortly before the onset of cardiac complications and 2 patients had a family history of premature ischaemic heart disease (one patient had a IIa hyperlipoproteinaemia and the other manifested a IIb pattern subsequent to being euthyroid (on thyroxine). This group contained all thyrotoxic patients with cardiac abnormality except 3 with radiological evidence of cardiomegaly. In 3 patients with cardiac enlargement (all male) no evidence of co-existent cardiac disease was found and screening for a family history of coronary artery disease or cardiomyopathy and for hyperlipoproteinaemia was negative. This offered suggestive evidence that cardiomegaly might be induced directly by thyrotoxicosis.

2. This possibility was examined further in a laboratory study.

ANIMALS AND METHODS.

18 male Wister rats were fed 360 μg m tri-iodothyronine daily in their drinking water for sixteen weeks. 18 controls received drinking water with no additive. The rats receiving the hormone showed little objective evidence of disability initially in the first month but subsequently they became sluggish and irritable.

6 rats on T3 were sacrificed at 16 weeks and weighed. The heart weights and D.N.A. and R.N.A. contents were measured. 6 controls were similarly treated.

6 rats on T3 were sacrificed and samples of left ventricle examined by electronmicroscopy for evidence of cellular structural disorder. A group of 6 control rats were similarly examined.
**TABLE 3B**

**CARDIAC PARAMETERS IN THYROTOXIC RATS**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>TREATMENT</th>
<th>TIME OF SACRIFICE (WEEKS)</th>
<th>L.V. WEIGHT (mg . SD)</th>
<th>LV WEIGHT / WEIGHT ANIMAL</th>
<th>D.N.A.CONTENT of L.V. mg. (SD)</th>
<th>R.N.A.CONTENT of L.V. mg. (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(n=6)</td>
<td>T₃</td>
<td>16</td>
<td>890 (12)</td>
<td>3.50 (0.19)</td>
<td>2.0 (0.21)</td>
<td>1.51 (0.4)</td>
</tr>
<tr>
<td>2(n=6)</td>
<td>0</td>
<td>16</td>
<td>600 (17)</td>
<td>2.50 (0.21)</td>
<td>1.20 (0.13)</td>
<td>1.00 (0.6)</td>
</tr>
<tr>
<td>3(n=6)</td>
<td>T₃ for 16 wks.</td>
<td>28</td>
<td>630 (21)</td>
<td>2.50 (0.11)</td>
<td>1.51 (0.16)</td>
<td>1.2 (0.2)</td>
</tr>
<tr>
<td>4(n=6)</td>
<td>0</td>
<td>28</td>
<td>612 (15)</td>
<td>2.49 (0.20)</td>
<td>1.21 (0.10)</td>
<td>1.02 (0.3)</td>
</tr>
</tbody>
</table>
ELECTRON MICROSCOPIC VIEWS OF LEFT VENTRICLE FROM THYROTOXIC RAT HEARTS.

(A) and (B) are general views of rat left ventricular sections after 3 months on 360 µg T₃ per day. (Magnification x 13,000).

The apparent mitochondrial disorganisation is probably artefact due to incomplete penetration of the glutaraldehyde preservative. There does, however, appear to be a consistent tendency to mitochondrial "clumping" in the hyperthyroid animals.

Myofibrillar structure and membrane organisation are maintained.

(C) and (D) are views of left ventricle from control (magnification x 6,000) and hyperthyroid (magnification x 8,400) rate left ventricle respectively.

Mitochondria are well preserved with intact cristae in the hyperthyroid specimen. Disorganisation in the control animal is probably artefact. The hyperthyroid specimen shows an erythrocyte in a capillary.

Overall structure is preserved.

(E) and (F) are further views of control (magnification x 6,000) and hyperthyroid (magnification x 10,750) rate left ventricle. Structural order is maintained except for mitochondrial cristae.

