THE LABORATORY ASSESSMENT OF THYROID FUNCTION WITH SPECIAL REFERENCE TO THE IMPORTANCE OF THYROID ABNORMALITY IN PSYCHIATRIC DISEASE

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SUMMARY

The aims of this study were to investigate the incidence and clinical importance of thyroid abnormalities in an acute psychiatric population and to use such information in the development of a laboratory diagnostic policy for the investigation of such groups. This involved the use of established *in vitro* assays for serum thyroxine (T^4), triiodothyronine uptake (T^3U), triiodothyronine (T^3) and thyroid stimulating hormone (TSH), together with the development and evaluation of both thyroxine binding globulin (TBG) and thyroxine binding prealbumin (TBPA) assays. For comparison, both the Wickham Survey (Tunbridge *et al.*, 1977) and data obtained from general medical and surgical patients, were used as reference.

The analytical study revealed that TBG measurement offered no advantage over T^3U in the routine assessment of thyroid function, but was only of value in cases where the T^3U was greater than 135-140%. However TBPA measurement was shown not to have any role in the evaluation of thyroid status, despite several theoretical advantages over both T^3U and TBG.

In a survey of 1544 patients referred for psychiatric care, the free thyroxine index (FTI) was initially employed for identifying patients with thyroid abnormalities. Equivocal FTI results were regrouped using serum TSH in patients with borderline low results, and serum T^3 for borderline raised values, together with a TBG assay if indicated. Drug interference was also identified as a possible cause of patient misclassification into myxoedemic or thyrotoxic groups.

The overall incidence of thyroid disease was found to be 2.65% and was unsuspected in 0.84% of all patients studied, with the major category being female hypothyroids over the age of 40 years. The clinical diagnosis was most often confused with paranoid schizophrenia and depressive illness.

This research has shown that the investigation of thyroid dysfunction in psychiatric patients represents an important area for consideration when developing a comprehensive hospital biochemistry service.

Key Words

Thyroid function tests; thyroxine binding globulin; thyroxine binding prealbumin; thyroid diseases; psychiatric disease.

DECLARATION

I hereby declare that the whole of the work submitted in this thesis is the result of my own investigation except where reference is made to published literature or where assistance is acknowledged.

Candidate P.G.H. Litherland

DECLARATION

I hereby declare that the work embodied in this thesis has not already been submitted in substance for any degree, and is not being concurrently submitted in candidature for any degree.

Candidate P.G.H. Litherland

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GLOSSARY OF ABBREVIATIONS

Ab	antibody
AC	adenylate cyclase
ADP	adenosine diphosphate
ANS	8-anilino -1- napthalene sulphonic acid
ATP	adenosine triphosphate
BMR	basal metabolic rate
BP	blood pressure
c-AMP	cyclic 3'5' adenosine monophosphate
cm	centimetre
mm	millimetre
CPBA	competitive protein binding assay
CV	coefficient of variation
DPH	diphenylhydantoin
ECT	electroconvulsive therapy
EDTA	ethylenediaminetetraacetic acid
ESR	erythrocyte sedimentation rate
FT^4	free thyroxine
FT^3	free triiodothyronine
FSH	follicle stimulating hormone
FTI	free thyroxine index
ɣ	gamma
g	gram
mg	milligram
µg	microgram
Hb	haemoglobin
HCT	human chorionic thyrotrophin
HMT	human molar thyrotrophin

HTF	heterotrophic factor
i	ionic strength
IgG	immunoglobulin G
Iu	international unit
KI	potassium iodide
l	litre
dl	decilitre
ml	millilitre
µl	microlitre
LATS	long acting thyroid stimulator
LATS-P	long acting thyroid stimulator protector
LSD	lysergic acid
m.amps	milli amperes
M	molarity of a solution
mol	moles of substance
mmol	millimoles of substance
µmol	micromoles of substance
nmol	nanomoles of substance
mS	milli siemens
M.Wt	molecular weight
NAD	nicotinamide adenine dinucleotide (oxidised form)
NADH	nicotinamide adenine dinucleotide (reduced form)
%	percentage
PBI	protein bound iodine
RIA	radioimmunoassay
RNA	ribonucleic acid
RT^3	reverse triiodothyronine
SD	standard deviation
SR	sinus rhyth m

T^3	triiodothyronine
T^4	thyroxine
T^3U	triiodothyronine uptake (T^3Uptake)
TBG	thyroxine binding globulin
TBPA	thyroxine binding prealbumin
TG	triglyceride
TRH	thyrotrophin releasing hormone
TSH	thyroid stimulating hormone
TSI	thyroid stimulating immunoglobulins
$\bar{x}$	mean of a population
>	greater than
<	less than

CHAPTER ONE

INTRODUCTION

1. General Introduction

The assessment of thyroid function is of importance to physicians in a wide range of specialities, to psychiatrists and also to clinical chemists and hospital physicists. This can be attributed to thyroid abnormalities being one of the most prevalent endocrine disorders and also to the often insidious nature of thyroid dysfunction, making early diagnosis and treatment of paramount importance. Thyroid disorders often present with a widely - varying range of symptoms because of the multifunctional role of the thyroid hormones. The biological effects of the hormones are therefore many and varied and there is often confusion with other diagnoses. (Wall and O'Flanagan, 1978).

The multiplicity of symptoms associated with thyroid-gland disorders include effects upon the central nervous system, and there is considerable evidence to suggest that an association exists between psychological disturbances and endocrine dysfunction. Psychiatric abnormalities are associated with thyroid disorders (Johnson, 1928; Asher, 1949; Whybrow et al., 1969) and also with diseases of the adrenal (Freedman et al., 1975) and pituitary glands. (Dissanayake and Lieberman, 1969). No specific psychological profile has been correlated with any endocrine dysfunction, so there may be no way of distinguishing psychiatric problems caused by purely emotional disturbances from those caused or aggravated by underlying endocrine disease.

With reference to thyroid abnormalities and in particular to hypothyroidism, delirium, depression, paranoid delusions and hallucinations have all been associated with psychiatric manifestations in at least 50% of all cases (Pitts and Guze, 1961). Many years previously, an eminent clinician, Asher (1949) had speculated that psychiatric hospitals were populated with many unrecognised hypothyroids.

In support of this view many psychological studies have suggested that the symptoms of myxoedema make an insidious appearance and are generally characterised by listlessness, lack of energy, slowness of speech, reduced sensory capacity, impairment of memory, somnolence,social withdrawal and an altered sleep pattern (Eayrs, 1960; Kales et al., 1967). Furthermore several of these psychological symptoms are commonly seen in depressed patients, many of whom are under psychiatric care.

There is indirect evidence suggesting that depressed patients may have lower thyroid hormone levels than are normally present. (Prange et al., 1969, 1970; Wilson et al., 1970) In fact Dewhurst et al., (1969) have observed abnormally high levels of thyroid stimulating hormone (TSH) in the blood of depressed patients, although it was suggested that the emotional stress associated with psychiatric illness might have been the cause of this hormonal increase. Thus, even though there is evidence to implicate thyroid dysfunction with depression, it is impossible to state whether abnormal thyroid function is the result or the cause of the psychiatric disorder.

The effect of stress upon TSH secretion has been studied in rats by Pamenter and Hedge (1980), where corticosterone was infused at different concentrations to establish various blood levels within the physiological range. At low levels of corticosterone infusion, significant inhibition of the TSH response to thyrotrophin releasing hormone (TRH) was observed, whereas at high levels of infusion no inhibition of this response was noted. TSH secretion was therefore shown to be modulated by physiological levels of the native hormone corticosterone and the authors related this effect to stress : little or no stress allowing operation of this regulatory mechanism, with major stress suppressing this regulation and thus leading to the associated

finding of elevated TSH levels. This supports the observations of Dewhurst et al., (1969) and may provide evidence into the underlying mechanisms involved in their findings.

In addition to the associated psychiatric disturbances with hypothyroidism, several studies have demonstrated that excessive thyroid secretion produces psychological symptoms including emotional lability, restlessness, irritability, over-reactiveness with predominent anxiety and tension (Eayrs, 1960; Whybrow and Ferrell, 1974). More severe mental disorders may also be seen in hyperthyroid patients if left untreated with antithyroid drugs. Psychoses of the acute organic type have frequently been encountered in severe cases and also during thyroid crisis. The incidence of other psychoses in hyperthyroids has been under some dispute (Bursten, 1961). Bursten also stated that thyrotoxicosis may masquerade under a variety of clinical psychiatric syndromes and even when the index of clinical suspicion is high, the diagnostic task is difficult. This fact may partly explain the varying incidence figures quoted for hyperthyroidism in psychiatric populations. At one extreme Lidz and Whitehorn, (1949) detected evidence of psychosis in 20% of thyrotoxic patients, whereas many workers maintain that it is rare to find psychoses in hyperthyroid populations (Katzenelbogen and Luton, 1935).

Further recognition of the association between thyroid abnormality and mental illness was provided by Clower et al., (1969) who stated that myxoedema and thyrotoxicosis constitute two of the most frequently overlooked causes of the so-called "organic psychoses". Asher (1949), observed that a psychosis associated with myxoedema may often be missed, because the textbook description of the disease is not the rule but the exception. Pitts and Guze, (1961) noted that a psychiatrist must always

be on guard for patients who may appear clinically depressed or delirious, with few, if any of the stigmata of the underlying myxoedema.

The confusion that exists between thyroid disease and psychiatric illness is evident, therefore, from this early work. Although the incidence of psychoses in a population of hyperthyroid patients was shown to be quite low (Clower et al., 1969), in the order of 1.2%, they concluded that for hypothyroid patients the incidence was approximately 17%. Previous workers (Lidz and Whitehorn, 1949) have also shown the incidence of myxoedema psychoses to be between 15 and 25%.

Little work has been carried out over the past 10-15 years on the association of mental illness with thyroid disease, although an article appeared in the British Medical Journal of Psychiatry entitled "Thyroid Dysfunction in Female Psychiatric Patients" by Nicholson et al., (1976). In this small study of 98 unselected female admissions to a large psychiatric hospital, biochemical screening for thyroid dysfunction led to the diagnosis of hypothyroidism in three cases, which could otherwise have passed unrecognised. They suggested that if all women in their study above the age of 40 were routinely screened for thyroid abnormality, the yield of significantly abnormal results would have been 8.0%.

More recently a study of chronically-ill psychiatric population by McLarty et al., (1978), suggested the prevalence of thyroid dysfunction in a total psychiatric in-patient population to be 1.2% (or 2% in females). The incidence of hypothyroidism was 0.5%, (five females, one male), but in only one patient was the diagnosis clinically obvious. Eight patients (all female) were clinically hyperthyroid (prevalence 0.7%) of whom six were previously undiagnosed. McLarty further stated that although the

incidence of hyperthyroidism was no higher than in the general population (Tunbridge et al., 1977), it was clear that the diagnosis may be overlooked in psychiatric patients, especially in females lacking the typical clinical features of the disease. In conclusion McLarty argued against there being a reservoir of clinical significant undiagnosed thyroid disease in psychiatric in-patients. In contrast however, in a survey of 50 patients admitted to the general psychiatric service at the Upstate Medical Centre, New York, Weinberg and Katzell (1977) found 6% of the patients to have thyrotoxic symptoms. This evidence conflicts with that provided by McLarty et al., who found an incidence of 0.7% in patients with thyrotoxicosis and associated psychoses.

In a more recent study Gold et al., (1981) evaluated the relationship between hypothyroidism and depression on 250 consecutive patients referred to a psychiatric hospital for treatment of depression or anergia. Twenty of the 250 patients (8%) had some degree of hypothyroidism : two patients were identified with overt, nine patients with mild, and ten patients with subclinical hypothyroidism. The authors suggested that their results illustrated a significant proportion of patients with depression may have early hypothyroidism and also that half of these cases were detected only by TRH testing (the subclinical group). Gold and his workers concluded that on the basis of their data, it appeared that most depressed, tired and anxious patients would be candidates for a comprehensive thyroid evaluation for hypothyroidism. They further stated that all patients with a poor response to traditional psychiatric treatments for depression and anergia, should have a comprehensive thyroid evaluation.

The above evidence therefore indicates the diversity of opinion that

exists regarding the prevalence of thyroid disorder within the general psychiatric population; widely differing figures being quoted by researchers in this area.

Further work is obviously necessary in this field to both ascertain the incidence of thyroid dysfunction in a psychiatric population and to determine the extent to which it passes unrecognised. Obviously the committing of a patient to a psychiatric institution for a part of his/her life, when the diagnosis may be of underlying thyroid dysfunction, is morally unacceptable, especially if the disorder will respond to treatment *per se*. A study has therefore been designed to evaluate the relationship between thyroid disorders and psychiatric disease and this work has been carried out on patients at a large psychiatric hospital (Highcroft Hospital, Erdington, Birmingham).

One of the major drawbacks relating to the investigation of thyroid dysfunction and severe psychological disturbance has been the limited range of biochemical investigations which have been employed in the majority of studies previously performed. It is now clear that overt thyroid disease is generally simple to diagnose clinically, and *in vitro* thyroid function tests are usually only needed in these cases to confirm the diagnosis and to provide a biochemical baseline for monitoring subsequent treatment. The introduction in the mid 1960's of clinically applicable methods for the measurement of serum total and free thyroxine (FT^4), effected a considerable improvement in the biochemical evaluation of thyroid abnormality. Since that time, there has been a proliferation of *in vitro* thyroid tests and a parallel decline in the use of *in vivo* procedures. Reasons for this are obvious since *in vivo* assays require the administration of potentially hazardous radioactive material, demand attendance of the patient at the laboratory and are reported to be less

accurate than *in vitro* studies, due mainly to variations in dietary iodine levels. Also the number of patients that can be screened by *in vivo* techniques is low and they cannot be automated or processed in large series.

As mentioned earlier, many of the signs and symptoms associated with thyroid dysfunction are non-specific and may be seen both in health and in many euthyroid diseases. Thus biochemical investigation is important for the diagnosis of mild or early thyroid disease, or when other symptoms atypical of thyroid disease may mask the underlying pathology, e.g. psychiatric disease. During the course of the current work, a thorough investigation was made of the various thyroid function tests routinely available, with a view to determining those most valuable in aiding diagnosis in the assessment of thyroid function in the psychiatric hospital.

The levels of thyroid hormones are partly controlled by the binding proteins, thyroxine binding globulin (TBG) and thyroxine binding prealbumin (TBPA). The assay of TBG has obtained considerable popularity recently, especially with reference to its value in the correction of the serum thyroxine (T^4) when binding protein levels are abnormal. The present study therefore investigates the contribution of TBG to the assessment of thyroid function and the potential value of TBPA assay is also examined. The role of both these proteins is evaluated in comparison with the conventional triiodothyronine uptake (T^3U), in order to establish an optimum test combination for differentiation of the various forms of thyroid disease.

CHAPTER TWO

THE DEVELOPMENT OF BIOCHEMICAL TESTS OF THYROID FUNCTION WITH REFERENCE TO THE HYPOTHALAMIC-PITUITARY-THYROID AXIS AND ITS CONTROL

Introduction

Thyrotrophin Releasing Hormone

Thyroid Stimulating Hormone

Human Thyroid Stimulators

Thyroid Hormones

"Free" Thyroid Hormones

Thyroid Binding Proteins

Thyroid Hormone Binding Tests

Thyroxine Binding Globulin and Thyroxine Binding Prealbumin Assay

2:1 Introduction

It is considered that a review of normal thyroid physiology and control is necessary to enable a full appreciation of both the limitations of current *in vitro* thyroid function tests and the basic concepts involved, in the evolution of these assays. It is proposed to initially discuss the various control mechanisms involved in thyroid hormone synthesis, followed by a detailed account of the inter-relationship of the different components of the hypothalamic-pituitary - thyroid axis with the various techniques used in the assessment of their functional capacity. Justification, when necessary, will be given for the inclusion of a particular *in vitro* assay or test combination, in the survey of thyroid dysfunction at Highcroft Hospital.

2:2 Thyrotrophin Releasing Hormone (TRH)

TRH is a tripeptide (L-pyroglutamyl-L-histidyl-L-proline amide) and is synthesised by the neurosecretory cells within the hypothalamus and stored in neuronal axons within the median eminence. From there it is released into the portal venous circulation passing down the pituitary stalk to the anterior lobe. Here it binds to membrane receptors on the thyrotroph cell, activating adenyl cyclase leading to the release and synthesis of thyroid stimulating hormone (TSH) (Wilber, 1971). The factors which control the synthesis and release of TRH itself in man are not clear and still await full ellucidation. Rupnow *et al*., (1979) using frog brain have described a macromolecule that yields TRH on chemical and enzymatic treatment and concluded that the precursor macromolecule was derived from ribosomal synthesis. However further work is required to support this theory relating to the biosynthesis of TRH. With recent advances in radioimmunoassay and immunohistochemical techniques, it is now thought that TRH also occurs in regions outside the hypothalamus.

Whereas hypothalamic TRH acts on the thyrotroph cell and stimulates TSH release in extrapituitary regions, there is evidence that TRH may have "neurotransmitter" or other nervous system functions quite apart from its role in the regulation of TSH secretion (Jackson and Reichlin, 1979).

Over the past ten years much information has been obtained on the biological importance of TRH in health and disease, especially since the hormone has been available in pure form. The response of normal human subjects to an intravenous injection of TRH is a prompt rise in plasma TSH peaking within 15-30 minutes and then a gradual decline to basal level over the next 150 minutes (Hershman, 1974). The TSH spike usually evokes a definite elevation in serum triiodothyronine (T^3), but infrequently any elevation of serum T^4 (Sterling and Lazarus, 1977).

The intravenous TRH test has proved a valuable tool in the diagnosis of thyroid disease (Ormston et al., 1971). Patients with primary hypothyroidism have an exaggerated and prolonged response, whereas patients with hyperthyroidism fail to respond to TRH because the raised circulating levels of thyroid hormone prevent release of TSH from the pituitary. However not all patients who fail to respond to TRH are hyperthyroid; absent or impaired responses are also seen in the majority of patients with opthalamic Graves' disease. Patients with secondary hypothyroidism usually fail to respond to TRH, whereas those with hypothalamic disease may show a characteristically delayed response (Hall et al., 1972)

Studies carried out by Synder and Utiger (1972) showed that the TSH response to intravenous TRH testing was substantially reduced by administration of small doses of exogenous hormones. The converse study by Vagenakis et al., (1974) showed increased sensitivity to TRH

after minimal lowering of the serum T^4 by iodide. These results show the predominating effect of thyroid hormone on the pituitary, with TRH perhaps exerting a fine control on TSH levels.

In summary the main value of the TRH stimulation test is (i) in the detection of early cases of hypothyroidism and (ii) in the investigation of some patients in whom thyrotoxicosis is suspected, but in whom the diagnosis remains unclear despite other test results.

The TRH test was not employed in the current survey, since it was thought that sufficient thyroid function tests were employed to render the use of this very sensitive index unnecessary. Also the test, being an *in vivo* procedure, is time consuming and was therefore considered impractical in view of the large numbers of patients that were to be screened.

The mechanism by which TRH stimulates TSH synthesis and release is complex. There is evidence in animals for catecholaminergic control of TRH production probably by noradrenalin, but support for this is lacking in man (Montoya *et al.*, 1979). TRH synthesis and/or release may be reduced by glucocorticoids and enhanced by cold, especially in children, and by thyroid hormones (Otsuki *et al.*, 1973; Kajihara *et al.*, 1972). The effect of cold exposure is known to increase the activity of TRH synthetase (Reichlin *et al.*, 1972). Thus there is monaminergic control of the synthesis and possibly, secretion of TRH and also the hormone has been found to be associated with subcellular hypothalamic particles similar in properties to those of synaptosomes containing norepinephrine and dopamine (Barnea *et al.*, 1975). It has been suggested that thyroid hormones act partially at the level of the brain possibly to modify the secretion of TRH. There is also some evidence

that thyroid hormones can also inhibit TRH secretion at the hypothalamic level (Kajihara and Kendall, 1967). However this early work is disputed and work reviewed by Reichlin et al., (1972) suggests a positive rather than negative feedback effect on the hypothalamus. This has been recently substantiated by Roti et al., (1978) who in the rat demonstrated a positive feedback of thyroid hormones on the hypothalamic production of TRH.

As mentioned earlier, after TRH has been produced, the mechanism by which TRH stimulates TSH synthesis and release is unclear. Early work by Florsheim (1958) on rats with anterior hypothalamic lesions gave an insight into the control of TRH release. He showed that much less T^4 was required to inhibit radioiodine discharge from the thyroid than in unlesioned control rats. It appears that as the amount of TRH delivered to the pituitary decreases the sensitivity of the adeno-hypophysis to feedback inhibition by thyroid hormones is increased.

The control of TSH release by TRH is also dependent on intracellular cation concentrations. Calcium ions are necessary for the potassium or TRH stimulated enhancement of TSH release to occur in vitro (Vale and Guillemin, 1967; Vale et al., 1968). TRH stimulates adenohypophyseal c-AMP even when calcium is removed (Zor et al., 1970) and thus it is not clear how the effect of TRH, impinging on the cell surface of a thyrotroph, is translated at the membrane into the production of c-AMP, which then stimulates the intracellular synthesis of TSH. The secretion of TSH is primarily regulated by the negative-feedback suppression of thyroid hormone at the level of the thyrotroph, whereas TRH functions as the major determinant of the "set point" of the interaction. Dopamine and somatostatin also have inhibitory effects on TSH secretion at the thyroid cell and reduce the degree of TSH release induced by TRH

(Scanlon et al., 1979; Tanjasiri et al., 1976).

It is still not clear which hypothalamic cell type synthesis TRH, although it may be neurons (McKelvy et al., 1975). Ependymal cells are not thought to be the cellular site of synthesis of TRH, although they are capable of the uptake of substances related to thyroid function and its control.

Evidence also exists for the possibility of a "short-loop" feedback mechanism existing between TSH and TRH (Hirooka, 1976; Roti et al., 1978); increased amount of TSH production by the thyrotrophic cells in the pituitary, causing a reduction in secretion of TRH. TRH may even be an inhibitor of its own secretion suggesting the possibility of an ultra-short feedback mechanism (Martini, 1973). Further work is however needed before the full elucidation of TRH production is obtained.

2:3 Thyroid Stimulating Hormone (TSH)

Human TSH is a glycoprotein (M.Wt. 28,000) consisting of two non-identical sub units designated α and β and formed by specific thyrotroph cells in the adenohypophysis under the influence of TRH.

It is generally agreed that TSH secretion is under negative feedback control by thyroid hormones (Dumont et al., 1976) yet details regarding the action of thyroid hormone on the pituitary cell and the relative importance of T^3 and T^4 in suppressing TSH secretion, are unclear. It is thought that the thyroid hormones may cause the formation within the pituitary of an inhibitory protein or polypeptide that interacts with TRH in regulating TSH metabolism. Whether T^3 and T^4 exerts the greater effect on pituitary feedback inhibition of TSH is still not certain. A study by Wenzel et al., (1975) assesses the problem in human subjects; different levels of T^3 and T^4 were administered prior to TRH

injection and the reduction in TSH response noted. Wenzel found that smaller doses of T^3 showed a faster and more pronounced inhibition of TSH release than with T^4. On the other hand, single intravenous injections of T^3 resulting in marked elevation of serum T^3 concentration may fail to alter TSH response to TRH administration for at least several hours (Wartofsky et al., 1974). Thus it can be seen that this problem of whether T^4 and T^3 exerts the greater effect on inhibition of TSH release is still unresolved.

The possibility of a direct feedback effect of the thyroid hormones upon the thyroid gland itself also exists and demonstration of the inhibition of the intrathyroidal enzyme, ornithine decarboxylase, has been shown by T^3 or T^4 pretreatment (Yu et al., 1976). Also thyroid hormones may directly inhibit TSH stimulated secretion by the thyroid at the level of adenylate cyclase (Takasu, 1974). However, the physiological role of such control in man is questionable since T^3 fails to inhibit TSH - mediated thyroid hormone release (Croxon et al., 1977). However there is no doubt that T^3 exerts a negative feedback action on TSH release at the level of the thyrotroph cell and nuclear receptors with a high affinity but low capacity for T^3 have been demonstrated in the pituitary (Wilber and Siebel, 1973). Thyroid hormone action on the thyrotroph cell leads to the production of an inhibitory protein which appears to inhibit the action of TRH on TSH synthesis and release in a dose-dependent manner.

2:3:1 TSH assay

The original methods for assaying TSH were biological procedures; the original method of Adams and Purves (1956) was an *in vivo* assay using guinea pigs and this was later modified for use in mice by

McKenzie (1958). The *in vitro* methods of Kirkham (1962) using guinea pigs and of Brown and Munro (1967) employing mice, are also of note. All these assays depended upon stimulation of the release of radioiodine from the thyroids of the experimental animals. In general the bioassays are time consuming, imprecise and lack sensitivity and have now been replaced by radioimmunoassay procedures which are highly specific (Hall *et al.*, 1971).

Serum TSH levels are raised in all patients with hypothyroidism due to primary thyroid disease(Steffes and Oppenheimer 1979), and TSH measurements have proved of great value in the diagnosis of thyroid failure. The more severe the thyroid failure, in general the higher the serum TSH level, though higher TSH levels tend to be found in young hypothyroid patients. Thus Evered and Hall (1972) classified hypothyroidism into several grades based on the clinical features and serum TSH levels. Measurement of the serum TSH level is also helpful in regulating thyroid hormone medication in hypothyroidism (Hall *et al.*, 1975). In hypothyroidism due to hypothalamic-pituitary disease, TSH levels are usually within the normal range. If there is other clinical and biochemical evidence of hypothyroidism, the finding of a "normal TSH value" indicates a pituitary or hypothalamic lesion.

In hyperthyroidism, the early routinely employed TSH assays were not sensitive enough to separate the low TSH levels in hyperthyroidism from those within the normal range, though TSH measurements after administration of TRH have proved to be of value. However recently, with the development of the more precise and sensitive assays for TSH, several workers have reported the value of finding a low serum concentration in hyperthyroidism (Rootwelt and Solberg, 1978; Wide and Dahlberg, 1979) The former workers stated that serum TSH had a predictive value in the

discrimination between euthyroidism and hyperthyroidism and a result in the upper half of the normal range made the diagnosis of thyrotoxicosis highly unlikely.

In non-toxic goitre the finding of a raised TSH level implies some degree of thyroid failure and indicates that the goitre can be reduced in size by thyroxine medication. The majority of patients with a goitre and a raised TSH level in Britain are found to have autoimmune thyroid disease; drug induced goitre and enzyme defects in the thyroid are more rare. Many patients with endemic goitres are also found to have a raised TSH level even when they are clinically euthyroid. (Hall et al., 1975).

From the above evidence it is clear that the assay of TSH is extremely valuable in the diagnosis of hypothyroidism and in the differentiation of primary from secondary thyroid hypofunction. The assay is also relatively simple to perform using RIA techniques and for these reasons was included in this study, according to the protocol described by Britton et al., (1975).

2:4 Human Thyroid Stimulators

Apart from TRH and TSH, other thyroid stimulators are known, but with the exception of human chorionic thyrotrophin (HCT) it seems unlikely that they have any role in normal physiology. They include human molar thyrotrophin (HMT), heterotrophic factor (HTF), which is probably follicle stimulating hormone (FSH), and thyroid stimulating immunoglobulins, namely long acting thyroid stimulator (LATS) and long acting thyroid stimulator protector (LATS-P).

The normal placenta produces two thyroid stimulators, HCT (Hershman and Starnes, 1969) and HMT (Hershman and Higgins, 1971). HCT shows

partial cross reaction with anti-human TSH antibodies whereas HMT shows no reaction. HCT and TSH have similar molecular weights (28,000) whereas HMT has a higher molecular weight (65,000) and a longer time course of action in vivo. HMT is also produced in large amounts in some patients with hydatidiform mole or choriocarcinoma, where it may be responsible for abnormal results of thyroid function tests and occasionally for the hyperthyroidism seen in these conditions. The role of these thyroid stimulators in normal pregnancy has not yet been established.

A considerable amount of research has been carried out in connection with LATS and LATS-P, since Adams and Purves first demonstrated the presence of LATS in the serum of some patients with Graves' disease in 1956. With the exception of TRH and TSH, LATS and LATS-P are now recognised as the more important stimulators found in thyroid disease and a complete account of the physiology and significance in the aetiology of thyroid disease can be found in Appendix 3

The role of TSI, LATS and LATS-P in the pathogenesis of thyroid disease, especially with reference to their significance in Graves' disease, is still unclear and further work is required before a complete understanding of the mechanisms involved becomes apparent. The value of LATS or LATS-P assay in clinical practice is thus very limited and at present is only available at certain research centres. These assays were therefore not included in this study, since it was considered that no further information would be obtained with reference to the specific diagnosis of hypo- or hyperthyroidism, in an individual patient.

2:5 Thyroid Hormones

2:5:1 Synthesis and metabolism

The details of synthesis of both T^3 and T^4 although pertinent to

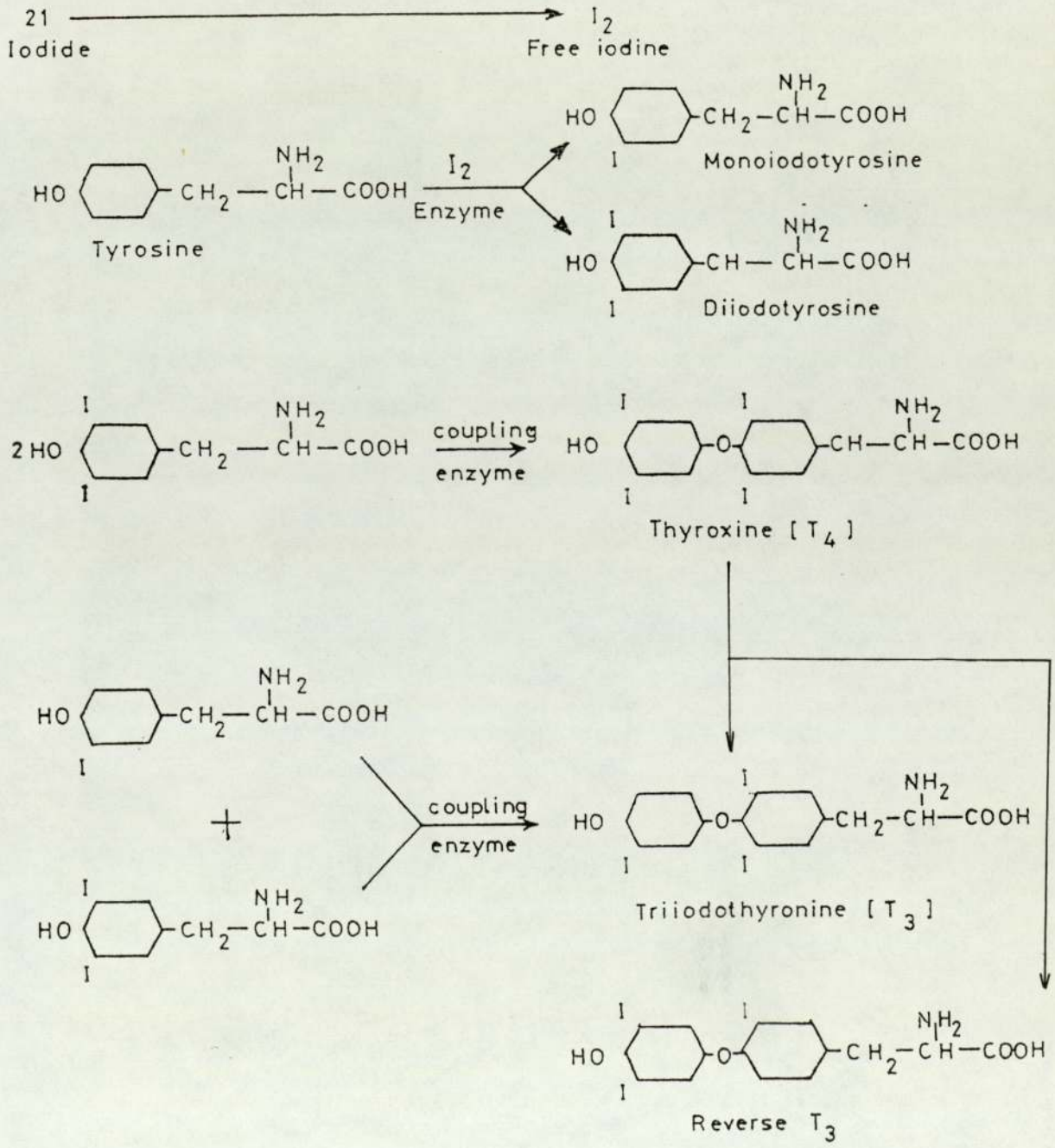
this current research will not be discussed in depth, but are diagramatically illustrated in Figure 1. It is known that the thyroid gland contains two separate groups of hormone synthesising cells. The parafollicular cells produce calcitonin, but the follicular cells produce T^3 and T^4 under the action of TSH via the second messenger c-AMP system. Plasma iodide is trapped and transported into the gland where it is oxidised to free iodine. The thyroglobulin precursor contains many tyrosine residues of which a few are iodinated at the colloid membrane to form mono- and diiodo-tyrosine. Coupling of mono, diiodo-tyrosine or of two molecules of diiodotyrosine to give T^3 or T^4 respectively, occurs within the thyroglobulin. The active hormones are then released from the gland after lysosomal hydrolysis of thyroglobulin; peripheral conversion of T^4 into T^3 takes place. Reverse T^3 (rT^3), which is inactive is probably produced instead of T^3 in conditions when continuing deiodination of T^4 to T^3 would upset the euthyroid status.

