PROBLEMS IN THE TREATMENT AND ASSESSMENT OF

PATIENTS WITH THYROTOXICOSIS:

A CLINICAL AND LABORATORY STUDY

by

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THIS THESIS IS DEDICATED TO MY

DEAR WIFE

AND

OUR DAUGHTER

This thesis originated from three years of personal involvement in the clinical assessment of a new radio-isotope (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 radio-iodine 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

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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.
These 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.

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. Lawrie 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-adrenoresceptor 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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PUBLICATIONS AND PRESENTATIONS OF WORK

PRESENTED IN THIS THESIS

The following publications concerning material presented in
this thesis have appeared or have been submitted to medical
journals.

PUBLICATIONS

I   Bremner, W.F., McDougall, I.R., Greig, W.R. (1971)
Results of Treating 297 Thyrotoxic Patients with 125_I
Lancet II, 281.

II   Bremner, W.F., Spencer, G.A. Ratcliffe, W.A., Greig, W. R.
Ratcliffe, J.G. (1976).
The Assessment of Iodine-125 Treatment of Thyrotoxicosis.
J. Clin. Endocrinol,5, 225.

III   Spencer, A., Bremner, W.F., Ratcliffe, W.A., Ratcliffe, J.G.
(1974).
Assessment of Thyroid Function in Patients Treated with
125-Iodine for Thyrotoxicosis.
J. Endocrinol 65, 18.

IV   Hillis, W.S., Bremner, W.F., Thomson, J.A., Lawrie, T.D.V.(197
Systolic Time Intervals in Thyroid Disease.
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V   Bremner, W.F., McDougall, I.R., Greig, W.R., Ratcliffe,J.G.
(1974).
Long Term Results of Iodine-125 Therapy for Thyrotoxicosis.
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Zinc and Thyroid Disease.
Submitted to Postgraduate Medical Journal.

VII   Bremner, W.F., Kennedy, J.S. (1976).
Radiological Effects of 125-I and 131-I on the Heperactive
Rat Thyroid.
Submitted to Journal of Pathology.

PRESENTATIONS AND ABSTRACTS

I   McDougall, I.R., Bremner, W.F., Ratcliffe, J.G., Greig, W.R.
Iodine-125 Therapy for Thyrotoxicosis.
Presented at the Scottish Society for Experimental
Medicine, October, 1974.

II   Spencer, C.A., Bremner, W.F., Ratcliffe, W.A., Ratcliffe, J.G.
Assessment of Thyroid Function in Patients Treated with
Iodine-125.
Presented at the Society for Endocrinology, November, 1974.

III   Hillis, W.S., Bremner, W.F., Thomson, J.A., Lawrie, T.D.V.
Systolic Time Intervals and Thyroid Disease.
Presented to the Scottish Association of Physicians,
September, 1974.

IV   Hillis, W.S., Bremner, W.F., Murray, R.G.,
      Tweddel, 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.
      Abstracted in Scottish Medical Journal, 1975 (in press).

V   Bremner, W.F., McDougall, I.R., Greig, W.R., Ratcliffe, J.G.
      Long-term Results of Iodine-125 Treatment for Thyrotoxicosis.
      Presented at 12th. International Symposium on Radioisotopes in
      Clinical Medicine and Research (1976).

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.   19

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.

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ABBREVIATIONS

LVET   LEFT VENTRICULAR EJECTION TIME

$PB^{127}I$ PROTEIN-BOUND IODINE-127

PEP   PRE-EJECTION PERIOD

QS_2 TOTAL ELECTROMECHANICAL SYSTOLE

STI   SYSTOLIC TIME INTERVAL

T_4 THYROXINE

TRH   THYROTROPIN-RELEASING HORMONE

TSH   THYROID STIMULATING HORMONE

T_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 tri-
iodothyronine) 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 factitiae (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-
dependant 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 ($\leq$ 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

Delayed
Hypersensitivity

T Lymphocyte

IgE
Anaphylactic
Pathway

Thyroid
Cell

B Lymphocyte ⟶

TSI Formation

Circulating TSI

T₄/T₃

Hyperthyroidism
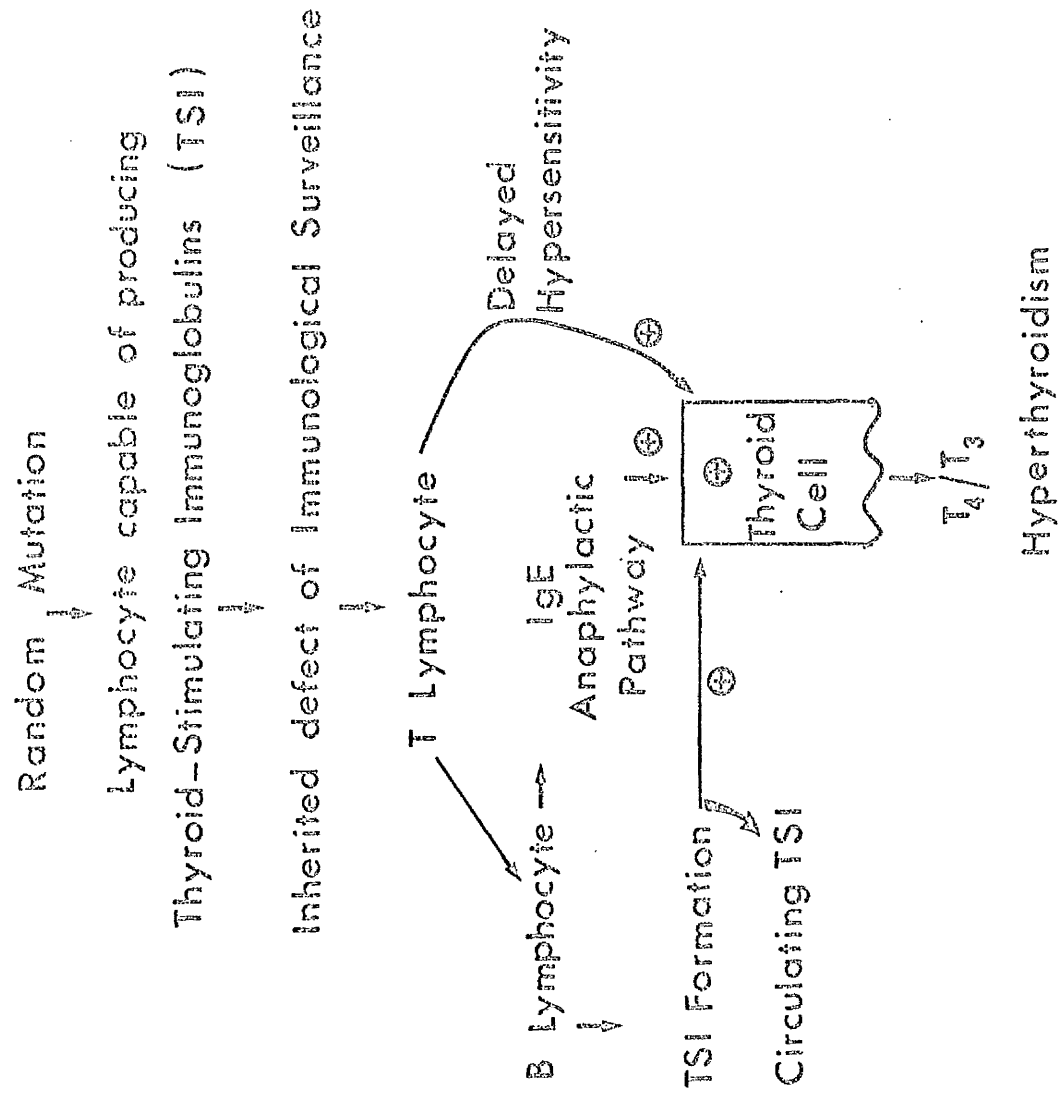
PART 1

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"   HYDRIOTAPHIA 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, 288-291) 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 Volpé et al. (291) and shown diagramatically opposite. According to Volpé'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 opthalmopathy to
formation of thyroid antigen-antibody complexes on extra-
ocular muscle membrane;   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

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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_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_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 0.8 - 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 μg. 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

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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 Thyroditis (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 87% 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 T_4 thyroxine administration) mitigating against a remission. Alexander et al (34) have used the T_3 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 granulocytopaenia 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 (46). 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).   Thyro-
cardiacs 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 Marshallese 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, -132-133,-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 goitous 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 neoplasia.

A 98% 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.
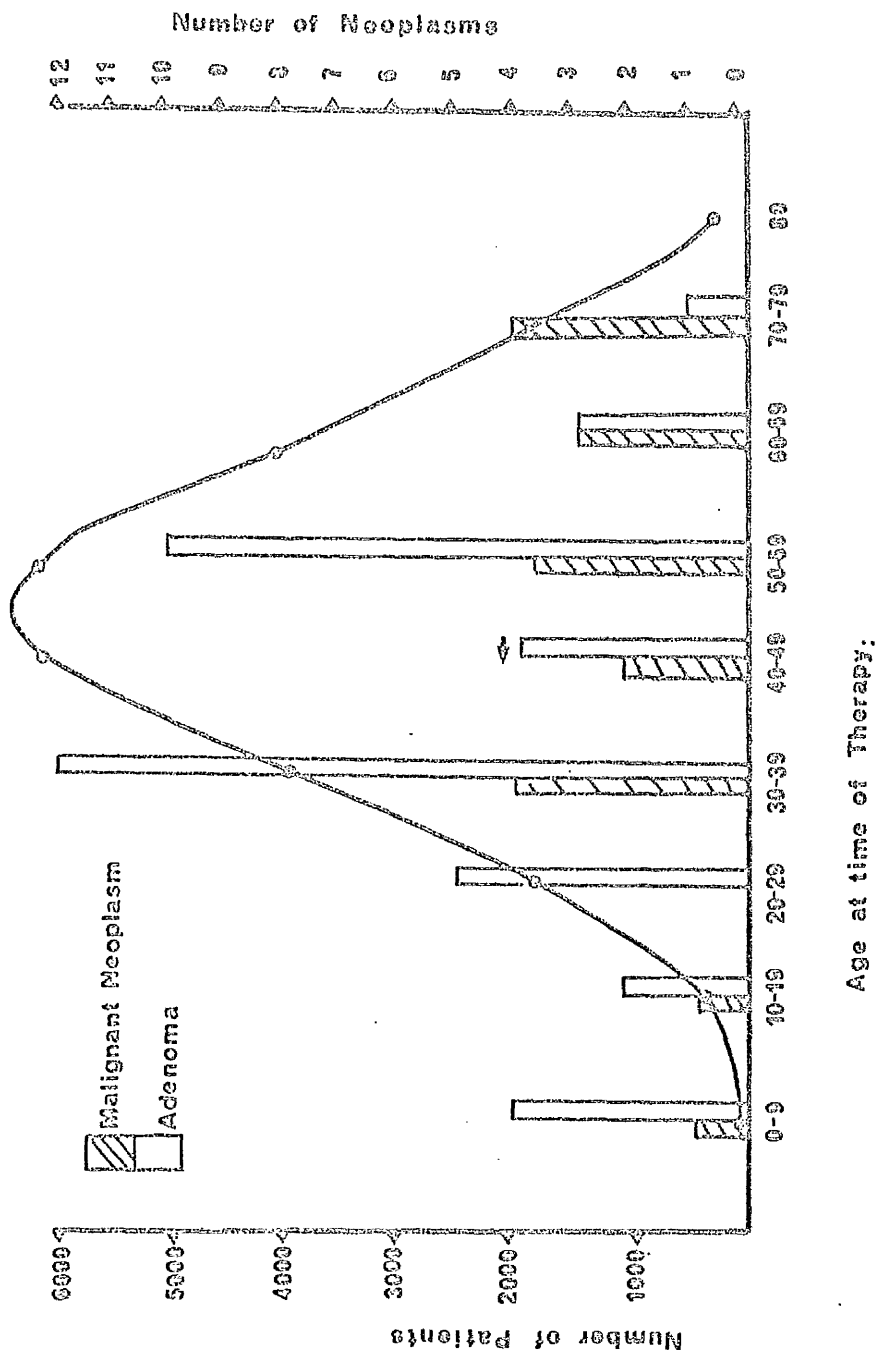
Raventos and Winship (232) suggested a mean interval of 10.9 years, while Dolphin (65) calculated the mean latent period to be 20 years minimum. In the Marshallese islanders, the first nodule was not found until 9 years after exposure and the first carcinoma not until the 11th year.

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.
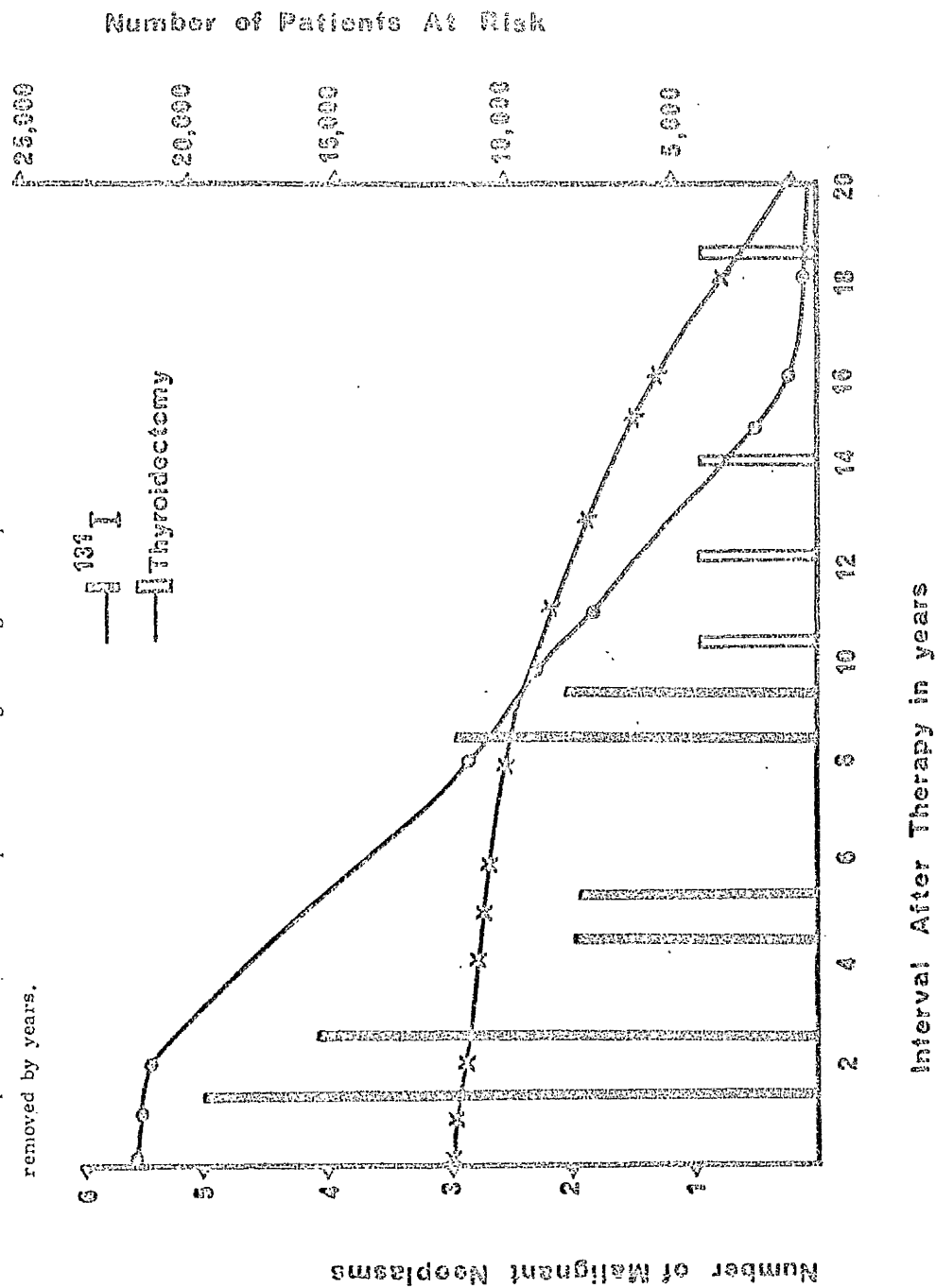
latent period for appearance of malignant neoplasm
is thought to be several years and so the majority
of these tumours were probably present at the time
of therapy.   After the 10th year, no new malignant
lesions were discovered in the iodine-131-treated
group even with 22,700 patient-years at risk after
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 β 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 E.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 amenorrhoea after cancericidal

therapy doses of iodine-131 but not after the amounts

used to treat thyrotoxicosis;   the amenorrhoea 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).
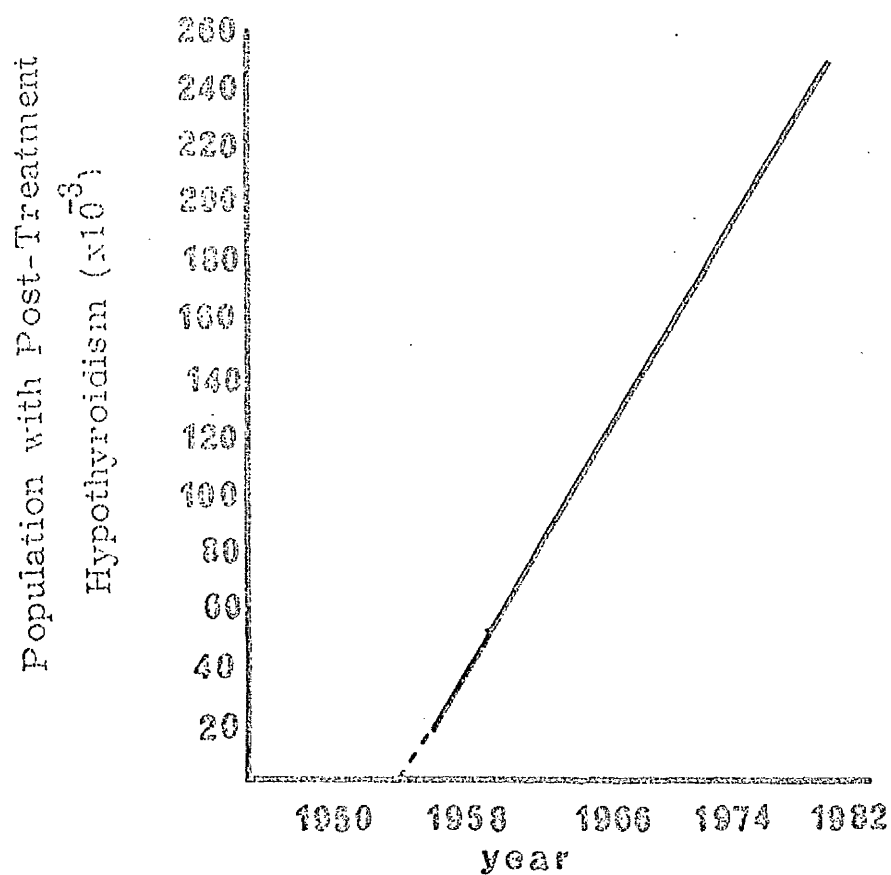
Fig. 4 Estimated total population with iatrogenic hypothyroidism after treatment of hyperthyroidism by radio-iodine or surgery.

○ follow up patients

○ new patients

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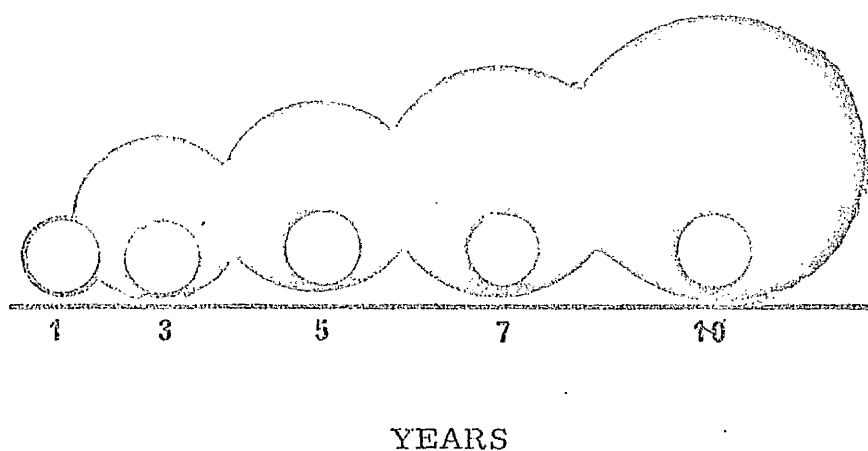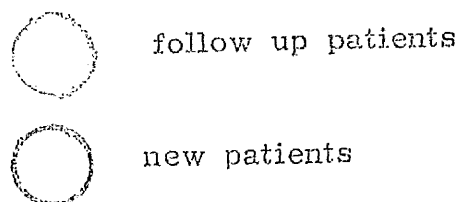
Fig. 5   Cumulative clinical load after radioiodine treatment of hyperthyroidism.   Although the number of new patients treated each year may remain constant (open circles), the number of patients at "at risk" of developing hypothyroidism accumulates and becomes part of the clinical load of a follow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

(From Hedley AJ; The use of a computer in patient follow-up in Scotland.   Scott Med J 15:395-399, 1970. By permission of E. & S. Livingstone.)