To obtain better fixation, several anaesthetised rats were submitted to thoracotomy and perfused with gluteraldehyde preservative via and aortic cannula at a pressure of 100mmHg. The cerebral vessel and distal aorta were ligated and the great veins sectioned just prior to retrograde coronary perfusion.
(G) (magnification x 22,750) and (H) (magnification x 22,750) are views from a control and hyperthyroid rat (720 µg T₃ per day), respectively. Mitochondrial architecture is better preserved with this method of fixation.

The presence of an erythrocyte in a capillary suggests still incomplete perfusion, and, possibly, prior administration of a vasodilator would enhance perfusion.

The overall appearances offer no evidence for thyroid hormone-induced structural damage. The mitochondrial clumping is possibly part of the adaptive changes of left ventricular hypertrophy.
6 rats on T₃ for 16 weeks were transferred back to normal drinking water and after a further 12 weeks were sacrificed with 6 control rats. Hearts and animals were weighed and heart DNA and RNA content measured.

Samples of left ventricle from rats on T₃ and from controls were stained with haematoxylin and eosin and examined for evidence of myocytic replication.

RESULTS

Treatment with T₃ for 16 weeks induced frank left ventricular hypertrophy (increase of~+ 50%) with concomitant increases in protein (i.e. weight), D.N.A. and R.N.A. content (Table 38). However, the mode of hypertrophy is questionable because no light microscopical evidence of myocyte replication (mitosis or polyploidy) was detected with only one possible multinucleate cell observed in 18 slides. This raises the possibility that hypertrophy is due to cell enlargement rather than division though Linzbach has shown fairly convincing evidence of cell division in hypertrophy due to hypertension (168a). However, D.N.A. content (mg./gm. tissue) is elevated and this suggests that division has occurred, though this may be limited to the connective tissue nuxity.

Electronmicroscopy failed to demonstrate any structural change. In particular, mitochondrial cristae were intact. It is known that thyroid hormone has potent effects on the mitochondrial respiratory enzyme change but no mitochondrial structural damage was observed (Figure 26).

Interestingly, there was evidence of regression of hypertrophy (though not to completely normal levels) after
cessation of tri-iodothyronine. This would support the thesis that thyrotoxic cardiomegaly may be a reversible entity and not necessarily an indicator of thyrotoxic cardiomyopathy. Further studies such as the use of tritiated thyridine to detect and localise nuclear changes would be of interest in this model.

The aetiology of the hypertrophy produced by thyroid hormone may be multiple. It may be due, for example, to the circulatory changes induced by the condition, with increased volume load, augmented contractility and tachycardia playing a part by imposing on the heart a demand for more energy output and, in turn, an adaptive growth response. Also direct metabolic effects of thyroid hormone may be involved with activation of D.N.A. and R.N.A. synthesis, stimulation of mitochondrial respiration and activation of membrane ATPase, all potential inducers of adaptive growth changes (50).

Faced with these many potential factors in producing cardiac hypertrophy, it is impossible to delineate an aetiology for the much rarer (and doubtful) entity of thyrotoxic cardiomyopathy. One may speculate that if the entity exists its rarity suggests the requirement of a further factor in addition to thyrotoxicosis — possibly a latent cardiac membrane or enzyme disorder which is activated by thyrotoxicosis and only then manifests its presence.

PART II. THYROID AND LIPID METABOLISM.

A. LIPID ABNORMALITIES IN THYROTOXICOSIS.

Measurement of total fasting plasma cholesterol levels in thyrotoxic patients (see Table 7) confirmed the frequently reported finding that cholesterol levels are significantly
lower than in euthyroid patients. These studies were extended to an investigation of free fatty acid levels and lipoprotein lipase levels in thyrotoxic patients.

PATIENTS AND METHODS

Ten female patients clinically and biochemically thyrotoxic in the age range 60-70 years were selected for study.