Within the serum, T^4 and T^3 are rapidly bound to proteins, and in order of decreasing affinity they are thyroxine binding globulin (TBG), thyroxine binding prealbumin (TBPA) and albumin. These account for approximately 60%, 30% and 10% respectively of the thyroxine binding capacity of serum. The affinity of T^3 for TBG is two - six fold less than that of T^4 for TBG. T^3 does bind to TBPA with an affinity similar to that for albumin.

Both T^4 and T^3 are metabolised in the peripheral tissues by deamination and decarboxylation to tetraiodothyroacetic acid or triiodothyroacetic acid. These metabolites are about one-quarter as active on a weight basis as their hormonal precursors although their onset of action is more rapid. Deiodination may also occur in the peripheral tissues, the liberated iodine being excreted in the urine.

Figure 1.

Synthesis and Structure of Thyroid Hormones



In the liver, thyroid hormone is rapidly conjugated with glucuronic acid and, to a lesser extent, with sulphate. Part of the conjugated T^4 apart from being excreted in the bile, may be reasorbed and transported to the kidney, where it may be deiodinated or excreted as intact conjugate.

2:5:2 The effect of organic iodide on thyroid hormone metabolism

The production of thyroid hormones is controlled through the hypothalamus and anterior pituitary by secretion of TRH and TSH. However, there is evidence that iodide influences thyroid hormone synthesis and secretion (Ingbar, 1972); these have been reviewed by Wolff (1969). Following acute exposure to large doses of iodide there is inhibition of thyroid hormone synthesis (Wolff and Chaikoff, 1948) and most of the iodide load is excreted by the kidney. Abassi and McKenzie (1967) showed that potassium iodide (KI) can impair thyroid function by a direct action, without altering pituitary TSH secretion. Iodine has an effect on thyroid vascularisation and this has been decreased following administration of KI for 10 - 14 days to patients with Graves' disease (Brownlie et al., 1977). The mechanism of this action remains unclear.

The reduction of thyroid hormone synthesis that occurs following iodide administration, only occurs with moderate doses of iodide, after a small transient increase of hormone release has depressed TSH secretion (Studer et al., 1976). The effect noticed by Wolff and Chaikoff (1948) should probably be defined in terms of the entire phase of iodine metabolism in which iodination increases in response to increasing doses of iodide rather than in terms of the quality of organic iodine formed. If iodide administration is prolonged, a decrease in iodide trapping results and is accompanied by a reduction in iodotyrosine coupling, which eventually leads to a loss of iodine from the thyroid and into the urine. Iodine deficiency induces autoregulatory enhancement of

iodine transport and increases the responsiveness of the iodine trap to the raised levels of TSH which follow the deficiency.

In 1969 Braverman et al., observed iodide induced hypothyroidism following inorganic iodide administration to patients rendered euthyroid, subsequent to treatment for hyperthyroidism.

TSH regulates thyroid function through the adenylate cyclase - cyclic 3'5' adenosine monophosphate (AC-cAMP) system (Herle et al., 1979) and the effect of iodide on this system has been extensively studied in the last years. The inhibitory action of iodide on AC or on c-AMP accumulation has been confirmed by several authors in different species, both in vitro (Sherwin, 1978) and in vivo (Rapoport, 1976). In Graves' disease patients, iodide or iodothyronines decrease c-AMP formation in response to TSH (Onaya et al., 1978).

The effect of iodide on RNA synthesis has also been studied (Pisarev and Klemman de Pisarev, 1980), and these authors concluded that the action of KI on RNA synthesis took place at the step of RNA transcription. In clinical studies in an endemic goitre area, Medeiros-Neto et al., (1975) showed a decrease in thyroid RNA concentration after the administration of iodized oil.

2:5:3 Protein Bound Iodine

Organic iodide therefore affects several aspects of thyroid metabolism and apart from its role in thyroid hormone control and homeostasis, is also involved in thyroid hormone synthesis, and early tests for thyroid function relied upon the quantitation of the serum protein bound iodine (PBI). The majority of the iodine in the serum of normal individuals, whose diet has not contained excess iodine, is to be found in both T^4 and T^3. However serum PBI measurements have proved

to be very unreliable and are less specific as an index of thyroid activity, than the assay of T^4 and T^3. Also falsely increased results for serum PBI have been reported in up to 25% of all patients and are usually due to one of the following causes:-

i) X-Ray contrast media

Most of these contain iodine and serum PBI may remain elevated after their use for months (e.g. "Biligrafin") or even years (e.g. "Myodil").

ii) Drug therapy

Several preparations currently available contain iodine compounds (e.g. many cough mixtures and intestinal sterilising agents - "Enterovioform").

iii) Thyroiditis or radiation therapy

Damage to the thyroid tissue may liberate thyroglobulin from the gland into the circulation.

iv) Dyshormonogenesis

This may occur in patients with rare enzymic defects in the synthesis of thyroid hormone. Mono- and di-iodotyrosines may be liberated into the circulation.

Levels of thyroid binding proteins also affect the serum PBI in a similar manner to that of T^4 and T^3 and this effect is not easily corrected. Therefore because of the non-specificity and many sources of interference, the serum PBI is now little used as an index of thyroid function and was thought to have no valuable role in the development of this current research.

2:5:4 Physiology of Thyroid Hormones

Over the past two decades there has been a dramatic increase in the number of diagnostic techniques available for the investigation of

suspected thyroid disease. Most emphasis has been placed upon the development of thyroid hormone assays; initially T^4 and more recently T^3. Measurement of total serum T^4 has become the most valuable single diagnostic test of thyroid function, levels being low in hypothyroidism and raised in hyperthyroidism, provided the binding proteins are normal. Recent data on thyroid hormone production and metabolism have altered concepts of normal thyroid physiology and much of this information has resulted from the development of methods for the assay of serum T^3. It has been calculated that T^3, although only representing 1-2% of the total circulating thyroid hormones, accounts for two-thirds of the total metabolic contribution of these hormones. Furthermore T^3 has a far greater biological activity than T^4 (3-4 times) and has a more rapid metabolic effect and turnover, largely because of decreased protein binding.

For more than 25 years, the thyroid gland has been known to produce T^3 (Gross and Pitt Rivers, 1952), but until recently it was thought that circulating T^3 was derived only from thyroid secretion. However it is now clear that most of the circulating T^3 is produced by monodeiiodination of T^4 in peripheral tissues, by iodothyronine deiodinases, which are found in kidney, liver, heart and fibroblasts (Pittman et al., 1971; Braverman et al., 1973). Hence T^4 can be considered in part as a prohormone and this led to the speculation that T^4 was itself biologically inactive (Oppenheimer, 1972). However the presence of nuclear receptors in peripheral tissues for both T^3 and T^4 (Sterling and Milch, 1975) and that several clinical conditions in which serum T^4 concentrations correlate better with clinical or physiological status, clearly indicate that T^4 may also have intrinsic biological activity.

Development of radioimmunoassay (RIA) techniques for the measurement of the alternative monodeiodination product of T^4,3,3'5'-triiodothyronine (reverse T^3 {rT^3}), have shown that this is present in the circulation and is mainly produced extrathyroidally (Chopra, 1976). However this hormone has little or no biological activity (Pittman et al., 1962) and is elevated by systemic illness, starvation,surgical stress and treatment with dexamethasone (Chopra et al., 1975, Burr et al., 1976), whereas T^3 levels are reduced by these conditions, partly because of impaired peripheral conversions of T^4 to T^3 (Bermudez et al., 1975)

2:5:5 Thyroid hormone levels in hyperthyroidism

In the majority of patients with hyperthyroidism, serum T^4 and T^3 concentrations are raised, but there is a disproportionate increase in serum T^3 concentrations and consequently a decreased T^4: T^3 ratio (Larsen, 1972). Often in early hyperthyroidism, the T^3 rises several months in advance of T^4 and therefore serum T^3 is a more sensitive indicator of thyroid hyperfunction and it has been proposed that it should be the first test applied when the clinical pattern fits that of hyperthyroidism. However, the advantage of sensitivity is hampered by a lack of specificity, because sometimes raised serum T^3 levels are also found in euthyroid patients previously treated for hyperthyroidism (Utiger, 1974; Marsden et al., 1975),or receiving preparations containing T^3. Therefore, the possible replacement of serum T^4 assay with that of serum T^3, as a routine test of thyroid function, has not been advocated. An increase in rT^3 also occurs in hyperthyroid patients (Nicod et al., 1976) and is approximately proportional to the increase in serum T^4 concentration.

Hyperthyroidism, when serum T^3 is elevated but total T^4 is normal is termed "T^3 - toxicosis" (Hollander,et al.,1972). However this can only be

used when the serum TBG concentration is normal, since a similar test pattern can occur in hyperthyroidism with TBG deficiency (Horwitz and Refetoff, 1977). Such patients have no distinctive or unusual symptoms or signs of hyperthyroidism (Hollander, et al., 1972). The possibility that there could be a syndrome of T^4 hyperthyroidism also exists, where serum T^4 is elevated, but with normal serum T^3 levels. As previously discussed patients with various acute and chronic illnesses have impaired extrathyroidal T^4 to T^3 conversion and there are reports of hyperthyroid patients with coincident non-thyroidal illness who had elevated serum T^4 levels with normal or low serum T^3 concentrations (Joasoo, 1975). In such patients preferential conversion of T^4 to rT^3 occurs and high levels of rT^3 have also been observed (Engler et al., 1978). As rT^3 is inactive, these dual pathways of T^4 metabolism may represent an important control mechanism in thyroid hormone economy, in patients with systemic illness.

In a recent study by Caro et al., (1980), a hyperthyroid patient was described with a normal total serum T^3 and T^4 concentration together with a normal thyroid binding protein concentration; the serum rT^3 and free T^4 (FT^4) however, were markedly elevated. The authors concluded that these findings, in the presence of clinical hyperthyroidism, were consistent with an impairment of peripheral conversion of T^4 to T^3, apparently in this case, due only to long-standing severe hyperthyroidism. The elevated serum FT^4 was due to a decreased binding affinity of TBG, although the reason for this was unclear, since binding protein concentration was normal.

In summary, T^4, T^3 and rT^3 levels are elevated in hyperthyroidism, although a disproportionate increase in T^3 relative to both T^4 and rT^3, occurs. Both the syndromes of T^3-toxicosis, associated with elevated T^3 levels and a normal serum T^4, and T^4-toxicosis where the reverse

is observed, have been described.

2:5:6 Thyroid hormone levels in hypothyroidism

Serum $T^3$and T^4 levels are characteristically reduced in patients with hypothyroidism, but up to 30% of clinically hypothyroid patients with reduced serum T^4 and elevated TSH levels, have normal T^3 values (Utiger, 1974). In certain patients with endemic goitre and associated hypothyroidism, elevated serum T^3 concentrations may even be found (Chopra et al., 1975). The presence of normal serum T^3 concentrations in some hypothyroid patients suggests that the failing thyroid gland can maintain T^3 production more readily than T^4 production, probably as a result of TSH stimulation. These findings argue that T^4 has intrinsic biological activity since normal serum T^3 concentrations alone are not sufficient to prevent either clinical hypothyroidism or increased TSH secretion.

In severe, acute or chronic non-thyroid diseases serum T^3 is often low partly due to impaired peripheral conversion of T^4 to T^3 and thus serum T^3 is not a reliable test for the diagnosis of hypothyroidism associated with other diseases. As discussed previously, in hypothyroidism T^3 levels may be normal or elevated, and therefore this provides further evidence of the limited role of serum T^3 assay in hypothyroidism. Serum rT^3 levels are low in hypothyroidism, as would be expected, since nearly all rT^3 is produced by peripheral conversion of T^4 (Nicod et al., 1976).

In summary, T^4,T^3 and rT^3 levels are reduced in hypothyroidism, although occasionally the serum T^3 is normal or elevated. Non-thyroid illness can cause a reduction in T^3 levels and thus the serum T^3 can be an unreliable indicator for diagnosis of myxoedema.

A review of thyroid diseases, encountered during the present study,

can be found in Appendix 1.

2:5:7 Thyroxine assay

Many techniques have been developed for the measurement of serum T^4 (Burke & Eastman,1974; Clark, 1965; Horn, 1975). Chemical methods involve column or thin layer chromatography or solvent partition; these methods determining hormonal iodine. Serum T^4 may be measured by competitive protein binding (Murphy & Pattee 1964) and this technique is available as a commercial kit. More recently however, radioimmunoassays have been developed for T^4 (Mitsuma et al., 1972; Burke and Eastman,1974; Ratcliffe et al., 1974). These tests are not affected by inorganic iodide, unlike previous PBI procedures, but they are affected by the level of thyroid binding proteins. With T^4 RIA, TBG must be taken into account since T^4 is bound more avidly and to a greater extent than T^3. Various releasing agents have been used, such as thimerosal and 8-anilino-1-naphthalene sulphonic acid (ANS). The effect of alteration in binding protein concentration, upon serum T^4 levels, is described later in this chapter on page 30.

2:5:8 Triiodothyronine assay

Since the identification of T^3 in blood and thyroid tissues by Gross and Pitt-Rivers in 1952, relatively little information had accrued until the latter part of the 1960's, concerning the role of this hormone in normal physiology and that of the thyroid gland. The major difficulty in obtaining this knowledge was the lack of simple, reliable, and specific methods for the quantitation of T^3 in blood and other biological fluids. Early methods include gas chromatography (Nauman et al., 1967) and saturation analysis techniques (Stering et al., 1969) although these techniques suffered from lack of precision and were complex and tedious to perform. However a significant advance in T^3 assay methodology was the production of specific T^3 antibodies by Brown et al., (1970) and subsequently the development of a sensitive, precise radioimmunoassay

for T^3 in serum (Brown et al., 1971) Other RIA procedures have been described by Lieblich and Utiger, (1972); Mitsuma et al., (1971); and Hesch and Evered. (1973).

The procedures employed are similar to those for the assay of serum T^4, and the RIA technique for T^3 is also affected by TBG concentration. The binding constant of this protein approaches that of the antibody used and releasing agents such as T^4 and its analogues have been used.

As previously described, T^3 estimation is probably most useful in diagnosis of hyperthyroidism, especially when used as described by Britton et al., (1975) and of less value in the diagnosis of hypothyroidism, where the serum T^4 usually falls before the serum T^3.

When considering the assay of serum T^4 and T^3, it is important to realise the effect of variation in the T^4: T^3 ratio upon interpretation of thyroid hormone tests. Thus a normal T^4 does not always indicate a euthyroid state - the patient may be hyperthyroid with "T^3-toxicosis" or may be hypothyroid, but taking oral contraceptives. This emphasises two important points (i) : the necessity for clinical assessment together with laboratory tests and (ii) the importance of using more than a single test of thyroid function.

2:6 "Free" thyroid hormones

The estimation of serum free T^4 (FT^4) and T^3 (FT^3) levels has until recently been unavailable for clinical evaluation. The assay of FT^4 is theoretically desirable over that of total T^4, since the free fraction is unaffected by factors affecting binding protein levels, particularly TBG. Many factors affect the TBG level (Page 30, Section 2:7) and this has also been shown by Young et al., (1975). The changes normally are

compensated for by similar changes in the total T^4 levels, as the negative feedback mechanism on the hypothalamic-pituitary-thyroid axis keeps the FT^4 concentration constant.

Recently many reports have appeared comparing the value of estimation of FT^4 with the more conventional assays for assessing thyroid function. Despite the theoretical advantages of measuring the free hormone fraction, a diversity of opinion exists concerning the value of its determination.

In a study by Tuttlebee and Bird (1981),serum FT^4 concentrations and FTI values were compared in 200 subjects. The authors found the FT^4 concentration was as good as the FTI in hyperthyroid, hypothyroid,elderly and acutely ill patients and a better diagnostic index of thyroid status in pregnancy and in oral contraception. A similar study carried out by Fyffe et al., (1980) comparing FT^4 and FTI (calculated using both an uptake test and thyroxine binding globulin), concluded that the FT^4 offered similar diagnostic efficiency to the other two calculated parameters. The effect of pregnancy and oral contraceptive therapy was not studied, since too few patients of this category were included in the survey.

Rootwelt and Solberg, (1981) using discriminant analysis compared FT^4 with other in vitro tests of thyroid function, in patients with thyroid and non-thyroid illness. Their results conflict with those obtained in the previous studies in that, as a single parameter, the FTI was found to be a marginally more efficient thyroid function discriminator than FT^4. The overall optimum test combination that was found for classification of thyroid abnormalities was serum total T^4, T^3 uptake and TSH, with serum T^3 replacing TSH, in distinguishing between

hyperthyroidism and euthyroidism.

Further evidence disputing the overall potential of FT^4 assay has been provided by Chopra et al.,(1980) who compared the assay of serum FT^4 by a variety of methods, in patients with thyroidal and non-thyroidal illness. Their study emphasised the diversity of results obtained for serum FT^4 concentration by using different assay techniques and how occasionally patients with non-thyroidal illness could be misclassified. The authors concluded that some disconcerting and unexplained inconsistencies occurred between serum FT^4 and the FTI and that further study was necessary to fully elucidate this phenomenon.

As for serum T^4, the determination of serum T^3 is influenced by alterations in thyroid binding proteins, and hence it has been reported that the assay of free T^3(FT^3) is valuable (Weeke and Orskov, 1975). However both tedious dialysis techniques and radioiodinated T^3 with a high specific activity are required, and therefore routine application of this test is difficult.

Free T^4 constitutes less than 0.1% of the total T^4 and thus attempts to assay the hormone have been rather unsuccessful. Christensen in 1959 used the technique of equilibrium dialysis of serum coupled with PBI values by Sterling and Hegedus, (1962). Further methods such as Sephadex filtration (Cavalieri et al., 1969; Lee et al., 1964),ultra-filtration (Schussler and Plager, 1967), charcoal absorbtion (Kumagai et al., 1967), electrophoresis (Schussler and Plager, 1967) and a combination of dialysis and gas chromatography (Petersen et al., 1977) have been used. However all these methods lack precision, are time consuming and thus not suitable for routine use.

RIA procedures are now available (Jiang and Tue , 1973) for the

assay of both FT^4 and FT^3 ; Chopra et al., (1980) has reviewed several methods for the assay of free T^4 in serum in thyroidal and non-thyroidal disease. However they are not as yet widely employed in clinical medicine, due to the limited research work correlating free hormone levels with the various thyroid states. The assays however give good precision and several commercial kits are now available e.g. "Amerlex^R Free T^4" (Radiochemical Centre, Amersham, U.K.) and "Corning Immo-Phase Free T^4" (Corning Medical, Essex), which are claimed to be fast, accurate and easy to perform.

Commencement of the present research began in 1976, at a time when the assay of FT^4 levels, apart from within research centres, was unavailable. At the time of writing, few laboratories in the U.K. offer FT^3 assay routinely and for these reasons the determination of free hormone levels was not employed in this study.

2:7 Thyroid binding proteins

Within the circulation, both T^4 and T^3 are transported in association with TBG, TBPA and albumin, as previously referred to on page 17 . Several factors can influence the levels of these proteins and are summarised in Table 1 .

As indicated in Table 1 , several drugs can affect both TBG and TBPA binding capacity and concentration and therefore indirectly affect serum T^4 and T^3 levels. These are further described in Appendix 2 Correction for the measured serum T^4 concentration in patients with alterations in thyroid hormone binding capacity can be provided by thyroid hormone binding tests, which estimate the residual binding capacity of the patients' serum. Therefore a binding test enables the total T^4 measurement to be interpreted, i.e. it indicates whether change

Table 1

Factors affecting binding capacity and concentration of TBG and TBPA

Increased TBG	Decreased TBG	Competition for binding sites
Pregnancy	Nephrotic syndrome	Phenytoin (limited effect)
Congenital	Liver failure	Salicylates
Oestrogens	Congenital	Phenylbutazone
Drug therapy	Drug therapy	Sulphonylureas
Phenothiazines (prolonged)	Androgens	
	Corticosteroids (large doses)	

Increased TBPA	Decreased TBPA
Analbuminaemia	Severe illness or trauma
Endocrine disease	Endocrine disease
Acromegaly	Thyrotoxicosis
Cushings syndrome	
Drug therapy	Drug therapy
Corticosteroids (large doses)	Dinitrophenol
	Salicylates

in T^4 is due to alteration in its binding or to change in T^4 secretion.

2:8 Thyroid hormone binding tests

The basis of the test is the in vitro addition of I^{125} - T^3 to a patient serum. The extent of binding of I^{125} - T^3 to the serum TBG depends on the degree of saturation of available binding sites by the patients T^4 : the remaining unbound I^{125} - T^3 is absorbed onto insoluble particulate material (resin or sephadex). When the serum T^4 is high as in hyperthyroidism, fewer binding sites are available on the serum TBG and more I^{125} - T^3 is taken up by the absorbent. If the TBG is increased in a euthyroid patient, then more binding sites are available and less I^{125} - T^3 is taken up by the absorbent. Results are expressed in different ways depending on the particular method used, and care should be exercised in interpretation.

The free thyroxine index (FTI)(Clark and Horn, 1965; Clark and Brown, 1970) is computed from the results of a T^4 estimation and a thyroid hormone binding test; this function being independent of alterations in TBG capacity. The FTI is of value particularly in pregnancy or in subjects receiving oestrogen therapy and shows good correlation with the FT^4 value (Wellby et al., 1966). Thus by calculating the FTI, a value for the serum T^4 corrected for alteration in binding proteins, is obtained.

2:9 Thyroxine binding globulin and thyroxine binding prealbumin assay.

Direct assay of thyroid binding proteins, especially of TBG, has received considerable attention over the past few years, due to the development of a monospecific antiserum to TBG by Bradwell et al., (1976). Several workers have subsequently described the advantages of determination of a T^4:TBG ratio over that of the T^4:T^3 Uptake (FTI) in the diagnosis of

thyroid disease (Burr et al., 1977; Attwood et al., 1978; McDowell, 1979) However the validity of their claims is disputed, with reference to the proposed replacement of the T^4:T^3Uptake with the T^4:TBG, in the routine assessment of thyroid disease. It is therefore proposed to review the assay of TBG and to critically evaluate its determination as an aid to the diagnosis of thyroid dysfunction. The possible replacement of the conventional FTI with the T^4:TBG ratio will also be discussed.

Assay of the thyroid binding protein TBPA has rarely been advocated as a thyroid function test, probably because it is a poor discriminator of different types of thyroid dysfunction. However the role of TBPA in thyroid hormone metabolism is of importance in normal individuals (Robbins et al., 1978) and in patients with non-thyroidal illness (Helenius et al., 1979),and one of the aims of this present investigation was therefore to develop a technique for the assay of TBPA in serum and to determine its possible value as a test of thyroid function, alongside those investigations currently available. Both TBG and TBPA determination will be included in the spectrum of tests employed in the psychiatric survey, if proved to be of diagnostic value in the assessment of thyroid disease.

CHAPTER THREE

LABORATORY METHODS FOR THE ASSESSMENT OF THYROID FUNCTION

Introduction

Initial Screening Tests

Haematological Assays

Biochemical Assays

Tests aimed at confirming/correctly classifying abnormal/ equivocal results on initial screening

Further tests carried out on specific patient groups

3:1 Introduction

In recent years ingenious biochemical procedures have been developed for the estimation of the concentration and biological effectiveness of thyroid hormones in blood. The relative merits and limitations of several procedures currently available has been reviewed by Evered, (1974)and Evered *et al.*,(1976). More recently much work has been published upon the value of certain tests and test combinations in order to obtain optimum diagnostic efficiency in assessing thyroid function. (Rootwelt and Solberg, 1978; Homburger and Hewan-Lowe, 1979; Liewendahl, 1977).

The use of radioactive iodine both for *in vivo* and *in vitro* studies of thyroid function has considerably broadened the understanding of thyroid physiology and pathology. However the administration of doses of potentially hazardous radioactive material to patients is not without danger as mentioned earlier. Therefore, there has not been a proliferation of *in vivo* procedures to the extent observed for *in vitro* studies employing radioactive tracers. Hence due to both theoretical and practical considerations, tests involving the administration of radioactive material to patients were not employed in this current research and therefore no details regarding their use will be given.

For the purpose of this study it is proposed to divide the assays employed into three groups, relating specifically to their use in the psychiatric survey. These are as follows:-

(i) Initial screening tests, involving both biochemical and haematological assays.

(ii) Tests aimed at both confirmation of abnormal results found on initial screening and also for aiding classification of

equivocal results.

(iii) Further tests carried out on specific patient groups selected for study. This group will include the thyroid binding proteins. (see Chapter 4).

An outline of the assay technique for each parameter is given, although precise technical details are omitted, i.e., volumes used etc. Technical details relating to the assay of the binding proteins will be given in Chapter 4, together with a complete account of their value in comparison with current tests of thyroid function.

3:2 Initial Screening Tests

3:2:1 Haematological Assays

The following two assays, namely blood haemoglobin (Hb) and erythrocyte sedimentation rate (ESR) were kindly performed by colleagues in the Haematology department at Good Hope Hospital, Sutton Coldfield, West Midlands.

3:2:2 Blood Haemoglobin

Determination of the Hb can be performed by several techniques including its measurement as oxyhaemoglobin, carboxyhaemoglobin, cyanme thaemoglobin, acid and alkaline haematin, by its oxygen capacity or its iron content. However the cyanmethaemoglobin method has now been adopted internationally as the approved standard method (Cannan, 1958; Crosby et al., 1954, Eilers, 1967). A cyanmethaemoglobin standard is now available and can be obtained from an appropriate certifying agency.

3:2:2:1 Principle

The Hb in whole blood is reacted with a reagent (Drabkins' solution) containing potassium ferricyanide, potassium cyanide and potassium dihydrogen phosphate. This oxidises the Hb to methaemoglobin and this is converted into the stable cyanmethaemoglobin by addition of potassium cyanide. The absorbance of cyanmethaemoglobin is measured at 540 nm, where a broad absorption peak is observed.

An automated method, based on the above principle, was employed for this study using the "Coulter Model S" automated haematological apparatus and a pre-calibrated whole blood standard provided by the manufacturer. Anticoagulated blood was used, 2.0 mls of sample being taken into a tube containing disodium ethylenediamenetetraacetic acid (E.D.T.A.).

3:2:2:2 Normal Values

The normal values quoted by our laboratory are 13.5 - 18.0 g/dl (Mean $\pm$ 2 SD) in adult males and 12.0 - 16.0 g/dl in adult females.

3:2:3 Erythrocyte Sedimentation Rate (E.S.R.)

The estimation of the erythrocyte sedimentation rate (E.S.R.) has been widely used in clinical medicine. The sedimentation rate of red cells in whole blood is influenced by plasma levels of fibrinogen and also by those of α^2 and γ globulins, to a lesser degree. The E.S.R. is raised therefore, in conditions associated with an increase of these specific proteins, such as infective, neoplastic and degenerative diseases. Extremely high values can be found in myelomatosis (carcinoma of the bone marrow) and macroglobulinaemia.

Many methods for the determination of the E.S.R. have been devised (Ham and Curtis, 1938; Nichols, 1942) and these differ according to the type of anticoagulants used, volumes of blood employed, assay tube dimensions, time allowed for sedimentation to occur, and the method of recording the results.

The method used for this study was that described originally by Westergren (1921).

3:2:3:1 Principle

The Westergren tube is a straight glass tube 30 cm. in length and 2.5 mm. in diameter; it is calibrated in mm. from 0 to 200 mm.

Venous blood was diluted with a one-fifth volume of 3.8% trisodium citrate as anticoagulant. The sample was well mixed and the blood then drawn up into a Westergren tube to the 200 mm. mark. The tube was placed

exactly vertical in a specially designed rack and allowed to stand for 60 minutes. The height of the clear plasma above the upper limit of the column of sedimenting red cells was then read. The figure obtained represented the E.S.R. in mm. per hour.

3:2:3:2 Normal Values

The normal ranges quoted by our laboratory are:-

3 - 5 mm. in 1 hour, for adult males;

4 - 7 mm. in 1 hour, for adult females.

3:2:4 Biochemical Assays

3:2:5 Serum Thyroxine

As mentioned in the introductory chapter many techniques have been devised for the assay of serum thyroxine (T^4). However, in recent years the two most widely used methods have been competitive protein binding assays (C.P.B.A.) and radioimmunoassays (R.I.A.). Many workers have developed their own assays for both procedures, although the majority of routine clinical chemistry departments have relied upon the use of commercially available kits, e.g. "Thyopac - 4" : a CPBA kit available from the Radiochemical Centre, Amersham; and "Immophase" an RIA kit available from Corning Medical, Halstead, Essex.

Competitive binding techniques (Murphy and Pattee, 1964; Ekins et al., 1969) have now been superceded by RIA methods, since the former suffer from several disadvantages, including non-reproducible recovery of T^4 after extraction (Chopra, 1972). In order to facilitate the understanding of the principle of the RIA technique for serum T^4 employed for this study, an outline of the basic steps involved for RIA procedures follows.

3:2:5:1 Basic Principles of Radioimmunoassay

The technique of RIA was first introduced by Yalow and Berson in 1960 for the assay of circulating levels of insulin and has been applied to the majority of peptide hormones. More recently radio-immunoassay has also been used for the estimation of steroids and other non-peptides.

Radioimmunoassays are based on competition between unlabelled and labelled antigen for a limited number of antibody binding sites. Thus conditions are chosen such that there is a relative excess of antigen, with the result that some of the antigen will be bound (B) while the rest remains free (F).

$$\underset{\text{(free fraction)}}{H + Ab} \underset{K^2}{\overset{K^1}{\rightleftharpoons}} \underset{\text{(bound fraction)}}{H : Ab} \qquad (1)$$

where:

H - free hormone

Ab - free antibody

H:Ab - hormone/antibody complex

K^1, K^2) - velocity of forward and backward reaction

Radioimmunoassays are based on determination of the percentage of the total antigen present in the antibody bound fraction and it will be apparent from equation (1) that only three factors can influence this.

(a) Energy of the reaction

(b) The percentage bound is directly proportional to the concentration of antibody present.

(c) The percentage of total hormone present in the bound fraction will decrease as the total concentration of hormone increases.

Only this third factor operates in a radioimmunoassay since the same antiserum is used throughout, and its concentration is kept constant. It follows, therefore, that the amount of hormone present can be calculated from its distribution between the antibody-bound and free fractions once equilibrium has been achieved. To do this, a tracer quantity of isotopically-labelled peptide is included in the reaction mixture, permitting the determination, using a scintillation counter, of the percentage of the total antigen that is present in the antibody-bound fraction, after separation by any of a number of physico-chemical or immunological techniques.

With reference to the assay of T^4, several variations in technique have been reported and these include:-

(a) Methods of extraction of the hormone from serum (Patel and Burger, 1973) and in certain cases the use of unextracted serum (Meinhold and Wenzel, 1974).