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(33,72,107,110,121,185,209) 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 β ray
emission which is absorbed over a path length of 400 to 2000
microns.   The γ emission is largely unabsorbed by the thyroid.
The β 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 8) 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 (82,125,161) 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 9 deals with the theoretical and experimental basis for
this proposal and with a trial of iodine-125 therapy in 360 patients.

FIG. 6

I-125 ELECTRONS - DOSE DISTRIBUTIONS
(SPHERES)

INFINITE PLANE SOURCE

70 μ SPHERE

35 μ SPHERE

DISTANCE FROM SURFACE (μm)
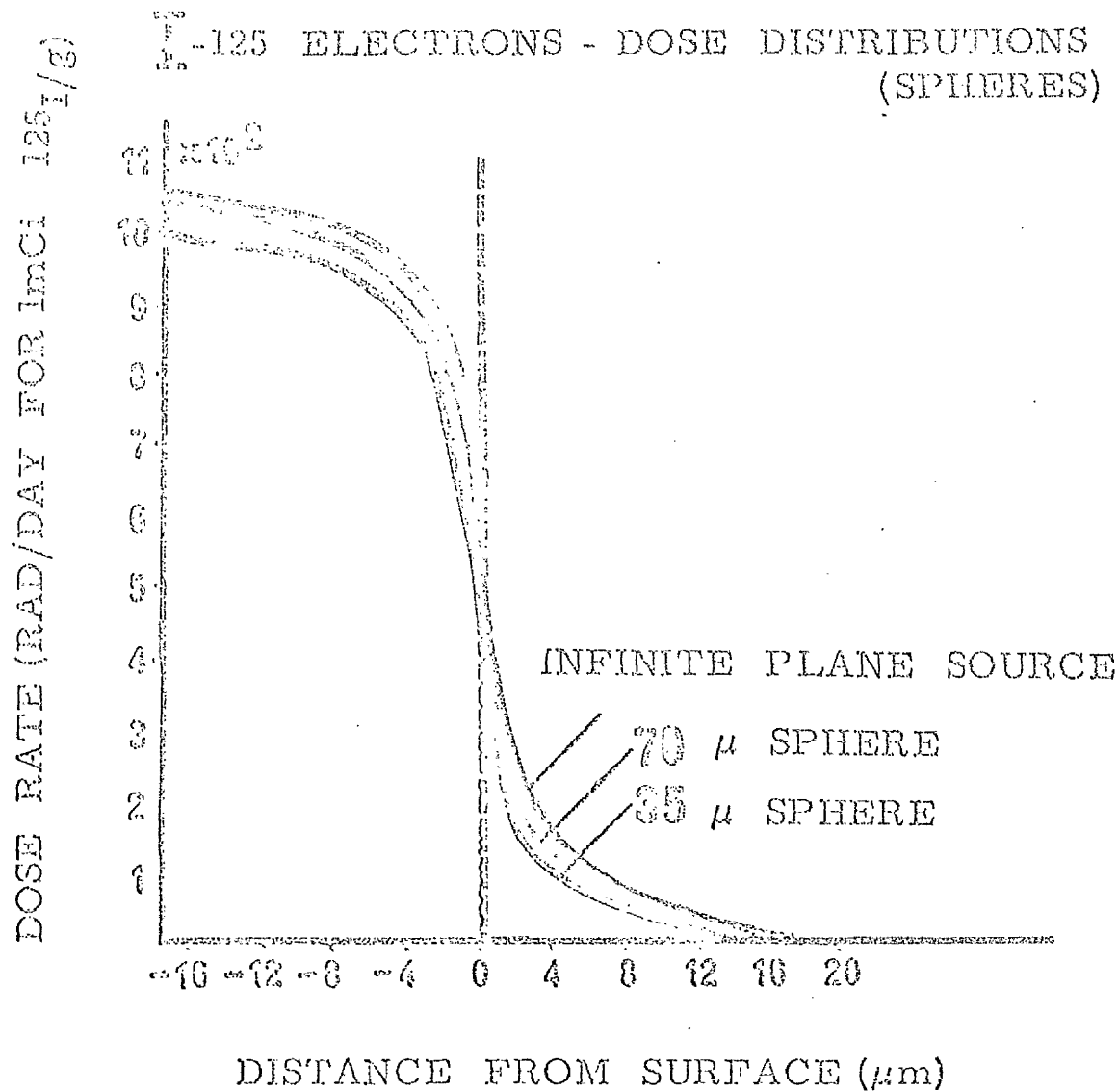
Rapid fall-off in dose rate across the boundary of a
sphere when all the iodine-125 is within the circumference
of the sphere.

THEORETICAL BASIS FOR THE USE OF IODINE-125.

Iodine-125, discovered in 1946, is one of the 24 radio-
nuclides of iodine.   Its decay is complex (112,259) occurring
by electron capture, γ emission and internal conversion with
production of soft x-rays and low energy β particles.   The
majority of the low energy x and γ rays pass through the thyroid
without dissipating energy.   Most of the energy deposited in
the thyroid is due to the low energy β 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

FIG. 7

I-125 ELECTRONS & X-RAYS - DOSE DISTRIBUTION. (Follicle)

A = all -125 in colloid
B = 90%   "   "   "
C = mean total gland dose

DISTANCE FROM CENTRE OF FOLLICLE (μm)
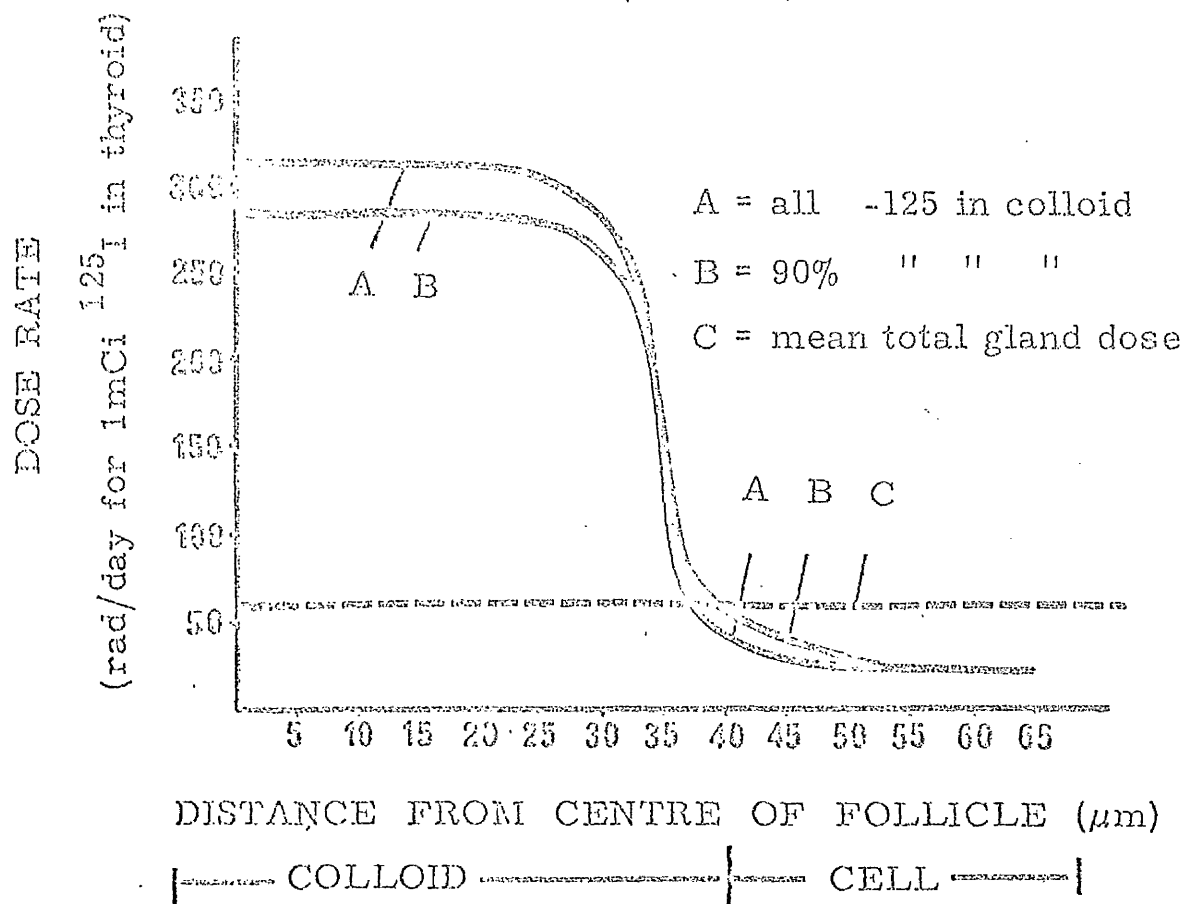
|— COLLOID —————————|— CELL —————|

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

10

FIG. 8

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

^{131}I COLLOID

^{125}I COLLOID

10μ

Nuclear irradiation high
Reproductive capacity
reduced.

Apical irradiation high
Functional capacity
reduced.
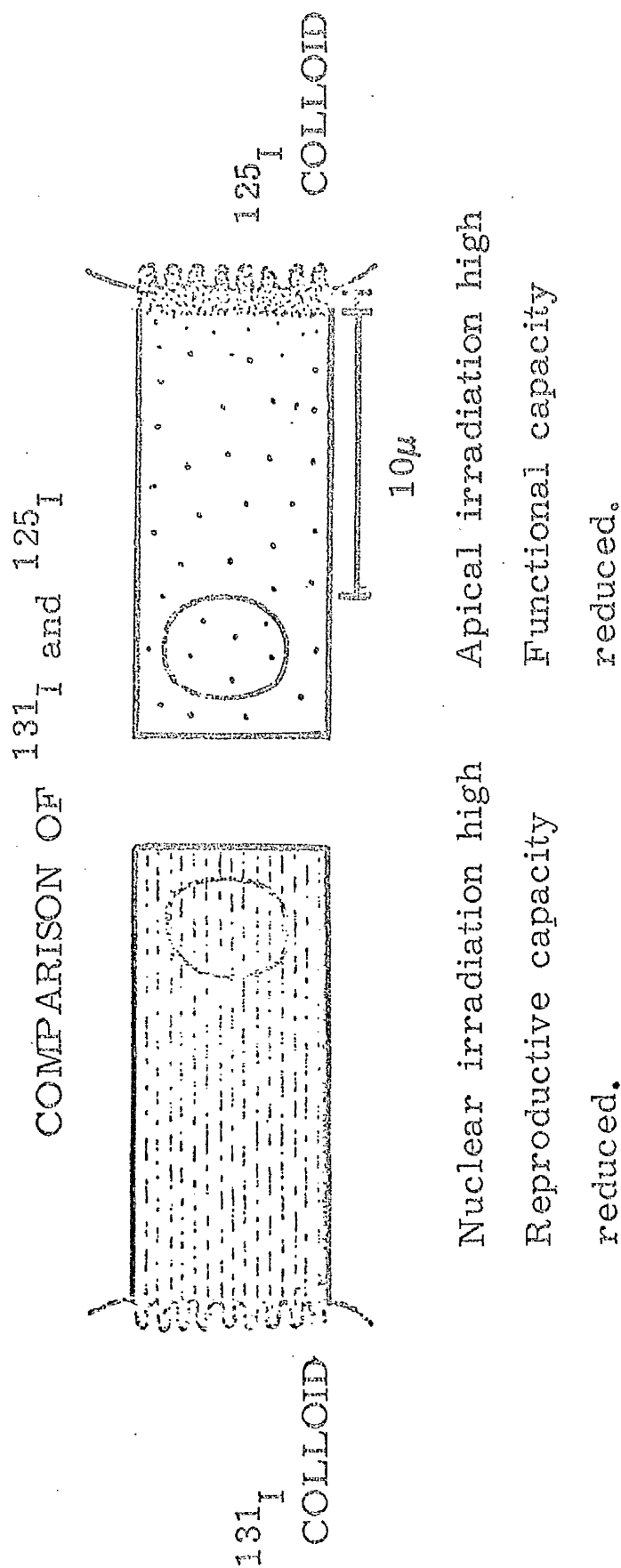
virtue of photon emissions, but the Rad dose at the colloid

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

6 times the nuclear dose (168).

Iodine-131 emits β and γ rays. The β rays have an average

energy of 187 KeV and are absorbed over a path length of 400 to

2000 microns. The γ 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

$T_4 + T_3$

Cells able to
divide but no
hormone
production (C)

Repair   Replication
(?Significance)

$T_4 + T_3$

Follicle cells
intact (D)

Division

$T_4 + T_3$

$T_4 + T_3$

131 I   C 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.

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 Sattler (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. (116) 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-I-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.

Lewitus 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
iodine-125 is efficacious though the need for further therapy
doses at this level suggested that efficacy might be less than ideal.

DESIGN 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 μCi) 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 8). From the calculations of

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FIG. 10
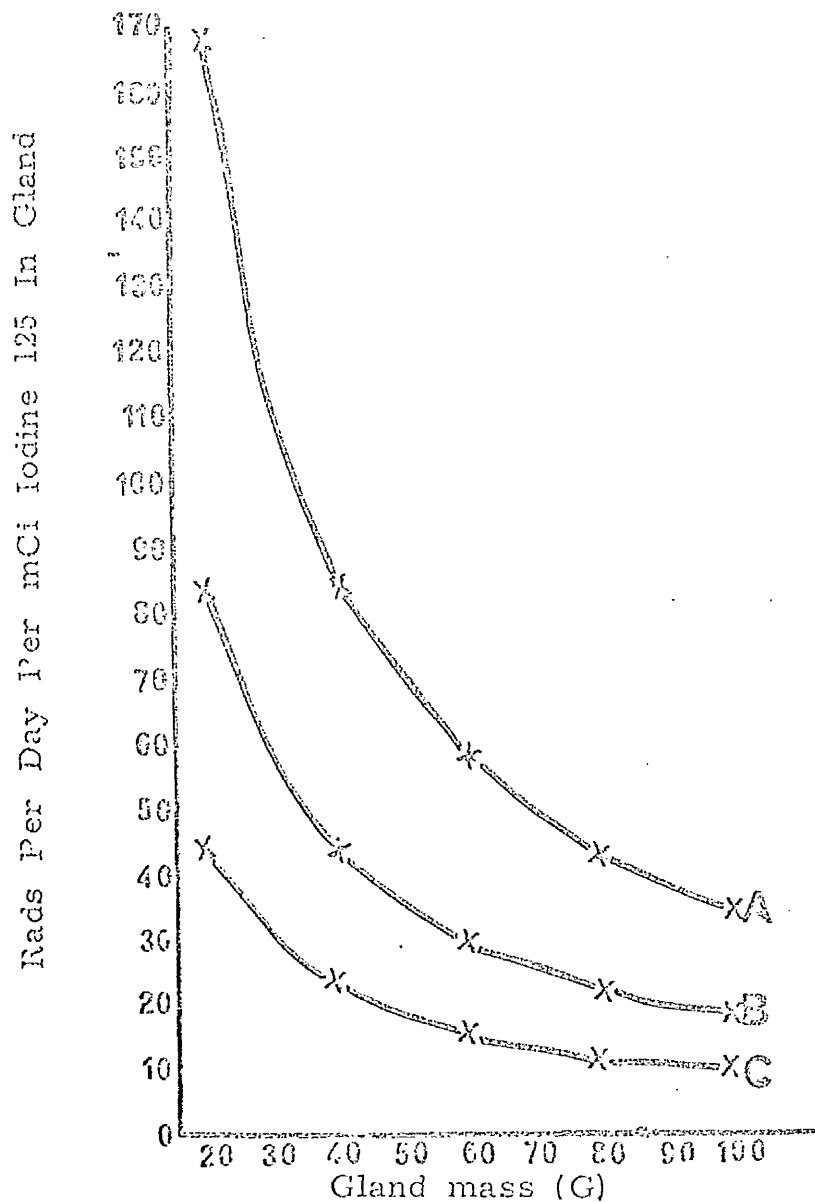
Dose rate (Rads) from 1mCi iodine-125 per day in
thyroid glands of masses from 20 grams to 100 grams

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:

$$\text{Rad Dose} = \frac{R \times \text{Therapy Dose (mCi)} \times \text{per cent thyroid uptake of dose} \times Te}{100}$$

where R is the reading from the graph and Te 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 (T_4, 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

308 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% of

patients are aged 40 to 59 years, only 1% 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 $\leq$ 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)

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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:

$$\text{Administered dose (mCi)} = \frac{\text{Desired dose } (\mu Ci/gram) \times \text{Thyroid mass (grams)}}{1,000}$$

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 μCi/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 1.

CLINICAL AND BIOCHEMICAL CONCORDANCE IN CLASSIFYING PATIENTS

CLINICAL STATUS	BIOCHEMICAL STATUS	NO. PATIENTS	SUBGROUP: BIOCHEMICAL STATUS	NO. PATIENTS	CLINICAL/BIOCHEMICAL AGREEMENT %
EUTH n = 126	EUTH	96	Normal TSH	83	76
			↑ TSH	13	
	TOXIC	20	$T_3 + T_4$ ↑	6	
			T_4 ↑	4	
			T_3 ↑	10	
	HYPO	10	$T_4 + T_3$ ↓	1	
			T_4↓ T_3 →	8	
			T_3↓ T_4 →	1	
TOXIC n = 30	TOXIC	25	$T_3 + T_4$ ↑	18	86
			T_4 ↑	2	
			T_3 ↑	5	
	EUTH	5			
MILD TOXIC n = 13	TOXIC	11	$T_3 + T_4$ ↑	6	
			T_3 ↑	5	
	EUTH	2			
HYPO n = 28	HYPO	12	T_4↓ T_3 →	3	43
			T_4↓ T_3 ↓	9	
	EUTH	16			

TABLE 2.

CLINICAL AND BIOCHEMICAL DISAGREEMENT IN PATIENT CLASSIFICATION

CLINICAL / BIOCHEMICAL

DISAGREEMENT AS % TOTAL BIOCHEMICAL STATE

BIOCHEMICAL STATE	% NOT DETECTABLE CLINICALLY
TOXIC	$36 \ (\frac{20}{56})^{\times}$
HYPO	$45.5 \ (\frac{10}{22})^{\times}$
EUTHYROID	$19.3 \ (\frac{23}{119})^{\times}$

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

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
(T_4 persistently in hypothyroid range) were classified as
clinically euthyroid while 57 per cent of patients classified
as clinically hypothyroid were, in fact, biochemically eutryroid.