Fasting venesamples were taken into E.D.T.A. anticoagulated tubes, 5000 units of heparin given intravenously and repeat blood samples taken exactly 15 minutes later. Samples were spun at 3000 r.p.m. immediately and the decanted plasma cooled to 4°C in an ice bath. The samples were transported to the laboratory at 4°C, and processed.

Fasting free fatty acid levels were measured by the Dole method (64).

Lipoprotein lipase activity was assayed by the method of Weir et al. (298), specimens being stored frozen until they could be processed together.

Pre- and post-heparin plasma samples were warmed to 37°C in an incubator over 30 minutes and lipase activity measured (see page77).

In the light of the findings these studies were repeated on 8 thyrotoxic patients in atrial fibrillation, all female, in the age group (65-76 years) prone to ischaemic heart disease, on digoxin but not on beta-adrenoreceptor blocking agents. All these patients were normotensive and had no evidence of rheumatic fever.

7 thyrotoxic patients presenting with congestive cardiac failure were similarly studied. These patients were on
**TABLE 39.**

FREE FATTY ACID LEVELS IN SELECTED GROUPS OF PATIENTS WITH THYROTOXICOSIS.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>NO. PATIENTS</th>
<th>F.F.A. μ Eq/L</th>
<th>CHOLESTEROL mmol/L</th>
<th>TRIGLYCERIDES mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.F.</td>
<td>8</td>
<td>905 ± 60</td>
<td>5.44 ± 0.91</td>
<td>1.02 ± 0.14</td>
</tr>
<tr>
<td>V.E.S.</td>
<td>7</td>
<td>890 ± 70</td>
<td>6.22 ± 1.55</td>
<td>1.12 ± 0.20</td>
</tr>
<tr>
<td>C.C.F.</td>
<td>6</td>
<td>935 ± 80</td>
<td>5.83 ± 0.78</td>
<td>1.21 ± 0.38</td>
</tr>
<tr>
<td>NO</td>
<td>10</td>
<td>925 ± 55</td>
<td>5.70 ± 0.85</td>
<td>0.97 ± 0.17</td>
</tr>
</tbody>
</table>
diuretics, digoxin (five) and were all female. The age range was 64-72 years.

RESULTS.

Fasting plasma free fatty acids in thyrotoxicosis were found to be elevated above the values of a euthyroid control group (same age range and sex) - \( p < 0.001 \). Similarly, post-heparin lipolytic activity was found to be augmented in the thyrotoxic group - \( p < 0.001 \).

These findings are detailed in the table opposite (Table 39). Patients in fibrillation or failure demonstrated similar findings and were not significantly different on measured parameters from the thyrotoxic group with no overt cardiac pathology.

DISCUSSION:

The possible significance of the increased fatty acid levels in thyrotoxicosis was evaluated as follows. Fatty acids are considered by some workers to be important dysrhythmic agents, particularly in the context of myocardial infarction, where Oliver et al. (210,155) contend that they play a major role in the causation of major dysrhythmias following infarction and so predispose to sudden death.

There is experimental evidence that very high levels of free fatty acids are arrhythmogenic in animals but the significance of the levels attained in humans after infarction (due to stimulation of lipolysis by catecholamines) is uncertain (212,213), as the levels attained are much lower than those produced in animals. Moreover, the reserve of albumin binding sites for mopping up released fatty acids is relatively enormous (266).
The significance of fatty acid levels in inducing atrial fibrillation in thyrotoxic patients was evaluated in the light of the preceding remarks.

No significant differences were, in fact, found between any of the three groups studied - in particular, patients presenting with atrial fibrillation were not significantly different in terms of circulating fatty acid levels or lipase activity from thyrotoxic patients with no overt cardiac pathology or thyrotoxic patients with cardiac failure.

These findings suggest that elevated fatty acid levels probably do not play a significant role in the causation of "thyrotoxic fibrillation". One might speculate that they are a relatively harmless aspect of the hypermetabolism and weight loss due to thyrotoxicosis.

PART II.