(b) Methods of blocking the effect of binding proteins. The most commonly used agent has been 8 - anilino - 1 - naphthalene sulphonic acid (ANS), but also salicylates and diphenylhydantoin have been used (Mitsuma et al., 1972; Larsen et al., 1970; Lieblich and Utiger, 1971).

(c) Incubation times for the assay vary from less than one hour (Sekadde et al., 1973) to several days (Beckers et al., 1974).

(d) Separation procedures for free and antibody bound hormone. These have included double antibody techniques (Beckers et al., 1974)

but polyethylene glycol (Sterling and Milch, 1974) and dextran coated charcoal (Mitsuma et al., 1972) have also been used.

3:2:5:2 Principle of the serum T^4 assay

The RIA technique used for this study was a commercially available kit manufactured by Corning Medical, referred to as the "Immophase T^4 RIA Test System". This technique employs thimerosal (sodium [(ocarboxyphenyl) thio] ethylmercury), as a blocking agent for binding proteins (Ratcliffe et al., 1974; Hüfner and Hesch, 1973). The antibody used in the system is obtained from the rabbit (rabbit anti-T^4 gamma globulin) and is covalently bound to porous glass particles. The use of this ingenious solid phase separation system of separating bound from free antigen, greatly simplifies the overall procedure. The porous glass particles have a large surface area and high relative density, the latter allowing a rapid and quantitative separation; the complete assay operational time being about 1½ hours.

Briefly the procedure employed for the assay was as follows:-

The antibody specific for T^4 was reacted with radiolabelled antigen (^{125}I-T^4) and a serum sample containing the unlabelled antigen (T^4) in known (standard or control) or unknown (patient) quantity. Thimerosal is used to release T^4 from binding proteins and thereby blocks the action of thyroxine-binding globulin (TBG). The unlabelled and labelled antigens compete for antibody binding sites and the more unlabelled antigen present, the less labelled antigen becomes bound to antibody and vice versa. A standard curve was obtained by holding the concentration of labelled antigen and antibody constant, while increasing the concentration of unlabelled antigen. Quantitation of the unknown sample was then obtained by interpolation from the standard curve.

The kit contained 500 ml. of rabbit T^4 antibody covalantly bound to porous glass particles, suspended in phosphate buffered saline which has bovine serum albumin and thimerosal added. Aliquots of 0.8 ml. of antibody are pre-dispensed into tubes to facilitate ease of analysis.

^{125}I labelled T^4 tracer is supplied in lyophylised form dissolved in phosphate buffered saline, containing bovine serum albumin, thimerosal and a red dye. The kit also contained six lyophylised standards in a protein based solution at 0, 32.2, 64.4, 128.7, 193.1 and 386.1 nmol/l. Two reference controls prepared from pooled tri-iodothyronine (T^3) - T^4 - free human plasma, were also provided.

Before employing the "Immophase" method it was necessary to carry out a short evaluation of the assay, by comparing it with the techniques used within the laboratory routinely. This was a commercially available CPBA, namely "Thyopac - 4" obtained from the Radiochemical Centre, Amersham. The standard deviation and coefficient of variation was calculated (as below) for the "Immophase" technique and a correlation coefficient determined for the two methods.

3:2:5:3 Calculation of standard deviation and coefficient of variation

At least forty duplicate determinations at varying levels of T^4 were performed using the "Immophase" technique - the calculations and results appear below:-

The standard deviation (SD) is given by

$$SD = \sqrt{\frac{\leqslant d^2}{n}}$$

where:

d = the difference between each pair of duplicates

n = the number of duplicates

The coefficient of variation is simply given by

$$CV = \frac{SD}{\bar{x}} \quad \text{or} \quad \frac{SD}{\bar{x}} \times 100 \quad \text{(expressed as a percentage)}$$

where

$\bar{x}$ = the mean value

The following values were obtained:-

$\bar{x}$ = 118 nmol/l T^4

SD = 9.8 nmol/l

CV = 8.3%

(NB: These results represent within assay variation)

A correlation graph plotting values of serum T^4 obtained using the Corning Eel kit versus the Radiochemical Centre's Competitive Protein Binding Kit gave an overall correlation coefficient of 0.96. Thus the serum T^4 values obtained using the Corning Eel kit correlated very well with a competitive protein binding assay. The assay was initially performed manually, although in November, 1976 the technique was automated by using Searle Instruments 'Analmatic' which is an automated diluting/dispensing station. This instrument was initially evaluated as before and gave an overall coefficient of variation of 7.9% and a correlation coefficient with the manual technique of 0.97.

It can be seen that no dramatic improvement in precision occured after automation of the method, yet it proved advantageous as operator fatigue and thus error were reduced and throughput of samples was increased with no loss in precision.

3:2:5:4 Normal Values

The normal values for serum T^4 quoted by our own laboratory are 58 - 148 nmol/l (mean $\pm$ 2 SD). This range applies to both adult males and females. Figures quoted by Corning Medical for a population of 325 patients, in whom physician diagnosis of thyroid state was not available, gave values between 58 and 167 nmol/l ($\bar{x} \pm 2SD$). Work published by Evered et al., (1976) using an RIA technique for serum T^4, gave a normal range of 62 - 154 nmol/l ($\bar{x} \pm 2SD$) and for an RIA method described by Black et al., (1975), a range of 71 - 174 nmol/l was quoted. Therefore the normal values given by our own laboratory for serum T^4 compare favourably with the published data.

3:2:6 Resin Uptake Test (T^3-Uptake)

An indirect assessment of serum T^4 levels can be provided by thyroid hormone binding tests which determine the residual binding capacity of the patients serum. The major use of such tests, however, is to correct the measured serum T^4 concentration for alterations in thyroid binding protein capacity.

In serum there is a dynamic equilibrium between free T^4 and bound T^4. In patients with normal TBG levels, the more elevated the T^4, the lower the proportion of available TBG sites and vice versa. The number of binding sites available is determined by the various T^3 uptake methods currently in use. T^3 is used most often due to its low affinity for thyroxine-binding pre-albumin (TBPA) and albumin, thus rendering it more specific for TBG sites. Also T^3 does not displace bound T^4 from TBG. On addition of $^{125}I - T^3$ to serum, a certain proportion, equivalent to the free binding sites, becomes bound to TBG. Various techniques have been used to separate the free and bound $^{125}I - T^3$ including red cells

(Hamolsky et al., 1957), ion exchange resins (Mitchell, 1960), and Sephadex (Hansen,1965). Determination of the free binding sites is calculated as being inversely proportional to the percentage of radioactivity bound to the secondary binder. The proportion of activity remaining in the serum however can be calculated as being directly proportional to the unbound sites.

The assay was originally described by Hamolsky et al., (1957), these workers using red cells for the uptake of ^{125}I - T^3. However later methods employed resins in either granular or sponge form as the secondary binders (Mitchell, 1958). Sisson,(1965) has summarised the many advantages of resins over red blood cells.

Confusion can arise with regard to the methods used for the presentation of results of serum uptake methods. The early red cell methods were reported as the percentage of the activity that was bound to the red cells and thus low values, compared to normal, were obtained for hypothyroid patients and high values for hyperthyroids. Later methods, including the assay procedure described shortly, were reported on the percentage of the activity that was bound to the patients own serum proteins, compared to a normal serum pool. Here, high values are obtained for hypothyroid patients and low values for hyperthyroids. Therefore when referring to serum uptake tests, it is essential not only to give the normal range, but also to state whether a raised value indicates hyper- or hypothyroid status.

3:2:6:1 Principle of the T^3 Uptake test

Several commercial kits are now available for the assay of T^3-Uptake in serum and this study utilised the "Thyopac-3" procedure marketed by the Radiochemical Centre, Amersham. This technique

utilises a modification introduced by Scholes (1962), whereby an absorbent which binds T^3 reversibly is used.

The radiolabelled $T^3(^{125}I - T^3)$ and a granular absorbent of unstated composition are supplied in vials to which either known (standard or control) or unknown (patients) serum was added. The vials were then mixed during which time an amount of active T^3 proportional to the free binding sites is removed from the absorbent and equilibrium is reached. The vials were then allowed to stand for several minutes until the absorbent settled and then the radioactivity of an aliquot of supernatant was determined using a γ-scintillation counter. The results were expressed in terms of a similarly treated manufacturers' reference serum. Different batches of reference sera carry a factor to allow comparison with the the midpoint of the normal range; this being given an arbitrary value of 100.

This method has been in use in the laboratory for several years and statistically has been proven to perform well (Bold and Browning, 1975) with a coefficient of variation of less than 5%.

3:2:6:2 Normal Values

The normal values quoted by the Radiochemical Centre have been adopted by our laboratory, namely 92-117% for adult males and females. Individuals with values less than 92% are classified as hyperthyroid and greater than 117% as hypothyroid.

3:2:7 Free Thyroxine Index

It was shown by Clark and Horn (1965) that a good index of thyroid status could be obtained by multiplying the protein bound iodine (or T^4) by the T^3 - resin uptake and they called this value

the "free thyroxine index" (FTI). The index was subsequently shown to correlate well with the direct estimation of free T^4 (Wellby and O'Halloran, 1966). This calculation gives a value for T^4 corrected for alterations in TBG capacity and is of value particularly in pregnancy or in subjects receiving steroid therapy, especially oestrogens. For example, pregnancy results in high levels of TBG and thus of serum T^4 due to pituitary feedback from the artificially lowered free T^4 serum concentration. T^3 uptake is therefore reduced due to the increase in free binding sites and when the two results are multiplied together, the alterations, as they are in opposite directions, tend to cancel each other out. The opposite phenomenon occurs in the nephrotic syndrome, with associated low levels of TBG.

For this study the F.T.I. was calculated as follows:-

$$\text{F.T.I.}(\%) = \frac{\text{Serum } T^4}{\text{Serum } T^3\text{- Uptake}} \times 100$$

3:2:7:1 Normal Values

The F.T.I. values approximate to the range quoted for serum T^4, namely 58 - 148 nmol/l for adult males and females. The function, as stated previously, is unlike the serum T^4, independent of alterations in TBG concentration.

3:3 Tests aimed at confirming/correctly classifying abnormal/equivocal results on initial screening

3:3:1 Lipid Studies

With the advent of more sophisticated tests of thyroid function, lipid analysis has now a very limited role to play in the assessment of thyroid function. Traditionally cholesterol has been assayed and more

recently triglycerides.

Many factors are known to affect cholesterol levels (Flynn and Hobbs, 1971) and therefore the results of this test must be interpreted carefully. A level of 8.0 nmol/l is found in over 80% of patients with primary hypothyroidism due to impaired catabolism and a fall in level in response to thyroid replacement therapy provides confirmatory evidence of the original diagnosis. (Hobbs et al., 1963). The test is of less value in the diagnosis of secondary hypothyroidism and is virtually useless in thyrotoxicosis because of the overlap with the normal range. Triglyceride levels tend to follow the same pattern as cholesterol - the inclusion of this assay and that of lipid electrophoresis in the study was hoped to yeild further information of the lipid changes in thyroid disease.

Cholesterol was estimated on the Auto Analyser (Technicon) using the colorimetric Liebermann-Burchard reaction. (Annan and Isherwood, 1969). Cholesterol and other sterols react at 37°C with a mixture of sulphuric acid, glacial acetic acid and acetic anhydride to yield a green colour which is measured colorimetrically at 625 nm.

Triglycerides were estimated enzymatically using the 'L.K.B.' reaction rate analyser. In the procedure used, the triglycerides were hydrolysed by a mixture of lipase and esterase. Subsequently, the glycerol released was determined kinetically by means of the coupled reactions:-

$$\text{Glycerol} + \text{ATP} \xrightleftharpoons{\text{Glycerol kinase}} \text{Glycerol - 3 - phosphate} + \text{ADP}$$

$$\text{ADP} + \text{Phosphoenolpyruvate} \xrightleftharpoons{\text{Pyruvate kinase}} \text{ATP} + \text{Pyruvate}$$

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightleftharpoons{\text{LDH}} \text{Lactate} + \text{NAD}^+$$

The conversion of $NADH_2$ to NAD was determined by measuring the

total decrease in absorption at 340 nm which was proportional to the amount of T.G. in the sample.

3:3:1:1 Normal Values

The normal ranges quoted in our laboratory for adult males and females are:-

Serum Cholesterol	:	3.0 - 7.3 mmol/l
Serum Triglycerides	:	Less than 1.8 mmol/l

3:3:2 Thyroid Stimulating Hormone

The value of thyroid stimulating hormone (TSH) assay has already been discussed at length in Chapter 2, page 13 and therefore will not be elaborated upon further.

As mentioned earlier the original bioassays for TSH (Adams and Purves, 1956) have now been replaced by more highly sensitive and precise radioimmunoassays (Odell et al., 1967; Hall et al., 1971). Several commercial kits are available and the Corning Eel, "Immophase Radioimmunoassay Test System" for the assay of TSH was chosen for this study.

This assay utilises a radioactive ^{125}I-anti TSH (TSH antibody), thereby avoiding the use of labelled TSH which is relatively unstable. TSH is quantitated in terms of ^{125}I - antibody bound to hormone. The bound radioactive antibody is separated from the free with a second anti-TSH which is covalently coupled to glass particles. This type of assay is referred to as a "Sandwich assay" and will be described further.

3:3:2:1 Principle of TSH "Sandwich" Technique

The radioactive ^{125}I - antibody (Ab*) specific for TSH, was

added to a known amount of TSH standard, control or patients serum (See Figure 2). The Ab* binds to any TSH present and forms a labelled complex. After a short incubation period, a second antibody, also raised against TSH, was added to the reaction mixture. This second antibody is covalently bonded to microscopic glass particles and serves to bind the labelled complex formed above. After binding the bound complex was separated by centrifugation and the precipitated complex counted in a γ scintillation counter. A standard curve was constructed of TSH concentration versus radioactivity and the TSH values for the patients read off the graph by interpolation.

The kit contained TSH antibody (rabbit anti-TSH) covalently bound to glass particles, suspended in phosphate buffered saline with bovine serum albumin added.

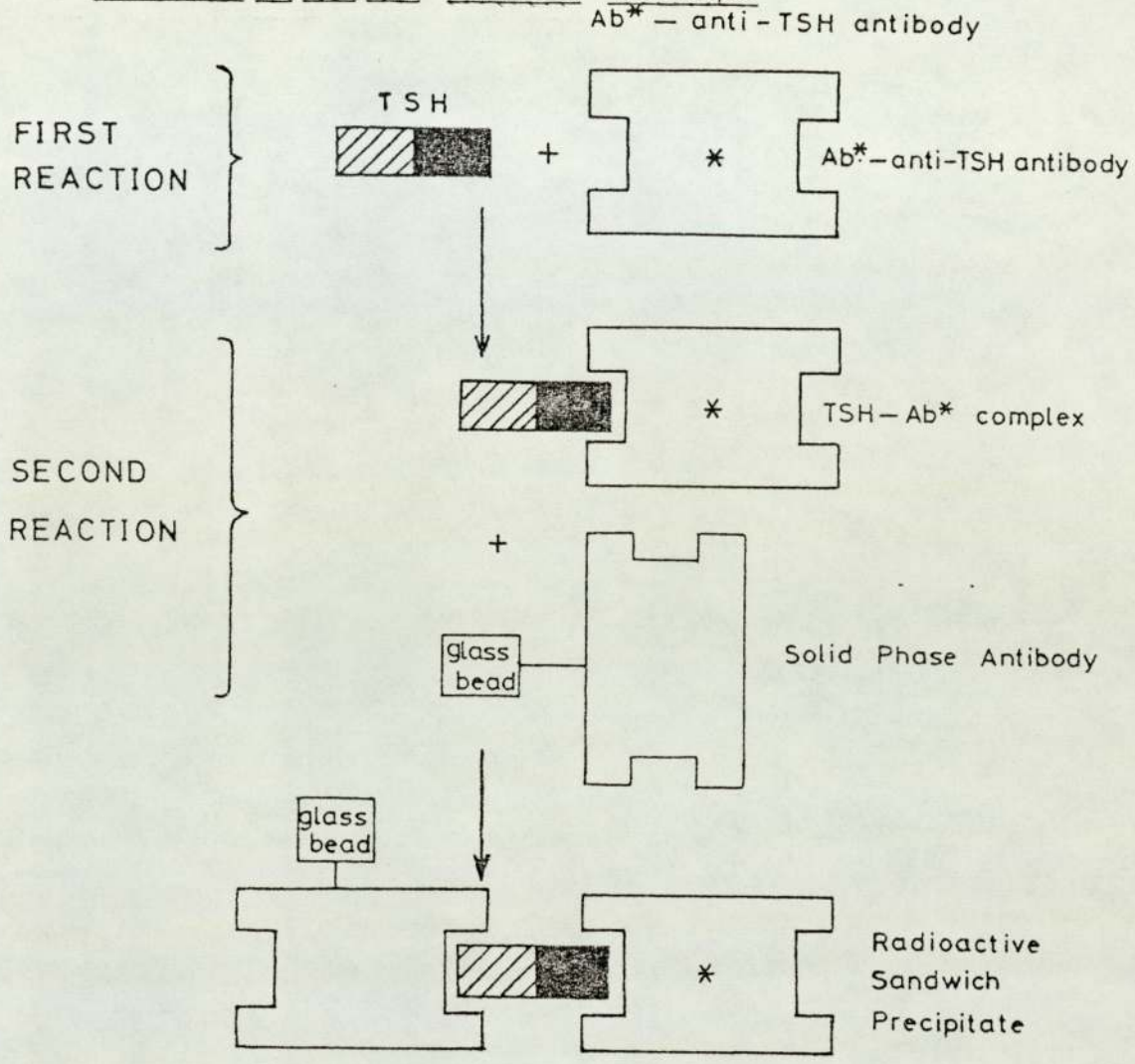
^{125}I - labelled rabbit anti-TSH antibody, dissolved in calf serum, is supplied in lyophylised form.

The kit also contained seven lyophylised standards in defibrinated porcine plasma, at 0, 1.5, 3.0, 6.0, 15.0, 30.0 and 60.0 μIu/ml. Two reference controls prepared in human serum were also provided.

An evaluation of the assay was initially carried out (as with the thyroxine assay), to determine the precision of the technique. An overall SD of 0.87 was obtained with a CV of 6.6%; these results representing a <u>within-assay</u> variation. It was not possible to perform an accuracy comparison as before (calculation of correlation coefficient), since this technique was new to the department. The manufacturers, however, quote a correlation coefficient of 0.924 against a reference method (not stated).

Figure 2

Principle of the RIA "Sandwich" Technique



3:3:2:2 Normal Values

The normal values quoted by our laboratory range from 1.0 - 8.0 μIu/ml (Mean ± 2 SD). These figures were obtained from our own data and show good correlation with other published values. Results quoted by Corning Medical for a population of 50 apparently normal patients (physician diagnosis of thyroid state was not available), gave values ranging from 1.8 to 10.1 μIu/ml ($\bar{x}$ ± 2 SD). Work published in 1981 by Rootwelt and Solberg using the "Radormon TSH-DA" RIA kit (from Kabi Diagnostica, Sweden) for serum TSH, quoted a normal range of 0 - 8 μIu/l.

3:3:3 Tri-iodothyronine

The early methods of measurement for T^3 have now been replaced by the development of precise and sensitive R.I.A. procedures (Gharib et al., 1972; Sekadde et al., 1973; Rastogi and Sawhney, 1974). For the R.I.A. of T^3 in serum, the effect of TBG upon the assay must be noted, since the binding constant of this protein approaches that of the antibody used. To overcome this effect several "releasing agents" have been utilised (Hufner and Hesch, 1973). Chemicals such as tetrachlorthyronine (Mitsuma et al., 1971), salicylate (Larsen, 1972), diphenylhydantoin (Hesch et al., 1972) and 8-anilino-1-naphthalene sulphonic acid (ANS) (Chopra, 1972; Eastman et al., 1973), have been used. Also various techniques for separation of bound from free hormone have been employed including ammonium sulphate precipitation (Chopra et al., 1972), coated charcoal adsorption (Ekins et al., 1969) and solid phase methods (Siegel et al., 1973). However the double antibody technique is one of the most common separation methods for T^3 RIA.

Several commercial kits are now available for the assay of T^3 in serum and the one chosen was the Corning Eel "Immophase T^3 Radio-immunoassay Test System", utilising a solid phase separation technique.

3:3:3:1 Principle of T^3 R.I.A.

An antibody specific for T^3 was reacted with a trace amount of radiolabelled antigen ^{125}I-T^3 and a serum sample containing the unlabelled antigen (T^3) in known (standard or control) or unknown (patient) quantity. ANS is used to release serum T^3 from serum binding proteins. The unlabelled and labelled antigen compete for antibody binding sites and the more unlabelled antigen present, the less labelled antigen becomes bound to antibody and vice versa. A standard curve was obtained by holding the concentration of labelled antigen and antibody constant, while increasing the concentration of unlabelled antigen.

Quantitation of the unknown sample was then obtained by interpolation from the standard curve.

The kit contained rabbit T^3 antibody covalently bound to porous glass particles, suspended in phosphate buffered saline, which has bovine serum albumin and thimerosal added. Aliquots of 0.8ml of antibody are pre dispensed into tubes to facilitate ease of analysis.

^{125}I - labelled T^3 tracer is supplied in lyophylised form dissolved in phosphate buffered saline, containing bovine serum albumin, ANS, thimerosal and a red dye. The kit also contained seven lyophylised standards in T^3-and T^4-free bovine serum albumin at 0, 0.38, 0.77, 1.54, 3.08, 6.15 and 12.31 nmol/l. Two reference controls prepared from pooled T^3-and T^4-free plasma were also provided.

An evaluation of the assay was initially carried out (as with both the T^4 and TSH assay), to determine the precision of the technique. An overall SD of 0.08 was obtained with a CV of 6.1%; these results representing a within-assay variation. Accuracy figures obtained by comparison with alternate methods were not derived, since no other method for T^3 was available in this department. However the manufacturers quote a correlation coefficient of 0.991 against a reference method (not stated).

3:3:3:2 Normal Values

The normal values quoted by our laboratory range from 0.5 - 2.9 nmol/l (Mean $\pm$ 2 SD). These figures were obtained from our own data and demonstrate good agreement with published work. Hesch and Evered, (1973) reported a normal range of 1.32 - 2.46 nmol/l while Evered et al., (1976) reported normal values ranging from 0.73 - 2.77 nmol/l.

3:4 Further tests carried out on specific patient groups

3:4:1 Thyroid Binding Proteins

Within the serum, T^4 is transported almost entirely in association with two proteins which act as specific carrier agents for the hormone. A glycoprotein which migrates electrophoretically in the region between the alpha^1 and alpha^2 globulins is designated thyroxine-binding globulin (TBG). Another protein, thyroid binding prealbumin (TBPA), is detectable electrophoretically just ahead of the albumin fraction. When large amounts of serum T^4 are present and the binding capacities of these specific carrier proteins are exceeded, serum T^4 is bound to serum albumin. TBG has a high affinity for binding to serum T^4, and a low affinity for T^3. TBPA has a lower affinity for serum T^4 and is less tightly bound; no T^3 is detectably bound to TBPA. In a normal subject only about one-third of the maximum binding capacity is utilised.

TBG and TBPA may be measured by their binding capacity for ^{125}I- T^4 or ^{131}I - T^4 (saturation analysis)(Robbins, 1956, 1963), or indirectly by the T^3-Uptake method (described previously), which reflects unoccupied binding sites. All these methods measure hormone binding capacity and not concentration and thus a method for the direct measurement of TBG and TBPA would be ideal and could provide very valuable information in appropriate cases, especially when binding protein levels are abnormal. An RIA technique for measuring TBG concentration has been described by Levy et al., (1971).

A method for the determination of TBG and TBPA was developed by Freeman and Pearson (1969) using the Laurell two dimensional immuno-electrophoretic method, as described originally by Clark and Freeman, (1967), coupled with autoradiography. They used this technique to precipitate the proteins in the form of Gaussian or near Guassian peaks.

whose area was proportional to antigen concentration. TBG was identified by saturating the precipitate with radioactive T^4, washing off the excess, then submitting the plate to autoradiography. However, the method is too cumbersome and costly in antiserum and other reagents for routine use. This technique however, was later modified by Drysdale et al., (1975), yet both methods require addition of radioactive tracer T^4 to serum for the identification of TBG and the assays take several days to complete - seven days for the latter method. Kranz et al., (1974) used an oligospecific antiserum prepared against α, electrophoretic fractions of serum proteins and was able to see the TBG precipitate after rocket electrophoresis, but confusion could occur with other serum proteins depending upon their relative concentrations.

Radioimmunoassay of TBG by Levy et al., (1971) needed radioisotopes and highly purified TBG for labelling. The method is unnecessarily sensitive, being able to measure 1 ug of TBG/l when the serum concentration is between 5 - 30 mg/l. The major problem in the past has been in the preparation of a high titre monospecific antiserum to TBG. This has not been generally available because of difficulties in preparing pure TBG for use as an antigen. However, recently this problem has been overcome by Bradwell et al., (1976) who used antigen/antibody coupling in agarose gel, which enabled them to separate TBG from contaminating proteins, yielding a high affinity monospecific antiserum.

3:4:1:1 Principles of Assay Procedure

The procedure employed for the quantitative determination of TBG/TBPA was that of electroimmunodiffusion (electroimmunoassay) but commonly recognised under the name of the "Laurel rocket technique". (Laurell 1965; 1966; 1972). The assay of these two proteins is fully described

in the following Chapter, where a comprehensive account of their value in the diagnosis of thyroid disease is discussed.

CHAPTER FOUR

THYROXINE BINDING GLOBULIN AND THYROXINE BINDING PREALBUMIN MEASUREMENT COMPARED WITH THE CONVENTIONAL T^3UPTAKE IN THE DIAGNOSIS OF THYROID DISEASE

Introduction

Selection of subjects for study

Methods

Results

Discussion

4:1 Introduction

The assays that are routinely used as tests of thyroid function are a combination of serum T^4, and T^3U and FTI. To clarify the diagnosis especially in equivocal cases, these are often followed up with serum T^3 and TSH estimation; occasionally a dynamic test of thyroid function (TRH stimulation) is also employed. With these tests it is possible to confirm the thyroid states of the majority of patients (Pain and Duncan, 1976; Evered et al., 1978)

However in a proportion of patients, particularly those who are taking oral contraceptives, are pregnant, have severe non-thyroid disease, are on antidepressant therapy, or are elderly, the above tests are more difficult to interpret due to the abnormal levels of the thyroid binding proteins. (This is more extensively reviewed later in this section). The derived index (FTI), relies upon the measurement of the serum T^4 combined with the T^3U, the latter representing an indirect measure of binding protein concentration. Initially this index was reported to compensate for abnormal levels of the thyroxine binding proteins (Howorth and MacLagan, 1969), but in recent years it has become recognised that the FTI may fail to correct the serum T^4 in the above cases. Reliance on this index therefore, may lead to a false diagnosis of thyroid status (Premachandra et al., 1976).

However, the development of a direct assay for TBG by Freeman and Pearson in 1969, represented a means by which the hitherto inherent problems associated with the FTI could be overcome. The present study was therefore designed partly to develop a method for the assay of TBG and to investigate its relationship with other tests of thyroid function. The value of the routine estimation of TBG and its aid in the

differentiation of thyroid disorders was also assessed.

The thyroid binding protein, TBPA, has received little attention in connection with its possible role as a test of thyroid function and as detailed later in this chapter, this study also investigates in depth a method for its determination and considers its clinical value in the diagnosis of thyrometabolic disease.

Both the assay of TBG and TBPA will be compared to the T^3U and hence to the FTI, in connection with their ability to differentiate between the various forms of thyroid dysfunction. An historical account of the development of these assays is necessary therefore, to enable a comprehensive understanding of the relative merits of each parameter.

The T^3U was originally introduced by Hamolsky et al., (1957) and represents a measure of binding protein capacity, rather than concentration. Originally red blood cells were used in the assay, but these have since been replaced by synthetic materials, mainly anion exchange resins (Mitchell et al., 1960) and Sephadex (Hansen, 1965). The T^3U was thought to reflect the level of free binding sites on TBG and has been shown to be related to the percentage free T^4 (Liewendahl, et al., 1971; Snyder et al., 1976). This relationship stimulated the introduction of free hormone indices, calculated from a measurement of total T^4 and T^3U. The FTI so derived, is able to correct for protein binding abnormalities which may occur e.g. in pregnancy or during oestrogen treatment, when elevated levels of TBG are present. Many investigators have since found the FTI to be of great clinical value especially in cases with associated abnormal protein levels (Liewendahl et al., 1971; Welby et al., 1974) and good correlation with free T^4 concentration has been shown (Olsen and Anderson, 1979;

Fyffe et al., 1980) T^3U tests are also capable of good precision and are simple to perform (Bold and Browning, 1975; McDowell, 1979).

The technique however, is subject to certain limitations and these have been extensively reviewed as detailed below. In pregnancy and in oral contraceptive therapy, the FTI does not accurately reflect free T^4 levels (Goolden et al., 1967, Souma, 1973; Felicetta and Green, 1980; Tuttlebee and Bird, 1981). The raised FTI found in certain of these cases demonstrates the inability of the T^3U to correct for the serum T^4, in situations where the TBG levels may be very high. In non-thyroidal illness, with decreased TBPA levels, the FTI occasionally underestimates the true free concentration (Tuttlebee and Bird, 1981). The reason for this is probably that the T^3U test does not correctly reflect variations in TBPA and albumin (together binding 30% of serum T^4), because of the relatively low affinity of these proteins for triiodothyronine (T^3).

Recently the potential value of direct assay of TBG levels has been suggested by several groups (Burr et al., 1977; Attwood and Probert, 1978; McDowell, 1979). Calculation of a T^4:TBG ratio has also been described (Attwood et al., 1978; McDowell, 1979, Raouf et al., 1980) and is stated to offer improved discrimation of various thyroid states in comparison to the FTI, specifically in cases where TBG levels are abnormal. Thus Burr et al., (1977) reported that the T^4:TBG ratio was able to differentiate between euthyroid subjects with normal TBG levels and those with thyrotoxicosis and myxoedema, at least as well as the FTI. However, in euthyroid patients with elevated TBG concentrations the T^4:TBG ratio gave values in the euthyroid range, whereas the FTI gave "thyrotoxic" results. The T^4:TBG ratio could not be applied in patients with absent TBG, but undetectable levels of TBG aided the interpretation of the low T^4 concentration.

McDowell, (1979) found a close correlation between T^4; T^3U ratio and T^4:TBG ratio and this has also been reported by Lecureuil et al., (1978). McDowell stated that the concomitant finding that the T^4:TBG ratio gave values within the euthyroid range for the ill, contraceptive and pregnant groups (that he studied), whereas the FTI results were outside this range, indicated the value of the TBG assay.

Attwood et al., (1978) concluded that the T^4:TBG ratio was of considerable help in interpreting serum T^4 values associated with abnormal TBG levels. They also found that the T^4:TBG ratio was also useful in differentiating the thyrotoxic from the normal subject; although in hypothyroidism, only marginal improvement in the differentiation from the euthyroid range, was found.

Recently,several reports have appeared that do not support the above studies and have shown the T^4:TBG ratio to be a poorer discriminator than the FTI (Roosdorp and Joustra, 1979; Fyffe et al., 1980; Rootwelt and Solberg, 1981). In a numerical comparison of the T^3U and T^4:TBG, Roosdorp and Joustra found, with respect to diagnostic potential, the T^4:TBG ratio to offer no advantage over the FTI, provided the index was based on T^3U measurements performed using a technique capable of high precision and accuracy. They concluded that the T^4-T^3U mapping technique (Mardell, 1978) should be used in place of the FTI and T^4:TBG, in all thyrodiagnostic procedures where more than a simple screening test was required.

Fyffe et al.,(1980) reported four methods of assessing the free T^4 status in patients with suspected thyroid dysfunction. They found that both the FTI and T^4:TBG ratio offered similar diagnostic efficiency, when amended reference ranges for euthyroid patients were employed.

Correlation between free T^4 levels (determined by two techniques) and both the above indices, was marginally improved for the FTI, compared to the T^4:TBG ratio.