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 these 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 hypo-
thyroid 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 3

VARIATION IN RESPONSE TO RADIOTHERAPY

	Thyroid size (gram)	% Tracer uptake	PBI-127	T_4	Dose (mCi)	Status at 3 Months
1.	25	75	819.5	221	5	Hypothyroid by 2 months ($T_4 = 12$)
2.	30	55	993	> 260	15	Toxic Repeat therapy at 4 months
					15	Toxic 8 months
					15	Toxic 6 months
					30	Toxic 3 months
					30	Toxic 6 months
					30	Toxic but well ($T_4 = 154$, $T_3 = 4.3$)
					135	

FOLLOW-UP DATA ON 303 PATIENTS

125I dose group	No. of Patients	Mean Age (yr)	Mean gland size (g)	Mean initial 125I dose (uCi/g thyroid)	Total No. of Additional Doses	% of group requiring further doses (a)	Average duration of follow-up (months)	Thyroid Status at Follow-Up (%)		
								Thyrotoxic	Euthyroid	Hypothyroid
I (≤200 uCi/g)	55	51	36	200	53	56	33	7	69	24
II (201-350 uCi/g)	87	52	42	330	63	47	30	12	67	21
III (351-500 uCi/g)	70	52	41	410	52	44	36	5	77	18
IV (501-750 uCi/g)	76	54	37	595	32	26	49	3	41	56
V (751-1600 uCi/g)	15	61	30	1100	1	7	54	0	40	60
	303									31.5%

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

FIG.12

POST 125 I:CLINICAL STATUS RELATED TO
DOSE OF 125 I
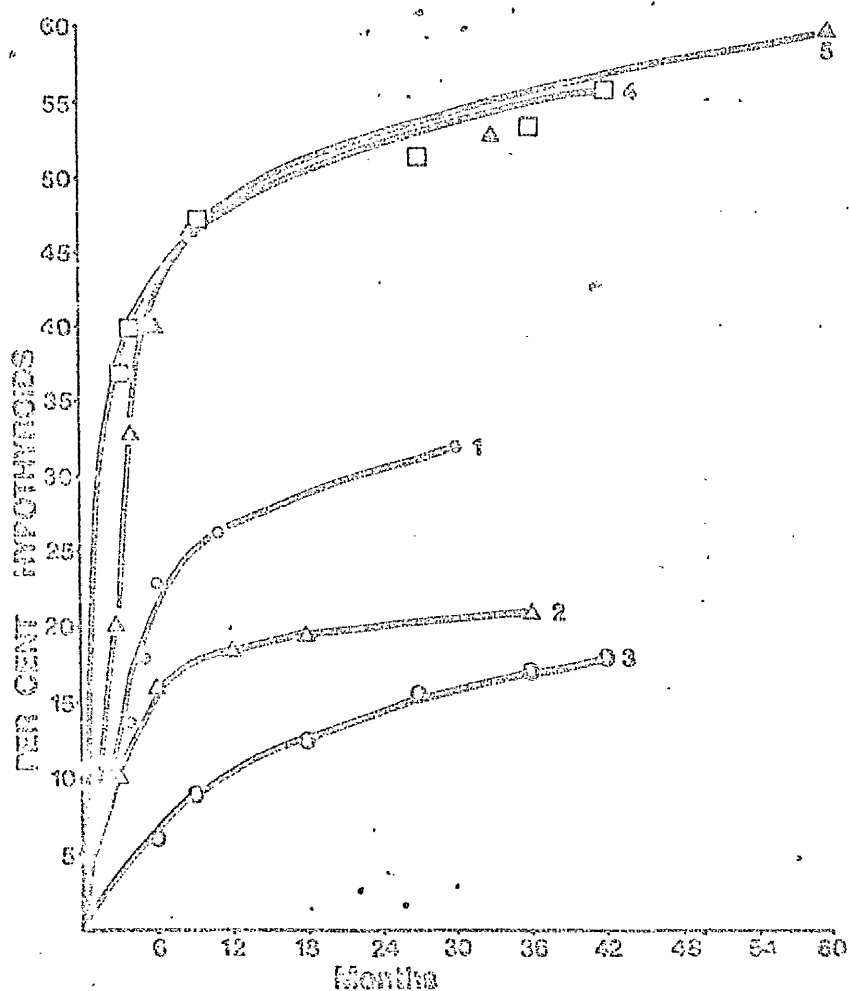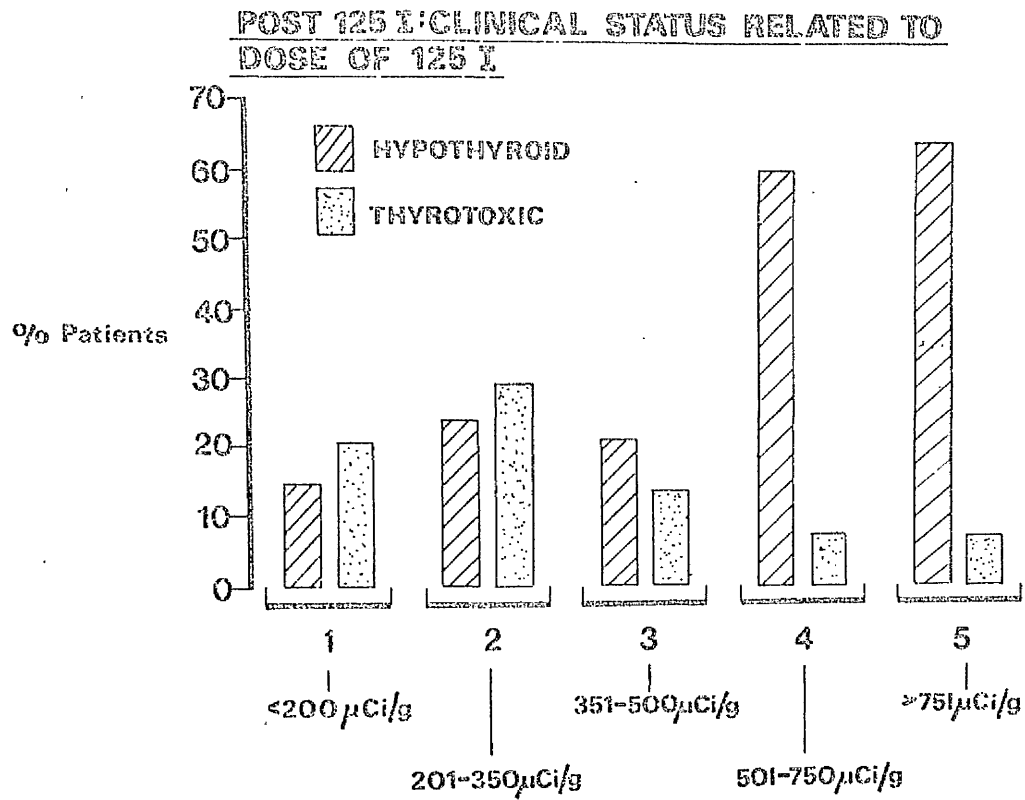
5. Gland Size and Response to Therapy.

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, large 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 these 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 erythematosis 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).

12.   Conclusion to Review of 303 Patients.

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 μCi/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 μCi/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 μCi/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.

13.   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 (λ).

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.

No   is the original number of patients.

D   is the number of therapy doses

λ the rate constant

Three sets of data are available (180,209,259) which give values of λ 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 μCi of iodine-131 to each gram of thyroid tissue (0.676). This indicates than an administered dose of 410 μCi/iodine-125 gram of thyroid is roughly as effective as 80 μCi 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 μCi/gram this figure is 0.6 which is less than the figure of 1.03 required for an iodine-131 therapy dose of 80 μCi/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. <u>Side Effects of Iodine-125 Therapy</u>

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)

GROUP	No.	
I	11	Patients euthyroid in 1971 and subsequently hypothyroid in 1973. 6 with normal TSH levels 5 with elevated TSH levels } in 1971
II	10	Patients euthyroid in 1971 and becoming hypothyroid prior to 1973. 6 with normal TSH levels 4 with elevated TSH levels } in 1971
III	40	Patients euthyroid in 1971 and still so in 1973. 32 with normal TSH levels in 1971 (6 now have elevated TSH levels) 8 with elevated TSH levels in 1971
IV	15	Patients mildly thyrotoxic in 1971 but well and receiving no further therapy. 9 patients seeming well and still biochemically mildly thyrotoxic 6 patients now completely euthyroid
V	28	Thyrotoxic patients in 1971 receiving further therapy 13 are now euthyroid (4 with elevated TSH) 9 are hypothyroid 3 remain thyrotoxic 3 are well but biochemically toxic

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 6

THYROID STATUS OF 164 PATIENTS REVIEWED 1971-73.

GROUP	NO. PATIENTS	TIME FROM LAST THERAPY TO REVIEW (MONTHS)	HORMONAL LEVELS IN 1971			HORMONAL LEVELS IN 1973			MEAN TIME TO HYPOTHYROID (MONTHS) FOLLOW-UP (MONTHS)
			T_4	T_3	TSH (range)	T_4	T_3	TSH (range)	
1	11 ⟍ 5 / 5	13 / 13	EUTHYROID 1971 / 58 ± 24.5 / 76 ± 23	1.94 ± 0.90 / 1.34 ± 0.60	1.0 (0-4) / 12.6 (6-22)	HYPOTHYROID IN 1973 / 38.6 ± 12.9 / 31 ± 12.9	1.12 ± 0.70 / 0.98 ± 0.70	30(15-60) / 35(30-60)	36 / 32
2	10 ⟍ 6 / 4	15 / 13	EUTHYROID 1971 / 98.0 ± 25.7 / 77.2 ± 25.6	HYPOTHYROID PRIOR TO 1973 REVIEW / 1.80 ± 0.60 / 1.20 ± 0.60	1.0 (0-4) / 12.0 (9-18)	27.0 (when hypo) / 25.7 (when hypo)	(when hypo) / (when hypo)		22 / 16
3	40 ⟍ 24 / 16	12 / 13	EUTHYROID 1971 EUTHYROID 1973 / 10.42 ± 25.0 / 79.8 ± 25.1	2.00 ± 0.70 / 1.50 ± 0.60	0.7 (0-2.2) / 9.8 (6-30)	86.2 ± 25.7 / 62.0 ± 25.0	1.66 ± 0.80 / 1.11 ± 0.60	1.9(0-4.5) / 15.0(6-30)	24 / 23
4	9 / 6	8 / 6	MILDLY TOXIC 1971 AND NOT REQUIRING FURTHER THERAPY / 157.3 ± 20.6 / 168.5 ± 23.4	3.37 ± 0.62 / 3.22 ± 0.65	0.2 (0-2.0) / 0.1 (0-1)	157 ± 19.3 / 126 ± 25.7	2.00 ± 0.36 / 1.64 ± 0.50	0 (0-4) / 2.0(0-5)	23 / 23
5	28		THYROTOXIC 1971 AND GIVEN FURTHER THERAPY/IES / 199.5 ± 51	5.24 ± 0.46	0.2 (0-3)	OUTCOME: 13 E → 9 TSH Normal, 4 TSH ↑ / 9 H, 3T, 3T but well			

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

68

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 7

DETAILS OF PATIENTS REVIEWED IN LATE 1974 (n = 257)

GROUP	CLINICAL STATUS	NO PATIENTS	% of TOTAL GROUP	T_4	T_3	FTI	THYOPAC 3	$PB^{125}I$	TSH	CHOLESTEROL
1	EUTHYROID	23	66	122.3 ± 37.3	2.82 ± 0.70	120.2 ± 43.8	105 ± 10	496 ± 165	2.05 ± 1.72	6.16 ± 1.04
	TOXIC	6	21	179.0 ± 45.0	3.99 ± 0.54	206.0 ± 56.6	88 ± 9	577 ± 71	1.20 ± 0.49	5.18 ± 0.7
	HYPO	5	15	-	-	-	-	-	-	-
2	EUTHYROID	34	47	106.8 ± 33.5	2.49 ± 0.58	100.4 ± 36.0	108 ± 8	433 ± 95	6.40 ± 13.46	6.35 ± 0.78
	TOXIC	22	29	168.6 ± 46.3	4.96 ± 1.86	189.2 ± 72.1	93 ± 13	717 ± 181	1.37 ± 1.52	5.02 ± 0.8
	HYPO	19	24	-	-	-	-	-	-	-
3	EUTHYROID	43	65	99.1 ± 28.3	2.45 ± 0.63	91.4 ± 29.6	108 ± 10	426 ± 110	7.25 ± 11.74	6.09 ± 0.78
	TOXIC	9	14	151.9 ± 32.2	4.13 ± 1.12	150.6 ± 41.2	99 ± 10	583 ± 87	1.66 ± 1.47	5.41 ± 0.6
	HYPO	14	21	-	-	-	-	-	-	-
4	EUTHYROID	21	31	94.0 ± 27.0	2.37 ± 0.55	87.5 ± 27.0	108 ± 10	449 ± 181	18.00 ± 27.6	36.99 ± 1.30
	TOXIC	5	7	151.9 ± 63.1	4.20 ± 2.27	191.8 ± 104.2	91 ± 21	646 ± 221	2.64 ± 2.55	-
	HYPO	42	62	-	-	-	-	-	-	-
5	EUTHYROID	4	29	84.9 ± 23.2	1.96 ± 0.48	81.1 ± 27.0	108 ± 12	268	35.80 ± 43.70	6.86 ± 1.0
	TOXIC	1	7	114.5	3.42	100.4	114	504	1.0	-
	HYPO	9	64	-	-	-	-	-	-	-

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

Normal T_4 and T_3 96

(TSH normal 83
(TSH elevated 13

Elevated T_3, normal T_4 11

Elevated T_4, normal T_3 4

Elevated T_4 and T_3 5

Low T_4, elevated TSH   9
                                                      ___
                                    TOTAL :   125
                                                      ===
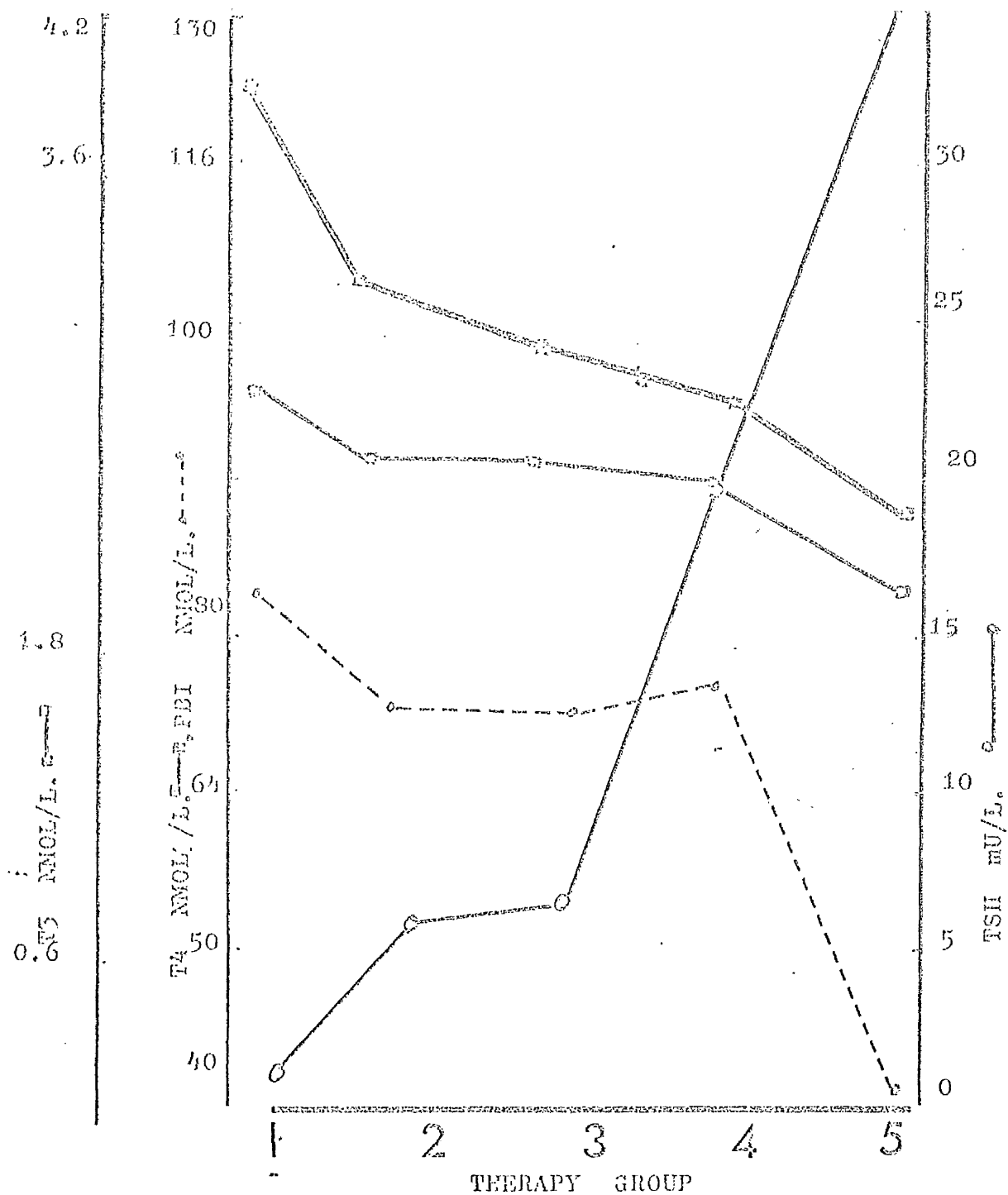
FIG.13 MEAN T_4, PBI, T_3 AND TSH LEVELS IN THE CLINICALLY EUTHYROID PATIENTS RELATED TO THERAPY GROUP. THE DOSE OF IODINE 125 GIVEN INCREASES FROM THERAPY GROUP 1 TO GROUP 5.

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 hypo-
thyroidism 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 9

REVIEW OF CLINICALLY EUTHYROID PATIENTS WITH ELEVATED TSH LEVELS

PATIENT NO.	AGE	INITIAL REVIEW			FOLLOW UP REVIEW				12m. FOLLOW UP REVIEW		
		T4 mm/1	TSH mu/1	Clin.Imp.	T4 mm/1	TSH mu/1	TRH Resp.	Clin.Imp.	T4 mm/1	TSH mu/1	Clin.Imp.
1	68	82	9.5	E	↓	LFU					↑
2	51	102	15.4	E	↓	DNA		↑	69	29.1	H
3	51	59	39.0	E	51	96	Exag.		43	39.0	E
4	56	77	25.0	E	78	26.0	Exag.		73	19.1	H
5	70	68	52.0	E	↓	DEAD					↑
6	65	93	40.0	E	117	42.5	Exag.		66	25.0	E
7	56	98	15.5	E	117	27.3	Exag.		95		E
8	53	68	15.5	E	↓	DNA		↑	63	23.1	E
9	53	88	28.5	E	111	18.0	Exag.		118	10.6	E
10	57	58	22.5	E	69	3.0	Exag.		60	21.7	E
11	70	74	41.9	E	↓	DNA		↑	65	13.8	E→H
12	55	134	21.3	E	109	32.0	Exag.		92	41.6	E
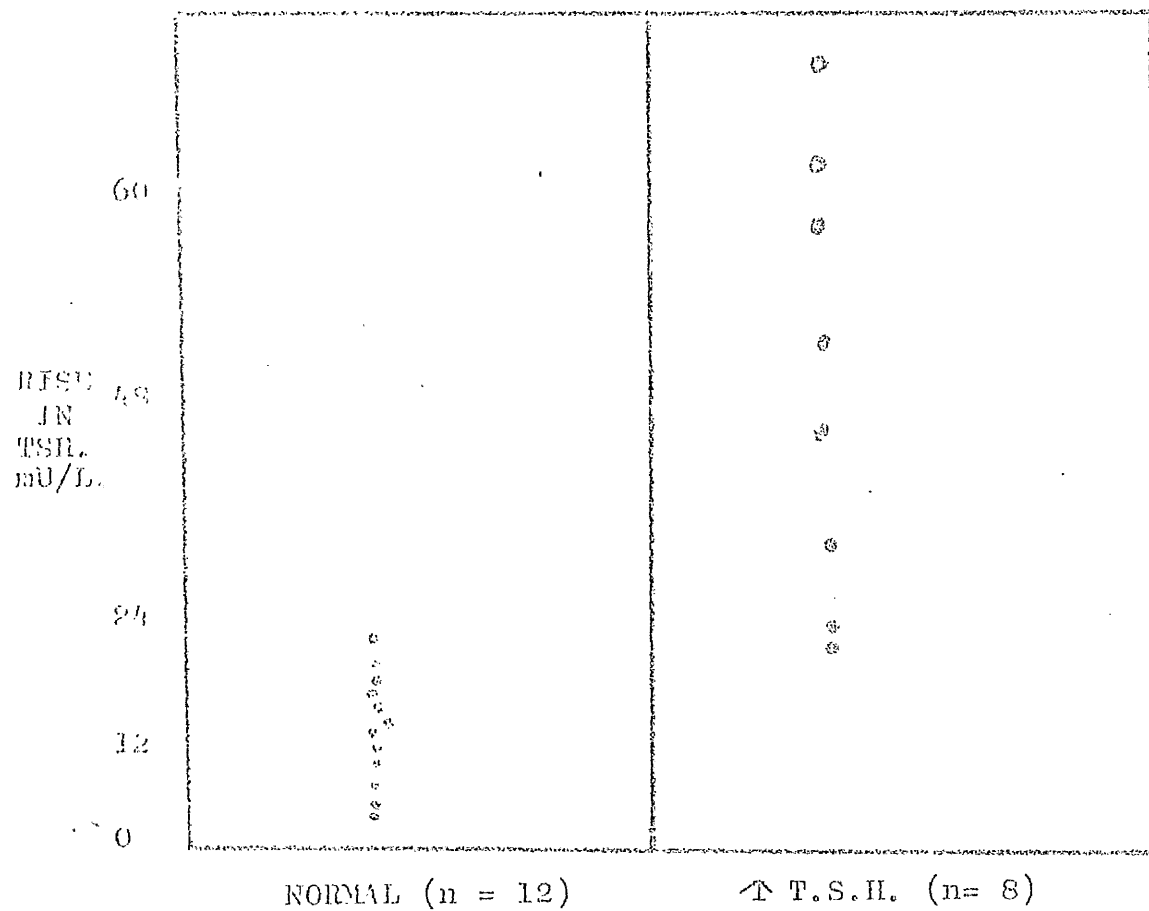
FIG. 14 T.S.H. RESPONSE TO T.R.H. IN PATIENTS
WITH "COMPENSATED EUTHYROIDISM".