8. THYROID AND Atherosclerosis.

It is wellknown that frank hypothyroidism is attended by an increased risk of accelerated coronary atherosclerosis. This is considered to be due to induced hypercholesterolaemia (79, 179, 93) and altered metabolism of potentially atherogenic material.

The author's interest in this aspect of thyroid disease was stimulated by clinical observations on a 51 year old woman. This patient presented in 1972 with angina of effort and was found on lipoprotein screening to have a Type IIa hyperlipoproteinaemia. She was treated with Trinitrin for angina and with a low cholesterol diet supplemented subsequently by the bile sequestrant cholestyramine. There was little response to this therapy over a two year period with serum lipoprotein parameters remaining persistently abnormal. The
<table>
<thead>
<tr>
<th>GROUP</th>
<th>CLINICAL STATUS</th>
<th>BIOCHEMICAL STATUS</th>
<th>HORMONAL LEVELS mean ± SD</th>
<th>CHOLESTEROL mean ± SD</th>
<th>TRIGLYCERIDE mean ± SD</th>
<th>INTRALIPID TOLERANCE TEST K2 (%/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 n=20</td>
<td>H</td>
<td>H</td>
<td>30+16 0.3±0.4 48±308</td>
<td>9 ± 1.1</td>
<td>3.41 ± 0.81</td>
<td>1.34 ± 0.24 n = 5</td>
</tr>
<tr>
<td>1 n=20</td>
<td>E</td>
<td>E</td>
<td>132±27 1.6±0.3 1±2</td>
<td>6.10±1.0</td>
<td>1.71 ± 0.43</td>
<td>3.86 ± 0.51 n = 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(on T₄)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 n=12</td>
<td>E</td>
<td>H</td>
<td>46±8 0.4±0.2</td>
<td>8.0 ± 0.9</td>
<td>2.71 ± 0.72</td>
<td>1.96 ± 0.43 n = 5</td>
</tr>
<tr>
<td>2 n=12</td>
<td>E</td>
<td>E</td>
<td>135±21 1.5±0.3</td>
<td>5.91±1.2</td>
<td>1.63 ± 0.51</td>
<td>3.92 ± 0.61 n = 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(on T₄)</td>
<td></td>
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</tbody>
</table>

VALUES IN S.I. UNITS.
The patient was seen by the author in 1974 and was considered to be possibly hypothyroid. A PBI-127 assay performed in 1972 as a routine screen gave a value of 284 nmol/L (within normal range). Full hormonal assay in 1974 gave frankly hypothyroid results -  

$$T_4 = 21 \text{ nmol/L, } T_3 = 0.10 \text{ nmol/L, TSH > 50 \text{ mU/L.}}$$

Thyroxine replacement therapy was cautiously instituted, eventually reaching a dose of 0.15 mg/day. Serum parameters on this dose are

$$T_4 = 162 \text{ nmol/L, } T_3 = 1.6 \text{ nmol/L, TSH 10 \text{ mU/mL.}}$$

Over a nine month period total serum cholesterol and LDL cholesterol fell progressively and are now well within the normal range. It is probable that rendering the patient virtually euthyroid has caused a reduction in her total body cholesterol mass with normalisation of serum values. The patient is at present being taken off cholestyramine to see if her values stay normal.

**PATIENTS AND METHODS**

Studies were performed on two groups (all female) of patients to characterise the significance of serum thyroid hormone levels in hypothyroidism. The first group consisted of 20 patients with clinically overt and biochemically confirmed hypothyroidism. The second group was composed of 12 patients clinically euthyroid but biochemically frankly hypothyroid (Table 40). Fasting lipoprotein profiles (Fredrickson classification) were performed on all these patients and, when abnormal, were repeated on at least one further occasion to confirm the findings.