Rootwelt and Solberg, (1981) in a recent study of several in vitro thyroid function tests by discriminant analysis, found that if a single parameter was used, then FTI was marginally more efficient than free T^4, which was better than the T^4:TBG ratio. Therefore, it was concluded that their study did not support reports in the literature showing the T^4:TBG ratio to be superior to the FTI.

Little work has been published relating to the value of TBPA in the assessment of thyroid function and this has probably been due to the fact that TBG is the major transport protein of thyroid hormones; TBG carries approximately 70-75% of the total circulating hormone, compared to a figure of 20-30% for TBPA (Gordon et al., 1952; Ingbar, 1958). Studies have also shown that TBG exhibits a higher affinity for both T^4 and T^3 than TBPA (Snyder et al., 1976; Koreek and Tabachnik, 1976; Cheng et al., 1977). However Robbins, (1975) concluded, after a study of transport protein function, that it was difficult to assert that one binding protein was more important than another. In fact he calculated, using the results of Hillier, (1971) on the dissociation rates of T^4 from TBG and TBPA, that both proteins contribute equally to the turnover of the metabolically active free T^4 pool mainly because that bound to TBPA is very labile. Robbins, (1973) has also shown that TBG is not essential for thyroid hormone action, through the study of euthyroid individuals with genetically absent TBG levels.

Current views suggest that thyroid binding proteins act as buffers in the circulation and modulate the transfer of thyroid hormones to

intracellular sites and prevent their excretion in the urine. TBPA probably represents a more readily accessible source of T^4 than TBG, due to its greater concentration and its lower binding affinity. An increase in free hormone occurs during acute illness due to the rapid diminution in binding capacity of TBPA (Helenius and Liewendahl, 1979). This phenomenon, which has also been observed by Bernstein and Oppenheimer, (1966), demonstrates the importance of TBPA in the contribution of free T^4 to the tissues. TBG is also obviously involved but to a lesser degree; Helenius and Liewendahl found decreased TBG capacity in serious non-thyroid illness, together with a reduced concentration. They concluded that due to the higher affinity of T^4 for TBG than TBPA, it was obvious that the decrease in TBG also contributed to the increased free T^4 fraction.

The above data therefore suggests that current tissue requirements of thyroid hormone are provided by release from TBPA with TBG representing a more stable store of hormone.

The assay of TBPA concentration in serum for the assessment of thyroid disease has received little attention as previously described and this study aims to determine the diagnostic value of TBPA, both as a single parameter assay and as a T^4 : TBPA ratio. The ability of TBPA to correct the serum T^4 for alterations in binding protein capacity, will therefore be studied and compared with the conventional T^3U and the more recently developed T^4:TBG ratio. Several groups of patients will be included in the survey, together with a randomly selected sample from the psychiatric population. The overall performance, represented by the ability of the above assays to aid in the assessment of thyroid dysfunction will be critically examined with special reference to the selection of the "ideal" binding protein analysis.

4:2 Selection of Subjects for Study

Subjects chosen for inclusion in the study were classified according to clinical diagnosis, sex and therapy as follows :

4:2:1 Euthyroid Group

200 "apparently euthyroid" individuals (100 male, 100 female) were selected. This group comprised patients whose blood samples had been received by the Biochemistry Department at Good Hope Hospital for routine thyroid function tests. Subjects with values falling outside the quoted normal range for the thyroid function parameters (in parentheses) namely, serum T^4 (58 - 148 nmol/l), serum T^3U (92 - 117%) and the derived index FTI (58 - 148), were automatically excluded, as were females who were either pregnant or on oral contraceptives. Borderline hypo and hyperthyroids, ie. patients with values between two and three standard deviations from the mean were further analysed by measurement of triiodothyronine (T^3) and thyroid stimulating hormone (TSH) assays respectively. This procedure has been recommended by Britton et al., (1975) who found that it considerably improved diagnostic efficiency, by reducing clinical uncertainty in borderline cases, to less than 2%. Patients with subsequent abnormal T^3 or TSH values were also excluded from this sample.

4:2:2 Hyperthyroid Group

This group consisted of 71 clinically and biochemically hyperthyroid patients (13 male, 58 female). These subjects were chosen at random, from both the in-patient and out-patient population at Good Hope Hospital. All patients had serum T^4 levels greater than 175 nmol/l and serum T^3 levels of greater than 4 nmol/l.

4:2:3 Hypothyroid Group

This group consisted of 44 clinically and biochemically hypothyroid

patients (6 male, 38 female). These subjects were chosen as described for the hyperthyroid group and all had serum T^4 levels below 30 nmol/l and serum TSH values of greater than 20 μIu/ml.

4:2:4 Pregnant Group

50 females were chosen who were between the second and third trimesters of pregnancy.

4:2:5 Contraceptive Pill users

18 females were chosen who were clinically and biochemically euthyroid and taking one of the oestrogen - containing oral contraceptives.

4:2:6 Psychiatric Group

A random sample of 378 acute admissions to Highcroft Hospital, Erdington, Birmingham (172 male, 206 female) were also chosen for study. This group consisted primarily of euthyroid patients, although a few individuals with abnormal function may also have been included.

4:3 Methods

In all the groups serum T^4, T^3 and TSH were assayed using the Corning Medical (Immunophase) radioimmunoassay test system and serum T^3U measured using the Radiochemical Centre (Thyopac - 3) protein binding assay. Both TBG and TBPA were assayed by the electroimmunoassay developed originally by Laurell (1966). Comprehensive details of the assay of serum T^4, T^3, T^3U and TSH have been previously described together with details of the quality control procedures employed.

The development of the TBG and TBPA assays is given below:

4:3:1 The Electroimmunodiffusion technique for the assay of TBG

This technique represents a simple, quick and reproducible method for the determination of a single protein in a protein mixture in a number of samples. The method is based upon the electrophoretic migration

of antigens in antibody containing gel and a specific immunoprecipitation of the antigens by means of the corresponding precipitating antibodies. Individual precipitates are formed for each antigen/antibody system present. The area enclosed by these precipitates is proportional to the antigen/antibody ratio. If suitable conditions for the electrophoresis are chosen, the major part of the antibody molecules will not move in the gel, while antigen molecules with electrophoretic migration different from that of the antibody molecules will move in the gel during the electrophoresis. At the beginning of the electrophoresis the antigen molecules migrate from the application in the antibody-containing gel. The number of antigen molecules exceeds the number of antibody molecules and small soluble immunocomplexes are formed. These continue migrating at a slower rate in the gel. As electrophoresis continues, more complexes are formed and finally fuse to form a precipitate. From this moment the position of the precipitate does not change, even if the electrophoresis is continued. The amount of antibody molecules which have combined with the antigen molecules are termed the 'equivalent amount'.

The area enclosed by the precipitate will depend on the concentration of the antigen and antibody in the system. This allows a method for quantitation of the protein under analysis. In the Laurell rocket technique accurate volumes of standards and test sera are applied to wells cut in an agarose gel containing the appropriate antibody in low concentration (usually approximately 1%).

Electrophoresis is performed, migrating the samples through the field of antibody. As antigens in the sample migrate, antigen/antibody complexes form and aggregate into visible, narrow stationary

precipitates when the equivalence ratio is reached. This precipitation occurs first as sidelines enclosing the antigen path. The successive consumption of antigen on sideline formation results in convergence of the precipitation lines to a peak. The height of this peak is proportional to the concentration of the antigen applied (Laurell, 1972).

4:3:1:1 Equipment

The following is a summary of the equipment which was used for the TBG assay.

4:3:1:2 Electrophoresis Apparatus

The power supply used was the Shandon 500 m. amps/500 volts power pack capable of supplying up to four electrophoresis tanks simultaneously. This pack has the facility to supply either constant current or constant voltage (the latter being used for the rocket technique), and also incorporating a switch to allow the user to change the polarity, enabling the same buffer to be used several times. A voltmeter with a pair of electrodes is to check the voltage in the gel, was also essential, as this needed to be set accurately for each electrophoretic run. The buffer tank used was a Chem Lab. Immuno-Electrophoresis Tank 269, capable of holding a total of six litres of buffer, thus giving sufficient buffer capacity essential for this type of electrophoresis. Up to three, 150 x 80 x 1 mm glass support plates could be run simultaneously enabling a high throughput of samples. The electrophoretic wicks were pure surgical lint cut to the exact width of the gel and approximately 10 cm in length.

A gel puncher, and template supplied by Hoechst were used for cutting wells in the agarose gel. Wells were cut to a diameter of 2 or 3 mm capable of holding up to 5 µl of sample in a 1.0 mm thick gel.

The glass plates were manufactured by Chance Proper and measured

150 x 80 x 1 mm. Other equipment consisted of a horizontal table for gel pouring; water bath at 56°C and 100°C for melting the agarose; staining dishes; hot air drier; various measuring pipettes were also used.

4:3:1:3 Reagents

(1) Buffer

Both the tank buffer and that used for the preparation of the agarose gel were prepared from the following stem solutions:-

Sodium Barbitone	($C_8H_{11}O_3N_2Na$)	206 g
Barbituric Acid	($C_8H_{12}O_3N_3$)	40 g
Sodium Azide	(NaN_3)	10 g

dissolved in distilled water, to a total volume of 10 litres, pH 8.6, ionic strength 0.1, specific conductivity = 7 mS (23°C). This solution can be used after many months storage at 4°C or several weeks at room temperature (20 - 26°C).

For use in both the tank and gel the above was diluted 1 in 2 to prepare a 0.05 ionic strength buffer.

(2) Agarose - Type 1

This was obtained from 'Sigma' Chemical Company. Ten g. agarose was added to 1000 ml of buffer. The solution was then brought to the boil to clarify it and was then divided into aliquots corresponding to the amount to be used for each plate. These were then stored at 4°C.

(3) TBG Antibody

This was obtained from Dr. A.R. Bradwell, Department of Medicine, University of Birmingham.

(4) Staining Solution

Coomassie Brilliant Blue R - 250 was used for staining the

precipitates and was prepared as follows:-

Coomassie Brilliant Blue	2.0 g
Distilled Water	600 ml
Methanol	400 ml
Glacial Acetic Acid	100 ml

(5) Destaining Solution

Prepared as above without addition of the Coomassie Brilliant Blue.

4:3:1:4 Procedure

The following is a description of the general procedure adopted, although certain modifications were made and these will be discussed later in this section.

The antibody containing gel was prepared by reheating to boiling point an aliquot of the pre-prepared agarose. This was then placed in a 56^{o}C water bath for several minutes to allow equilibration of the agarose. Initially a 1% concentration of antibody in the gel was prepared and the correct volume of anti-TBG to give this concentration, was added to a 25 ml glass universal container. This was also allowed to equilibrate to 56^{o}C. Meanwhile a 150 x 80 x 1 mm clean glass plate was placed in a 70^{o}C drying oven, as preheating the plate considerably aids the even distribution of the gel. The levelling table was also set up and two glass rods placed on it to support and insulate the plate from excessive cooling by the table. At this stage the preheated glass plate was placed on the levelling table and the agarose and antiserum mixed by pouring the contents from one tube to the other, several times. A measuring pipette preheated to between 50 and 60^{o}C was then used to transfer a set volume of the agarose/antiserum mixture to the glass plate. The mixture was poured onto the centre of the plate and

allowed to run out to the periphery so forming an even gel layer. Any bubbles were removed with a hot wire or by passing a bunsen flame across the surface of the plate. The agarose was then left to set for 5 - 10 minutes. After congealing of the agarose the wells were punched out 2 cm from the 150 cm end of the plate, using the gel puncher and template. The glass plate was then placed on the electrophoretic apparatus with the gel face down on the cotton wicks. The plate was orientated such that the row of wells were nearest to the cathodic side of the tank. The current was then switched on and the voltage across the tank adjusted to the desired level. (This was monitored using the voltmeter.)

The appropriate standards and tests were then applied to the wells, using a microlitre syringe,with the current on (to avoid diffusion rings around the applications). The lid was then placed on the tank and electrophoresis was carried out for 16 hours (overnight). After this time the current was switched off and the plate removed from the tank. The gel was then pressed according to the technique of Laurell:-

The gel was covered with a layer of wet filter paper after filling the wells with buffer. The trapping of air bubbles was avoided and several layers of soft blotting paper were then placed on the filter paper. A slight pressure was then sustained by means of inverting the levelling table and placing it over the layers of paper. After 10 - 15 minutes the pressure and blotting paper were removed, care being taken not to peel the thin layer of gel from the glass plate. The plate was then either washed in 0.1 M sodium chloride followed by distilled water for several minutes (to facilitate removal of non-precipitated proteins), or immediately dried using a hot air drier until glossy

and clear.

The plate was then stained for about 10 minutes and destained at least three times for periods of about 10 minutes. It was then finally dried in hot air.

Measurement of peak height was then carried out using millimeter paper, with the gel side of the plate facing the paper (to avoid the error of parallax). This enabled the height to be measured to approximately 0.3 mm. If this technique gave a non-linear standard curve, a more exact quantitation would be carried out; this giving an approximation of the area enclosed by the peak. The peak height and width at half peak height, were measured and multiplied together. The results were then plotted on graph paper, i.e. peak height x width versus TBG concentration. Interpolation of the test peak height x width ratios from the standard curve enabled the TBG concentration in each test sera to be calculated.

4:3:1:5 Standardisation of the TBG Assay

No pure TBG standard was available in the U.K. when the assay was initially carried out and therefore the following approach was adopted:-

It was decided to prepare a 'pool' of human sera obtained from pregnant patients at Good Hope Maternity Hospital, Sutton Coldfield, 0.1% of sodium azide was added as a preservative and the sera was aliquoted and deep frozen at -20^{o}C. A sample of the 'pool' was sent to the Department of Medicine, University of Birmingham, for standardisation against a sample of pure TBG, work initially carried out by Drs. J. Robbins and M. Gershengorm, (Arthritis, Metabolism and Digestive Diseases Unit, N.I.H., Bethesda, Md., U.S.A.).

This serum pool was assayed several times and was found to have a value of 34.0mg/l and this material was used for the preparation of a standard curve as follows:-

Standard Concentration (mg/l)	6.8	13.6	20.4	27.2	34.0
Volume of Buffer (0.05 i)	0.4	0.3	0.2	0.1	0.0
Volume of Standard (serum pool)	0.1	0.2	0.3	0.4	0.5

(N.B. All volumes are quoted in millilitres)

Quantity control of the assay was performed using a separate serum pool, which was deep frozen in 1 ml aliquots. This material was included in each assay run and provided a precision check on the technique.

Several assay runs were necessary before well defined rockets, with heights varying between 10 and 50 mm (the reproducibility of the method is best with peak heights between these limits) were obtained. Various modifications to the originally described procedure were necessary to achieve the above results; the final "optimal" assay conditions chosen, were as follows:-

Agarose gel concentration : 1.0%

Antiserum concentration in the gel : 1.25%

Gel thickness : 1.0 mm

Antigen well size : 2.5 mm

Voltage applied across the gel : 25 - 30 volts (constant current)

Electrophoresis time : 16 hours

Staining time : 10 - 15 minutes

No washing procedure was employed for the removal of background staining, as this had been reduced by the pouring of thin gels.

4:3:1:6 Statistical Evaluation of the Assay

The precision of the assay was determined by assaying one hundred samples, at varying concentrations of TBG, in duplicate. From this a within batch $\bar{x}$ of 18.16 mg/l with an SD of 0.52 and a CV of 2.86% was obtained; this data comparing well with that quoted by Bradwell et al.,(1976) who obtained a CV of 2.9% for the assay "under routine conditions"

The normal values for this assay and their derivation will be described later in this chapter.

4:3:2 Thyroxine Binding Prealbumin

The "Laurell rocket technique" , as previously described,was the method chosen for the quantative determination of TBPA. This technique has already been explained in detail and thus only an outline of the method, together with any modifications, will be given here. With reference to the TBG assay, it was necessary to make several complete runs, before acceptable results were obtained. Experience gained from the latter technique proved invaluable for the development of the TBPA method.

4:3:2:1 Technique

Rocket immunoelectrophoresis was carried out using a 1% agarose gel with a sodium barbitone/barbituric acid buffer, pH 8.6. ionic strength 0.1 and a specific conductivity of 8 mS ($23^{o}C$). The buffer was diluted 1 in 2 to give an ionic strength of 0.05 for both the gel and electrophoretic tank. Sample wells of 2.5 mm diameter were cut in a 1 mm thick agarose gel containing 2.0% of the TBPA antiserum (0.24 mls of TBPA antiserum were added to 12.0 mls of agarose). Samples, standards and controls were pre-diluted in buffer, 1 in 5, and applied,

in 3ul aliquots, to each of the appropriate wells. Electrophoresis was allowed to proceed for 16 hours at 30 volts, without cooling. The gel was then press dried and stained with Coomassie Brilliant Blue R-250 and the peak heights measured against the standards, using a calibration curve. Samples falling outside the range of the standard curve were either further diluted in buffer and re-assayed, or a lower dilution employed.

4;3;2;2 Standardisation of the TBPA Assay

The method utilised a serum protein standard, "Protein Standard Serum B" and also TBPA specific antiserum. This material was manufactured by Behringwerke AG, (West Germany), and is available in the U.K. from Hoechst Pharmaceuticals, Hownslow, Middlesex.

Three standards were supplied by Behringwerke and this material was obtained from a serum pool of healthy adults. All these solutions were ready to use and typical concentration values for TBPA were 11.0, 21.5 and 42.0 mg/dl.

The TBPA specific antiserum was obtained from rabbits by immunisation with highly purified human plasma TBPA, isolated from the blood of healthy donors and subsequently stabilised. The antiserum was also supplied ready for use.

Quantity control of the assay was performed using stabilised human serum, also manufactured by Behringwerke (Standard-Human-Serum, Stabil). This serum had a quoted value for TBPA concentration and was included on every electrophoretic plate analysed.

4:3:2:3 Statistical Evaluation of the Assay

Seward Laboratories, Blackfriars Road, London, manufacture a reference serum, obtained from a serum pool of healthy adult donors.

This was employed, for the determination of precision and accuracy of the method. A sample of this reference serum was assay on 100 batches of tests and gave the following results:-

$\bar{x}$ = 34.00 (Actual Value - 33.9 mg/dl)

SD = 1.94

CV = 5.7%

The above results represent a between-batch variation.

4:4 Results

4:4:1 TBG and TBPA Concentrations

The distribution of both the TBG and TBPA levels in the euthyroid group, for males and females, is shown in figures 3 and 4. The mean concentration ($\pm$ 1 standard deviation {SD} of the mean {$\bar{x}$}) for the TBG in euthyroid males was 12.11 $\pm$ 2.55 mg/l and in euthyroid females 13.24 $\pm$ 2.31 mg/l. Corresponding results for TBPA in males were 25.40 $\pm$ 7.55 mg/dl for females and 23.01 $\pm$ 5.7 mg/dl.

An interesting phenomenon of these results was the reciprocal relationship that occurred between the male and female mean value for both TBG and TBPA; i.e. with reference to TBG, the observed female mean concentration was of higher value than that for males; the reverse occuring for TBPA. A possible explanation of this observation will be discussed later.

For clinical use, the spread of results for both males and females in the TBG and TBPA assays was such that in practical terms the sex difference could be ignored. A composite normal range, calculated as the mean $\pm$ 2 SD, was therefore 7.01 - 17.86 mg/l for TBG and 10.3 - 40.5 mg/dl for TBPA.

Women who were pregnant had a mean TBG level of 31.9 $\pm$ 4.2 mg/l, which was significantly different from the euthyroid mean. The mean value for TBPA in this group was 22.5 $\pm$ 2.9 mg/dl, this parameter showing no significant difference from the euthyroid mean.

The group of women who were receiving oral contraceptive therapy had a mean TBG level of 14.35 $\pm$ 2.18 mg/l and for TBPA 23.92 $\pm$ 3.72 mg/dl. Both these protein values were similar to those found for the euthyroid population and the considerable elevation of the TBG concentration that occurred in the pregnant group was not observed.

Figure 3

Distribution of Serum TBG Levels in the Male and Female Euthyroid Group

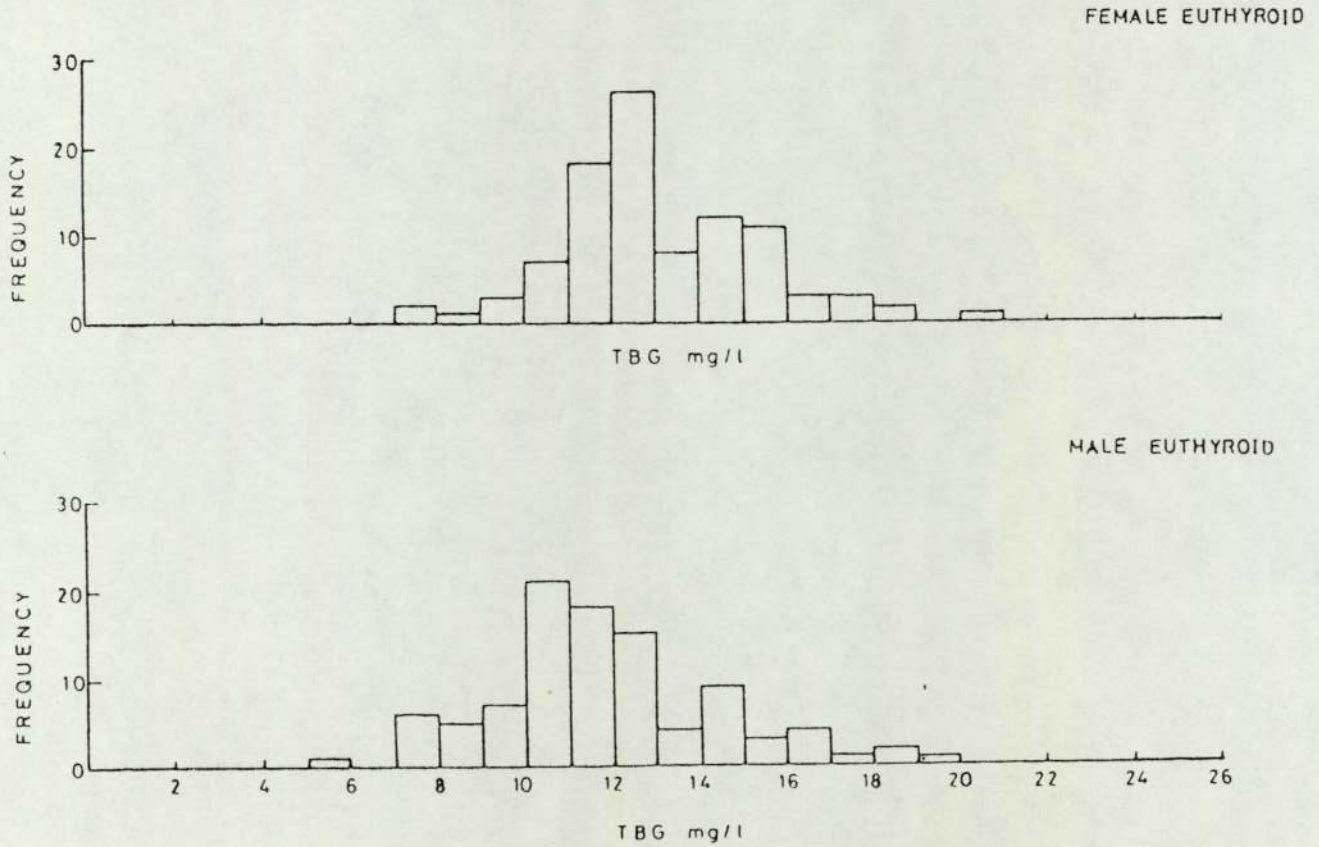
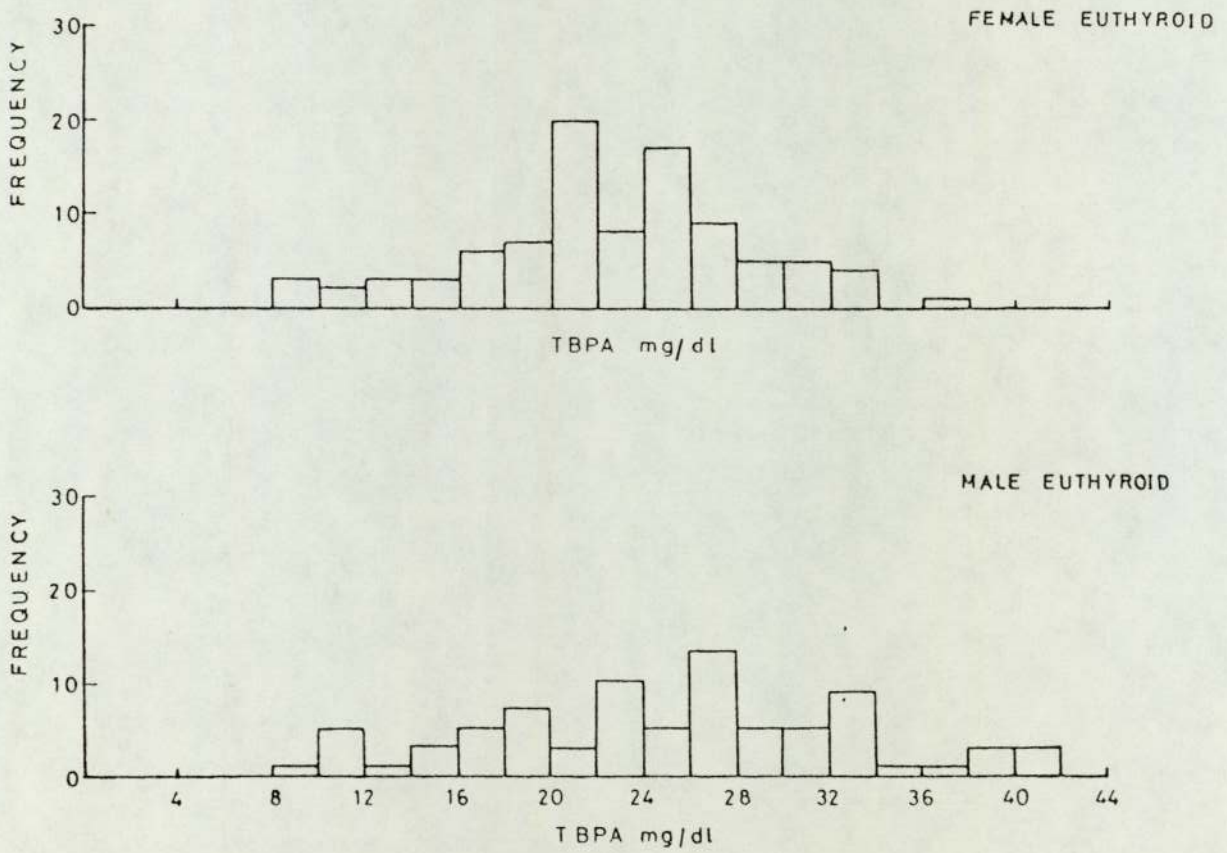


Figure 4

Distribution of Serum TBPA Levels in the Male and Female Euthyroid Group



The psychiatric group had a mean TBG concentration of 14.40 $\pm$ 5.75 mg/l and the values for TBPA were 25.07 $\pm$ 6.98 mg/dl. Both these parameters gave results which showed no significant difference from the mean values observed for the euthyroid population.

The accuracy and precision of both TBG and TBPA assays has already been reported in the previous chapter, and compares favourably with figures obtained by other workers (Attwood and Probert, 1978; Ingenbleek et al., 1972)

Figures 5 and 6 compare values for TBG and TBPA, in both hypo-and hyperthyroidism, with those in the euthyroid group. The $\bar{x}$ concentrations of TBG and TBPA (in parentheses), for the hypo-and hyperthyroid groups were 15.82 $\pm$ 3.09 mg/l (26.39 $\pm$ 5.52 mg/dl) and 13.35 $\pm$ 4.77 mg/l. (18.12 $\pm$ 5.43 mg/dl) respectively. Slightly improved discrimination was observed between the hypo-and hyperthyroid groups, when TBPA was measured, compared to a poorer discrimination with TBG. However the figures show that there is considerable overlap with the normal range in both cases. although the mean values are distinct, particularly with reference to the TBPA assay.

The mean and 1 standard deviation (SD) values with a 2 SD range, for serum TBG and TBPA (including also data on serum T^4 and T^3U) in all groups studied,are summarised in Table 2 . The six groups with the exception of the psychiatric group, can be seen to be statistically different on the basis of the serum T^4 and T^3U values, this being less marked for both TBG and TBPA.

4:4:2 Comparison of T^3U with TBG and TBPA

The TBG and TBPA levels in each of the six groups were correlated with the T^3U and T^4 data, by evaluating the constants of the linear

Figure 5

Distribution of Male/Female Serum TBG and TBPA Levels in Hypo- and Hyperthyroidism compared with The Euthyroid Range

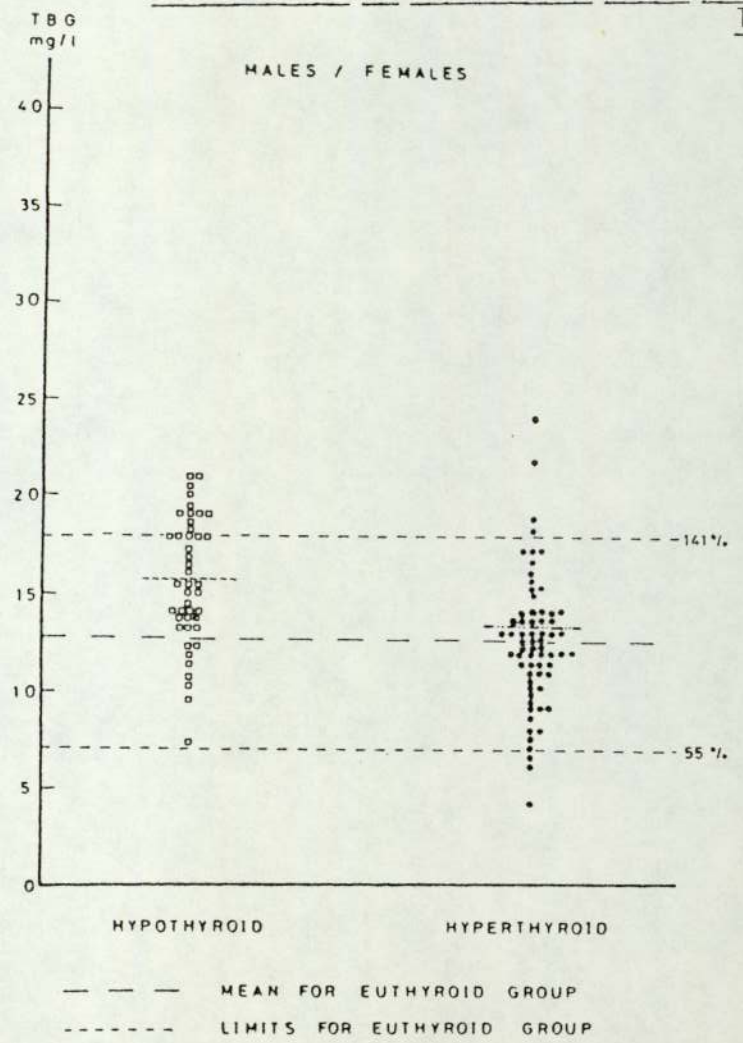


Figure 6

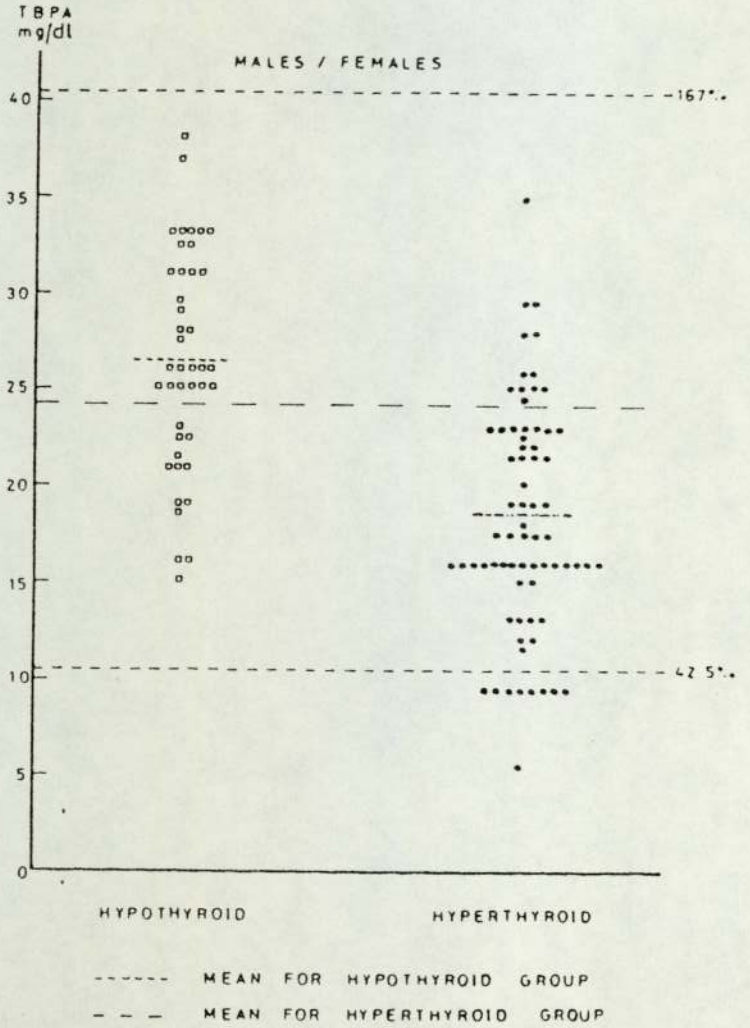


TABLE 2

Mean and one standard deviation values with two S.D. range for Serum T^4 and T^3 Uptake, TBG and TBPA in the groups studied

Assay		Euthyroid Male	Euthyroid Female	Hypothyroid	Hyperthyroid	Oral Contraceptive	Pregnant	Psychiatric
T^4 (nmol/l)	Mean	97.46	101.82	19.22	227.21	106.0	152.3	107.54
	S.D.	20.65	21.66	13.58	47.58	16.20	22.3	25.06
	Range	56.16-138.76	58.5-145.14	0-46.38	132.05-322.37	73.6-138.4	107.7-169.9	57.42-157.66
T^3U (%)	Mean	104.83	107.32	124.77	91.01	111.60	137.4	107.78
	S.D.	7.15	7.16	10.74	14.15	7.20	5.7	8.20
	Range	90.53-119.13	93-121.64	103.29-146.25	62.71-119.31	97.2-126.0	126.0-148.8	91.38-124.18
TBG (mg/l)	Mean	12.11	13.24	15.82	13.35	14.35	31.9	14.11
	S.D.	2.25	2.31	3.09	4.77	2.18	4.2	2.80
	Range	7.01-17.21	8.62-17.86	9.64-22.0	3.81-22.89	9.99-18.71	23.5-40.3	8.51-19.71
TBPA (mg/dl)	Mean	25.40	23.01	26.39	18.12	23.92	22.5	25.07
	S.D.	7.55	5.70	5.52	5.43	3.72	2.9	6.98
	Range	10.3-40.5	11.61-34.41	15.35-37.43	7.26-28.98	16.48-31.36	16.7-28.3	11.11.39.03

regression equation : $y = mx + c$ (where x = TBG or TBPA). Table 3 shows the correlation coefficient with its statistical significance, for each group studied. It can be seen that in each group, TBG levels are well correlated with the T^3U, reflecting the physiological basis of the T^3U test.