TABLE III

POST 125Ĩ RESPONSE TO TRH IN PATIENTS WITH NORMAL T_4, T_3
AND ELEVATED T.S.H.

NO.	BASAL TSH mU/L	POST TRH LEVEL mU/L	$\triangle$ TSH mU/L
1	15	39	24
2	96	170	74
3	130	210	80
4	60	110	50
5	75	163	88
6	74	100	26
7	11.5	47.6	36%
8	50	108	58

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(158), 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 10

LIPOPROTEIN PARAMETERS IN "COMPENSATED EUTHYROIDISM"

	EUTHYROID c ↑TSH	CONTROL GROUP (TSH normal)	UPPER LIMITS OF NORMAL (FREDRICKSON)
No.	12	10	–
Age range	40–49	40–51	40–49
Total chol. (± SD)	* 7.25 ± 1.17	6.35 ± 1.04	8.03
LDL chol.	** 4.14 ± 1.04	3.11 ± 0.78	4.92
Trig.	Ø 1.58 ± 0.14	1.24 ± 0.17	1.81 ± 0.2
VLDL Chol.	Ø 0.78 ± 0.26 N.S.	0.57 ± 0.36	0.91

* 2 values outwith normal.   Ø 2 values outwith normal.

Cholesterol values are S.I. units   (mean ± SD)

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 sino-
atrial 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

PATIENT NO GROUP	Q_2S_2I	$LVET_I$	PEP_I
(1) ↑ TSH (m = 8)	539 ± 15 *	403 ± 8 *	138 ± 15 *
(2) Normal TSH (m = 10)	535 ± 8	404 ± 4	128 ± 4
P	NS	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"

PATIENT GROUP	K2 (% m $^{-1}$)
* EUTHYROID c $\uparrow$ TSH (n = 6)	2.98 $\pm$ 0.30
* CONTROL (TSH normal) Clin. euthyroid (n = 5)	3.91 $\pm$ 0.40
	S p $<$ 0.01

* same age range.

of Intralipid (a soyabean extract available commercially from A.C.Vitrum, Sweden) is administered as an intravenous bolus and venesamples obtained at 10 minute intervals over the subsequent hour from a Venflon 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 λ 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, K2 (% / min), is the required parameter.

The parameter K2 is a measure of the body's capacity to remove chylomicra from plasma, i.e. is a measure of lipid clearing capacity. The K2 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 E.D.T.A. anticoagulated blood. The samples were frozen directly and assayed with control samples from 10 normolipaemic, 6 Type IV hyper-triglyceridaemic 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. (298). The incubation mixtures were immediately assayed in triplicate by extraction with Dole reagent (64) and measurement of total free fatty acid levels by micro-titration 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

GROUP	NO. SUBJECTS	UNESTERIFIED FATTY ACIDS μeq/L Plasma		LIPOPROTEIN LIPASE ACTIVITY μeq/min/100 ml. Plasma	
		PRE-HEP	POST-HEP	PRE-HEP	POST-HEP
COMPENSATED EUTHYROIDISM	7	0.4 ± 0.4	2.7 ± 0.5	3.0 ± 0.7	12.0 ± 3.2
NORMALS	10	0.4 ± 0.3	1.1 ± 0.4	1.2 ± 0.7	12.2 ± 2.1
TYPE IV	8	0.5 ± 0.4	3.9 ± 0.5	4.7 ± 0.9	12.1 ± 3.8
TYPE II	6	0.4 ± 0.25	1.2 ± 0.5	1.1 ± 0.6	11.9 ± 2.1

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 hypertriglyceridaemia
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

PATIENT	PRE-IODIDE			6 WEEKS			16 WEEKS		
	T_4	T_3	TSH	T_4	T_3	TSH	T_4	T_3	TSH
1	43	1.34	39	70	1.30	17	59	1.32	29
2	73	1.61	19	84	1.50	4	76	1.49	12
3	92	1.21	42	100	1.30	11	89	1.29	31
4	60	1.15	22	65	1.20	13	64	1.18	20
MEAN n = 4	67	1.33	30.5	80	1.33	11.3	72	1.32	23

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 16

REVIEWS OF CLINICALLY EUTHYROID – BIOCHEMICALLY T_3 TOXIC PATIENTS

PATIENT NO.	INITIAL REVIEW			7 MONTHS FOLLOW UP REVIEW				12 MONTHS FOLLOW UP REVIEW		
	T_4	T_3	Clinical Impression	T_4	T_3	TRH response	Clinical Impression	T_4	T_3	Clinical Impression
1	127	3.42	E	173	5.45	None		113	3.68	E-mild T
2	126	3.67	E	176	4.78	None		115	3.10	E
3	106	3.52	E	↙	LFU			↗		
4	90	3.39	E	89	2.78	None		74	2.74	E
5	139	3.47	E	106	3.54	Exag.		96	2.83	E
6	109	3.20	T	106	3.54	Exag.		94	2.71	E
7	124	3.85	T	127	4.78	None		106	3.76	E
8	67	3.60	T	↙ D.N.A. ↗				91	2.16	E
9	83	3.36	E	112	3.39	None		91	2.62	E
10	139	3.45	E	120	3.23	None		63	2.32	E

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 I-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"

PATIENT	PRE-IODIDE			6 WEEKS			15 WEEKS		
	T_4	T_3	TSH	T_4	T_3	TSH	T_4	T_3	TSH
1	127	3.42	0	180	3.90	0	135	3.91	0
2	106	3.62	0	176	4.10	0	160	3.70	0
3	90	3.39	1	114	3.40	0	95	3.36	0
4	139	3.47	1	173	3.46	1	141	2.01	2
5	67	3.60	0	96	4.10	0	80	3.70	0
Mean n = 5	106	3.50	0.4	148	3.79	0.2	122	3.34	0.4

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 (281) 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).

T_3 nmol/l.

400
300
200
100

F_4 F. T. I.

4   10   20   40

LEGEND   No.

Thyrotoxic   28   x
Compensated
Euthyroid   11   ⊗
T_3-toxic   20   ⊙
Euthyroid   48   •
Hypothyroid   12   ⊡
T_4-toxic   7   2
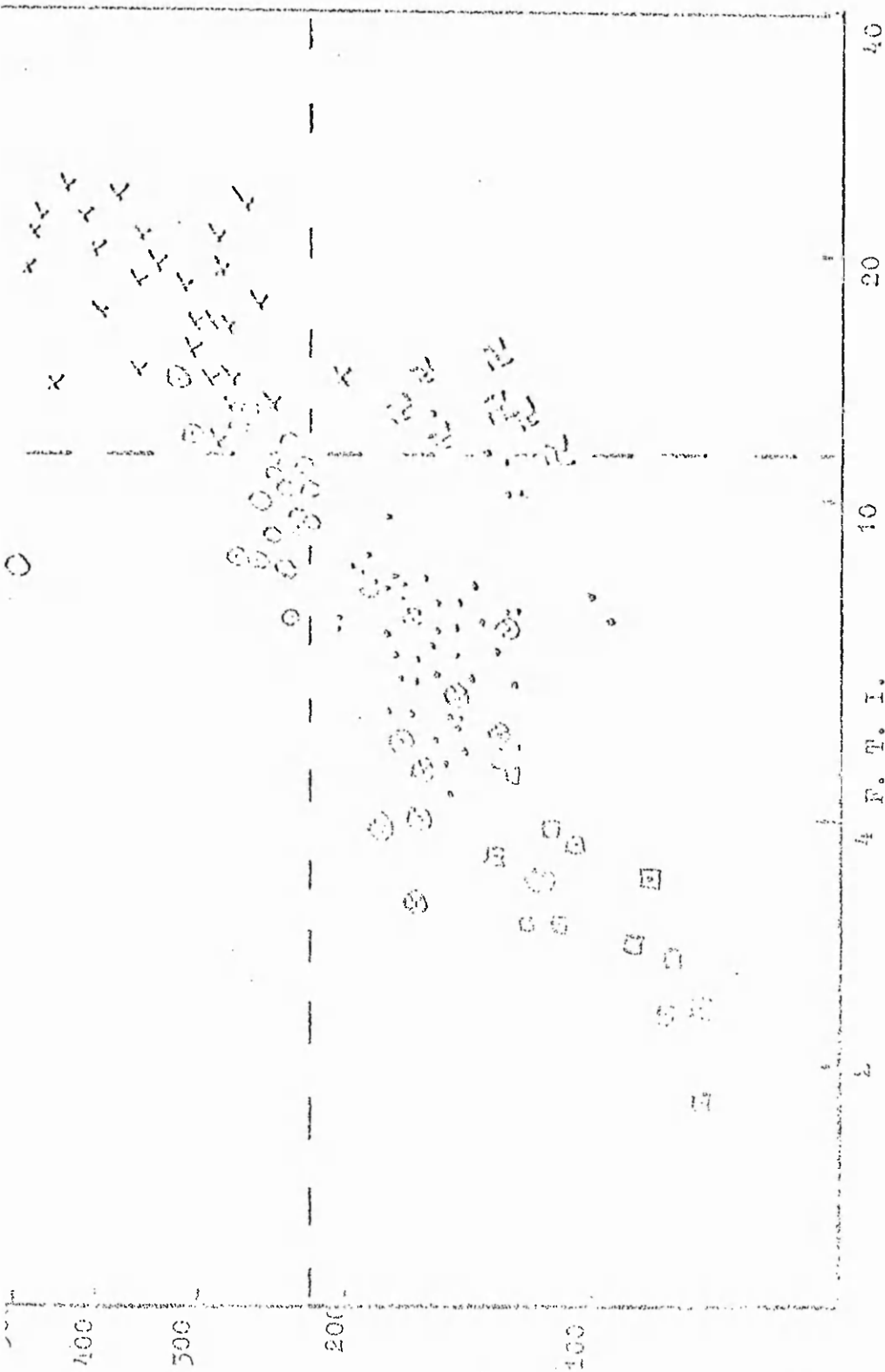
FIG. 15   LOG-LOG SCATTERGRAM DEMONSTRATING PROBABLE EXISTENCE OF T_4-TOXICOSIS AS A CLINICAL ENTITY.

It has also been suggested that T-4 toxicosis is a relatively common finding in elderly patients with thyroid disease (32,38) 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 6 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 6 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 125^I-
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 - Saberi 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 T_4 and T_3
inhibit the T.S.H. response to T.R.H. and interpret the
delay in onset of action of T_4 as indicating that the intra-
pituitary inhibition is due to T_3, time being required for
de-iodination of T_4 to T_3. Biochemical studies, however,
indicate high affinity specific T_3 receptors in pituitary,
but also, in some species, low affinity specific T_4 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 "T_4 - 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 131^I 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 125^I therapy.

TABLE 18

TRH TEST POST-125^I IN CLINICALLY AND BIOCHEMICALLY EUTHYROID PATIENTS

GROUP	NO. PATIENTS	T_4	T_3	TSH	$\triangle$ TSH $\bar{c}$ TRH at 20 min.
1 No response	7	114.5 ± 21.9	1.66 ± 0.6	0.1 ± 0.2	0.4 ± 0.6
2 Response inadequate	10	110.7 ± 27.0	1.61 ± 0.5	0.9 ± 2.0	4.5 ± 2.3
3 normal response	7	104.2 ± 21.9	1.55 ± 0.4	1.9 ± 2.1	14.0 ± 1.5
Group I 9 months later	n = 3	108 ± 20.6	1.58 ± 0.7	0.9 ± 1.0	7.1 ± 3.0

Prior to T.R.H. injection (200 μg. intravenously in the morning) full hormonal measurements were performed (T_4, T_3, 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 T_4 and T_3 levels.

When the responses to T.R.H. ($\triangle$ T.S.H.) was graphed against time since last therapy (it was not possible to define accuratelythe onset of thyrotoxicosis in this group), therewas 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 T_4 and/or T_3 levels may produce pituitary thyrotroph suppression and they may not recover for some time when T_4 / T_3 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 T_4 and T_3 levels.

WHY DID THE TRIAL FAIL ?

> "But facts are chills that
>
> winne ding, an' canna 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

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

* +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 $PB^{127}I$ and TSH levels
in the sera of rats given oral TRH (100 or 1000 μg/kg. for
32 days), while Chopra 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 (78).
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 anaesthaesia 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 $PB^{127}I$.   The results are detailed
in Table 20.

FIGURE 17

HISTOLOGY OF THYROID IN RATS GIVEN
TRH FOR THREE MONTHS.
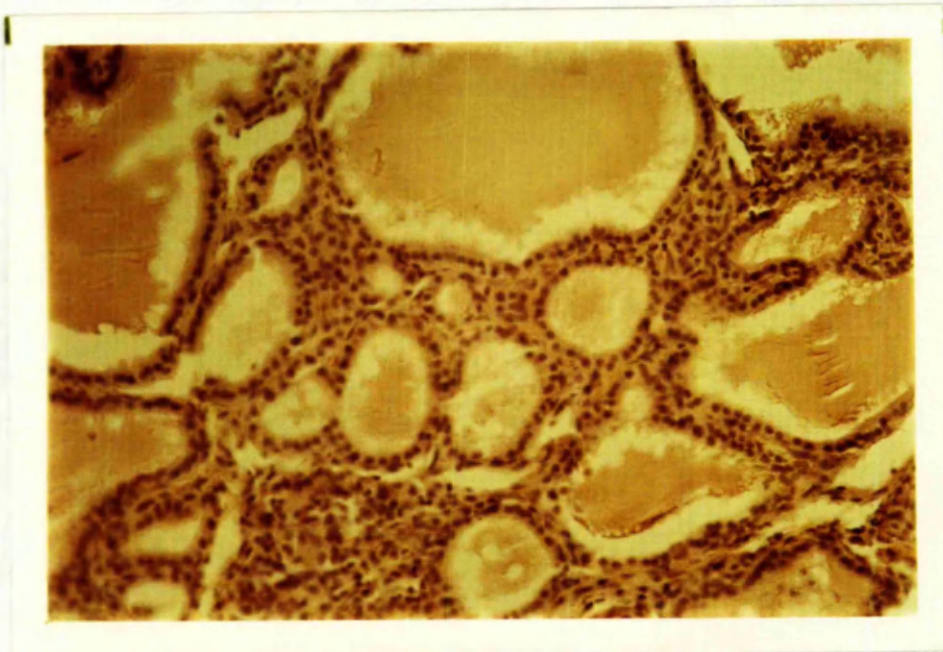
(A)   GENERAL VIEW OF HYPERACTIVE THYROID
(H. and E. x 220).
ACTIVE COLLOID RESORPTION IS EVIDENT.
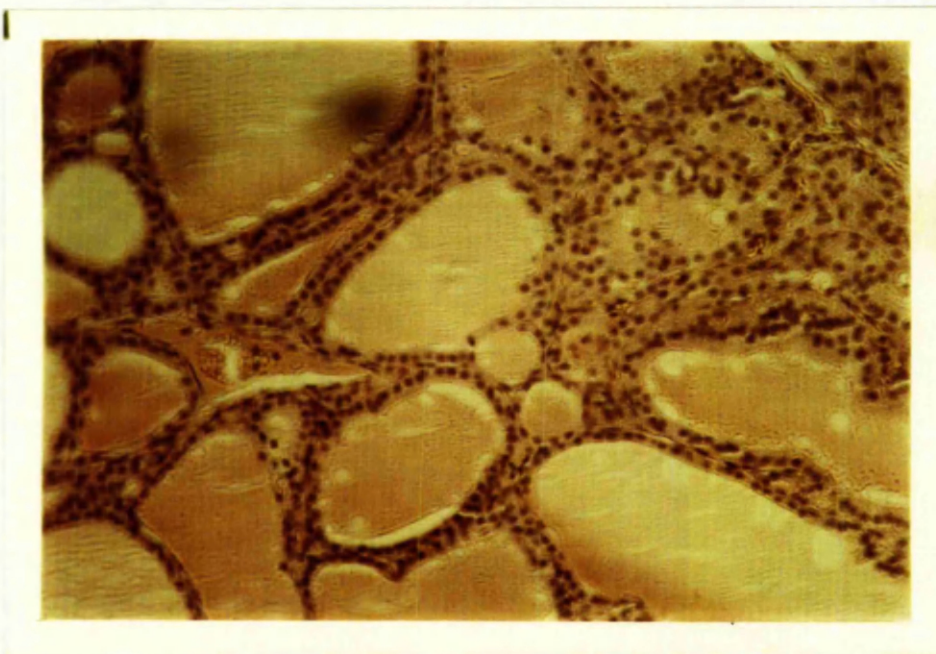
(B)   VIEW OF CONTROL GLAND.
(H. and E. x 560).
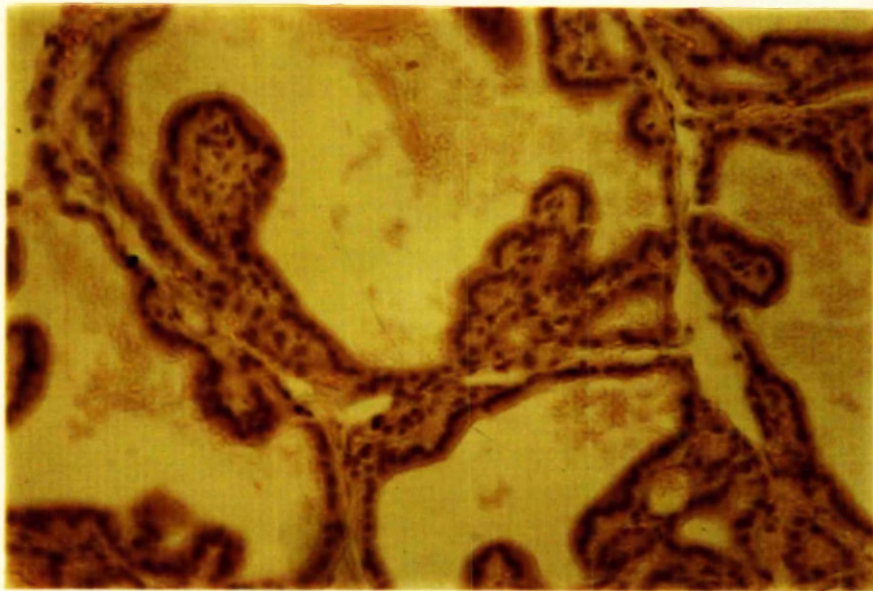
(c) ANOTHER VIEW OF HYPERACTIVE RAT THYROID.
(H. and E. x560).

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 Wister 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, Askanazy change (cell cytoplasmic structural
change) and extravasation of follicular colloid into the
intravascular space.