Lipoprotein classification of fasting plasma was performed by the standard method of ultracentrifugation (92) and assay of cholesterol in the subfractions by a modification of the Pearson reaction as described below.
The reagents consist of diluent composed of 25% acetic acid (50 ml.) in acetic anhydride (150 ml.) with 7.5% toluene-p-sulphonic acid as colour stabiliser. The acid reagent is 15% sulphuric acid in diluent (4.5 ml. conc. H₂SO₄ + 25.5 ml. diluent).

The standard cholesterol solution consists of 209.5 mg. cholesterol in 100 ml. diluent. This allows for the final volume after colour development being 4.4 ml. for the standard and 4.2 ml. for the serum. The standard cholesterol solution is 200 mg./100ml.

(The reagent is dangerous and must be pipetted carefully). Standards and blank are set up thus:

<table>
<thead>
<tr>
<th>TEST</th>
<th>STANDARD</th>
<th>BLANK</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2 ml. serum</td>
<td>0.2 ml. standard</td>
<td>2 ml. diluent</td>
</tr>
<tr>
<td>2 ml. diluent</td>
<td>0.2 ml. water</td>
<td>2 ml. diluent</td>
</tr>
</tbody>
</table>

There is a time lag of several seconds before a reaction takes place causing generation of considerable heat; the contents of each tube are mixed by careful shaking and allowed to cool to 37°C in a water bath. Add 2 ml. acid reagent and again mix contents of the tube.

There is an initial precipitation of protein and this redissolves readily. The tubes are all incubated in a water bath at 37°C. for 10 minutes. A green colour develops which is stable for about 10 minutes and thereafter begins to fade slowly. For this reason it has been found best to run
determinations in batches of eight. If more than one batch has to be carried out a standard 8 blank should be included each time. The optical densities are read against the blank at 425 mgs. on an SP 600.

**CALCULATION**

\[
\text{mgm cholesterol/100 ml. serum} = \frac{\text{OD Test sol.}}{\text{OD Standard Sol.}} \times 200
\]

Total serum triglyceride was assayed by the method of Kessler and Lederer (148)

The intravenous Intralipid tolerance test was performed as previously described (p.75) in 5 patients from each group.

These studies were repeated when patients were euthyroid on thyroxine replacement therapy (0.15 - 0.20 mg./day).

**RESULTS**

15 of the 20 clinically and biochemically hypothyroid patients had hyperlipoproteinemia results - 8 showed a Type IIa abnormality, 2 a IIb pattern and 5 a Type IV pattern. All 20 patients had abnormal Intralipid results indicating a decreased utilisation of triglyceride.

Repeat lipoprotein profiles three months after full replacement therapy had been instituted, showed normal profiles for all but two patients - one had a mild Type IV abnormality (probably related to obesity) and one a continuing IIa pattern. This last patient has a family history of ischaemic heart disease and is now on a low cholesterol diet.

Of the 12 patients with biochemical but not overtly
clinical hypothyroidism, six had abnormal phenotypes -
two a IIa pattern, 2 a IIb pattern and 2 a Type IV pattern.
The mean levels for total cholesterol and triglyceride and
subfractions were lower than in Group I paralleling
the degree of thyroid hormonal abnormality. All these
abnormalities reverted to normal with adequate replacement
therapy. Seven patients had abnormal Intralipid
tests.

Those results emphasise the importance of routine
hormonal screening to detect subclinical hypothyroidism
which may be a significant factor in producing clinical heart
disease by accelerating the atherosclerotic process. Bastenie
et al. (11,12,57) have emphasised this point and claim to
have detected a significant incidence of thyroid abnormality
in patients sustaining myocardial infarctions.

The data presented here is sufficient to emphasise the
dangers of inadequate review of treated thyrotoxic patients.

THE SIGNIFICANCE OF "COMPENSATED EUTHYROIDISM"

There is some dispute as to the significance of an
elevated TSH value in a clinically euthyroid individual with
thyroxine and tri-iodothyronine values in the normal range.

One report suggested that, in post-therapy patients, this
finding could persist for several years and did not indicate an
increased risk of the patient developing frank hypothyroidism
(274) - see page 72.