Figure 7 demonstrates the relationship between the T^3U and TBG in the euthyroid and pregnant group. For values of TBG less than 20 mg/l, a good correlation was noted ($r = 0.91$), however for TBG values above this figure, the relationship becomes non-linear and a plateau is reached at T^3U concentrations of between 135 and 140%. This phenomenon has been previously observed by Burr et al.,(1977) and was reported to be due to an inability of the T^3U to measure high TBG concentrations.

The relationship of T^3U and TBG for the hypo-and hyperthyroid population is shown in Figure 8 . As illustrated by Attwood and Probert, (1978), a greater displacement from the euthyroid correlation appears to exist for the hyperthyroid group, than is evident for the hypothyroid group. The correlation coefficient between T^3U and TBG for the hyperthyroid population gave a value of 0.82, this figure representing the best correlation found in any group (Table 3).

Figure 9 illustrates the relationship between T^3U and TBPA in the euthyroid and pregnant group. The latter population shows complete positive displacement from the euthyroid relationship and this reflects the failure of the TBPA concentration to increase during pregnancy, as previously stated. Values for T^3U are increased however, as demonstrated by the graph.

The correlation between T^3U and TBPA for the hypo-and hyperthyroid population is shown in Figure 10. Significant correlation occurred only

Figure 7

Relationship Between Serum T^3U and TBG in the Euthyroid and Pregnant Groups

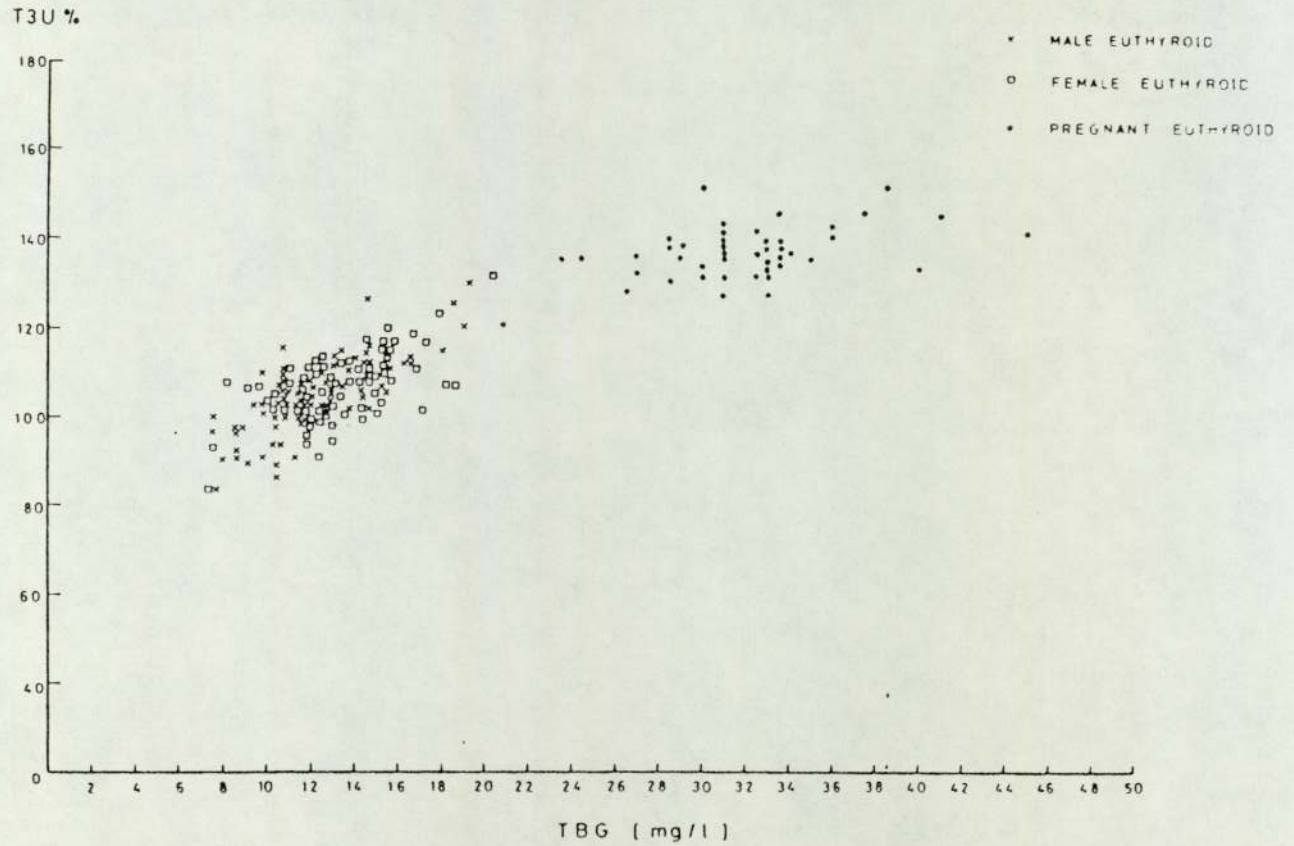


Figure 8

Relationship Between Serum T^3U and TBG in the Hypo- and Hyperthyroid Population
(Regression Line for Euthyroid Group shown as -)

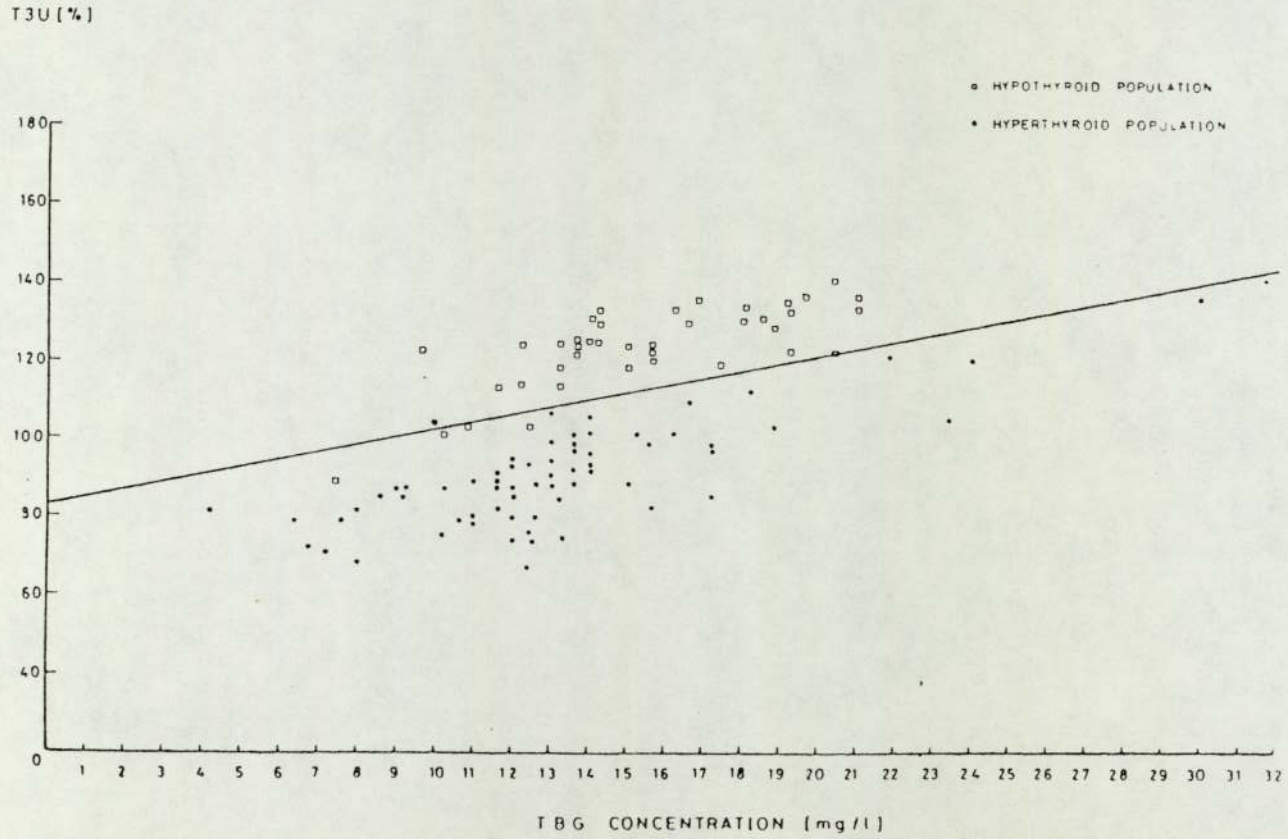


TABLE 3

Regression between serum TBG and TBPA against serum T^3U and T^4 for all six groups

(figures shown are coefficient of correlation x = TBG/TBPA

	GROUP	EUTHYROID	HYPOTHYROID	HYPERTHYROID	ORAL CONTRACEPTIVE	PREGNANT	PSYCHIATRIC
1.	TBG : T^3U	0.69 (0.001)	0.69(0.001)	0.82(0.001)	0.76(0.001)	0.52(0.001)	0.75(0.001)
2.	TBPA : T^3U	0.13 (NS)	0.34(0.05)	0.47(0.001)	0.35 (NS)	0.08 (NS)	0.11 (NS)
3.	T^3U : T^4	0.14 (NS)	0.19 (NS)	0.23(0.05)	0.12 (NS)	0.20 (NS)	0.16 (NS)
4.	TBG : T^4	0.38(0.001)	0.06 (NS)	0.17 (NS)	0.11 (NS)	0.56(0.001)	0.44(0.001)
5.	TBPA : T^4	0.035 (NS)	0.11 (NS)	0.09 (NS)	0.12 (NS)	0.03 (NS)	0.04 (NS)

(Statistical significance for each group is shown in parentheses)

Figure 9

Relationship Between Serum T^3U and TBPA in the Euthyroid and Pregnant Groups.

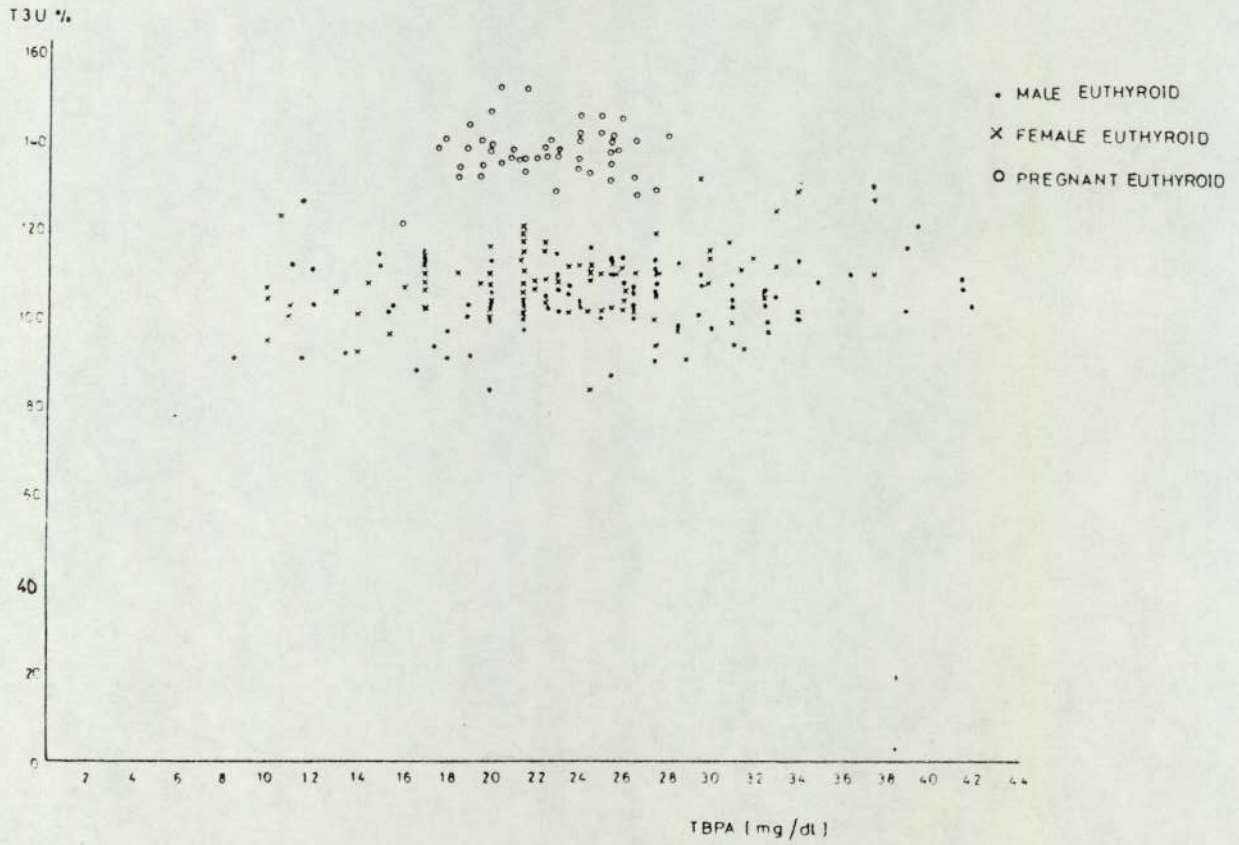
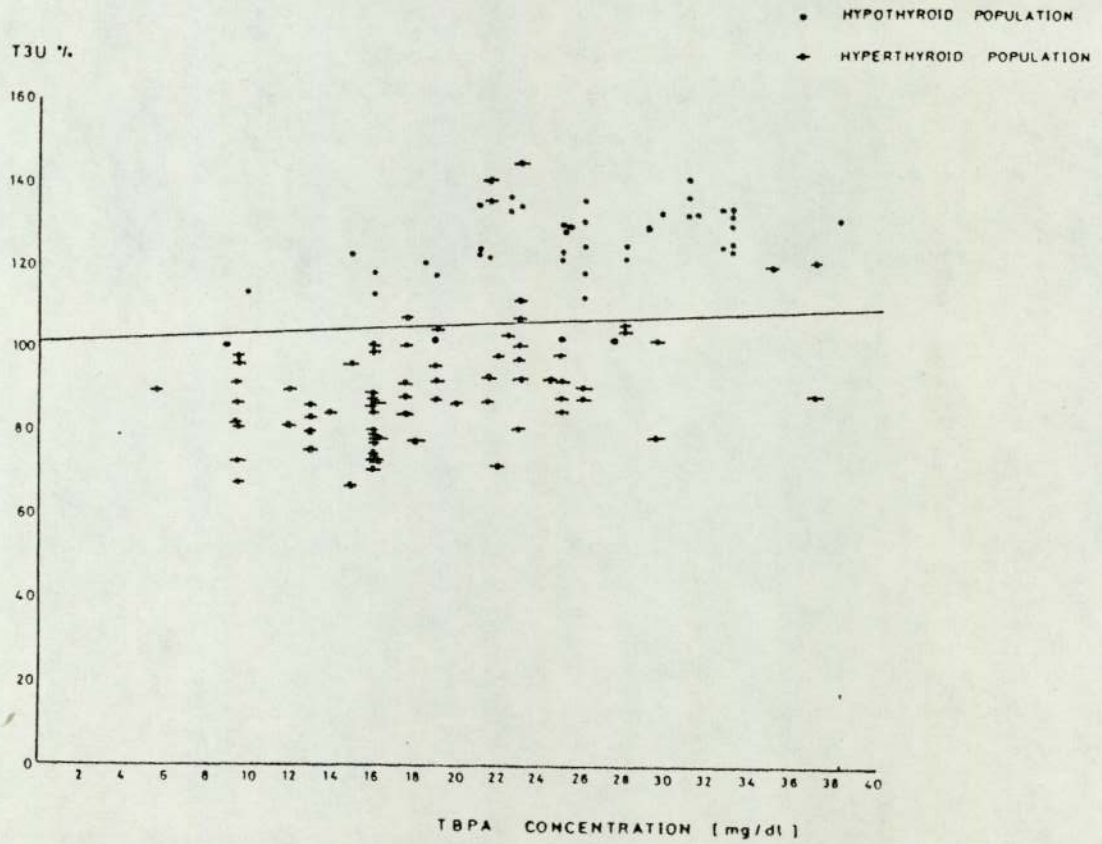


Figure 10

Relationship Between Serum T^3U and TBPA in the Hypo- and Hyperthyroid Population
(Regression Line for Euthyroid Group Shown as -)



in these two groups (Table 3); however the discriminating power for the separation of myxoedema from thyrotoxicosis appeared less efficient than that shown for TBG versus T^3U.

4:4:3 Comparison of T^3U, TBG and TBPA with serum T^4.

The thyroid mapping technique, as described by Mardell, (1978) was used to correlate T^3U, TBG and TBPA with serum T^4.

Figure 11 shows the relationship between T^3U and T^4 in the euthyroid, hypo-, hyperthyroid and pregnant population. The discriminatory power of serum T^3U and T^4 to distinguish between these groups, when the results are plotted graphically, is shown and a good separation was obtained. Significant correlation between T^3U and T^4 only occurred in the hyperthyroid group (Table 3); this finding probably reflecting the high degree of correlation found between TBG and T^3U.

The relationship between TBG and T^4 in four out of the six groups studied, is shown in Figure 12. When plotted graphically, these two parameters appear to distinguish between euthyroid, hypo-, hyperthyroid and pregnant groups at least as well as T^3U versus T^4. Significant linear correlation between TBG and T^4 occurred in both the euthyroid and pregnant groups. This finding is probably due to the parallel increase in T^4 levels with increasing TBG values; a phenomenon only observed in the latter two groups.

Figure 13 shows the relationship between TBPA and T^4 in several groups. The hyperthyroid group are poorly differentiated from the pregnant group, reflecting the absence of rise in TBPA concentration during pregnancy, in contrast to that observed for TBG. However, the hypo- and hyperthyroid groups are clearly differentiated from the euthyroid population, although no significant correlation occurred in any group (Table 3).

Figure 11

Relationship Between Serum T^3U and T^4 in the Euthyroid,Hypo-,Hyperthyroid and Pregnant Population

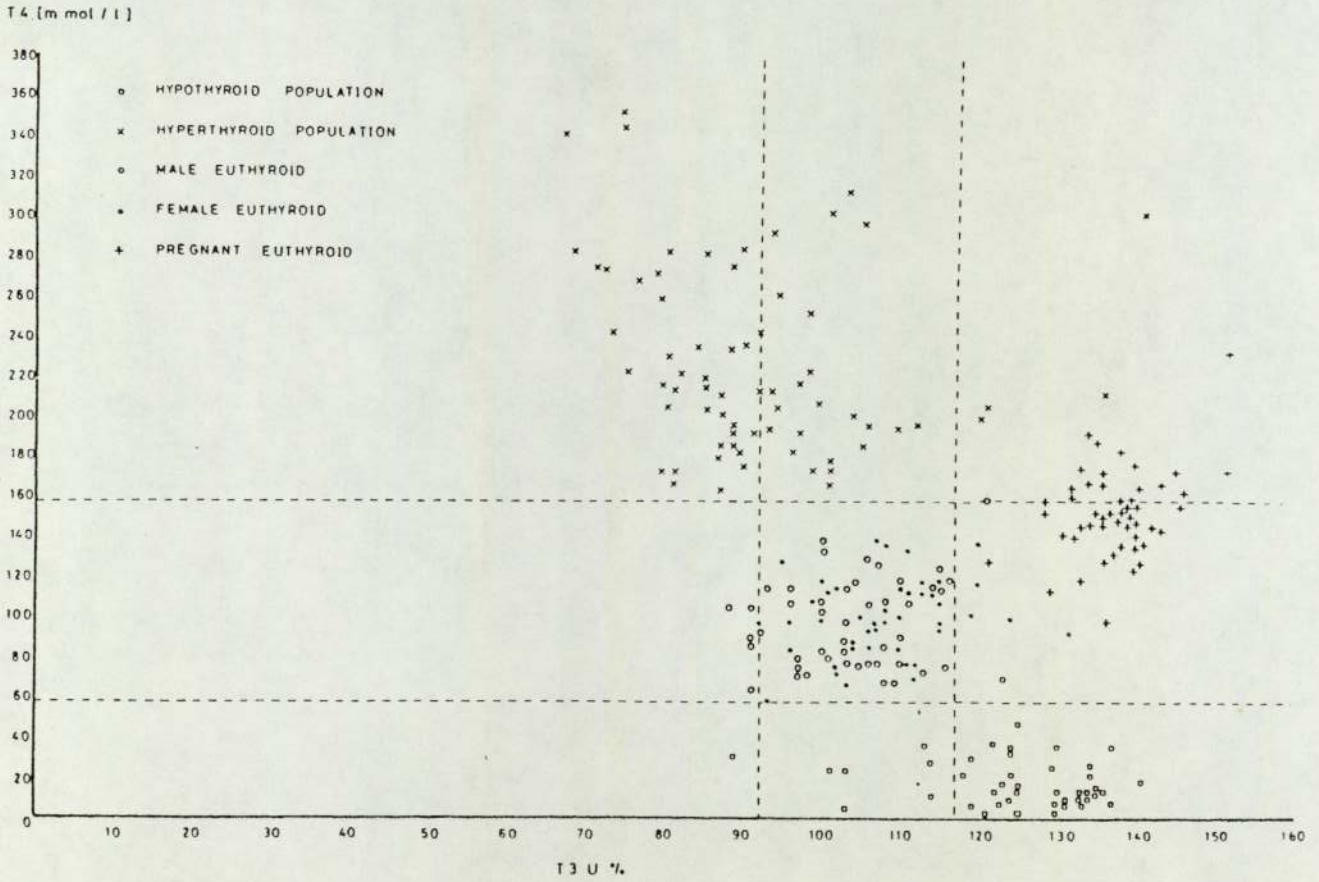


Figure 12

Relationship Between Serum TBG and T^4 in the Euthyroid, Hypo-, Hyperthyroid and Pregnant Population

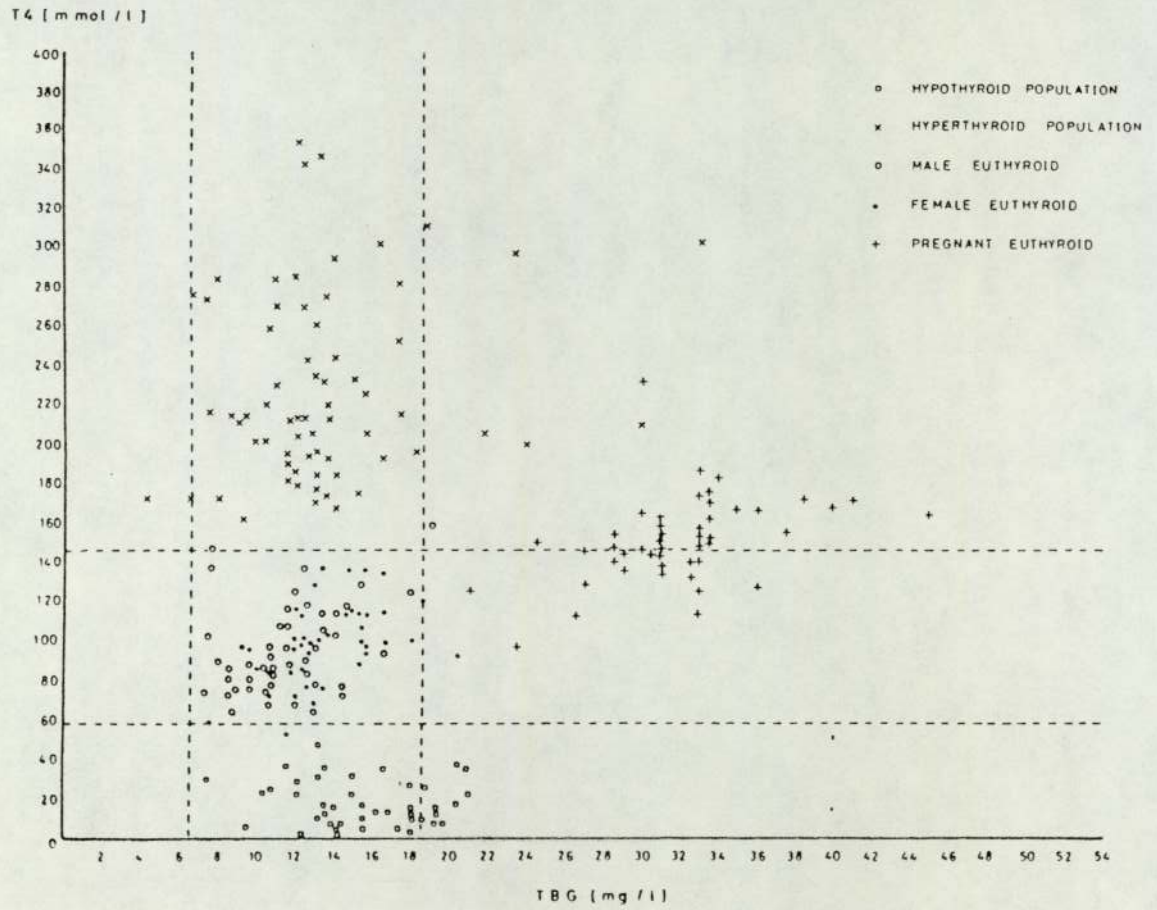
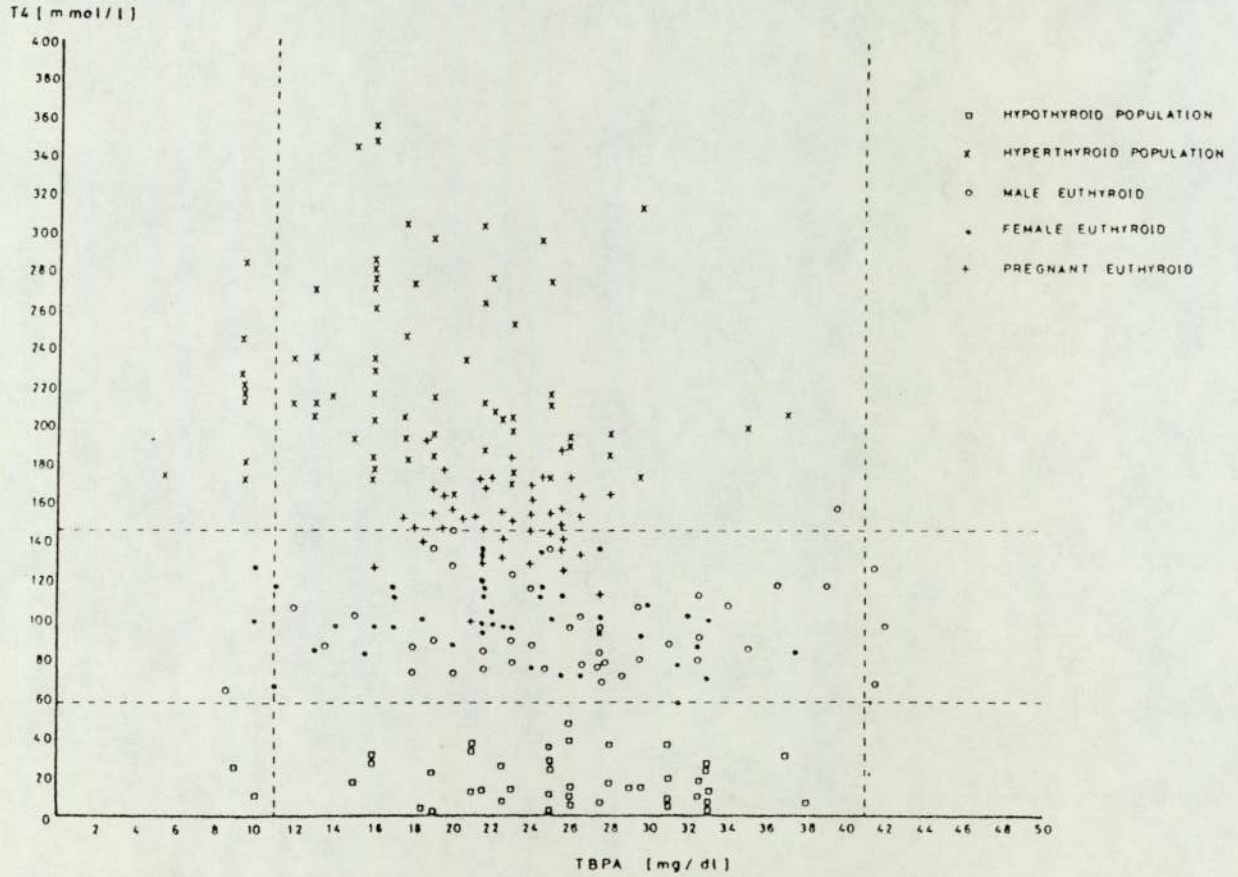


Figure 13

Relationship Between Serum TBPA and T^4 in the Euthyroid,Hypo-,Hyperthyroid and Pregnant Population



4:4:4 FTI. T^4:TBG and T^4:TBPA ratio

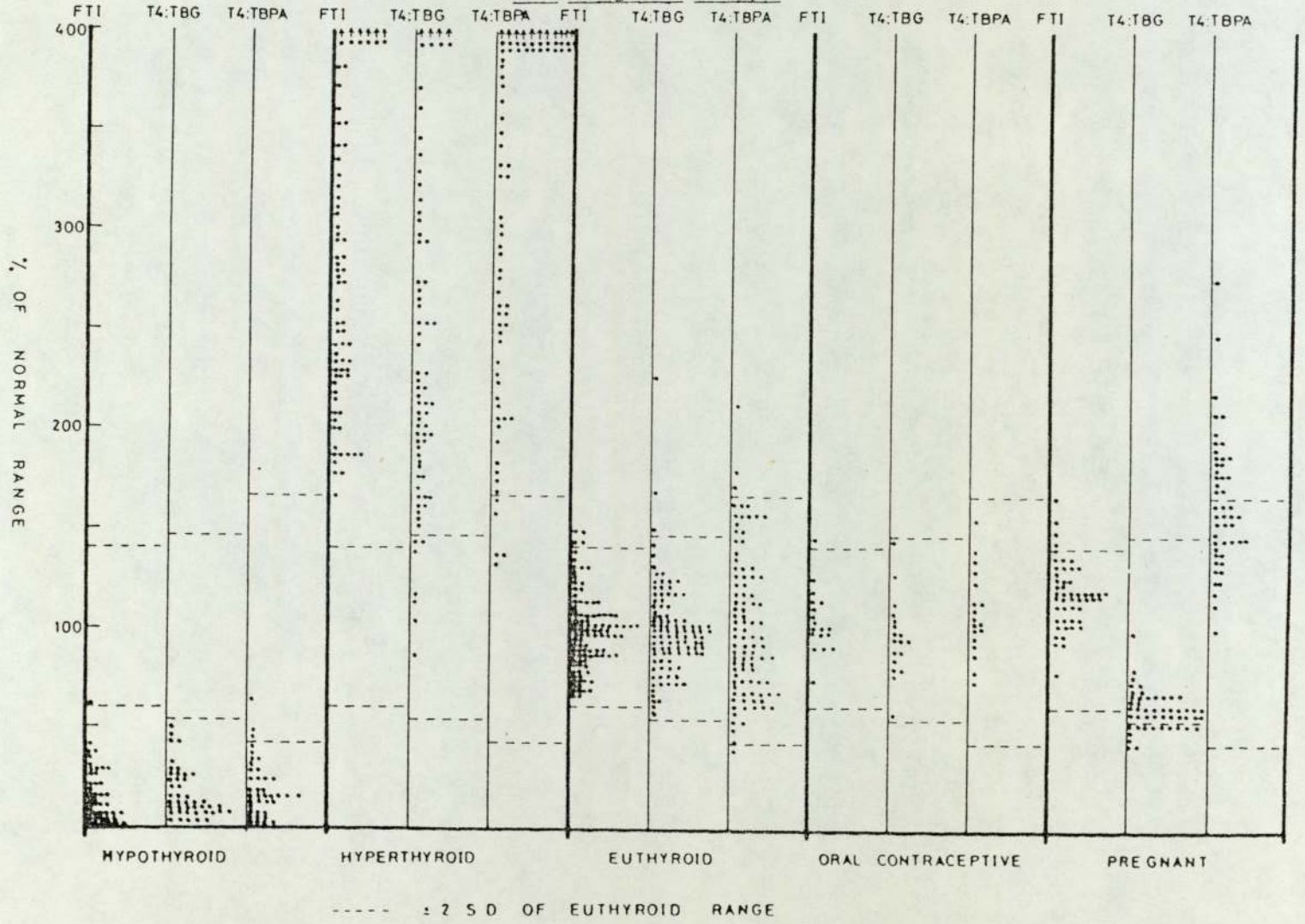
Figure 14 shows a comparison of the calculated ratios FTI, T^4: TBG and T^4: TBPA, for the groups illustrated. To facilitate ease of comparison, the mean of the normal ranges for all the parameters has been equalised by determination of the "percentage of reference range mean", for each individual value plotted graphically.