RADIOBIOLOGICAL ACTIONS OF ^{125}I AND ^{131}I ON TRH-STIMULATED RAT THYROID

ISOTOPE	DOSE μCi	TIME AFTER ADMINISTRATION (DAYS)	No. RATS STUDIED	MEAN THYROID WEIGHT (mg)	PBI μg/100ml	AUTORADIOGRAPHIC PATTERN		ASKANAZY CHANGE	HISTOLOGY FOLLICULAR DAMAGE	EXTRA-FOLLICULAR COLLOID
						RADIAL	ANNULAR			
^{125}I	10	1	2	35	–	–	–	–	–	–
	40	1	5	34	5.1	+	+	–	–	++ n=4
	100	1	2	29	5.3	+	+	–	+	++
^{131}I	10	1	2	34	–	+	+(n=1)	–	–	–
	40	1	2	32	–	+	+	–	–	+
	100	1	2	32	5.3	+	+	–	–	+
^{125}I	2.5	5	1	31	–	–	–	–	–	–
	10	5	3	32	–	–	–	–	–	–
	40	5	2	–	5.5	+n=1	–	–	–	–
	160	5	1	27	6.8	+	–	–	–	–
^{131}I	10	5	1	31	–	–	–	–	–	–
	40	5	1	30	5.4	–	–	–	–	–
	100	5	2	31	6.7	+	+	+n=1	+	+
^{125}I	2.5	9	1	34	–	–	–	–	–	–
	10	9	1	33	5.0	–	–	–	–	–
	160	9	3	25	7.2	+	+	+	+	+
^{131}I	10	9	1	31	–	–	–	–	–	–
	40	9	2	30	5.9	+	+	+	+	+
	100	9	2	28	6.9	+	+	–	+	+
^{125}I	40	16	3	30	5.0	+	+	+	+	+
	160	16	1	22	7.0	++	++	++	++	++
^{131}I	10	16	1	31	–	+	+	–	–	–
	40	16	3	30	5.2	+	+	–	+	+
	100	16	1	22	6.8	+	+	+	+	+

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}I 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 100μCi^{131}I with no frank structural or
autoradiographic changes at 40 μCi^{131}I.   The ^{125}I-treated group

FIGURE 18

APPEARANCES OF IRRADIATED GLANDS IN RATS
GIVEN T.R.H.
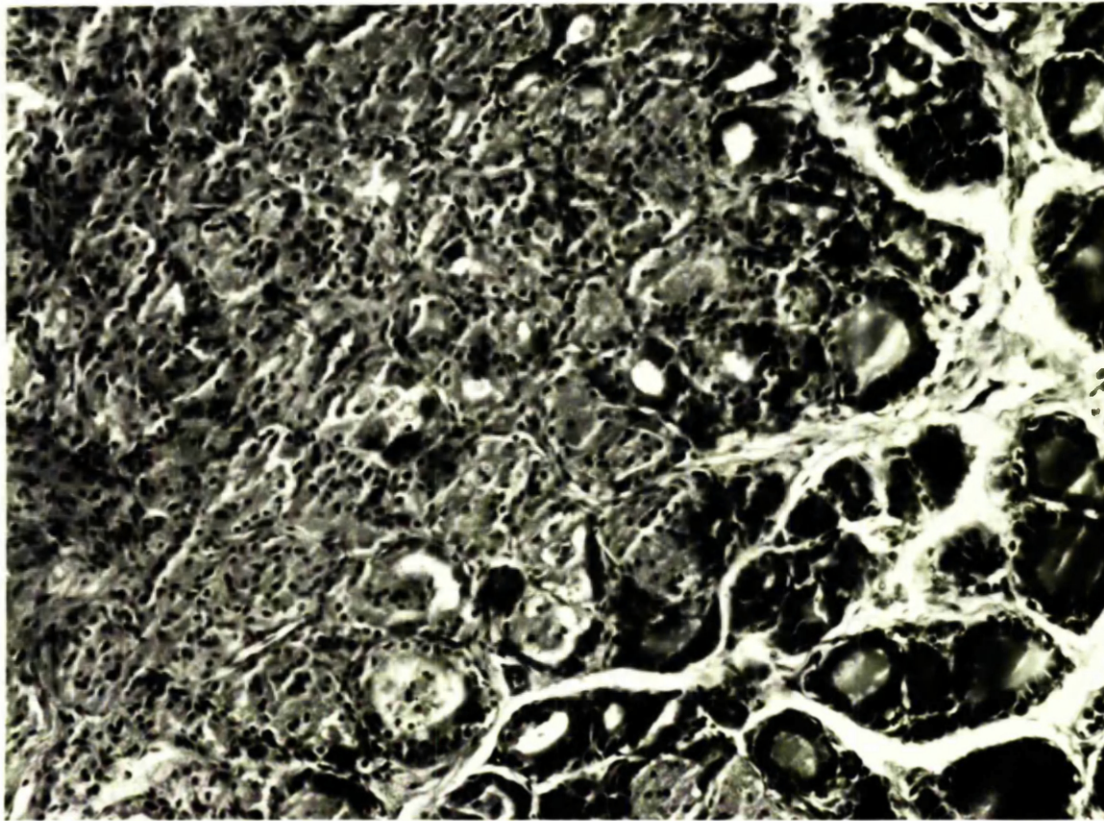
(A)

FRANK FOLLICULAR DAMAGE (UPPER HALF OF FIELD)
DAY 16 DOSE – 160 μc ^{125}I
(H. and E. × 225)
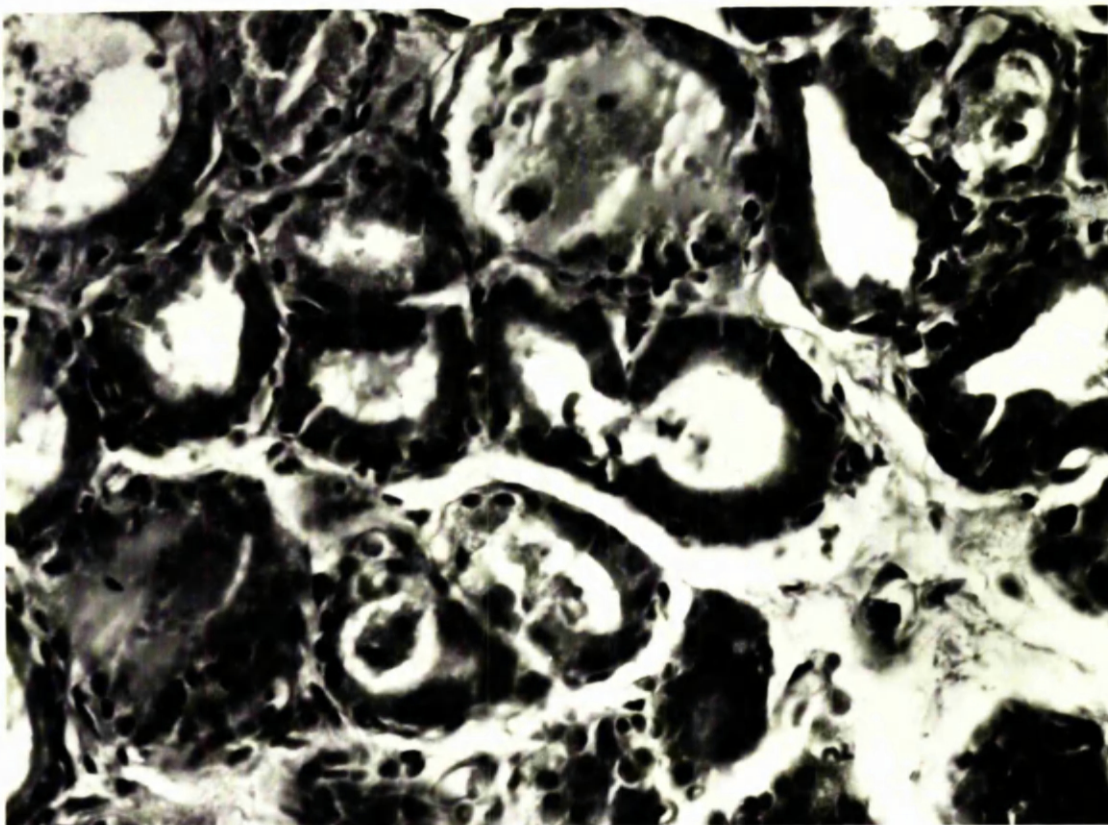
(B) ASKANAZY CHANGE. THE THYROID EPITHELIAL CELLS ARE LARGER THAN NORMAL, CUBICAL, HAVE A GRANULAR CYTOPLASM WHICH IS EOSINOPHILIC.
DAY 16 DOSE 160 μc ^{125}I H. and E. x 560.
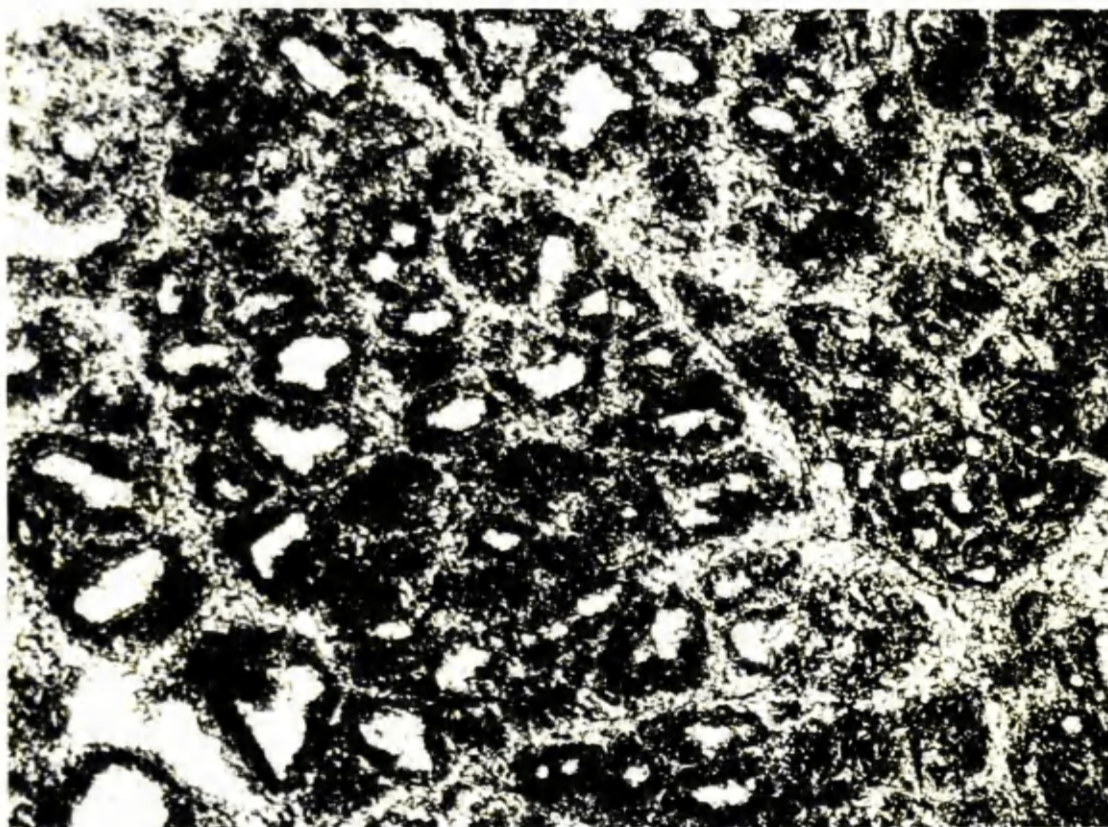
(C) AUTORADIOGRAPH SHOWING CONSIDERABLE LOSS OF LABELLED COLLOID FROM FOLLICLES INTO PERIFOLLICULAR TISSUE.
DAY 9 DOSE 160 uc ^{125}I.   Ilford 45 x 225.
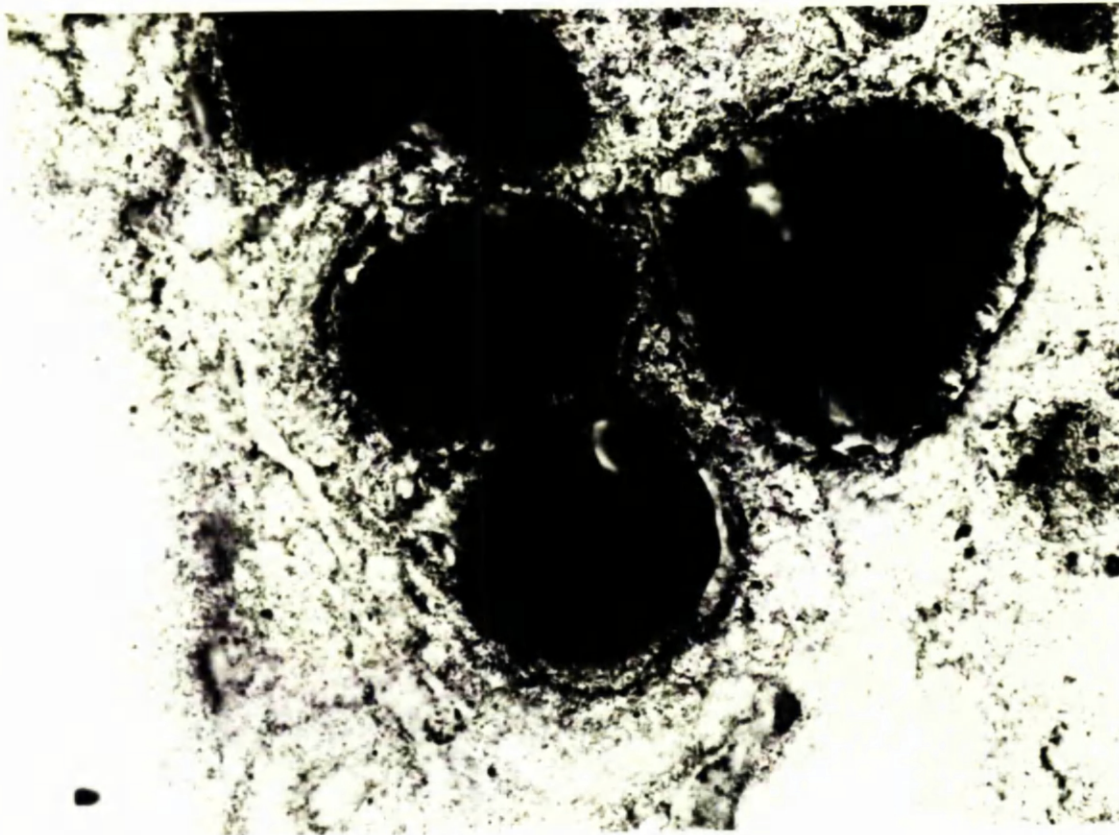
(D) AUTORADIOGRAPH SHOWING RADIAL AND ANNULAR PATTERNS OF LABELLED COLLOID LE
Ilford 44 x 560.
DAY 9   DOSE 160 μc ^{125}I.

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 I 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 auto-
radiographic 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}I producing an effect earlier than ^{131}I. The dose ratio for similar effect would appear to be of the order of 4:1 ^{125}I : ^{131}I. There is no demonstrable sparing effect of ^{125}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}I in 360 thyrotoxic patients, where there is no demonstrable reduction in the rate of hypothyroidism at three years after ^{125}I therapy and where the dose ratio of ^{125}I to ^{131}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}I or ^{131}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 qualitive 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 specious differences with subsequent goitrogen
administration. The therapeutic irradiation from ^{125}I is of
low energy and so its effect will be confined to follicles
where it is takenup - i.e. non-stimulated follicles will be
protected. ^{131}I, 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}I (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}I therapy has been shown to be due to
a damaged capacity of follicular cells to replicate (5).

Konecny (151) observed a striking increase in gland weight
in rats after ^{125}I treatment (detected after Day 30 and peaking
at Day 60) and interpreted this effect as due to a nuclear
sparing action of ^{125}I and retained capacity to respond to
TSH: this, however, is explicable in terms of the lowish dose
of 125_I administered (50 µCi). Damage was not apparent on
histological examination until Day 15 - in accord with the
findings demonstrated here for 40 µCi ^{125}I.

The present findings agree with those of Jongejan and van 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 T_4 levels (an apical function) and histological evidence of cell death 100 days after isotope administration show no evidence for a differential action of ^{125}I 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.

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

Neither the data presented here, nor the results of the clinical trial of ^{125}I in humans support the claims for a differential effect with ^{125}I.

TABLE 19

RESULTS OF PREVIOUS TRIALS OF IODINE 125 THERAPY FOR THYROTOXICOSIS

REF.	NUMBER OF PATIENTS TREATED	DOSE (mC) mean	range	THYROID STATUS (% OF GROUP) EUTHYROID	TOXIC	HYPOTHYROID	DURATION OF FOLLOW-UP (MONTHS)
1	45	2.5	-	42	31	7	-
	11	5.0	(4-6.5)	54	37	9	-
2	30		4-9.5*	73	20	7	
	21		10-19.5*	43	38	19	mean 34
	12		20-35*	50	42	8	
	63		4-36*	60	30	10	
3	42	*22	(6-60)*	71	24	5	12
	28	*10	(2.8-32)*	43	53	4	9

REFERENCES: 1. Lewitus et al.(1971) (168)
2. Weidinger et al.(1974) (297)
3. Siamson et al. (1974) (258)

* 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.

β-ADRENORECEPTOR 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 - adrenoreceptor 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-adrenoreceptor 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 55/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 β-blocking drugs to thyrotoxic
patients and suggested that a more critical evaluation of the
action of β-adrenoreceptor blocking agents was required.
Obviously, if there is an element of adrenergic overactivity
in hyperthyroidism then a β-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 β-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
β -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 β -adrenoreceptor
drugs.

Moreover, cardiac glycosides are known to inhibit the
N_a, K,- ATP-ase 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

98
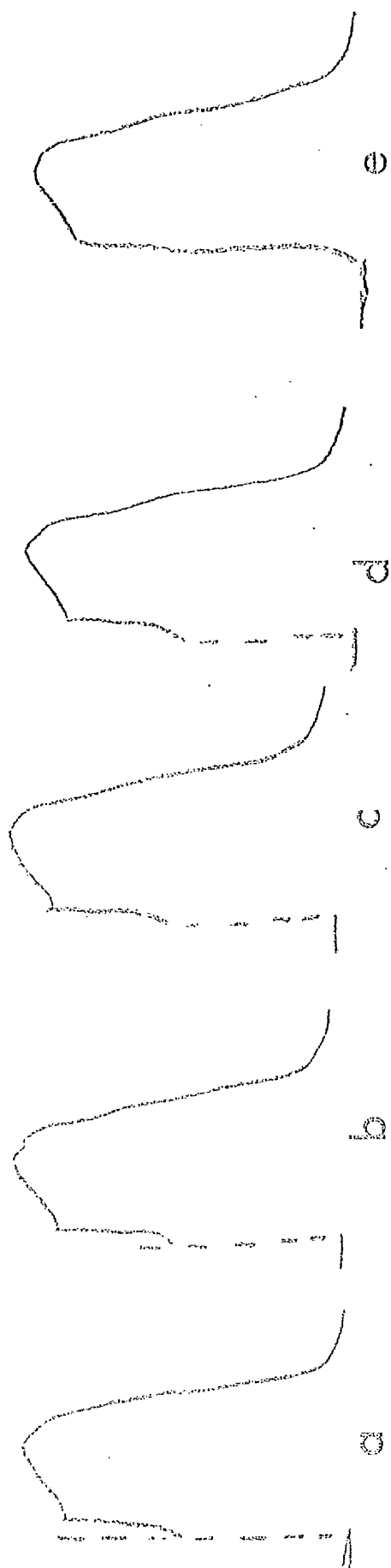
FIG. 12 ACTION POTENTIAL RECORDED IN CALF PURKINJE FIBRES IN PRESENCE OF ADRENALINE (5.5 x 10⁻⁵M). THE Na⁺ CURRENT WAS PROGRESSIVELY BLOCKED WITH INCREASING DOSES OF TETRODOTOXIN: (a) CONTROL; (b) 5 x 10⁻⁵M; (c) 5 x 10⁻⁷M; (d) AND (e) 5 x 10⁻⁶M. NOTE COMPLETE ABSENCE OF INITIAL SPIKE IN (e). (CARMELIET AND VEREECKE 1969).