This, however, was a retrospective study with the potential
for bias in patient selection. The author's prospective study
of a group of these patients carried out over a period of one
year suggest they do, in fact, develop hypothyroidism at a
greater rate than patients with normal TSH values. This suggests
that such patients should perhaps be commenced on thyroxine replacement therapy at this stage.

As this state of "compensated euthyroidism" is probably an intermediate stage on the way to frank hypothyroidism, it is interesting to search for any metabolic abnormalities that may indicate a degree of hormonal insufficiency even though this condition is termed "compensated".

A significant incidence of abnormality was noted, particularly with the intravenous Intralipid test (page 75). This supports the contention that such patients may be at risk from abnormal intermediary metabolism and may run an increased risk of atherosclerosis. It would appear wise to commence such patients on replacement therapy as soon as TSH levels are raised.

Furthermore, it has been claimed that women with elevated TSH levels have elevated prolactin levels (due to TRH stimulation) and run an increased risk of promotion of carcinoma of the breast (96, 197), though this is still an unsettled issue (307). This may be another valid argument for advocating early replacement therapy in female patients with "compensated euthyroidism".

**CONCLUSION TO PART IIB.**

These studies indicate that thyroid disorder is a continuous spectrum with large parts of the spectrum dependent on full hormonal characterisation for detection. These occult abnormalities have been demonstrated by the author to carry a high incidence of disordered intermediary metabolism. This underlines the importance of adequate hormonal assessment of patients with possible thyroid disease and the need for prompt hormonal replacement therapy where indicated.
TENTATIVE CONCLUSIONS TO THIS STUDY

$^{131}I$ therapy is the potential treatment of choice for the majority of thyrotoxic patients by virtue of its efficacy, simplicity of administration and lack of serious side-effects, particularly in regard to carcinogenesis. The major drawback to its use is the risk of hypothyroidism. The present study has demonstrated fairly conclusively that iodine-125 offers no obvious advantage in this regard. The question therefore arises, can we improve the mode of usage of iodine-131?

Throughout the Trial of iodine-125, the author found only one patient who, when started on thyroxine replacement therapy, failed to take it and this failure was the result of the patient's genuine misunderstanding of the lifelong nature of such replacement therapy. Similarly, two years' attendance at a general thyroid clinic confirmed the impression that patient non-compliance was very rare. This accords with the findings of Safa who found 91.5% of patients treated with iodine-131 and given replacement therapy were still taking it, all the others but one having stopped on the advice of their physician(250a). This indicates that thyroxine replacement therapy is simple and well adhered to. Furthermore, with present day assays of $T_4$, $T_3$ and TSH, it is possible to gauge replacement dose accurately so as to give doses in the euthyroid range and avoid subclinical hyper- or hypo- thyroidism. Also, the use of zinc clearance measurements or, more so, STI measurement permit a measure of the cardiac response to thyroid hormone levels and may assist in adjusting replacement therapy.

It seems an attractive policy to deliberately adopt thyroid ablation as the goal of radioiodine therapy and to destroy
the thyroid as soon as feasible with largish dose(s) of iodine-131, so as to permit early institution of replacement therapy, prompt discharge of the patient from the clinic and reduction of time lost off work, patient inconvenience and thyroid clinic load. The potential dangers of subclinical hypothyroidism of "compensated euthyroidism" and the author's experience in cardiology clinics where there is a steady trickle of treated thyrotoxic patients presenting with cardiac complications of undetected hypothyroidism, support this approach to thyrotoxicosis. Studies are needed of the optimum dose regimes that should be adopted in such an approach. Careful monitoring would be required to detect any increase in the incidence of carcinoma and leukaemia that might result from the increased body irradiation (1.7 Rads to the marrow per millicurie) from higher therapy doses.
REFERENCES


B.


152
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REFERENCES (Contd.)

REFERENCES (contd.)


REFERENCES (Contd.)


J.


K.

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N.


O.


P.