The hypothyroid group were clearly differentiated from the euthyroid state, when using both the FTI and T^4: TBG. An overlap with the normal range occurred however for the T^4:TBPA, thus reflecting the wide scatter of values observed for this index in the euthyroid group.

For the hyperthyroids, the FTI showed improved discrimination from the euthyroid range, when compared to both the T^4:TBG and T^4:TBPA, where a considerable overlap occurred. However, for the T^4:TBPA ratio, a wide separation from the normal range occurred for approximately one third of all the values. This may be explained on the basis that TBPA concentration is most reduced in hyperthyroid patients, when compared to that seen for both the T^3U and TBG.

In the oral contraceptive group, all calculated parameters gave values within the normal range, with the exception of one patient who had an FTI just above the upper limit of normal. With reference to the FTI, all the values obtained were distributed equally either side of the mean of 100%; however for the T^4:TBG approximately two thirds of all the values were below 100% and for the T^4:TBPA approximately two thirds of all the values recorded were above this figure. The inverse relationship between the T^4:TBG and T^4:TBPA spread of results presents an interesting feature and probably reflects the slightly greater increase in TBG levels found in this group, compared to that noted for TBPA.

Figure 14 Relationship Between FTI, T_4 :TBG and T^4:TBPA in the Hypo-, Hyperthyroid, Euthyroid, Oral Contraceptive and Pregnant Groups



For subjects with considerably elevated TBG levels, i.e. those in the euthyroid pregnant group, none of the three calculated parameters gave all values in the euthyroid range. Four individuals were misclassified as thyrotoxic by the FTI; thirteen as hypothyroid by the T^4:TBG and twenty one as hyperthyroid by the T^4:TBPA. The T^4:TBG $\bar{x}$ value was considerably lower than that of the FTI and the T^4:TBPA $\bar{x}$ value was considerably higher. This variation will be discussed later.

Patients within the psychiatric group gave values for all the three calculated ratios within the euthyroid range. On subsequent evaluation of the randomly selected group, no single patient studied had abnormal thyroid function when examined by clinical or biochemical investigations.

4:5 Discussion

The normal values (i.e. those obtained in the euthyroid group) for both TBPA and TBG are comparable with those reported by other workers (Ingenbleek et al., 1975; Hesch et al., 1976; Burr et al., 1977). The absence of an international standard for TBG makes direct comparison with other workers difficult. However, similar results for TBG levels were found in the present study to those reported by Burr et al., (1977) with an identical TBG reference preparation. No significant difference in TBPA and TBG concentrations between males and females was noted, although the recripocal relationship that occurred between the male and female $\bar{x}$ - value for both TBG and TBPA, was of interest.

TBG levels increased in pregnancy, in contrast to those of TBPA which showed a slight decrease. The inverse relationship between levels of TBG and TBPA referred to above and also the effects noted in pregnancy can be explained by the observations of Ingenbleek, et al., (1980). They suggested that this was due to a stimulation of hepatic synthesis of TBG (Glinoer et al., 1977) and depression of TBPA (Sakurada et al., 1967) by oestrogens; androgens having the opposite effect (Feldman and Carter, 1960; Federmann et al., 1958). This phenomenon has also been observed by Robbins et al., (1978), where a 2.75 fold increase in TBG production within the first twenty four hours following oestrogen administration was noted in animals. Robbins concluded that this effect was mainly brought about by a change in synthesis and secretion of TBG by the liver.

TBG values in the oestrogen treated female group were intermediate between those of the euthyroid and pregnant population, as would be expected. However this result was not observed for TBPA, although the numbers in this group were relatively small. The psychiatric group gave a mean value for both TBG and TBPA of no significant difference to that

obtained for the euthyroid population. On subsequent biochemical and clinical evaluation of the randomly chosen psychiatric patients, it was found that no single patient had abnormal thyroid function and therefore the above finding was not unexpected.

Both TBG and TBPA levels varied only slightly from the euthyroid means, in hypo- and hyperthyroidism. Values for TBG in hypothyroidism were slightly higher than those found in hyperthyroidism, but neither group had a mean value below the euthyroid mean. TBG results in the literature for hypo- and hyperthyroidism are somewhat conflicting. Levy et al., (1971) reported low levels in hyperthyroidism and normal levels in hypothyroidism. Conversely, Hesch et al., (1976) reported high TBG values in mild thyrotoxicosis and raised TBG in overt hypothyroidism. Attwood and Probert, (1978) found the TBG to be raised in myxoedema and lowered in thyrotoxicosis, a finding also observed by Burr et al., (1977).

TBPA levels in hypothyroidism were only slightly increased compared to the euthyroid mean, although a more significant decrease was seen for the TBPA level in hyperthyroidism.

On the basis of the above findings, both the TBG and TBPA assay, when used in isolation are of limited clinical value in the diagnosis of thyroid disease. The wide overlap with the normal range for both myxoedematous and thyrotoxic TBG and TBPA values, reflects the continuous spectrum of thyroid disorders. McDowell, (1979) also concluded that serum TBG clearly did not aid the diagnosis of hyperthyroidism, since the mean value of the hyperthyroid group he studied showed no significant difference from that of the euthyroid population. In the hypothyroid patients, McDowell reported a higher TBG level than the euthyroid mean although the range of values overlapped to such an extent with the normal, that the assay was also of limited value in the diagnosis of myxoedema. Both the

findings are supported by this present study and the TBG assay is only of value when used in conjunction with other tests.

The relation between the T^3U and TBG concentration showed good correlation for all groups, although at higher values of TBG (20 mg/l) and of T^3U (135%), the rate of increase of T^3U with increasing TBG concentration was lowered. This resulted in the T^3U tending to underestimate the TBG concentration and explains why the FTI may give inappropriately elevated levels in patients who are taking the pill, are on oestrogen therapy, or pregnant (Burr et al., 1977). This inability of the T^3U to measure high concentrations of TBG was reported by Burr and his colleagues as a methodological rather than a physiological problem, although they postulated that the free thyroxine concentration may actually be raised in patients with elevated TBG levels.

T^3U and TBG levels correlated well in both hypo- and hyperthyroidism; the displacement from the expected value for a particular TBG level being very much greater in hyperthyroidism. This may be explained by the marginal increase observed for TBG levels in thyrotoxicosis.

Correlation between T^3U and TBPA was much worse than that for T^3U and TBG; only the hypo- and hyperthyroid groups showing significant values. Discrimination between these two populations was therefore reduced, when compared to that achieved by T^3U v TBG.

The inability of the T^3U to measure elevated levels of TBG has been used by previous workers to condemn the T^3U as a routine test of thyroid function, especially when used to calculate the FTI. Burr et al., (1977) placed considerable emphasis on the value of the T^4:TBG ratio in the interpretation of serum T^4 levels associated with inherited high levels of TBG. They stated that thirteen patients with congenitally high levels

of TBG had been misclassified using the FTI, all of whom were correctly classified by the T^4:TBG. Significant correlation occurred between serum T^4 and TBG in both the euthyroid and pregnant population, suggesting that the serum TBG is the major determinant in total serum T^4 concentration in both euthyroid and pregnant subjects. The linearity of the T^4:TBG relationship for euthyroid and pregnant individuals compared to the non-linear relationship of the T^4 and T^3U (as shown by the lower level of correlation in these groups), reflects the ability of the T^4:TBG ratio to correct for high levels of TBG as opposed to the failure of the FTI.

Burr et al., (1977) reported twenty one patients with high levels of TBG in twelve months, which was stated by Mardell (1977) to represent a very low incidence in comparison with the area covered by his laboratory. He suggested therefore that the validation of an assay, using a minority group, was unfounded,and further stated that in a study of 2,400 patients, only 0.5% of T^3U results were greater than 135%. None of these patients had serum T^4 levels high enough for misclassification into the thyrotoxic group to occur on the basis of an FTI calculation.

In our laboratory, the incidence of patients with levels of greater than 135% for T^3U is 0.6%; this data supporting the findings of Mardell. Comparison of the FTI and T^4:TBG ratio by the present study, for the oral contraceptive and pregnant group, i.e. in subjects with elevated TBG levels, gave results in which only four out of sixty eight individuals were labelled "thyrotoxic" by the FTI, compared to thirteen individuals being misclassified as hypothyroid by the T^4:TBG ratio. The T^4:TBPA ratio failed to classify correctly just less than 50% of this group, demonstrating the limited value of the TBPA to correct the

serum T^4 in subjects with abnormal protein binding. Only in the hyperthyroid group was the T^4:TBPA found to give improved discrimination from the euthyroid range when compared to the T^4:TBG ratio. These findings reflect the poor correlation that was observed between serum T^4 and TBPA in all groups. Also the failure of the TBPA concentration to rise in pregnancy compared to the increase noted for the T^3U and TBG levels, further confirmsthe poor discrimination demonstrated by the T^4:TBPA ratio.

Attwood et al., (1978) drew no direct comparison between his investigation of the T^4:TBG ratio and the FTI. In fact these workers demonstrated poorer discrimination of hypo- and hyperthyroidism from the euthyroid range than others have shown for the FTI. In comparing the FTI with the T^4:TBG and T^4:TBPA ratios in these two groups, the present study suggests that for hypothyroidism, the diagnostic efficiency of the three parameters is FTI > T^4:TBG > T^4:TBPA and for hyperthyroidism : FTI > T^4:TBPA > T^4:TBG. The FTI therefore is shown to be a more powerful discriminator, especially for hyperthyroidism where seven clinically proven thyrotoxics were misclassified as euthyroid by the T^4:TBG and five patients were similarly misclassified by the T^4:TBPA.

An alternative to calculation of the FTI has been described by Mardell, (1978) in which serum T^4 is plotted graphically against T^3U; He demonstrated the value using this thyroid mapping technique for correlation of serum T^4 with T^3U and suggested that improved classification of thyroid abnormality was obtained. In a study of 8,000 patients over an eighteen month period, he found four euthyroid patients with T^3U values over 130% where an FTI would have resulted in a false classification of thyrotoxicosis. By using the mapping technique

of serum T^4 versus T^3U, in conjunction with a T^4 assay, correct classification into the euthyroid range was obtained.

The mapping technique was used to correlate serum T^4 with T^3U,TBG and TBPA in the hypo -, hyper -, euthyroid and pregnant individuals. Improved separation of these groups was obtained for the serum T^4 versus T^3U plot, compared to serum T^4 versus TBG or serum T^4 versus TBPA. These findings further support the value of using the serum T^4 in conjunction with the T^3U, in preference to either TBG or TBPA.

Roosdorp and Joustra, (1979) in a computerised study of this technique, stated that with respect to diagnostic potential, the T^4:TBG did not appear to offer a real advantage over the FTI, provided this index was based on T^3U measurements of good quality. In a recent publication by Cusick (1979) the T^4:TBG ratio was found to be misleading in assessing thyroid status in patients with reduced TBG levels. The T^4:TBG ratio gave very high results in patients with undetectable levels of TBG, and was near the upper limit of normal for those patients with markedly lowered levels. The FTI, however, produced results which although were below the reference range, proved of greater clinical value. Further support for the FTI in patients with elevated levels of TBG has been given by Sheridan et al., (1978) using a simple modification of the FTI. These workers found considerably improved classification of patients with abnormal levels of TBG, using both the modified and conventional FTI.

The data obtained from the randomly selected psychiatric patients was unable to offer any further insight into the value of the FTI over that of the T^4:TBG or T^4:TBPA ratio, as no abnormal thyroid function results were obtained in this group. Therefore on the basis of the information already obtained, it was decided to abandon the use of both

the T^4:TBG and T^4:TBPA ratios for routine screening of these patients in the psychiatric survey (reported in the following chapter), and to adopt the FTI as the calculated index.

In summary the results of this study suggest that the TBPA is of little value in the routine assessment of thyroid disease, especially with regard to correction of the serum T^4 in those patients with abnormal binding protein levels. The T^4;TBG ratio is of value only in patients with a raised serum T^4 with an elevated T^3U value above 135%, or a TBG assay in patients with lowered serum T^4 and T^3U values. As previously mentioned the former situation rarely occurs ; the incidence in this laboratory being in the order of 0.59%. Although a number of laboratories now routinely employ direct measurement of TBG as part of their thyroid function test profile, it is clear from the findings of this study, that for the majority of patients encountered by a routine clinical chemistry department, calculation of the FTI still offers the best overall diagnostic discrimination.

CHAPTER FIVE

THE EVALUATION OF THYROID DISEASE IN A LARGE PSYCHIATRIC HOSPITAL

Introduction

Selection of patients and sampling procedure

Results

Discussion

5:1 Introduction

For many years psychiatric abnormalities have been associated with thyroid dysfunction and this has been reviewed in Chapter 1. However, few studies which evaluate this relationship have been carried out within psychiatric hospitals. Thus the present work investigates the prevalence of thyroid disorders in patients admitted for assessment to a large psychiatric hospital and determines the extent to which patients with a thyroid abnormality are passing unrecognised. The importance of recognition of thyroid dysfunction in psychiatric patients cannot be over emphasised, as treatment may dispel the psychiatric symptoms and allow the patient to be released from psychiatric care.

Previous surveys have been hindered by the unreliability of clinical criteria in diagnosing thyroid disease in psychiatric patients and the limited range of laboratory investigations available as an aid to confirming the diagnosis. The present study involves a standard method of assessing an individuals psychiatric state together with haematological assays and recently developed biochemical tests for the assessment of thyroid function. Patients found to have previously undiagnosed thyroid disease will be studied in depth and case histories presented.

The various lines of investigation that are to be followed briefly comprise :

i) Determination of the prevalence of thyroid abnormalities in the psychiatric population, for both previously diagnosed cases and those recognised during the survey. The effect of age and sex will also be observed.

ii) Assessment of the most effective form of treatment for a patient

with previously unrecognised thyroid dysfunction; i.e. whether treatment of the thyroid disorder per se or of the psychiatric abnormality was required to effect a marked improvement in the patients clinical state.

iii) The relationship between thyroid function and various psychiatric disorders.

iv) Recommendations and proposals for future study.

For comparison of both biochemical data and prevalence rates, two other distinct populations will also be selected for study and are outlined later in this chapter. Previously published work will also provide information regarding the prevalence of thyroid disorders in the general population and the effects of age, sex, etc. on various thyroid parameters.

5:2 Selection of patients and sampling procedure

The study was conducted over a two year period on patients who had been referred for psychiatric assessment to the Highcroft Hospital, Erdington, Birmingham, by their general practitioner. This group therefore represented an acute psychiatric population and it was estimated that between twenty and twenty five subjects per week would be screened.

For each individual admitted for assessment, a standard questionnaire was completed and this is shown on pages 103 - 107. This required personal details and any previous history or family history of thyroid disorder, together with any treatment or investigations performed. An initial psychiatric evaluation was recorded and subsequently a more detailed investigation of symptomology using a psychiatric checklist. A physical examination was performed and details recorded, with particular emphasis being placed on those symptoms and signs specific for thyroid disease. Current medication was also recorded, since many

QUESTIONNAIRE FOR THYROID SURVEY

A. GENERAL PATIENT DETAILS

Name:..... Age:..... Sex: M/F

Married/Single/Divorced/Widowed

Last Menstrual Period:..... Age at onset of menopause:.....

Cycle: ..... Recent Pregnancy: YES/NO
When: .....

Oral Contraceptive Pill: YES/NO Which: .....

Hospital No: ..... Trial No:.....In-Patient/Out-Patient

Date of Admittance: ..... Date of Discharge: .....

History of Past Thyroid Disorder: YES/NO .....

.....

Treatment: .....

B. INITIAL PSYCHIATRIC EVALUATION

Patients Main Problem and Psychiatric Symptoms: .....

.....

.....

.....

.....

Diagnosis (if made): .....

C. PSYCHIATRIC SYMPTOM CHECK LIST

MOOD:	Anxious/agitated	YES/NO
	Depressed	YES/NO
	Diurnal variation in mood	YES/NO
	Elated	YES/NO
	Suspicious	YES/NO
	Irritability	YES/NO
	Other	YES/NO
	.....	
SLEEP:	Difficulty getting off to sleep	YES/NO
	Frequent waking	YES/NO
	Early morning wakening	YES/NO
EATING:	Appetite increased	YES/NO
	Appetite decreased	YES/NO
	Anorexic	YES/NO
	Recent loss of weight	YES/NO
	Slow speech (psychic retardation)	YES/NO
	Slow movements (motor retardation)	YES/NO
	Apathy	YES/NO
	Drowsiness	YES/NO
	Over-active	YES/NO
	Delusions	YES/NO
	Hallucinations : visual	YES/NO
	auditory	YES/NO
	olfactory	YES/NO
	tactile	YES/NO
	gustatory	YES/NO
Orientated:	for time	YES/NO
	for place	YES/NO
	for person	YES/NO
Memory:	loss of recent memory	YES/NO
	loss of distant memory	YES/NO
Insight	.....	YES/NO

D. PHYSICAL SYMPTOMS

CLINICAL FEATURES

1.	Sensitivity to heat/cold	YES/NO
2.	Weakness	YES/NO
3.	Tiredness	YES/NO
4.	Stiffness	YES/NO
5.	Constipation/Diarrhoea	YES/NO
6.	Hair loss	YES/NO
7.	Dry skin	YES/NO
8.	Disturbed periods	YES/NO
9.	Deafness	YES/NO
10.	Hoarseness	YES/NO
11.	Muscular and joint pains	YES/NO

E. PHYSICAL EXAMINATION

Pulse .....Blood Pressure.....J.V.P.....

Signs of C.C.F .....

Patients looks myxoedematous .....

Patients looks thyrotoxic .....

E.C.G. abnormalities if any .....

Chest X-ray abnormalities .....

Photomyogram .....

F. DRUGS

On Admission	Length of Time
Lithium ..... YES/NO	.....
Thyroxine ..... YES/NO	.....
Anti-thyroid drugs .....YES/NO	.....
1.	.....
2.	.....
3.	.....
4.	.....
5.	.....
6.	.....
7.	.....

Effect of Thyroid/Psychiatric Therapy

G. INITIAL LABORATORY TESTS

Haemoglobin .....Erythrocyte Sedimentation Rate.....

Serum Thyroxine ..... T^3 Uptake.....

F.T.I .....

Further Investigations: NO / YES If YES see accompanying proforma entitled "Laboratory Investigations"

H. LABORATORY INVESTIGATIONS

Patient's Name: ..... Age: .....

Sex: ..... Ward (In/Out).....

Date Admitted to Study: .....

Clinical Details (Summary): .....

.....

Treatment (To Date of Admission): .....

.....

Biochemical/Haematological Investigations		Normal Range
Serum Thyroxine	nmol/l	58 - 148 nmol/l
T^3 Uptake	% normal	92 - 117%
Free Thyroxine Index		58 - 148
Haemoglobin.....	g/dl.	13.5-18.0g/dl (Male);12.0-16.0g/dl (Female)
E.S.R.: .....mm/hr.		3 - 5 mm (Male); 4 - 7 mm (Female)

Provisional Diagosis

Euthyroid
Hyperthyroid
Hypothyroid

Further Investigations

NO
YES

Thyroid Studies	Normal Range
Thyroid Stimulating Hormone (TSH)	Less than 8.0 μIu/ml.
Real T^3	0.5 - 2.9 nmol/l
Thyroid Binding Globulin (TBG)	7.01 - 17.86 mg/l.
Thyroid Binding Pre Albumin	103.0 - 405.0 mg/l.

Lipid Studies	Normal Range
Cholesterol.....	3.0 - 7.3 nmol/l.
Triglycerides	Less than 1.8 nmol/l.

Lipid Electrophoresis

Other Tests

Lithium.....

Final Diagnosis

Effect of Thyroid and/or Psychiatric Therapy

Patient To Be Included in Final Statistics

YES

NO

"

drugs are known to affect thyroid function and reference to these is made in Appendix 2 Finally the questionnaire included a summary of the various biochemical and haematological investigations that were performed on each patient included in the study.

Blood samples were taken initially for the following biochemical and haematological investigations : serum T^4, T^3U and FTI, Hb and ESR. No further laboratory tests were conducted if the results of these assays were found to be normal; however if an abnormal result was obtained the following tests were performed : a repeat serum T^4,T^3U and FTI together with a TSH assay if hypothyroidism was suspected and a T^3 assay if hyperthyroidism was suspected. Previous workers have shown T^3 to be a poor discriminator of hypothyroidism (Patel and Burger,1973) and TSH a poor discriminator of hyperthyroidism (Hoffenberg, 1973). Lipid studies including serum cholesterol and triglycerides were also carried out on this abnormal group.

For equivocal cases a similar strategy to that outlined by Britton et al., (1975) was adopted for improving diagnostic efficiency. This procedure follows that described previously for patients with abnormal results on initial testing, except that a borderline FTI is used as the basis for further tests. Britton using this procedure at the Middlesex Hospital, London, found the clinical uncertainty in distinguishing borderline cases was less than 2%, whereas previously this had been as high as 47%.

Technical details relating to the assay of all these parameters have been fully described in Chapter 3; strict quality control was maintained on all laboratory investigations. Patients with abnormal thyroid function diagnosed using the above criteria, were re-assessed after an interval of approximately one month and certain patients were studied in detail

during the entire survey period. A diagram illustrating the procedure adopted on admission, for each psychiatric patient admitted to the survey, is shown in Figure 15 This process of continuous assessment enabled the patients progress to be monitored, especially with respect to the value of using psychiatric and/or thyroid therapy. Case histories together with follow up data are presented for those subjects with previously undiagnosed thyroid dysfunction.

Thyroid binding protein analysis was carried out on a randomly selected group of patients and the results of this study have been presented in Chapter 4.

5:2:1 Psychiatric disorders encountered during the survey.

A broad spectrum of abnormal mental phenomena will be encountered during the survey and therefore an understanding of both the basic concepts involved and the classification of thyroid disorders is pertinent to this research. A brief description of mental disorders is given in Appendix 4.

The relationship between thyroid hormone levels and psychiatric diagnosis was also investigated as previous workers (Rybakowski and Sowinski, 1973; McLarty et al., 1978)have reported conflicting results. Six distinct groups of psychiatric patients were chosen and these categories conform to the World Health Organisation "Glossary of mental disorders and guide to their classification, for use in conjunction with the International Classification of Diseases" (8th revision, Geneva,1974). The categories chosen for study were :

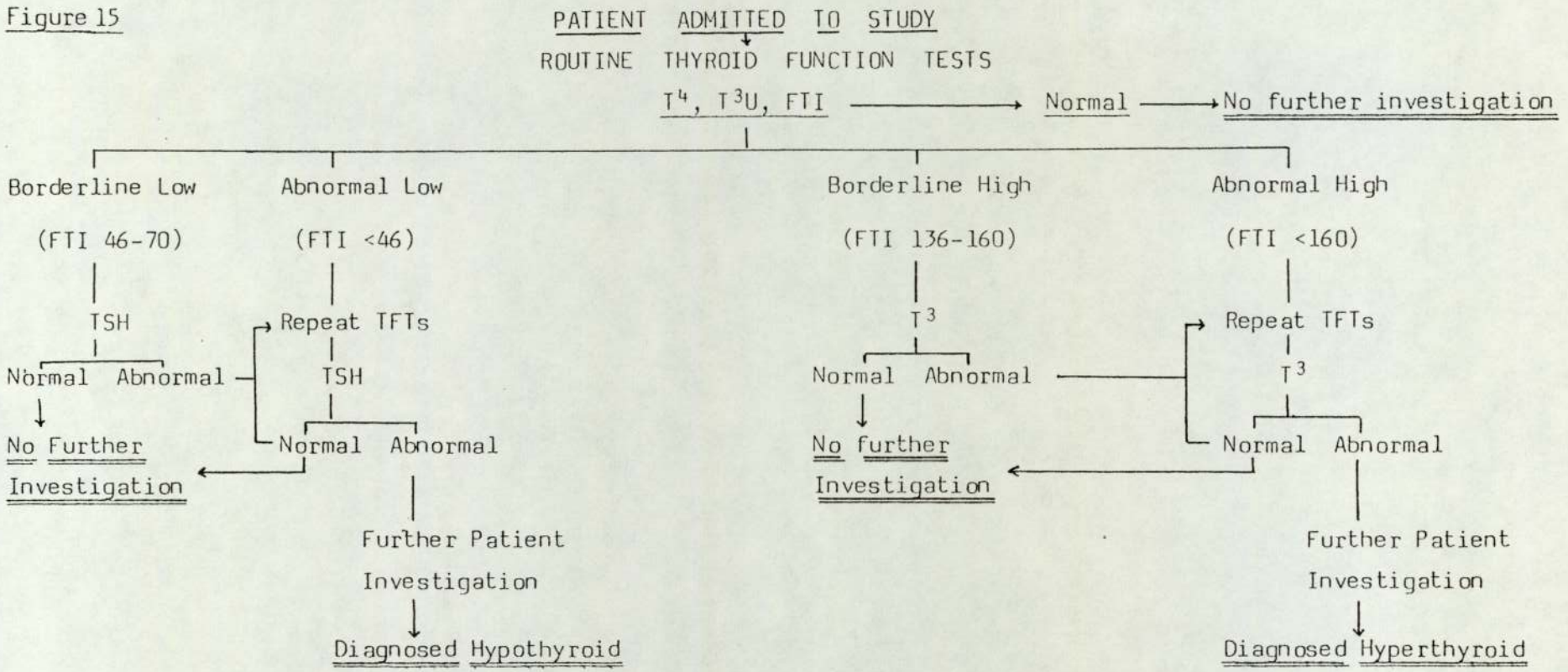
i) Schizophrenic psychoses

ii) Affective psychoses and other non-organic psychoses (including depression)

iii) Neurotic disorders

iv) Organic psychotic conditions (Senile dementia)

Figure 15



Test Procedure Adopted for each Patient on Admission to the Psychiatric Survey.

v) Other organic psychotic states (Epilepsy)

vi) Alcohol and drug psychoses.

In all these groups serum T^4, T^3U and FTI were assayed and the results compared, with a view to further establishing the association between thyroid function and psychiatric disease.

The effect of various physical factors upon thyroid metabolism have been well documented and this can partly be explained by their interaction with human TSH; factors such as cold exposure (Gale,1973), stress (Gregermann, 1971), age and sex (Ingbar,1976; Tunbridge et al., 1977; Evered et al., 1978) have all been implicated in effecting a change in thyroid hormone levels. Hence it was decided to study the seasonal changes in thyroid function, in an attempt to ascertain whether this had any significant effect upon the detection of individuals with thyroid abnormality. Patients admitted to the study during the two winter months, January and February and the two summer months, July and August all had blood samples taken for the assay of serum T^4, T^3U and FTI.

Comparison of these three parameters for the winter and summer months yielded no significant differences in either mean concentration or spread of results. Subsequent study of this specific subject was therefore felt unnecessary and will not be reported on further.

5:2:2 Selection of control groups for comparison.

The selection of an 'ideal' control group presented many difficulties, especially due to the ethical problems encountered. Control subjects should ideally be matched for age and sex with the psychiatric group, before any accurate comparisons can be made. This however proved to be impractical and therefore it was necessary to devise a suitable regime which could provide adequate control data.

The Wickham survey does provide a comprehensive study of thyroid disease in the general population (Tunbridge et al., 1977) and the effect of age, sex, illness and medication on thyroid hormone concentrations has been observed by Evered et al.,(1978) However for a more direct comparison, two distinct groups of subjects were chosen as follows:-

i) The first group comprised patients whose blood samples had been received by the Biochemistry department of Good Hope Hospital for thyroid function tests. One hundred male and one hundred female patients, subsequently found to be both clinically and biochemically euthyroid, were chosen after exclusion of any females who were either pregnant or on oral contraceptives. The binding protein study, discussed in chapter four., similarly employed this group for a reference euthyroid population.

ii) The second group comprised patients whose blood samples had been received by the Biochemistry department for routine biochemical profiles. Two hundred and fifty-five patients were selected after exclusion of any individuals known to have thyroid disease. The samples were matched on a male to female ratio as that occuring in the psychiatric study and were of a similar age range.

The first control group served as a reference euthyroid population for all the following parameters : Serum T^4, T^3U, FTI,TSH and T^3.