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 β-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 β-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 β-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

DIAGRAM OF THE POSTULATED INTERRELATIONSHIPS BETWEEN ACTION POTENTIAL DURATION, CONTRACTION AND INTERNAL CALCIUM.
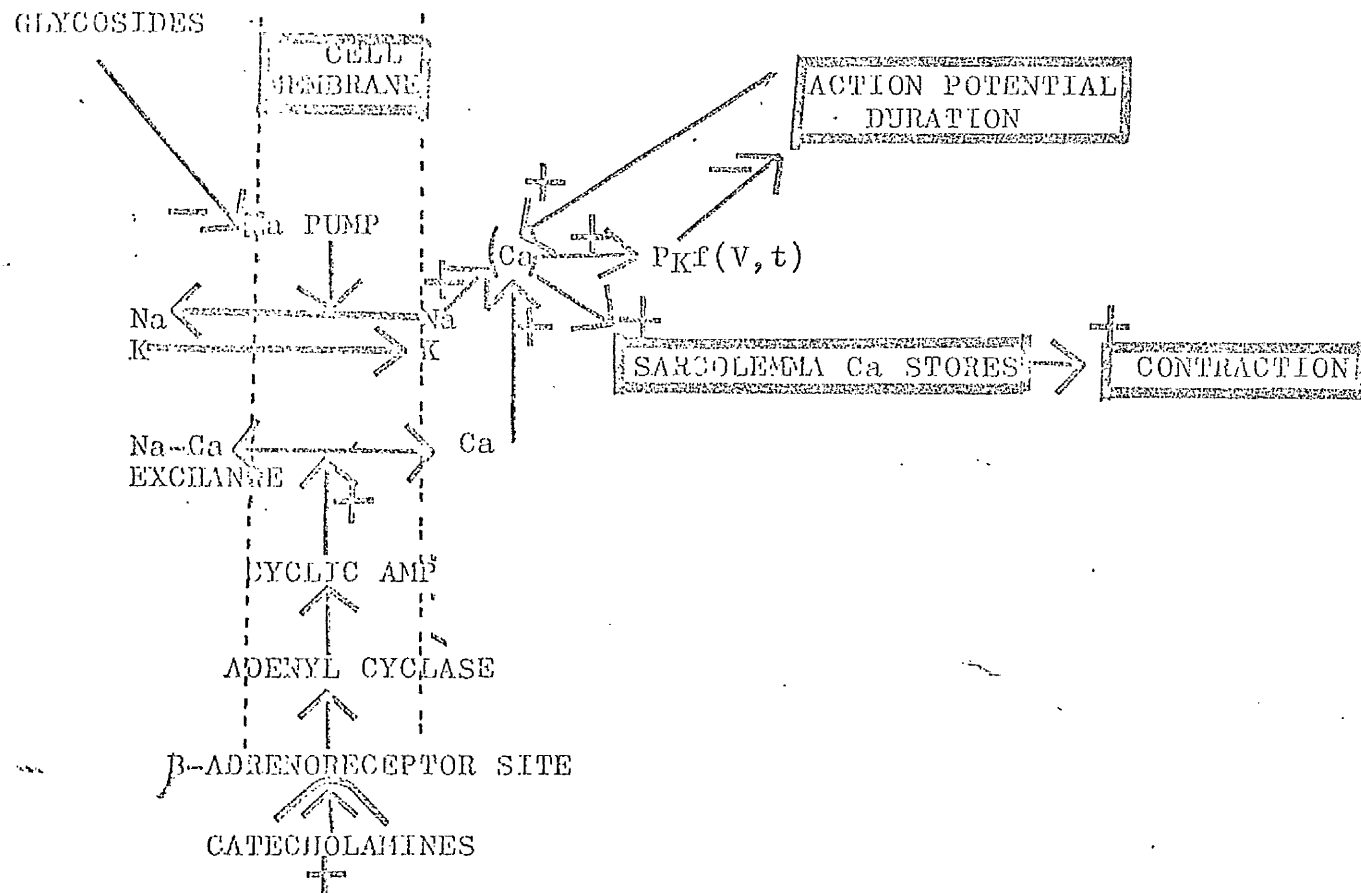
An increase in intra-cellular calcium augments myocardial contract by stimulating sarcolemmal calcium uptake and release. Intra-cel calcium is increased in turn by direct inflow of calcium (slow cal current) shown here as part of the Na-Ca exchange, which in turn i stimulated by cyclic AMP produced by catecholamine action at the B-adrenoreceptor site. Intra-cellular calcium is increased indire 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 outw potassium current (shown here as a function of membrane potential and time), thereby shortening the action potential duration, redu 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 studes, 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 cardio-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 (28,104), practolol which
is a cardio-selective beta-blocker, has some intrinsic
sympathomimetic activity, and which has some benefit clinically
in toxic patients (30,205), oxprenolol which has some intrinsic
sympathomimetic action and is of little benefit clinically in
hyperthyroidism (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 proprenolol on
the effect of a cardiac glycoside (ouabaine) 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-blockade 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.

(6) 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 p-mole/cm^2 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.
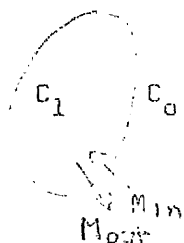
Let such a cell of area A and volume V,
and with an internal concentration of C_1
be suspended in a solution containing the
same ion at a concentration C_0. For
experimental purposes some of the external
ions are replaced by the labelled ions C_0^x.
The specific activity of the bathing fluid
is then 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_{in} and M_{out} for the influx

and the efflux respectively. The labelled fluxes of the

ion are then M_{in}^{x} and M_{out}^{x} respectively.

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

$M_{in}A - M_{out}A$ therefore the concentration changes by

$M_{in}A/V - M_{out}A/V$ in unit time.

i.e. $d/C_1)\,dt = M_{in}\,A/V - M_{out}A/V \ldots\ldots\ldots\ldots(1)$

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:-

$$d(C_1^{x})/dt = M_{in}^{x}A/V \ldots\ldots\ldots\ldots\ldots\ldots\ldots(2)$$

Rearranging $M_{in}^{x} = d(C_1^{x})/dt.V/A \ldots\ldots\ldots\ldots\ldots\ldots(3)$

M_{in} is then obtained by multiplying M_{in}^{x} by C_o/C_o^{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 $M_{in}^{x}A/V$ in equation (1) becomes very

small (theoretically zero), and so

$$D(C_1^{x})/dt = -M_{out}^{x}A/V \ldots\ldots\ldots\ldots\ldots\ldots\ldots(4)$$

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_{out} is proportional to (C_1)

or $M_{out} = K_2 C_1$ $\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots$(5)

where K_2 is a constant.

Therefore from equation 4:-

$$d(C_1^x)/dt \quad = \quad -A/V \cdot K_2 \cdot (C_1^x) \dots\dots\dots\dots(6)$$

$A/V \cdot K_2$ can conveniently be replaced by k giving,

$$d(C_1^x)/dt \quad = \quad -k \cdot (C_1^x) \dots\dots\dots\dots\dots(7)$$

$$\text{or,} \quad (C_1^x) \quad = \quad (C_1^x)_{t=o} \cdot \exp \cdot -kt \dots\dots\dots\dots(8)$$

where (C_1^x) is the activity left in the cell after time t, where $(C_1^x)_{t=o}$ was the original activity at time zero.

A more convenient form of this equation is obtained by taking the log. when $\quad \ln. (C_1^x) = \ln.(C_1^x)_{t=o} - kt \dots\dots\dots$(9)

$$\text{or} \quad \log_{10} (C_1^x) = \log_{10}(C_1^x)_{t=o} - kt \cdot \log_{10} \dots(9a)$$

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 $\log (C_1^x)_{t=o}$ and slope proportional to k.

k in these 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/time (i.e. min.$^{-1}$ etc.)

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FIGURE 20.

Flow Diagram For Measurement of Intracellular Ion Concentrations and Influxes.

Concentration

10^5 cells/plate

3 plates
in soak sol^n
±isotope ± drug

Wash ×4 in Na$^+$, K$^+$-free,
Isotonic KREBS at 0°C

TRYPSINIZE 0.5ml of 0.25% w/v
± inhibitor
± isoton

PIPETTE ×6

Make up to 25ml

Count cell N^o Count Isotope
(Coulter Counter)   (Tricarb)
                    =

ION CONCENTRATION

Influx

3 plates in soak sol^n
± drug.(no isotope)

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

^{22}Na / 30 Secs ^{86}Rb \ 10 mins
        Later   Later

3ml
of sol^n

Wash×4 in Na$^+$ K$^+$-free Isotonic KREBS
at 0°C

Count Isotope   Assay Na,K
(Tricarb)   (Photometer)

TRYPSINIZE
± inhibitor
± isoton

PIPETTE

Make up to 25ml

Count Cell N^o Count Isotope
              =
30 second ^{22}Na influx

FIGURE 2.

Flow Diagram For Efflux Measurements.

PLATES OF CELLS EQUILIBRATED IN $^{22}Na/^{86}Rb$ MEDIUM $\pm$ DRUG.

Wash x4 in Non-Radioactive Soak Sol.

$\pm$ Drug for 30 Seconds

Leave in Soak Solution

1 min (^{22}Na)
$9\frac{1}{2}$ min (^{86}Rb)

2 mins (^{22}Na)
$29\frac{1}{2}$ in (^{86}Rb)

5 mins (^{22}Na)
$59\frac{1}{2}$ mins (^{86}Rb)

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

^{22}Na Content
t = 1.5 min

Measured. (10 Plates per Assay)
t = 2.5 min

t = 5.5 min

^{86}Rb Content (3 Plates per Assay)
t = 30 min

t = 30 min

t = 60 min

Residual
at t = o
or

Residual

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

$$k = M_{out}/C_1 \cdot A/V \qquad \ldots\ldots\ldots\ldots\ldots(10)$$

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

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 $k \cdot T_{\frac{1}{2}} = \ln.2$ (i.e. 0.693), where $T_{\frac{1}{2}}$ is the halftime of the exponential process, equation 11 becomes:-

$$M_{out} = \ln.2/T_{\frac{1}{2}} \cdot V/A \cdot (C_1) \quad \ldots\ldots\ldots\ldots\ldots(11a)$$

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 $moles/cm^2$. 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-adrenoreceptor 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-l Propranolol, Oxprenolol, Practolol and Tolamolol, in concentrations from 0.1 ugm/ml. to 100 ugm/ml. The effect of Propranolol on Ouabaine-induced ionic changes were measured in the presence of Ouabaine with or without 5 ugm/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 10^6 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 2.5×10^4 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-adrenoreceptor 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 Ouabaine-induced ionic changes, plates were incubated for four days and then the medium was replaced with growth medium

* where appropriate

TABLE 22.

EXPERIMENTS TO STUDY EFFECT OF PROPRANOLOL ON
OUABAINE-INDUCED IONIC ALTERATIONS

No.	Drug Combinations	Times of Exposure to Drug (Hours)
1	Ouabaine alone	24
2	Ouabaine and Propranolol	24 last 12
3	Ouabaine and Propranolol	24 last 1/2
4	Propranolol and Ouabaine	24 last 12

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 Rb^{86} (which behaves as ^{42}K, but
is more convenient to handle by virtue of its longer half-life (170)).
Concentrations of isotope 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

1. Measurement of cell volume.

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 μ) apart. Since trypsinised cells are spherical in shape, the volume may be calculated from cell volume, $V, = \frac{4}{3} \pi r3$ 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.

2. Measurement of cell surface area.

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)).
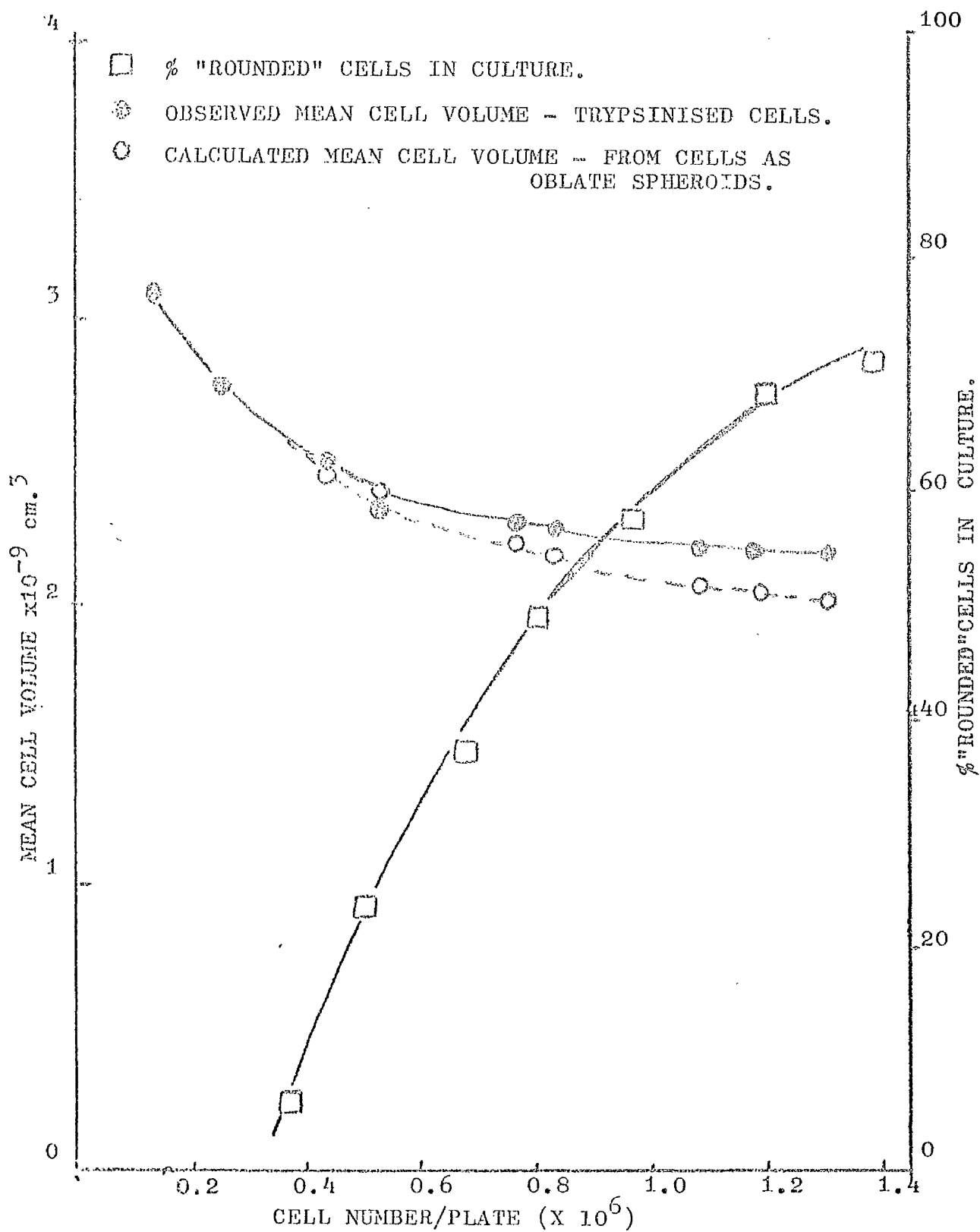
FIG. 22 SHOWING THE MEAN CELL VOLUME, CALCULATED REGARDING
THE CELLS AS OBLATE SPHEROIDS, COMPARED TO MEASURED
MEAN CELL VOLUME OVER A RANGE OF CELL POPULATIONS.

A total of 50 cells from a thickly populated monolayer were
so measured, 50 cells from a thinly propulated 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. 200m M 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 $O_{p1} \times W_1 = O_{p2} \times W_2$ (equ. 1)
where O_p and W are the cell water and osmatic pressures in
Kreb (suffix 1) and hyperosmolar Krebs(suffix 2). On transfer,
the cells lose water $(W_1 - W_2)$.

$$D + W_1 = V_1 \qquad V_1 \text{ - volume in solution 1}$$

$$D + W_2 = V_2 \qquad V_2 \text{ - volume in solution 2}$$

$$\therefore W_1 - W_2 = V_1 - V_2$$

and from this, W_2 is substituted in equ.1 and the volume of cell
occupied by water is given by $W_1 = \dfrac{V_1 - V_2}{\left(1 - \dfrac{O_{p1}}{O_{p2}} \right)}$

TABLE 23.

IONIC PARAMETERS IN THE PRESENCE OF B-ADRENORECEPTOR BLOCKING DRUGS

Drug	Drug Conc. (ug/ml).	Intracellular Ion Conc. (meq/l cell H_2O)		Ion Influx (p-mole/cm^2 sec.)		Ion Efflux (p-mole/cm^2 sec.)	
		K	Na	K	Na	K	Na
Propranolol	0.1	163±4	26±8	11±3	22±8	12±3	32±8
	0.5	167±4	30±8	13±3	20±8	11±3	36±8
	1.0	159±5	27±8	13±3	20±8	12±3	33±8
	2.0	160±4	27±8	11±3	21±8	13±3	32±8
	3.0	159±4	26±8	12±3	20±8	14±3	29±8
	5.0	161±4	27±8	14±3	21±8	11±3	32±9
	50.0	120±8	50±10	20±5	41±11	18±5	48±10
	100.0	100±8	62±10	36±8	55±11	31±7	60±10
Practolol	0.5	159±4	26±8	13±3	20±7	12±3	34±8
	1.0	163±4	30±8	13±4	22±8	14±3	–
	5.0	165±4	26±8	12±3	18±8	12±3	–
	100.0	90±8	70±11	40±7	60±11	32±8	–
Oxprenolol	0.5	155±4	27±8	13±3	22±5	12±3	–
	1.0	159±4	22±8	11±3	20±8	11±	–
	5.0	160±4	26±8	14±3	21±8	–	–
	100.0	105±6	60±10	35±8	50±11	30±8	–
Tolamolol	1	158±5	27±8	12±3	21±8	12±3	–
	2	166±4	22±8	11±3	21±7	–	–
	3	160±4	23±8	10±3	25±8	11±3	–
	5	159±3	25±8	13±3	22±8	–	–
	100	110±6	61±10				

TABLE 24

B-ADRENORECEPTOR BLOCKING DRUG ACTION AND DIGOXIN

DRUG	OUABAINE CONC. (M)	INTRACELLULAR ION CONC. (meq/1 cell H_2O)		ION INFLUX (p-mole/cm^2 sec.)		ION EFFLUX (p-mole/cm^2 sec.)	
		K	Na	K	Na	K	Na
OUABAINE for 24 hours	0	160 ± 4	24 ± 8	11 ± 3	20 ± 8	12 ± 3	32 ± 8
	10^{-8}	150 ± 4	46 ± 8	10.5 ± 3	30 ± 8	–	–
	5.10^{-8}	40 ± 6	130 ± 10	90 ± 3	40 ± 10	3.5 ± 2	44 ± 12
OUABAINE + 5 ug/ml PROPRANOLOL	10^{-8}	148 ± 4	45 ± 8	10 ± 3	33 ± 8	12 ± 3	32 ± 8
	5.10^{-8}	42 ± 6	128 ± 10	85 ± 3	42 ± 7	3.5 ± 3	40 ± 12
OUABAINE (24 hrs) + 5ug/ml PROPRANOLOL for 30 minutes	10^{-8}	151 ± 4	46 ± 7	105 ± 3	32 ± 8	–	–
	5.10^{-8}	35 ± 6	125 ± 9	90 ± 3	41 ± 7	3.4 ± 2	38 ± 10
5 ug/ml PROPRANOLOL (24 hrs.) + OUABAIN for 12 hrs.	10^{-8}	150 ± 4	45 ± 8	10 ± 3	30 ± 8	–	–
	5.10^{-8}	45 ± 6	120 ± 10	8.5 ± 3	42 ± 10	3.0 ± 2	40 ± 10

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 5×10^{-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.

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Using such a preparation, Nayler 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 <u>not</u> depress isolated cardiac muscle function when used in <u>clinically attainable concentrations.</u>

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-adrenoreceptor 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 betablocker.

The experiments described here demonstrate that beta-adrenoreceptor 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 ine (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

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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 (278)

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 dysrhythmia 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

fluxes 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

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fibres may be quite different from their action on cardiac muscle fibres. However, El Shahawy 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 β-blockade in a thyrotoxic patient, are evidence for a single cardiac site of action of β-blockers -- the sympathetic receptor site and a lack of sympathetic hyperactivity in thyrotoxicosis. This would suggest that the effect of β-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 β-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 β-blockade will be at best only partial and, therefore, treatment with β-blockade alone (as has been advocated prior to surgery (195) is potentially hazardous.