The second group was designed to enable the prevalence of thyroid disorders in a non psychiatric hospital population to be assessed and served as a direct means of comparison.

5:3 Results

5:3:1 Control group data

The first control group comprised of 100 males and 100 females who were euthyroid and selected by the procedure described previously in this chapter. The mean, SD and two SD range for the serum T^4, T^3U, FTI, TSH and T^3 is shown in Table 4. The values obtained demonstrated close correlation with those quoted by our laboratory as have been previously described in Chapter 3. All parameters, with the exception of serum TSH, were normally distributed and thus calculation of the 2SD range described above was statistically valid. However, a skewed (non-Guassian) distribution was observed for serum TSH and this together with a reduced assay sensitivity for low concentrations of TSH rendered calculation of a similar range difficult. Calculation of 2 standard deviations includes 95% of all values obtained for a specific parameter, and therefore in order to obtain comparable data for the TSH assay, the 95% range was determined and is shown in Table 4.

The second control group comprised of 100 males and 155 females representing 39% and 61% respectively of the total population studied. Subjects were matched with a male/female ratio, identical to that encountered in the psychiatric population, with a view to obtaining a more realistic index of the prevalence of thyroid disorders in this control group. The division of individuals into two categories, i.e. previously diagnosed thyroid abnormality and identified in survey, proved impractical since only a limited amount of clinical information was available on a particular patient. The following data on prevalence rate therefore utilises both categories of patient in its compilation.

TABLE 4

Mean and one standard deviation values, with two SD range, for serum T^4 T^3U, FTI, TSH and T^3 in the reference euthyroid population (i.e. the first control group)

Assay		Euthyroid Population		
		$\bar{X}$	SD	Range ($\bar{x} \pm 2$ SD)*
T^4 (nmol/l)	Male	97.5	20.7	56.2 - 138.8
	Female	101.8	21.7	58.5 - 145.1
T^3U (%)	Male	104.8	7.2	90.5 - 119.1
	Female	107.3	7.2	93.0 - 121.6
FTI	Male	92.5	18.6	55.3 - 129.7
	Female	94.8	17.7	59.3 - 130.2
TSH (μIu/ml)	Male	3.90	not applicable	1.4 - 8.0
	Female	5.10	not applicable	2.2 - 9.6
T^3 (nmol/l)	Male	1.73	0.39	0.95 - 2.51
	Female	2.08	0.44	1.20 - 2.96

* $\pm$ 2 SD range does not apply to TSH assay, where 95% range is quoted.

5:3:2 Prevalence of thyroid disorder in the second control group.

5:3:2:1 Hypothyroidism

Three patients, consisting of two females and one male were identified as hypothyroid. The prevalence of hypothyroidism was therefore 1.29/100 females and 1/100 males with an incidence in the whole population of 1.18% when both males and females were included.

5:3:2:2 Hyperthyroidism

Two patients, both female, with hyperthyroidism were identified in the control group. The prevalence of hyperthyroidism was therefore 1.29/100 females with no males being identified. The incidence in the whole population was therefore 0.78%

5:3:3 Statistical evaluation of psychiatric patients included in the survey

The total number of acute psychiatric admissions to Highcroft Hospital during the two years of the survey was 1895, of which 1828 patients were sampled; 67 individuals were therefore lost from the study. The reasons for this were several fold as follows:-

i) Patients were discharged within twenty four hours, before the blood samples were taken.

ii) Patients refused to participate in the survey by not allowing blood to be taken.

iii) Blood samples were spoiled in transit between the hospital and laboratory.

Thus 97% of all the patients initially admitted were included in the survey.

There were 1122 females of which 181 were readmitted during the two year period (16.1% readmission rate), giving the actual number of females screened as 941. Thus a total of 706 males were screened of which 103

were readmitted (14.6% readmission rate) giving the actual number of males surveyed as 603. Therefore the total number of 'new' patients that were included in the two year survey was 1544, with female and male admissions representing 69% and 31% of the total respectively and this figure is typical for all psychiatric hospitals.

The age and sex distribution of the patients is shown in Figure 16

5:3:3:1 Serum T^4, T^3U and FTI concentrations

During the latter 18 months of the survey 1064 patients were selected, with a male to female ratio representative of the total psychiatric population (415 males and 649 females), to represent the distribution of serum T^4, T^3U and FTI (Figures 17-22). All parameters were found to be normally distributed in the population studied and the mean ($\pm$ 1SD) for serum T^4 was 107.5 $\pm$ 25.1 nmol/l, T^3U 107.8 $\pm$ 8.2% and FTI 100.4 $\pm$ 24.0. No significant difference in concentration for any of these parameters was noted between males and females.

5:3:3:2 Serum TSH and T^3 concentrations

From the previous group of 1064 patients, 185 were randomly selected for analysis of their TSH and T^3 levels and the distribution of the values obtained is shown in Figures 23 and 24 The distribution of TSH was non-Gaussion being markedly positively skewed and the mean concentration was 3.49 µIu/ml. A 95% range was calculated and gave values between 1.0 and 7.6 µIu/ml. A normal distribution was observed for serum T^3 with a mean ($\pm$ 1SD) of 1.85 ($\pm$ 0.39) nmol/l.

5:3:4 The prevalence of previously diagnosed thyroid disorders and those identified by the psychiatric survey.

5:3:4:1 Total incidence of thyroid abnormality

The total incidence of both previously diagnosed and undiagnosed

Figure 16

Age and Sex Distribution of the 1544 Acute Psychiatric Patients Studied (941 Females, 603 Males)

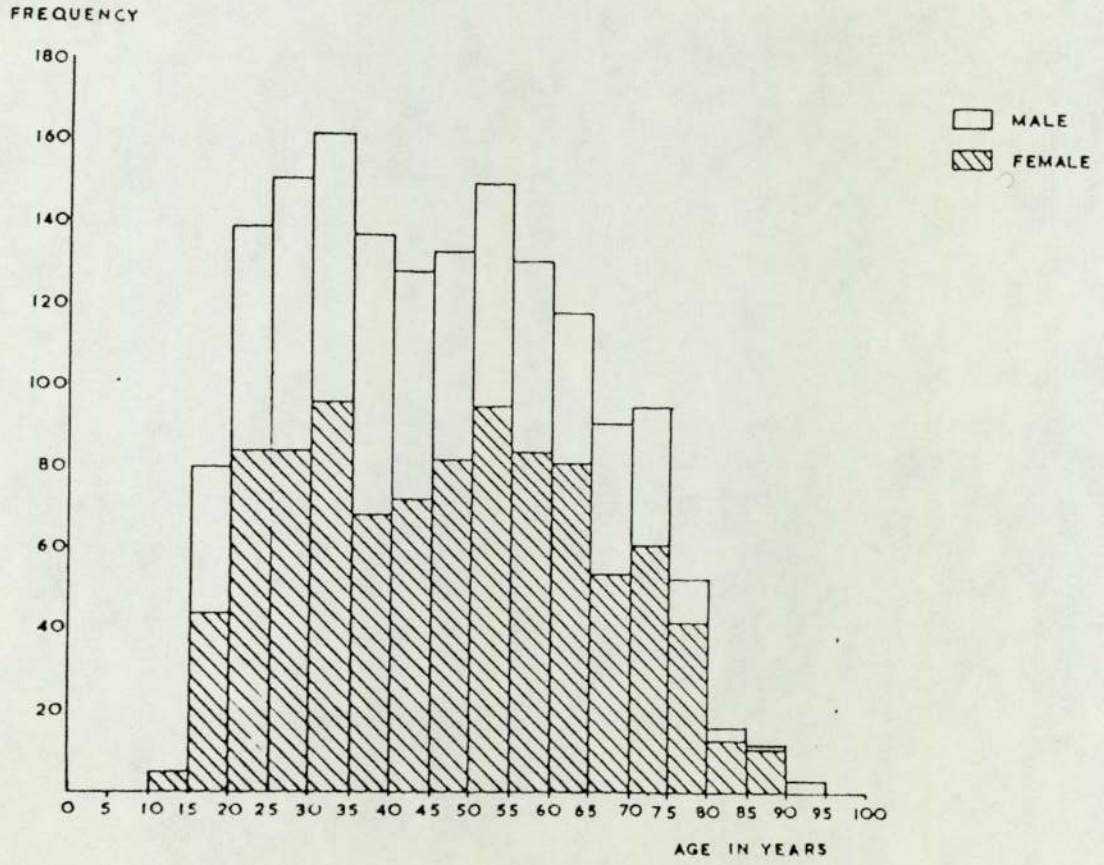


Figure 17

Distribution of Serum T^4 in the Male Psychiatric Group

(n = 415) ($\bar{x}$ Serum T^4 Concentration = 108.6 nmol/l)

SD = $\pm$ 22.68

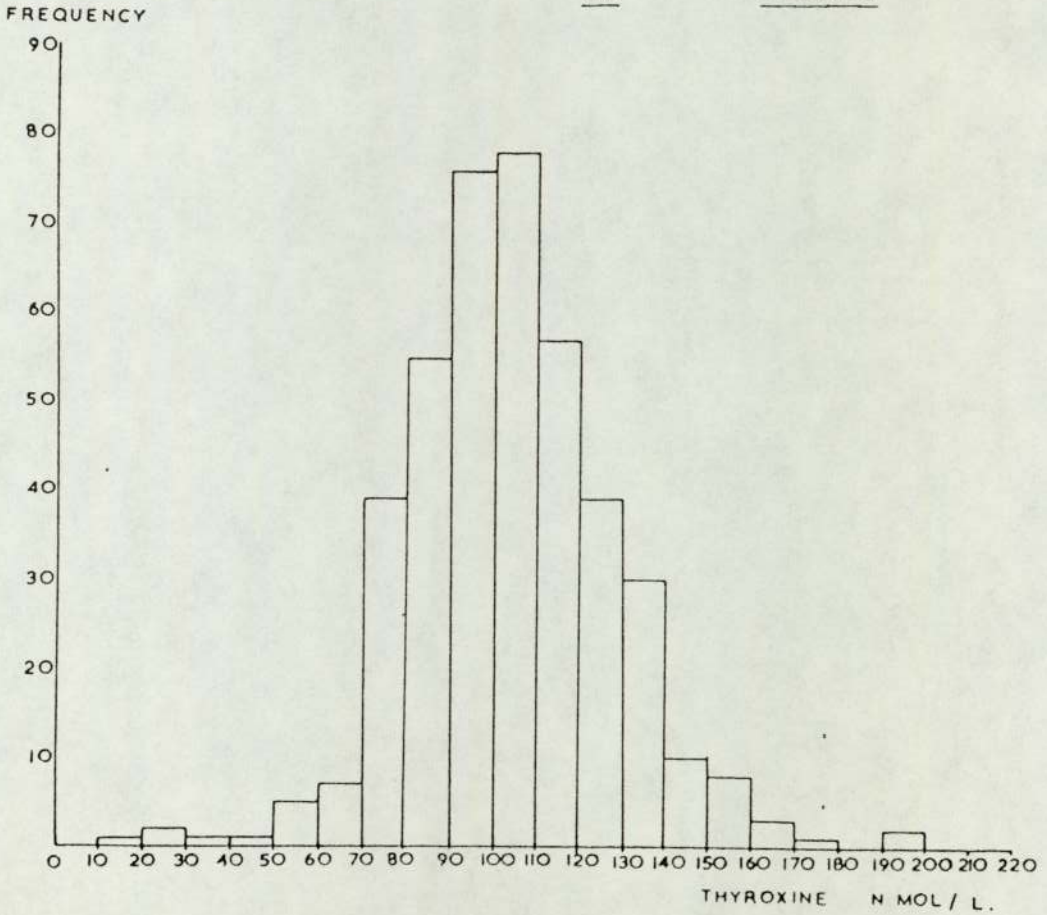


Figure 18

Distribution of Serum T^4 in the Female Psychiatric Group
(n = 649) ($\bar{x}$ Serum T^4 Concentration = 106.8 nmol/l)
SD = ± 26,89

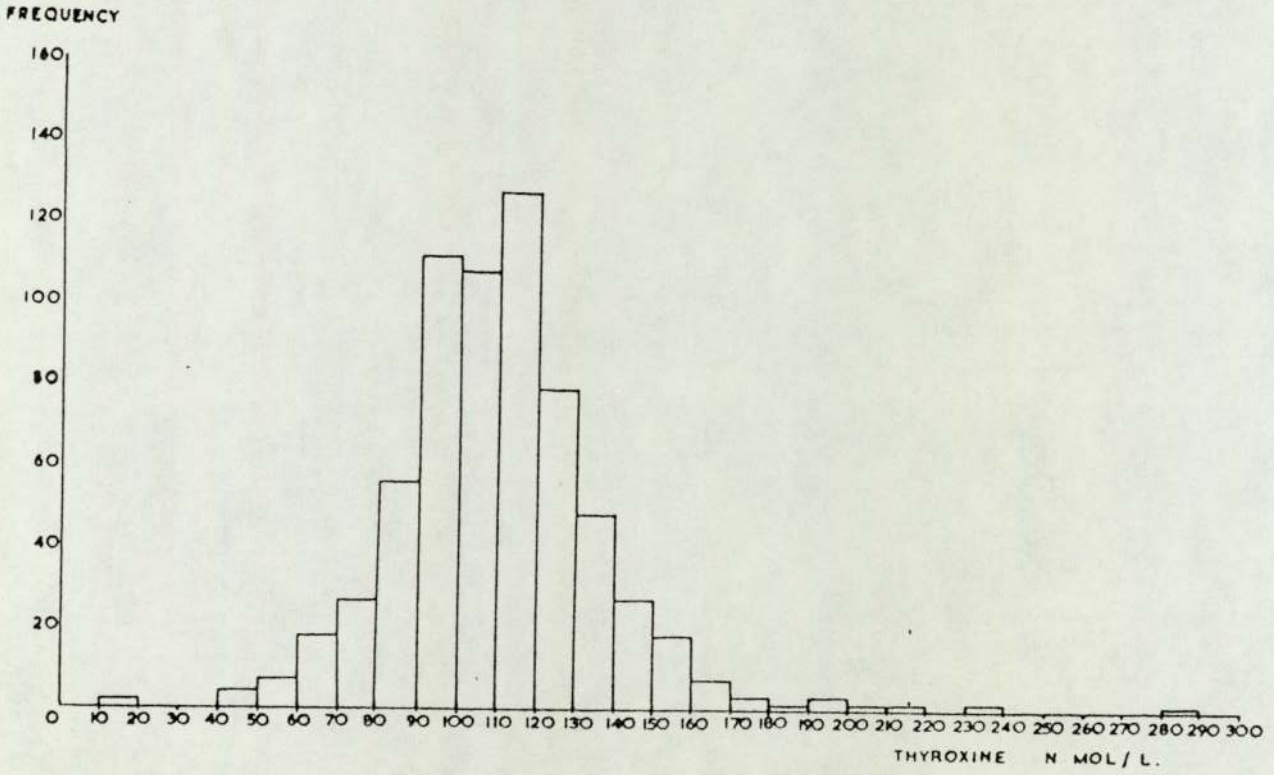


Figure 19

Distribution of Serum T^3U in the Male Psychiatric Group

(n = 415) ($\bar{x}$ Serum T^3U Concentration = 106.1%)

SD = $\pm$ 7.16

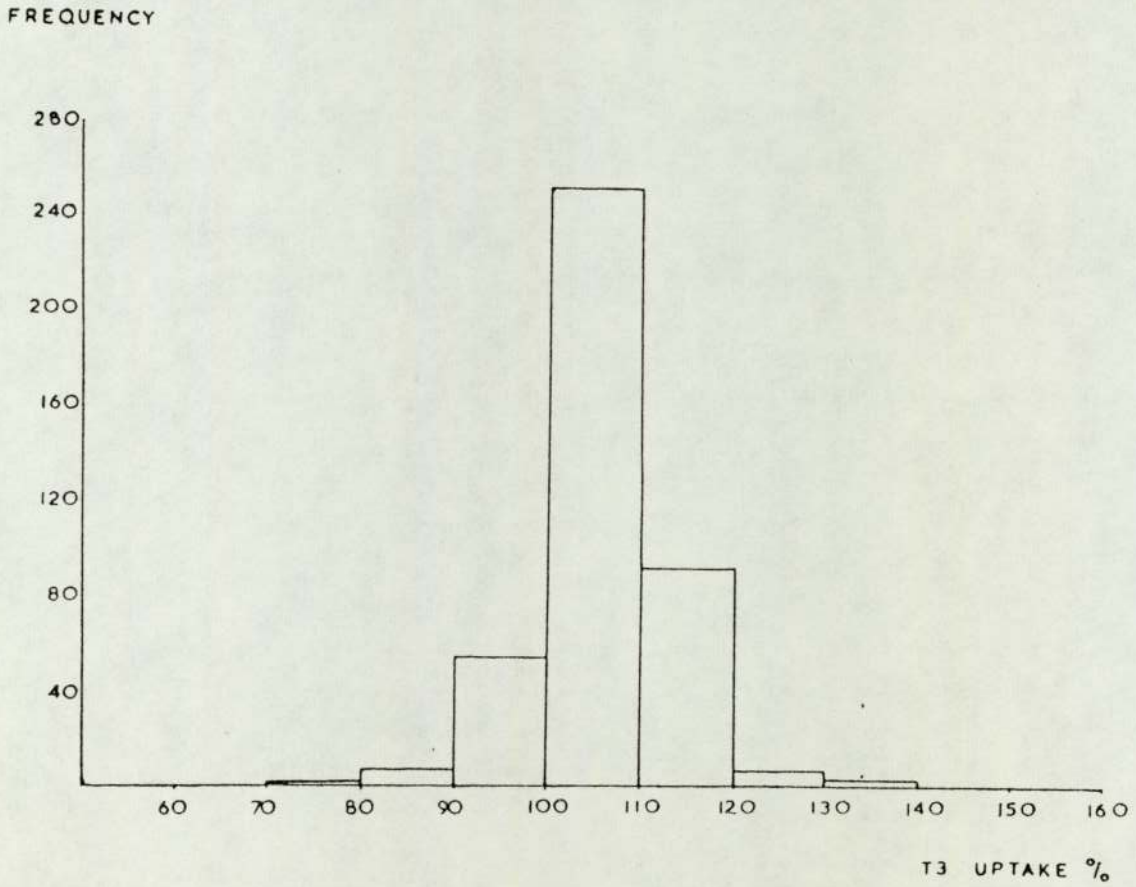


Figure 20

Distribution of Serum T^3U in the Female Psychiatric Group

(n = 649) ($\bar{x}$ Serum T^3U Concentration = 108.5%)

SD = $\pm$ 11.28

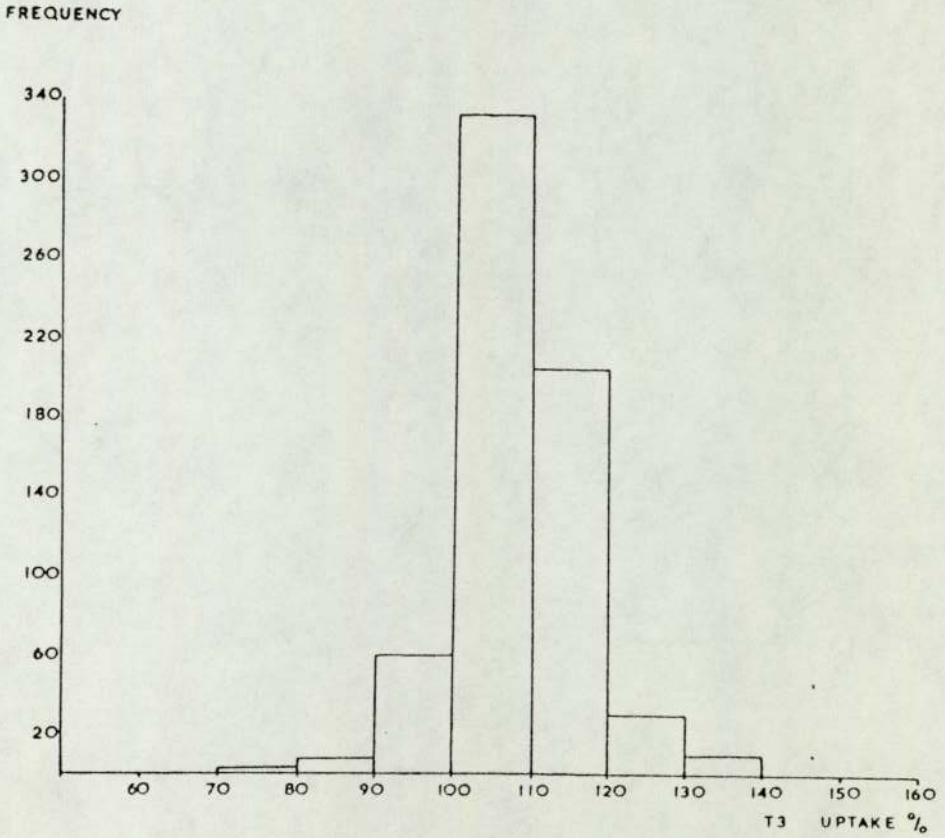


Figure 21

Distribution of Serum FTI in the Male Psychiatric Group
(n = 415) ($\bar{x}$ Serum FTI Concentration = 102.1 $\pm$ 21.2

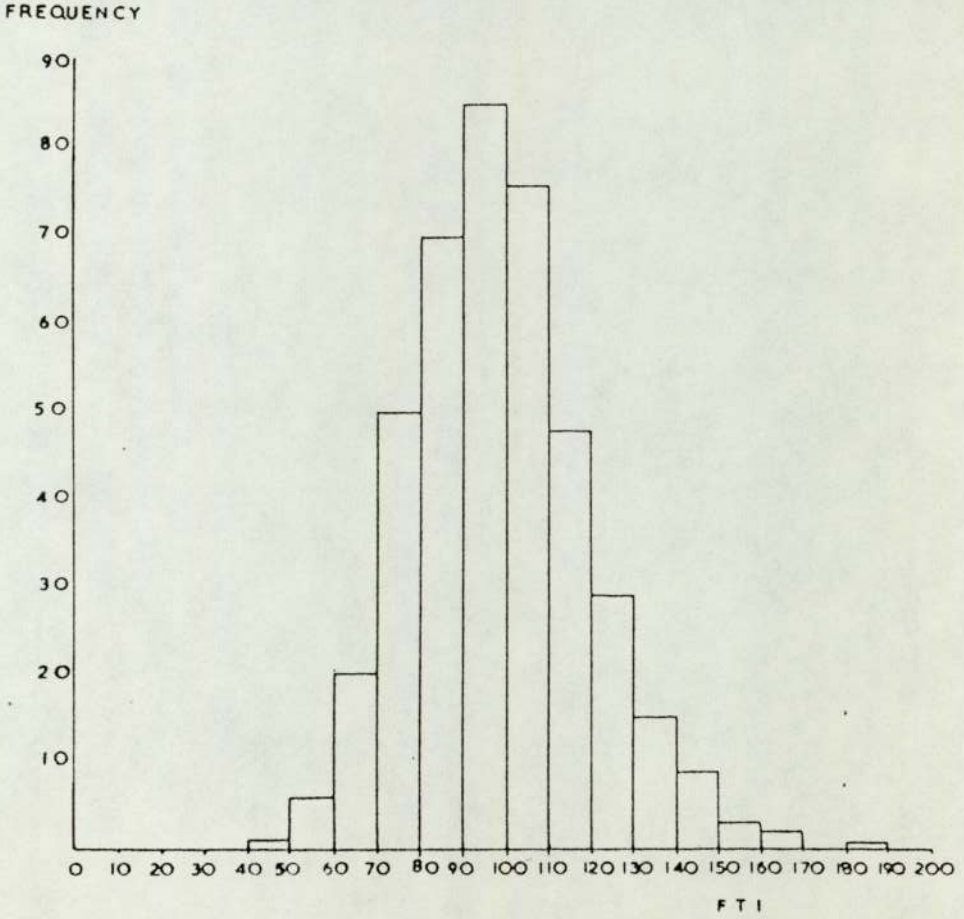


Figure 22

Distribution of Serum FTI in the Female Psychiatric Group

(n = 649) ($\bar{x}$ Serum FTI Concentration = 95.5)

SD = ± 25.8

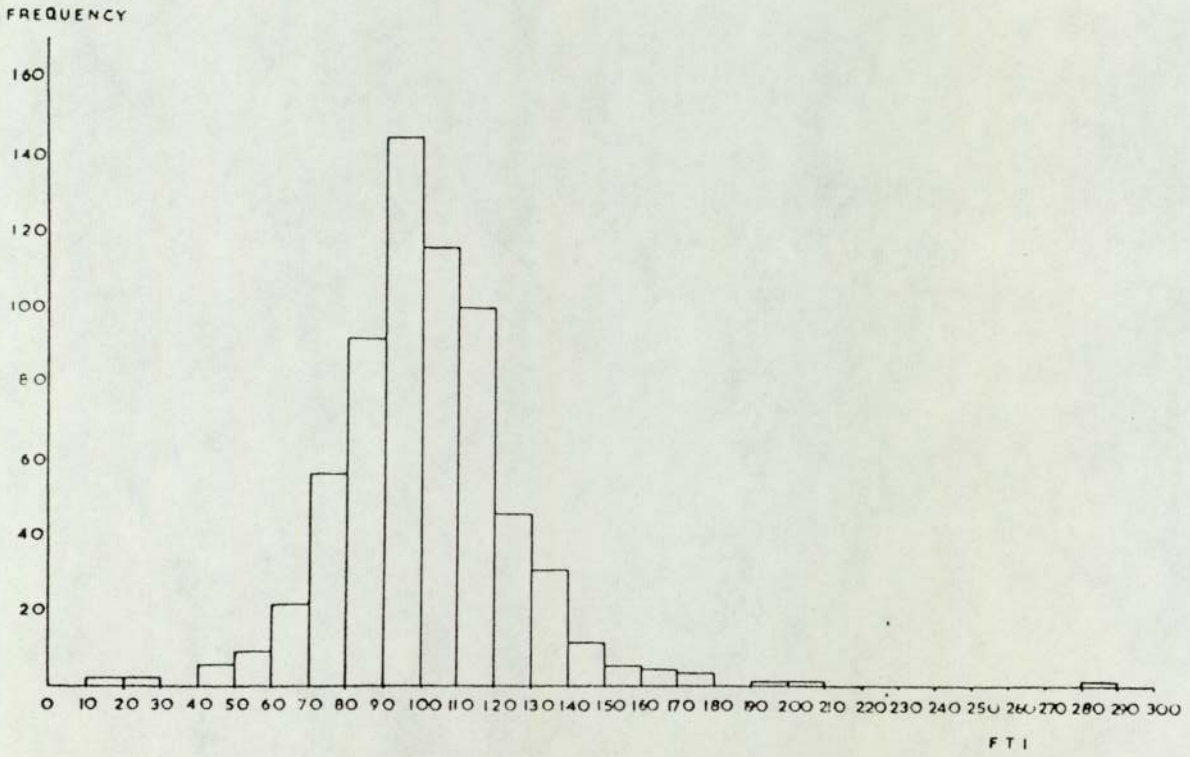


Figure 23

Distribution of Serum TSH in the Male and Female Psychiatric Group
(n = 185) ($\bar{x}$ Serum TSH Concentration = 3.49 µIu/ml)

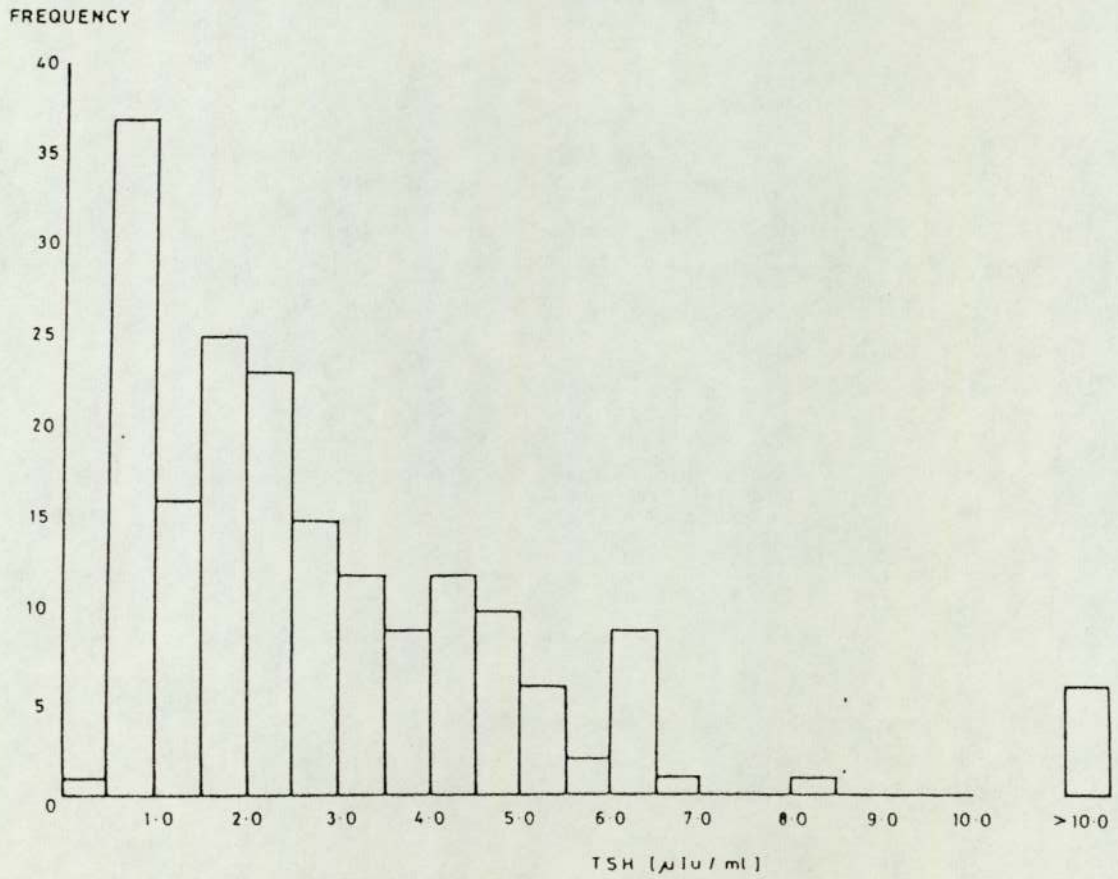
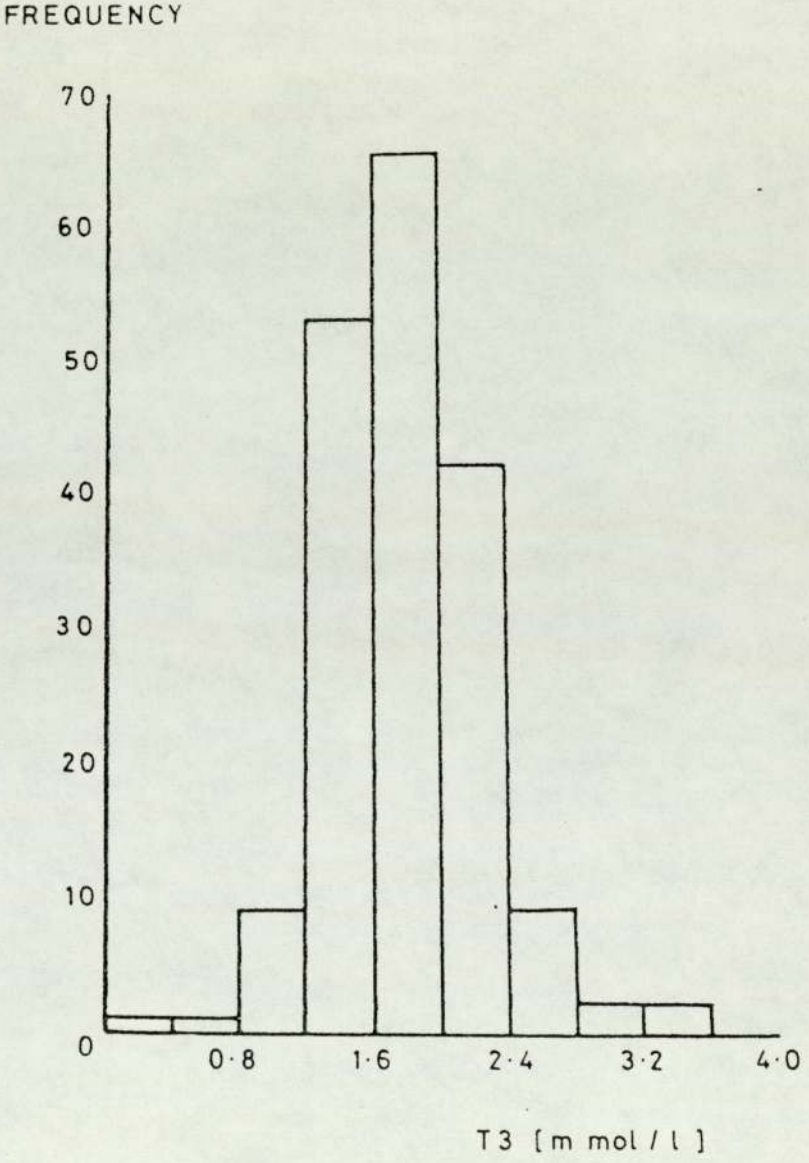


Figure 24

Distribution of Serum T^3 in the Male and Female Psychiatric Group (n = 185)

($\bar{x}$ Serum T^3 Concentration = 1.85 nmol/l)

SD = $\pm$ 0.39



thyroid disease in the psychiatric population studied, represented 4.14% and 0.34% of the female and male population respectively, with an overall figure of 2.65% for the whole population. This is illustrated in Table 5 and illustrated in Figure 25

Nineteen patients were hypothyroid (18 female, 1 male) representing 1.91% of the female and 0.17% of the male population, with an overall figure of 1.23% for the total population. Nineteen patients were hyperthyroid (18 female, 1 male) and the incidence rates were therefore as for hypothyroidism. Three patients (all female) were diagnosed euthyroid, although clinically an abnormal gland was detected. The incidence was therefore 0.32% of the female population and 0.19% of the total population.