It is pertinent to observe that while β-blockade reduces pulse rate, tremor, stare and skin conductance (117,178,281), the effects of such therapy in one trial, were not sufficient to allow patient preference for β-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 25

CLINICAL AND BIOCHEMICAL DISCORDANCE IN A RADIO-IODINE
TREATED PATIENT

PATIENT	SEX	AGE (YEARS)	CLINICAL STATUS		THERAPY	TIME	HORMONAL LEVELS			TRH TEST
			WAYNE INDEX	COMPARED TO PRE-THERAPY		SINCE THERAPY (MONTHS)	T_4	T_3	TSH	$\triangle$TSH
W.L.	F	52	E	E	12 mCi ^{131}I	12	273	5.60	0	0

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 (237,256).

In collaboration with Dr. W.S.Hillis, 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 Garrod (1874)(97) but it was with the detailed studies of Wiggers in 1921 (311,312) that the foundations were laid for the present 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 divisable 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

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I.V.C.T. 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, there~~~ ~~, primarily affect I.V.C.T.

1) Preload which is t~~ ~~astolic muscle fibre length, or
   the end-diastolic v~~ ~~me.

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

3) Myocardial contractility.

4) Ventricular dyssynergy.

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.

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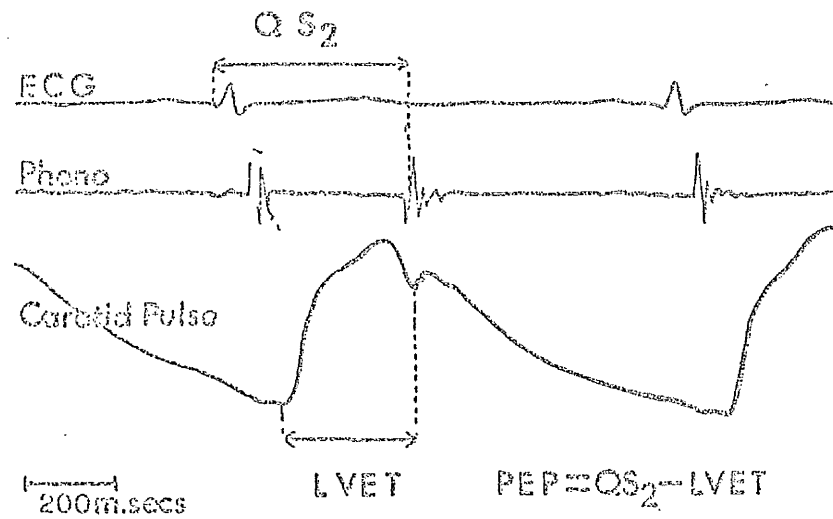
QS₂ Total Electromechanical Systole
LVET Left Ventricular Ejection Time
PEP Pre Ejection Period

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 (Elema 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 (Mingograf 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 Elema Schonander EMT 510 C transducer and its amplifier is greater than 80 Herz.

The intervals measured were (1) the total electromechanical systole (QS_2) 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.

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FIGURE 24

Systolic Interval	Sex	Regression Equation	SBI Index	S. Deviation
QS$_2$	M	QS$_2$ = -2.1 x HR + 546	546 ± 14	
	F	= -2 x HR + 549	549 ± 14	
EXP	M	EXP = -0.4 x HR + 131	131 ± 13	
	F	= -0.4 x HR + 133	133 ± 11	
LVET	M	LVET = -1.7 x HR + 413	413 ± 10	
	F	= -1.6 x HR + 418	418 ± 10	

PEP/LVET ratio = 0.350 ± 0.040

The duration of the QS_2, 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 1) 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 hyper-thyroid patients (Table 26) and 15 hypothyroid subjects (Table 27).   The diagnosis of hyperthyroidism was made on clinical examination and confirmed by $PB^{127}I$ estimation (values $\geqslant$ 670 nmol/l) and ^{131}I uptake studies (24 hour thyroidal uptake $>$ 50%), PBI^{131} 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 electro-cardiography, 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 $PB^{127}I$ 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 $549 \pm$, 1/p < 0.001) resulting from a normal left ventricular ejection time, 418 ± 20 (calculated value 417 ± 1,) and

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STUDY 1   ESTIMATED PARAMETERS   TABLE 26.

Patient No.	Age Years	Sex	Heart Rate /min	QS2 Meas.	QS2 Calc.	QS2 1 Meas.	QS2 1 Calc.	LVET Meas.	LVET Calc.	LVET 1 Meas.	LVET 1 Calc.	PEP Meas.	PEP Calc.	PEP 1 Meas.	PEP 1 Calc.	PEP/LVET 1
1	54	M	97	300	335	491	546	250	258	468	418	53	92	96	131	0.2000
2	48	F	104	330	741	538	549	265	252	431	418	65	91	105	132	0.2452
3	44	F	111	310	327	527	549	255	240	432	418	55	89	99	133	0.2236
4	58	F	113	285	523	511	549	280	237	402	418	65	88	110	133	0.2954
5	28	F	89	350	371	528	549	260	276	422	418	70	97	105	133	0.2566
6	51	F	69	400	411	538	549	330	308	440	418	70	105	98	133	0.2121
7	47	M	72	360	405	504	549	300	293	425	418	60	104	89	133	0.2000
8	42	F	67	335	415	519	549	315	315	418	418	70	106	101	133	0.2222
9	52	F	102	360	345	564	549	295	270	443	416	65	96	101	133	0.2202
10	37	F	107	300	335	514	549	240	247	411	418	60	90	103	133	0.2500
11	58	F	90	320	369	500	549	250	274	394	418	70	97	106	133	0.3000
12	35	F	89	370	371	549	549	315	276	457	418	55	97	91	133	0.1745
13	46	F	80	370	389	530	549	320	290	443	418	50	101	82	133	0.1862
14	57	F	127	280	295	574	546	230	225	435	418	50	82	101	133	0.2173
15	24	F	82	310	374	492	549	275	274	414	418	35	88	88	132	0.1272
16	49	F	79	360	391	518	549	290	292	416	418	70	101	102	133	0.2413
17	65	F	95	315	359	505	549	240	265	392	448	75	95	113	133	0.3125
18	55	F	71	345	407	487	549	280	304	394	418	65	105	93	133	0.2361
19	47	F	96	355	357	527	549	260	264	424	418	75	95	113	133	0.2600
20	19	M	92	295	353	486	546	230	257	377	413	65	94	102	131	0.2526
						517±91	51.9±1			419±26	417±1			100±8	132±1	0.2312±0.0

Patient No.	Age Years	Sex	Heart Rate/ minute	CS2 Meas.	CS2 Calc.	CS2 1 Meas.	CS2 1 Calc.	LVET Meas.	LVET Calc.	LVET 1 Meas.	LVET 1 Calc.	PEP Meas.	PEP Calc.	PEP 1 Meas.	PEP 1 Calc.	PEP/LVET
1	57	F	53	445	437	554	546	300	325	390	413	144	110	165	131	0.4800
2	71	F	68	420	414	555	549	300	309	409	418	120	106	147	133	0.4000
3	48	F	63	450	423	576	549	320	317	421	418	130	108	155	133	0.4062
4	69	F	70	410	407	550	549	285	304	397	418	125	105	153	133	0.4385
5	63	F	68	420	413	560	549	300	309	409	418	120	105	147	133	0.4000
6	58	F	74	430	401	578	549	285	293	403	418	145	103	175	133	0.5087
7	35	F	53	475	443	581	549	340	333	425	428	135	112	156	133	0.3970
8	62	M	55	455	431	570	546	305	315	399	413	150	109	172	131	0.4918
9	65	F	67	400	415	554	549	250	310	357	418	150	106	177	133	0.6000
10	58	F	63	450	423	576	549	320	317	421	418	130	108	155	133	0.4062
11	40	F	80	400	389	560	549	270	290	398	418	130	101	162	133	0.4524
12	52	F	84	395	381	563	549	280	284	414	428	115	99	149	133	0.4107
13	59	M	54	430	433	543	546	290	321	582	413	140	109	162	131	0.4827
14	56	F	64	440	421	563	549	310	316	412	418	130	107	155	133	0.4193
15	55	M	55	470	431	586	546	320	325	414	413	150	109	172	131	0.4687
						565 ± 14	548 ± 1			402 ± 17	416 ± 2			161 ± 10	132 ± 2	0.4527 ± 0.056
						$P < 0.01$				$P < 0.01$				$P < 0.001$		$P < 0.001$
																Calc. value 0.3500 ± 0.04

- Fig. 28

HYPERTENSIVE (GROUP A) AND NORMOTENSIVE (GROUP B) PATIENTS PRE- AND POST-TREATMENT

Patient No.	Age Years	Sex	Dose Rate/Minute	CO2 1 Meas.	Calc.	PaP 1 Meas.	Calc.	EVTM 1 Meas.	Calc.	EVTM/EVTV
A										
1	54	M	57	538	545	128	131	407	413	0.35
2	43	M	50	530	549	127	133	407	418	0.35
3	44	F	72	524	549	124	133	490	418	0.33
4	16	F	84	538	549	129	133	409	418	0.35
5	28	F	64	543	549	136	133	407	418	0.36
				534±7		128±4		405±4		0.346±0.001
B										
1	57	M	79	576	546	137	131	439	413	0.34
2	71	M	68	535	549	122	133	413	418	0.32
3	48	F	73	556	549	139	133	417	418	0.37
4	69	F	75	560	549	175	133	455	418	0.30
5	63	F	95	525	549	125	133	402	418	0.34
				550±20		130±3		421±15		0.333±0.003

Values before treatments

Hyper Group A			513±18		103±6		418±16		0.242±0.051
Hyper Group B			559±10		155±7		405±12		0.424±0.034

Patient No.	Age	Sex	Heart Rate/ minute	QS₂ 1 Meas.	Pred.	LVET 1 Meas.	Pred.	PEP 1 Meas.	Pred.	PE
1	48	F	74	518	549	398	418	120	133	C
2	37	M	115	510	546	411	413	111	131	C
3	49	F	93	546	549	439	418	103	133	C
4	50	F	88	576	549	491	418	86	133	C
5	51	F	81	472	549	335	418	88	133	C
6	43	F	59	533	549	414	418	119	133	C
7	44	F	81	512	549	405	418	107	133	C
8	49	F	103	526	549	420	418	106	133	C
9	57	F	138	546	549	451	418	95	133	C
10	62	F	107	524	549	406	418	118	133	C
11	46	F	85	520	549	421	418	99	133	C
12	53	F	103	526	549	420	418	106	133	C
13	41	F	106	517	549	418	418	102	133	C
14	56	F	67	509	549	412	418	97	133	C
15	55	F	111	542	549	433	418	109	133	C
16	23	F	146	532	549	429	418	103	133	C
17	51	F	67	539	549	427	418	112	133	C
18	47	F	91	542	549	431	418	111	133	C
19	61	F	130	560	549	448	418	112	133	C
20	53	F	115	565	549	444	418	121	133	C
21	32	F	83	536	549	416	418	120	133	C
22	56	F	88	526	549	411	418	115	133	C
23	51	F	85	515	549	406	418	109	133	C
24	56	F	122	499	549	395	418	104	133	C
25	51	F	113	506	549	406	418	100	133	C
26	51	F	100	525	549	415	418	110	133	C
27	63	F	87	494	549	384	418	110	133	C
28	31	F	102	519	549	413	418	106	133	C
29	49	F	83	531	549	418	418	113	133	C
30	52	F	80	510	549	428	418	82	133	C
31	44	F	107	524	549	421	418	103	133	C
32	53	F	92	514	549	437	418	77	133	C
33	44	F	100	460	549	370	418	90	133	C
34	57	F	74	518	549	413	418	103	133	

Patient No.	Age	Sex	Heart Rate/ minute	CO_2 Meas.	CO_2 Calc.	IVEDP Meas.	IVEDP Calc.	DEP 1 Meas.	DEP 1 Calc.	PEP/CO₂
1	56	M	70	550	549	397	418	193	133	0.58
2	57	F	66	567	549	418	418	149	133	0.41
3	54	M	57	570	549	427	413	143	132	0.36
4	60	F	82	554	549	410	418	133	133	0.47
5	61	M	66	568	549	346	413	162	132	0.59
6	52	F	60	535	549	391	418	144	133	0.41
7	46	M	84	532	549	424	418	149	133	0.41
8	44	F	54	543	549	382	418	162	131	0.48
9	61	F	93	605	549	429	418	177	133	0.50
10	49	F	81	577	549	430	418	147	133	0.38
11	50	F	64	568	549	412	418	156	133	0.42

PREDICTIVE STUDY : STANDARD SUBJECTS   TABLE 31.

Patient No.	Age	Sex	FEV/ FVC minute	VC 1 Meas.	VC 1 Pred.	FEV 1 Meas.	FEV 1 Pred.	PEF 1 Meas.	PEF 1 Pred.	PEF/FEV
1	45	F	85	517	549	418	418	126	133	0.34
2	45	F	80	552	549	418	418	137	135	0.36
3	54	F	69	540	549	413	418	127	133	0.33
4	45	F	85	500	549	416	418	134	133	0.32
5	60	F	63	541	549	406	418	135	135	0.36
6	51	F	88	533	549	410	418	123	133	0.33
7	35	F	111	567	549	428	418	139	135	0.38
8	45	F	82	554	546	406	413	123	151	0.35
9	62	M	75	510	549	409	415	150	133	0.36
10	45	F	69	556	549	412	418	126	133	0.33
11	46	F	73	526	549	402	418	124	153	0.33
12	75	M	60	530	546	406	418	122	135	0.32
13	55	M	69	550	549	412	413	123	133	0.37
14	60	F	85	528	549	398	418	128	133	0.36
15	55	M	85	536	546	409	415	127	131	0.33
16	54	F	63	531	549	404	418	127	133	0.34
17	55	F	94	543	549	410	418	135	133	0.37
18	48	F	66	569	549	435	418	131	133	0.32
	47	F	69	573	549	440	418	133	133	0.32

EXERCISE STUDY : DISCORDANT DIAGNOSIS   TABLE32.

Patient No.	Age	Sex	Body Entry Glands	I32 1 Meas.	I32 1 Pred.	FREE 1 Meas.	FREE 1 Pred.	FDB 1 Meas.	FDB 1 Pred.	FDB/LIVER	DIAGNOSIS E.T.I.	DIAGNOSIS Clinical & biochemical	Remarks
1	50	F	72	544	549	415	418	129	133	0.33	Euthyroid	Hyper	T_4, T_3, FT_3
2	51	F	80	522	549	458	418	152	133	0.33	Hypo	Euthyroid	T_4, T_3, FT_3 TSH
3	41	F	91	522	549	386	418	136	133	0.42	Euthyroid	Hyper	^{131}I uptake, T_4 = gland
4	42	F	65	570	549	424	418	146	133	0.38	Hypo	Euthyroid	T_4, T_3, TSH normal

shortening of the pre-ejection period 100 ± 8 (calculated

value 132 ± 1, $p < 0.001$).   The PEP/LVET ratio was

reduced 0.231 ± 0.04 (calculated ratio 0.340 ± 0.40,

$p < 0.001$).(Table 26).

The hypothyroid subjects showed prolongation of the

total electro-mechanical systole 563 ± 14 (calculated

value 548 ± 1, $p > 0.01$) which resulted from shortening

of the left ventricular ejection time 402 ± 17 (calculated

value 416 ± 2, $p < 0.01$) and marked prolongation of the

pre-ejection period 161 ± 10 (calculated value 132 ± 2, $p < 0.$

The PEP/LVET was increased 0.459 ± 0.056 (calculated value

0.3500 ± 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_2 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.

Patient No.	Sex	Heart rate/min	CC 1		IWRT 1		PEP 1		PEP/min	
			Mean	Pred.	Mean	Pred.	Mean	Pred.		
1	F	44	548	549	410	418	138	133	0.35	q_4, q_3, q_2, q_1^{127}
2	F	70	530	549	403	418	170	133	0.31	results in normal
3	F	109	573	549	429	418	140	133	0.35	ranges
4	F	76	512	549	382	418	135	133	0.40	
5	F	107	500	549	395	418	123	133	0.37	
6	M	82	522	546	381	413	135	131	0.40	

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 1968 (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-iodothyromine
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 myxoedema 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 (85a, 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 T_3 toxicosis requires the radio immunoassay of tri-iodothyromine, an expensive, time-consuming technique not generally available. In addition, there is the group of patients with "compensated" euthyroidism, i.e. with normal T_4 and T_3 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 "haemodynamic" 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

TABLE 36

THYROID HORMONE AND SYSTOLIC TIME INTERVALS PARAMETERS IN 20 PATIENTS

I	II	III	IV	V	VI	VII	VIII	IX	X
T_4 μg/100 ml.	T_3 mg/ml.	HR	QS_2 msec	QS_{2_I} msec	LVET msec	$LVET_I$ msec	PEP msec	PEP_I msec	PEP/LVET
8.04	1.04	73	380	526	285	402	101	124	3333
16.2	3.50	107	310	524	235	406	75	118	3191
9.8	2.38	81	350	512	275	405	75	107	2727
7.6	1.30	80	395	555	290	418	105	137	3620
18.8	3.70	107	310	524	250	421	60	103	2400
16.0	3.10	72	400	544	300	415	100	129	3333
4.6	1.48	80	380	540	285	413	95	127	3333
10.8	2.24	85	410	580	310	446	100	134	3225
11.2	3.80	83	360	526	265	398	95	128	3584
20.0	2.60	100	260	460	210	370	50	90	2380
13.4	3.00	74	370	518	280	398	90	120	3214
5.2	1.70	82	350	522	250	381	100	133	4000
12.7	2.30	67	375	509	305	412	70	97	2298
10.0	1.39	86	375	547	280	418	95	129	3392
9.9	1.80	69	405	550	295	412	110	138	3728
15.4	3.12	88	400	516	350	491	50	86	1428
23.7	5.58	93	360	546	280	439	70	107	2413
12.2	0.58	--	--	--	--	--	44	85	--
12.6	3.25	67	375	509	305	412	70	97	2298
22.5	3.25	93	360	546	290	439	70	107	2413

NOTE: Hormonal data was processed when thyroxine was assayed as
μg/100 ml. and triiodothyronine as mg/ml.

TABLE 37.

SIGNIFICANT RELATIONSHIPS FOR T_4, T_3 and STI's OF THE FORM,

$$STI = aT_4 + bT_3 + C$$

S.T.I.	RELATIONSHIP	ACCURACY OF FIT (ASSESSED AS ROOT MEAN SQUARE/MEAN VALUE OF STI) AND EXPRESSED AS A PERCENTAGE.
H.R.	$HR = 67.5 + 1.34\ T_4 - 0.57\ T_3$	3.2
P.E.P.	$PEP = 100.9 - 3.43\ T_4 + 6.69\ T_3$	6.3
QS_2	$QS_2 = 401 - 3.92\ T_4 + 5.49\ T_3$	8.7
LOG PEP	$\dfrac{LOG}{PEP} = 2.04 - 0.02\ T_4 + 0.05\ T_3$	14.7
$\dfrac{PEP}{LVET}$	$\dfrac{PEP}{LVET} = 3850 - 63.6\ T_4 - 31.8\ T_3$	17

$Y = aZ + bZ^2 + cZ^3 +$ 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.

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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 & 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 34

SIGNIFICANCE OF RELEVANT PARAMETERS MEASURED IN GROUPS STUDIED

Thyroid Status	Thyrotoxic		Euthyroid		Hypothyroid
No. Patients	24		10		10
Heart rate mean (SD)	100 (12)	*$P < 0.001$	70 (8)	P NS	65 (15)
Fasting serum cholesterol mean (SD) mmol/l.	5.44 (1.04)	$P < 0.05$	6.22 (0.78)	$P < 0.005$	7.51 (0.91)
Serum thyroxine mean (SD) nmol/l.	194 (23)	$P < 0.001$	105.5 (17)	$P < 0.001$	27 (13)
Serum tri-iodothyronine mean (SD) nmol/l.	3.0 (1.2)	$P < 0.01$	1.7 (0.2)	$P < 0.001$	0.58 (0.21)
Plasma zinc mean (SD) umol/l.	14.74 (2.23)	P NS	14.24 (2.50)	P NS	14.12 (2.39)
Zinc clearance mean (SD) ml/min.	0.70 (0.33)	$P < 0.001$	0.34 (0.12)	P NS	0.32 (0.05)
Zinc clearance / Creatinine clearance x 100	0.636 (0.30)	$P < 0.001$	0.309 (0.110)	P NS	0.32 (0.041)

* 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-iodothyromine 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-iodothyromine (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

PARAMETER	CORRELATION COEFFICIENT
Heart rate (resting)	0.87
Rate of weight loss	0.92
Serum thyroxine	0.71
Serum tri-iodothyronine	0.64
Serum cholesterol	0.60
Wayne Index	0.61
Severity/acuteness score	0.86
Plasma zinc	0.42

FIGURE 25

RATING SCALE TO ASSESS ACUTENESS AND SEVERITY OF METABOLIC RESPONSE TO THYROTOXICOSIS

PARAMETER Heart Rate.	SCORE	PARAMETER Serum Cholesterol mmol/l	SCORE	PARAMETER Serum Thyroxine mmol/l	SCORE	PARAMETER Serum Tri-iodothyro-nine nmol/l	SCORE	PARAMETER Rate of weight loss Kg/week	SCORE
70 - 80	0	2.59	5	154	0	2.2 - 2.5	0	0.27	1
81 - 90	1	2.59 - 3.63	4	154-180	1	2.6 - 3.0	1	0.28 - 0.32	2
91 - 100	2	3.64 - 4.66	3	181-206	2	3.1 - 3.5	2	0.33 - 0.36	3
101 - 110	3	4.67 - 5.70	2	207-232	3	3.6 - 4.0	3	0.37 - 0.41	4
111 - 120	4	5.71 - 6.73	1	233-257	4	4.1 - 4.5	4	0.42 - 0.45	5
121	5*	6.74	0	258	0	4.6 - 5.0	5	0.46	6
						75.0	6		

* 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

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.... a highly significant correlation with the rating

devised to estimate the acuteness and severity of the

body's metabolic response to thyrotoxicosis.

No significant correlations were found in the ten hypo-

thyroid 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 α_2 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 physicians's assessment
of his patient.

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 38 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 cardio-
myopathy.

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
* 24 were women, 16 men.

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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 μgm. 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 T_3 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 T_3 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.

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TABLE 38

CARDIAC PARAMETERS IN THYROTOXIC RATS

GROUP	TREATMENT	TIME OF SACRIFICE (WEEKS)	L.V.WEIGHT (mg .SD)	LV WEIGHT / WEIGHT ANIMAL	D.N.A.CONTENT of L.V. mg.(SD)	R.N.A.CONTENT of L.V. mg. (SD)
1(n=6)	T_3	16	890 (12)	3.50(0.19)	2.0(0.21)	1.51(0.4)
2(n=6)	0	16	600 (17)	2.50(0.21)	1.20(0.13)	1.00(0.6)
3(n=6)	T_3 for 16 wks.	28	630 (21)	2.50(0.11)	1.51(0.16)	1.2 (0.2)
4(n=6)	0	28	612 (15)	2.49(0.20)	1.21(0.10)	1.02(0.3)

FIGURE 26.

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 T_3 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 erythro-
cyte 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 sub-
mitted to thoracotomy and perfused with gluteraldehyde preservative
via and aortic cannula at a pressure of 100mm.Hg. 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 T$_3$ 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.

A

B

C

D

E

F

G

H
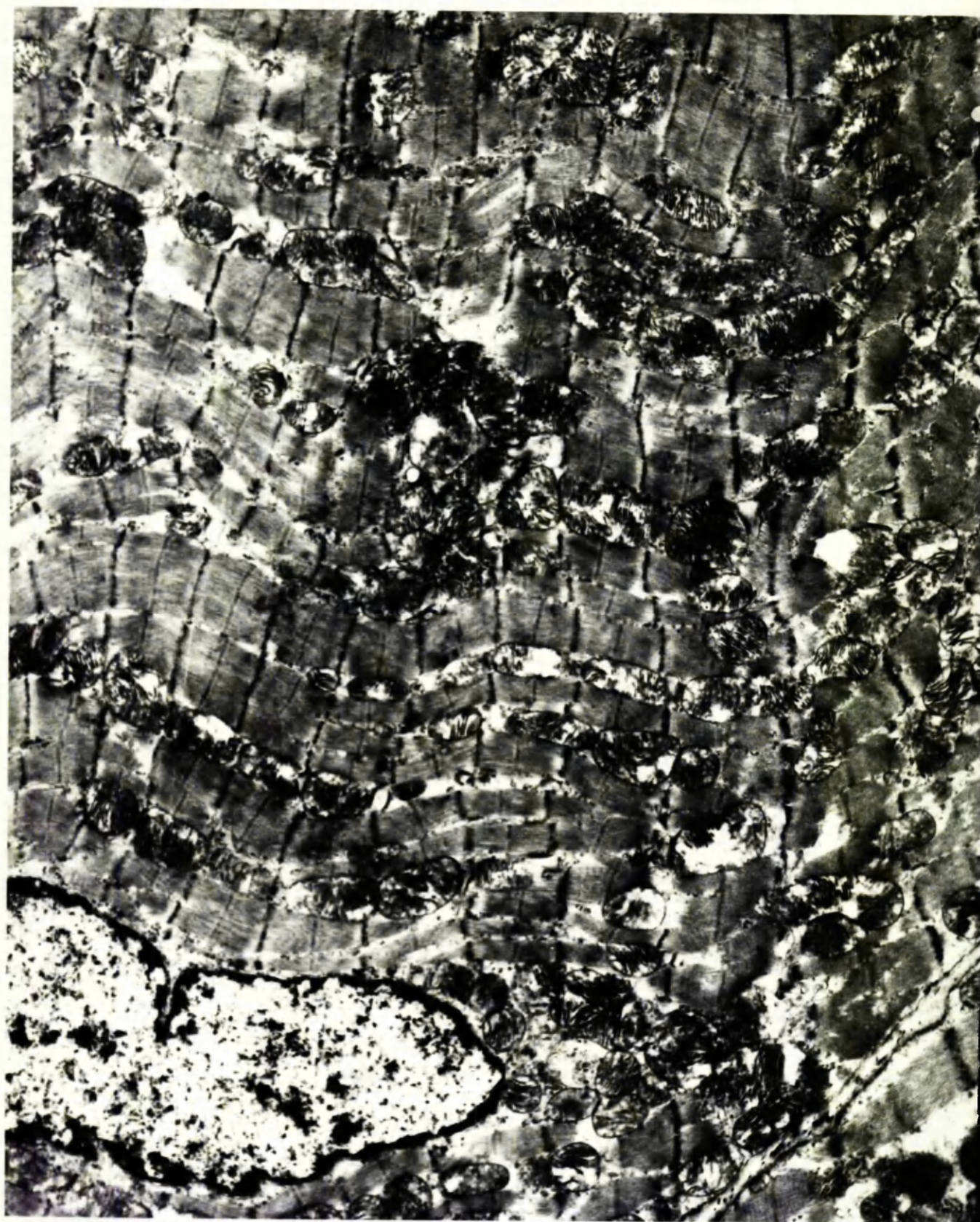
6 rats on T_3 for 16 weeks were transferred back to
normal drinking water and after a further 12 weeks were
sacrificed with 6 control rats.   Hearts and animals
were weighed and heart DNA and RNA content measured.

Samples of left ventricle from rats on T_3 and from
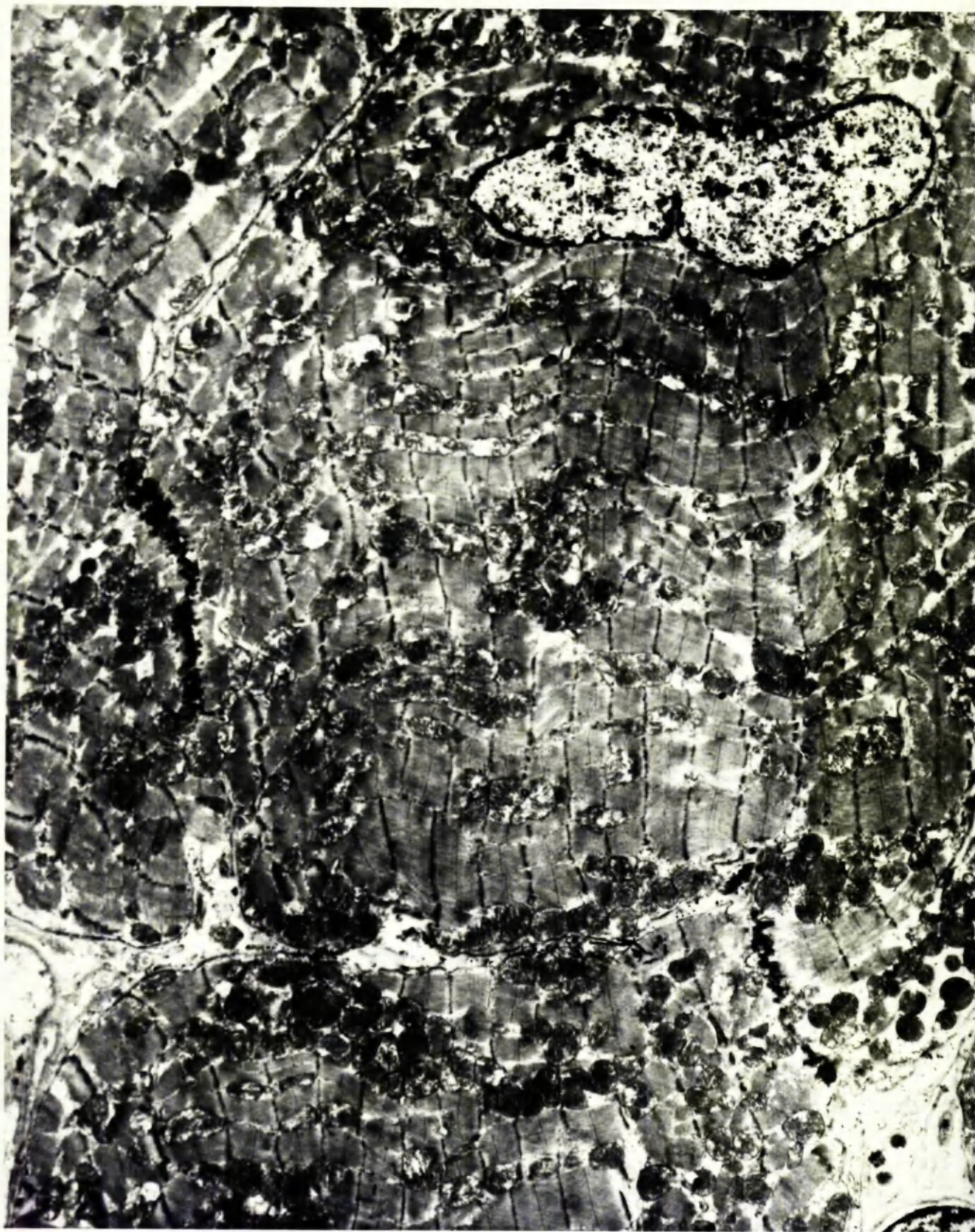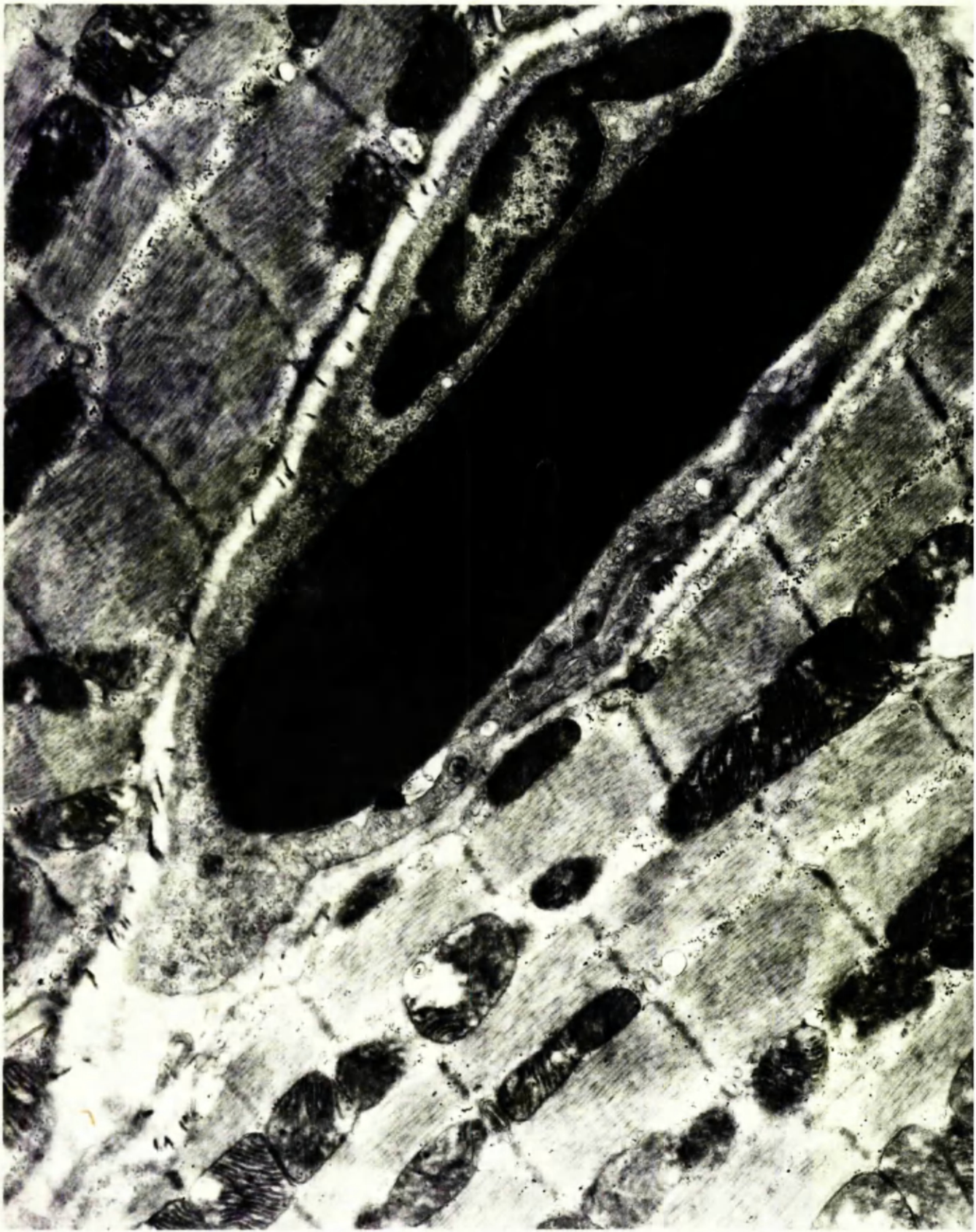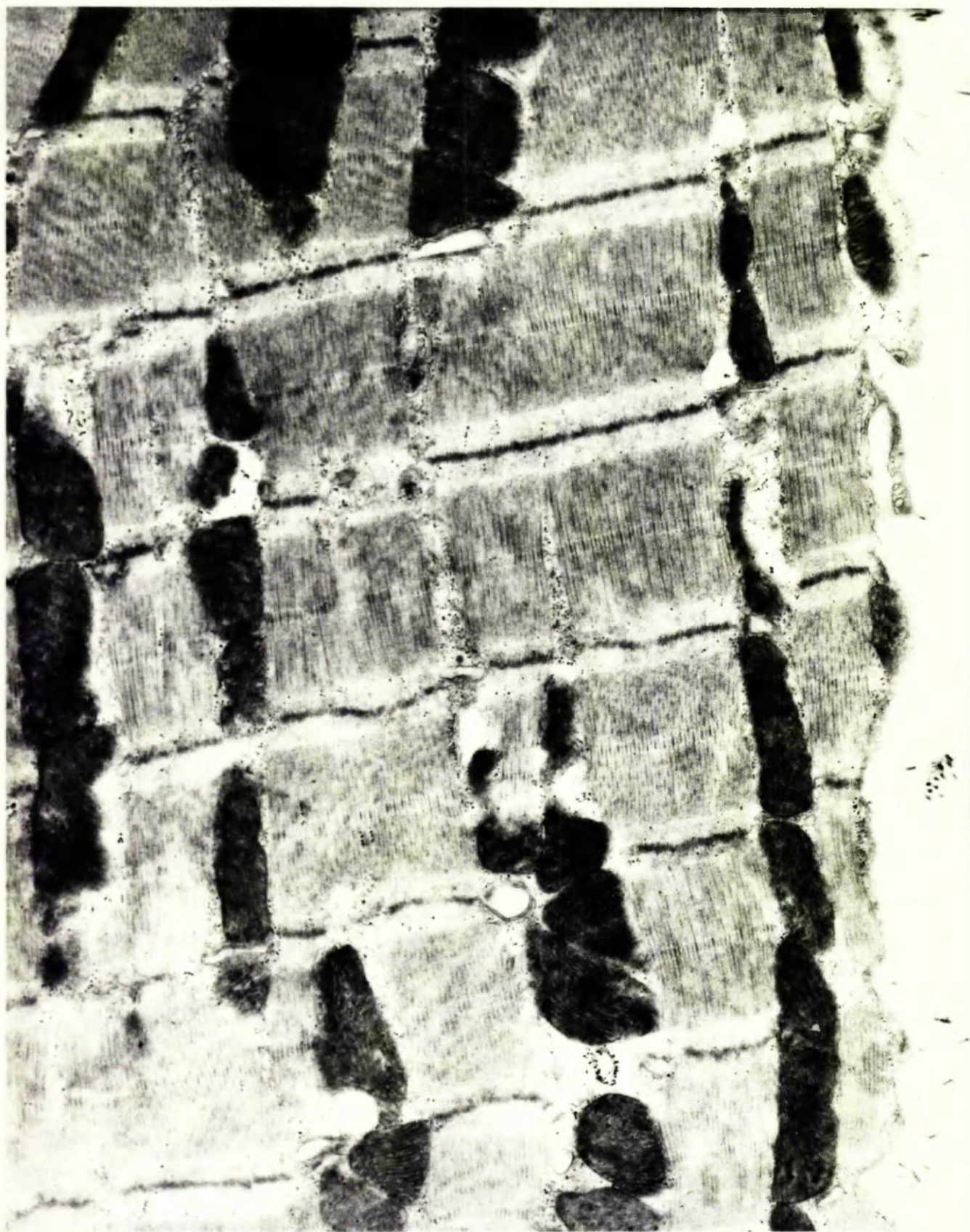controls were stained with haematoxylin and eosin and
examined for evidence of myocytic replication.

RESULTS

Treatment with T_3 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 moiety.

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 page 77).

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.

GROUP	NO. PATIENTS.	F.F.A. μ Eq/L	CHOLESTEROL mmol/L	TRIGLYCERIDES mmol/L
A.F.	8	905 ± 60	5.44 ± 0.91	1.02 ± 0.14
V.E.S.	7	890 ± 70	6.22 ± 1.55	1.12 ± 0.20
C.C.F.	6	935 ± 80	5.83 ± 0.78	1.21 ± 0.38
NO COMPLICATIONS	10	925 ± 55	5.70 ± 0.85	0.97 ± 0.17

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.

B.   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 40

LIPID AND LIPOPROTEIN STUDIES IN HYPOTHYROID PATIENTS

GROUP	CLINICAL STATUS	BIOCHEMICAL STATUS	HORMONAL LEVELS mean ± SD			CHOLESTEROL mean ± SD	TRIGLYCERIDE mean ± SD	INTRALIPID TOLERANCE TEST K2 (%/min)
			T_4	T_3	TSH			
1 n=20	H	H	30±16	0.3±0.4	48±308	9±1.1	3.41±0.81	1.34±0.24 n=5
1 n=20	E	E (on T_4)	132±27	1.6±0.3	1±2	6.10±1.0	1.71±0.43	3.86±0.51 n=5
2 n=12	E	H	46±8	0.4±0.2		8.0±0.9	2.71±0.72	1.98±0.43 n=5
2 n=12	E	E (on T_4)	135±21	1.5±0.3		5.91±1.2	1.63±0.51	3.92±0.61 n=5

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 - $T_4 = 21$ nmol/L,
$T_3 = 0.10$ nmol/L, TSH $>$ 50 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 nmo/L, T_3 - 1.6 nmo/L, TSH 10 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. H_2SO_4 + 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:

TEST	STANDARD	BLANK
0.2 ml.serum 2 ml. diluent	0.2 ml.standard 0.2 ml.water 2 ml. diluent	2 ml.diluent

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° 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 hyperlipoproteinaemia 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.

These 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 emphase 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 (page75). 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.

[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.

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