Thyroid dysfunction was detected over a wide age range (20-80 years) and the relationship of thyroid abnormality with age is shown in Figure 26

5:3:4:2 Previously diagnosed thyroid disorders

The total number of patients with previously diagnosed thyroid abnormalities was 28, consisting of 26 females and 2 males. The prevalence of established thyroid disorders was therefore 2.76/100 females and 0.33/100 males, with an overall incidence of 1.81% in the total population.

5:3:4:3 Hypothyroidism

Nine patients, consisting of 8 females and 1 male had been previously diagnosed and treated for hypothyroidism. The mean age of this group on admission to the survey was 57.5 years (range 28-75 years). Of these, 4 patients were suffering from depression, 2 from schizophrenia, 2 from myxoedema psychosis and 1 from senile dementia.

The prevalence of established hypothyroidism was therefore 0.85/100

Table 5 The incidence of recognised and unrecognised thyroid disorders in the psychiatric population (n = 1544)

(Actual numbers of patients are shown in parentheses)

	Previously diagnosed (recognised)			Identified In Survey (unrecognised)			Overall		
	Male	Female	Whole Pop^{n}.	Male	Female	Whole pop^{n}.	Male	Female	Whole Pop^{n}.
Group									
Hypothyroid	0.17 (1)	0.85 (8)	0.58 (9)	nil (0)	1.06 (10)	0.65 (10)	0.17 (1)	1.91 (18)	1.23 (19)
Hyperthyroid	0.17 (1)	1.59 (15)	1.04 (16)	nil (0)	0.32 (3)	0.19 (3)	0.17 (1)	1.91 (18)	1.23 (19)
Euthyroid (With goitre)	nil (0)	0.32 (3)	0.19 (3)	nil (0)	nil (0)	nil (0)	nil (0)	0.32 (3)	0.19 (3)
Total Incidence In Group	0.34 (2)	2.76 (26)	1.81 (28)	nil (0)	1.38 (13)	0.84 (13)	0.34 (2)	4.14 (28)	2.65 (41)

The above figures are representative of the incidence (%) of thyroid disease in the specific population quoted.

Figure 25

The Incidence of Previously Diagnosed and Undiagnosed Thyroid Disorders, Identified in the Psychiatric Population (n = 1544)

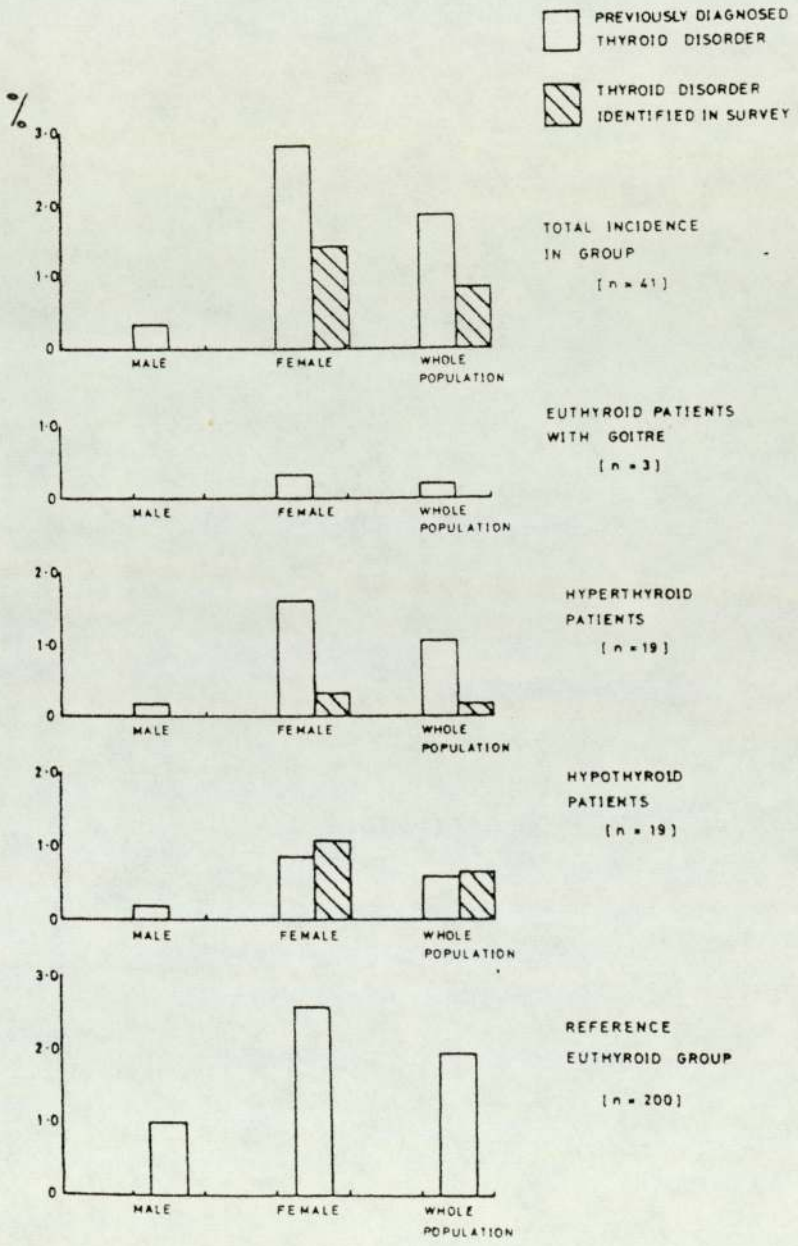
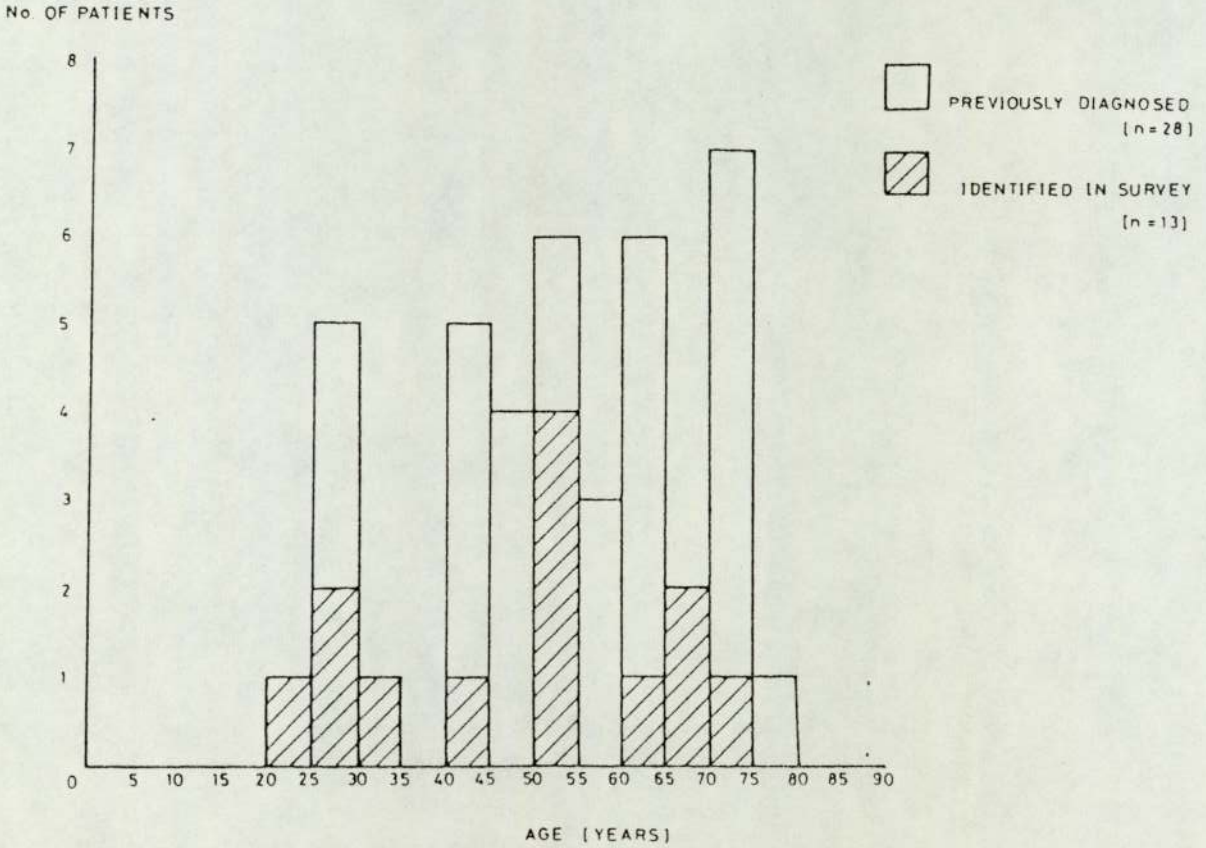


Figure 26

Relationship Between the Number of Patients Presenting with Thyroid Abnormality, and Age, in the Psychiatric Population (n = 41)



females and 0.17/100 males. The incidence of recognised hypothyroidism in the whole population was thus 0.58% when both males and females were included.

5;3:4:4 Hyperthyroidism

Sixteen patients, consisting of 15 females and 1 male, had been previously diagnosed and treated for hyperthyroidism. The mean age of this group on admission was 55.4 years (range 30-80 years). With reference to psychiatric disorder, 11 patients were suffering from depression, 2 from schizophrenia, 2 from senile dementia and 1 from phobic anxiety.

The prevalence of established hyperthyroidism was therefore 1.59/100 female and 0.17/100 males. The incidence of recognised hyperthyroidism in the total population was thus 1.04% when both females and males were included.

5:3:4;5 Euthyroid with an abnormal gland

Three patients (all female) had been previously diagnosed euthyroid, although clinically an abnormal thyroid gland was detected. Two subjects were shown to have a non-toxic goitre and 1 to have a TB gland. The mean age of these patients on admission to the survey was 53.7 years (range 30-73 years) and all were suffering from depression.

The prevalence of a euthyroid thyroid disorder was therefore 0.32/100 females and the incidence in the whole population was 0.19%

5:3:4:6 Thyroid disorders identified by the survey

The total number of patients with thyroid abnormalities identified by the survey was 13, all of whom were female. The prevalence of unrecognised thyroid disorders was therefore 1.38/100 females, with an overall incidence of 0.84% in the whole population. The incidence of

unrecognised thyroid disorders relative to age is illustrated in Figure 27

5:3:4:7 Hypothyroidism

Ten patients (all female) with previously undiagnosed hypothyroidism were identified by the survey. The mean age of this group on admission was 53 years (range 30-73 years). With reference to psychiatric disorder, 5 patients were suffering from depression and 5 from schizophrenia.

The prevalence of unrecognised hypothyroidism was therefore 1.06/100 females with no males being identified. The incidence of unrecognised hypothyroidism in the whole population was thus 0.65%, all of whom were female.

5:3:4:8 Hyperthyroidism

Three patients (all female) with previously undiagnosed hyperthyroidism were identified in the survey. The mean age of this group on admission was 39.7 years (range 24-69 years). With reference to psychiatric disorder all three patients were suffering from depression.

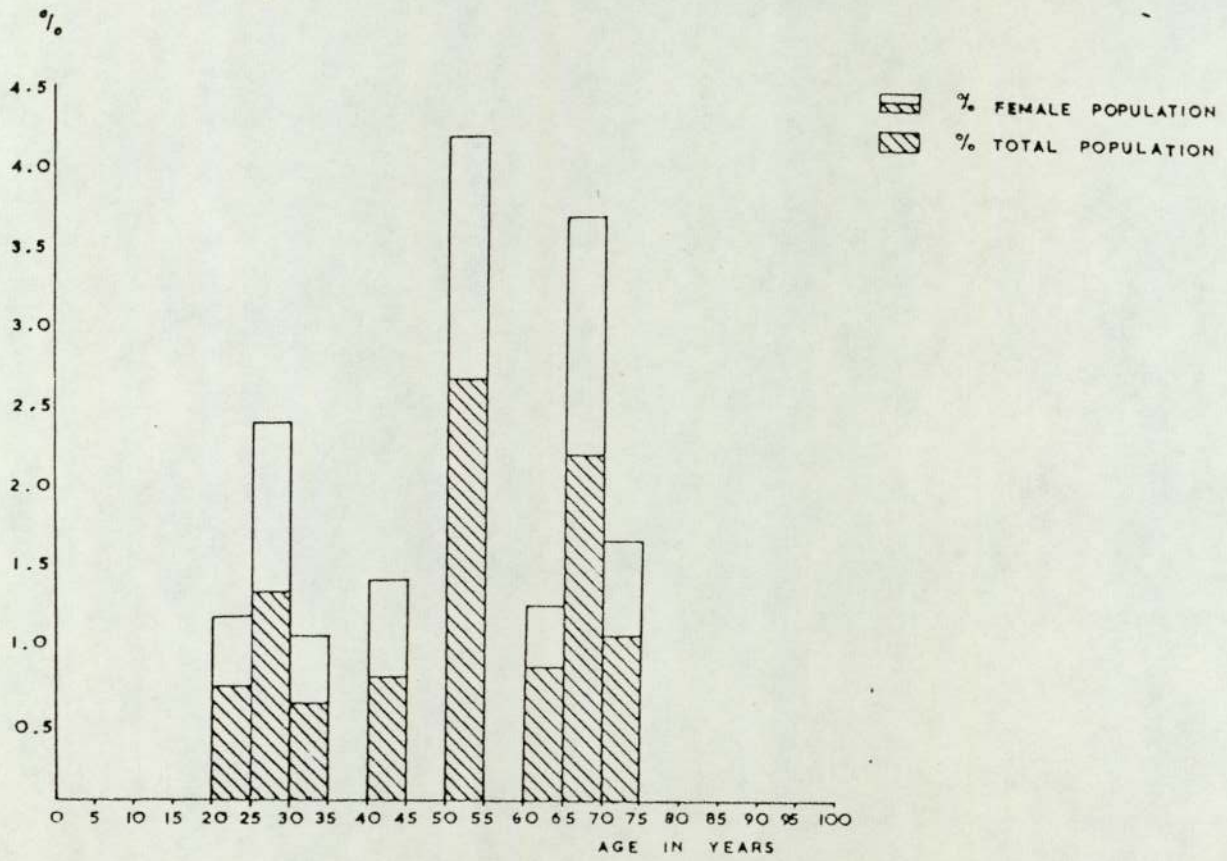
The prevalence of unrecognised hyperthyroidism was therefore 0.32/100 females with no males being recorded. The incidence of unrecognised hyperthyroidism in the total population was thus 0.19%, all of whom were female.

5:3:5 The effect of chemotherapeutic agents on the population studied, with reference to alteration of thyroid function.

Various drugs are known to affect thyroid hormone levels directly or indirectly and this aspect has been discussed further in Appendix 2 With reference to this, all the patients included in the study were questioned regarding past and present medication, and any subject found to have disturbed thyroid function, as a direct consequence of drug therapy, was excluded from the statistical evaluation of the survey.

Figure 27

The Incidence of Unrecognised Thyroid Disorders Relative to Age in the Psychiatric Population (n = 13)



However, in an attempt to demonstrate the marked effect certain drugs can have upon thyroid metabolism and how often the psychiatrist/clinician may be misled by the biochemical evidence, a case history of a patient who overdosed on salicylate is presented (case No.3).

5:3:6 Case Histories

For the thirteen patients with previously unrecognised thyroid dysfunction, detailed case histories were prepared and are presented.

CASE HISTORY No. 1.

Mrs. T.R., a divorced 32 year old white female, was admitted on 18th March 1976.

On admission she was unable to give a clear or logical account of herself and had marked thought disorder, with delusions and hallucinations. Initially she required large doses of phenothiazines to stabilise her aggressive and uncontrolled behaviour. Her past psychiatric history included a short admission under Section 29 of the Mental Health Act, to another psychiatric hospital for a similar episode. She had occasionally taken lysergic acid (LSD) several years before admission.

On examination she presented as an obese lady with symmetrically and equally depressed tendon reflexes. Her blood pressure (BP) was 100/60 mm Hg with a pulse of 88 SR regular; otherwise physical examination was normal. Routine biochemical investigations were normal and her haemoglobin (Hb) was 13.5 g/dl, with an erythrocyte sedimentation rate (ESR) of 10 mm/hr. Thyroid function tests at the time of admission indicated gross hypothyroidism with a serum T^4 of 27 nmol/l, a T^3U of 127%, an FTI of 21, and a TSH of greater than 64 μIu/ml. The serum was turbid and the fasting cholesterol was 7.3 nmol/l and triglycerides were elevated at 3.4 nmol/l; the β and pre-β lipoprotein factions were also raised. An initial provisional diagnosis of schizophrenia was changed to myxoedema psychosis and T^4 in addition to depot major tranquillisers was prescribed. On this treatment regime she improved sufficiently to be discharged two months after admission.

She was readmitted two weeks later in a disturbed state and threatening suicide, but without psychotic symptoms. Non-compliance with treatment was the most likely cause for readmission. She was

discharged one month later with normal thyroid function tests and T^4 was recommended indefinitely. She has not since needed psychiatric supervision.

CASE HISTORY No. 2.

Mrs. A.M., a 23 year old separated white female, was admitted on 2nd February 1976, following a domiciliary visit by a Consultant Psychiatrist at her General Practitioner's request.

On admission she complained of anxiety and depression which had gradually increased over the previous seven months. She said her problems were due to worry over her husband's physical and mental abusiveness towards her, and had reached a peak when he finally left her. There was no previous physical illness.

On examination she presented as a thin, anxious and restless woman and her BP was 120/88 mm Hg with a pulse of 76 SR regular; an ejection systolic murmur was heard at the apex of the heart. There was no tremor of the hands, exopthalmos or any other clinical features of disturbed thyroid function, besides those noted above. Routine biochemical investigations were normal and her Hb was 15.3 g/dl with an ESR of 5 mm/hr. Thyroid function testing indicated a markedly elevated serum T^4 of 238 nmol/l, a T^3U of 101%, an FTI of 234 and a T^3 of 3.56 nmol/l. Cholesterol, triglycerides and lipid electrophoresis were within normal limits. A diagnosis of hyperthyroidism with reactive anxiety and depression was made and treatment with tricyclic antidepressants started.

During the time after admission, her thyroid function tests settled to within the "high normal range" and she improved sufficiently to be discharged to the out-patient department. In the ensuing two months there occurred two episodes of deliberate overdosing with prescribed

medications. Admission to a general hospital on these occasions revealed her thyroid function still to be in the high normal range and she appeared clinically euthyroid. She continued to receive psychiatric therapy and also received support from the Social Services. In August of the same year she suffered a recrudescence of her anxiety and also developed a swelling of the neck. On examination she appeared to be mildly thyrotoxic and biochemical testing again revealed elevated thyroid function tests. Antithyroid therapy was commenced and she was treated with carbimazole. When she had returned to a euthyroid state in October, a massive pulsatile thyroid was removed at operation and histology confirmed a severe toxic gland. She made an excellent recovery from her thyroidectomy and since operation has not required psychiatric care.

CASE HISTORY No.3.

Mrs. B.R., a Caucasian woman aged 29 years, was admitted from a district general hospital following self-poisoning with nitrazepam, amitryptyline, chlorpromazine and procyclidene. On admission she complained of recurrent depression over the previous nine years, with a history of weight loss, poor appetite and suicidal tendencies. There had been several previous attempts at suicide necessitating admissions to general and psychiatric hospitals. At the time of admission she was clearly depressed and voiced suicidal ideas and during her stay as an in-patient, took a further overdose of salicylates.

Physical examination was unremarkable, as were routine biochemical and haematological investigations. The results of thyroid function testing were a serum T^4 of 53 nmol/l, a T^3U of 128% an FTI of 41 and a TSH of 23 μIu/ml, indicating mild hypothyroidism. However her thyroid disturbance was not specifically treated, since it was thought that the overdose of drugs, especially chlorpromazine, prior to admission,

resulted in her slightly depressed thyroid function. One week after admission, she overdosed on salicylates which also are reported to reduce serum T^4 levels. Therefore further thyroid function tests were performed and revealed a serum T^4 of 49 nmol/l, a T^3U of 124%, an FTI of 39 and a TSH of 30 μIu/l. Again no specific thyroid therapy was given and she was treated with various antidepressants and E.C.T. making a slow and uneventful recovery during the following year.

Five months after admission her thyroid function tests showed a serum T^4 of 78 nmol/l, a T^3U of 120%, an FTI of 64 and a TSH of 2.0 nmol/l. The return of her thyroid function to normal, without specific treatment, suggested that her thyroid underactivity was probably due to drug effects. However, she was referred to a Consultant Physician for a further independent assessment of her possible thyroid dysfunction and was finally diagnosed as mildly hypothyroid. Subsequently she was treated with T^4 and currently no longer requires psychiatric care.

CASE HISTORY No.4.

Mrs. M.T., aged 48 years was admitted with depression and suicidal ideation. She had a previous history of psychiatric hospital admissions with hypomania and depression.

Physical examination, routine biochemical and haematological investigations were all normal. Thyroid function testing revealed a serum T^4 of 55 nmol/l, a T^3U of 118% an FTI of 46 and a TSH of 11 μIu/ml.

No specific thyroid therapy was given as the biochemical results were equivocal, although mild hypothyroidism was suspected. She was therefore treated with antidepressants and E.C.T but subsequently developed hypomania. This necessitated a change of medication to chlorpromazine and lithium carbonate and there followed a slow recovery, although she

relapsed on several occasions.

At follow up one year later, while on prophylactic lithium, her thyroid function tests revealed a serum T^4 of 48 nmol/l, a T^3U of 127% an FTI of 38 and a TSH of greater than 64 μIu/ml. The effect of lithium upon thyroid metabolism was considered but due to the marked elevation of her serum TSH level, she was diagnosed as hypothyroid. Treatment was commenced with T^4 and a rapid improvement in her mental state occured with no further relapses. Her psychiatric therapy was subsequently reduced and repeat thyroid function tests were normal.

CASE HISTORY No.5.

Mrs. K.T., aged 55 years was admitted with paranoidal depression.

Physical examination and routine biochemical and haematological investigations were normal. Thyroid function testing indicated a serum T^4 of 63 nmol/l, a T^3U of 114%, an FTI of 55 and a TSH of 25 μIu/ml.

A diagnosis of mild hypothyroidism was made and treatment included antidepressants, major tranquillisers and E.C.T; T^4 was added at a later date. When seen in follow up, one year later, she was completely symptom-free and her thyroid function tests were normal.

CASE HISTORY No.6.

Mrs. W.W., a widow aged 75 years was admitted with endogenous depression. She had been admitted on two previous occasions with a similar complaint.

Physical examination and routine biochemical and haematological investigations were normal. Thyroid function was found to be reduced and indicated a serum T^4 of 31 nmol/l, a T^3U of 114% an FTI of 27 and a TSH of 114 μIu/ml. An ECG also showed typical myxoedematous changes.

A diagnosis of hypothyroidism was made and treatment was commenced with antidepressants and T^4 was added at a later date. There followed a slow improvement in her mental state and at follow-up two years later she was symptom-free and her thyroid function tests were normal.

CASE HISTORY No. 7.

Mrs. M.J., aged 64 years, was admitted following a domiciliary visit, with a history suggestive of late onset schizophrenia with auditory hallucinations, agitation and insomnia during the previous two weeks. She had also been admitted twice previously with similar symptoms.

Physical examination and routine biochemical and haematological investigations were normal. Thyroid function was markedly depressed with a serum T^4 of 22 nmol/l, a T^3U of 119%, an FTI of 18 and a TSH of 37 μIu/ml.

A diagnosis of hypothyroidism was made and treatment was commenced with T^4 and a small dose of chlorpromazine. At follow up three years later she remained well and symptom-free; thyroid function was also normal.

CASE HISTORY No.8.

Mrs. B.H., aged 32 years was admitted under Section 26 of the Mental Health Act with acute florid paranoid schizophrenia.

Physical examination and routine biochemical and haematological investigations were normal. Thyroid function was marginally depressed with a serum T^4 of 60 nmol/l, a T^3U of 112%, an FTI of 54 and a TSH of 55 μIu/ml.

Hypothyroidism was diagnosed and treatment was commenced with T^4 and major tranquillisers. Although she remained psychotic there was some improvement in her mental state and she became composed and largely symptom-free. One year after admission her thyroid function tests were normal.

CASE HISTORY No.9.

Miss A.M., aged 25 years was admitted from the out-patient department complaining of agitation,weepiness and depression. She came from an unstable background, her parents having divorced, and had a history of many abortions during the past seven years. On several previous occasions she had attempted suicide, by either self-poisoning or gassing.

Physical examination and routine biochemical and haematological investigations were normal. Thyroid function tests at the time of admission showed a serum T^4 of 192 nmol/l, a T^3U of 111%, an FTI of 172 and a T^3 of 3.3 nmol/l.

Mild hyperthyroidism was diagnosed, although the patient lacked any clinical features of the disease and therefore no specific thyroid treatment was given. Initially, only a slight improvement in her psychiatric state occured, despite treatment with antidepressants and tranquillisers. Further thyroid function tests, 11 months later, still indicated mild thyrotoxicosis although no typical features of hyperthyroidism were seen. She was therefore referred to a Consultant Physician for confirmation of this diagnosis, and was finally treated with the antithyroid drug, carbimazole. No marked improvement in her psychiatric state occurred and she was further readmitted after 3 months suffering from anxiety and depression. Shortly following readmission, she voluntarily discharged herself from psychiatric care and was lost from further study. Her thyroid function tests, prior to discharge, still indicated mild thyrotoxicosis and her mental state had not improved.

CASE HISTORY No.10.

Mrs. E.B., was admitted following a domiciliary visit at her General Practitioner's request. Over the previous several months there had been increasing self-neglect, depression, agitation and confusion at home. There was no previous psychiatric history.

Physical examination revealed a 69 years old widow. Her BP was 190/110 mm.Hg. and a soft pan systolic murmur was detected in the mitral area; a goitre was also found. Routine biochemical and haematological investigations were normal. Thyroid function testing revealed a serum T^4 of 173 nmol/l, a T^3U of 104% an FTI of 166 and a T^3 of 3.3 nmol/l. X-ray of the thoracic inlet demonstrated a large goitre extending retrosternally to the aortic knuckle and causing deviation to the left of the cervical trachea.

She was diagnosed mildly hyperthyroid and treatment was commenced with carbimazole and propranolol. During the following year her mental state improved markedly, although she remained mildly agitated and confused on occasions. Her thyroid function tests returned to normal within this period. Psychiatric therapy, with antidepressants and major tranquillisers, was commenced five months after admission.

CASE HISTORY No.11.

Miss C.K., aged 43 years, was admitted with endogenous depression and obsessional symptoms.

Physical examination and routine biochemical and haematological investigations were normal. Thyroid function testing indicated a serum T^4 of 49 nmol/l, a T^3U of 113%, an FTI of 43 and a TSH of 20 μIu/ml.

A diagnosis of hypothyroidism was made and she was treated initially with tricyclic antidepressants and E.C.T; T^4 was added at a later date.

There followed an uneventful recovery and she was discharged well. At follow-up one year later she remained symptom-free and her thyroid function tests were normal.

CASE HISTORY No.12.

Mrs. C.F., aged 56 years, was admitted following a domiciliary visit under Section 25 of the Mental Health Act, with a history of paranoid schizophrenia. She had been admitted ten years previously with schizophrenia, being discharged well from out-patients two years later.

Physical examination, routine biochemical and haematological investigations were normal. Thyroid function testing revealed a serum T^4 of 70 nmol/l, a T^3U of 123% an FTI of 56 and a TSH of 26 μIu/ml.

Typical features of myxoedema were not detected and the patient therefore received no thyroid therapy, despite her depressed thyroid function. She was treated with chlorpromazine and E.C.T. and later was maintained on Modecate. She was readmitted one year later with a recrudescence of her symptoms. Thyroid function results showed a serum T^4 of 64 nmol/l, a T^3U of 128%, an FTI of 50 and a TSH of greater than 64 μIu/ml. She was therefore diagnosed hypothyroid, but unfortunately moved away from the area before treatment was commenced. Further follow-up has been subsequently unsuccessful.

CASE HISTORY No. 13.

Mrs. F.H., aged 56 years was admitted complaining of depression with a previous history of epilepsy and recurrent falls.

Physical examination revealed a pale,thin and undernourished lady, due to self neglect. Routine biochemical investigations were normal but her Hb was 7.6 g/dl, indicating severe anaemia. The results of thyroid function testing were a serum T^4 of 52 nmol/l, a T^3U of 94%, an FTI of 54 and a TSH of 18 μIu/ml.

An initial diagnosis of mild hypothyroidism was made, although no specific treatment was given. Prior to admission she was not receiving phenytoin for her epilepsy and therefore the possibility of drug interference was eliminated. Treatment for her epilepsy was commenced and antidepressants were added. She was subsequently treated with antiepileptic and antidepressant drugs but unfortunately she died shortly after discharge from hospital and therefore further follow-up studies were not possible.

5:3:7 Relationship between psychiatric diagnosis and thyroid hormone levels

A comparison of mean ($\pm$ 1SD) concentrations for serum T^4 , T^3U and FTI in male and female patients with schizophrenic psychoses, affective disorders including depression, neurotic disorders, senile dementia, epilepsy and alcohol/drug psychoses in shown in Table 6 One thousand and ninety four subjects were included in the above categories and are representative of all the patients encountered during the survey with those specific disorders. The effect of drugs upon the thyroid has been discussed in Appendix 2 and the results of any patients receiving agents known to affect thyroid function were carefully interpreted.

Similar serum T^4 and FTI mean values were observed for males and females with schizophrenic psychoses, affective psychoses and senile dementia, although when compared to the reference euthyroid group, these values were significantly higher. Mean values for patients suffering from neurotic disorders were significantly lower than the previous three categories and reduced further in the epileptic group. Only in the latter group were the mean T^4 and FTI values lower than those found in the euthyroid group.

Serum T^3U levels were similar for all psychiatric groups with the exception of the senile dementia and alcohol/drug abuse patients, where a slight reduction in serum T^3U was observed. T^3U levels in the reference euthyroid group compared favourably with those of all the psychiatric categories.

The distribution of the serum FTI, compared to the reference group for all the psychiatric categories is shown in Figure 28

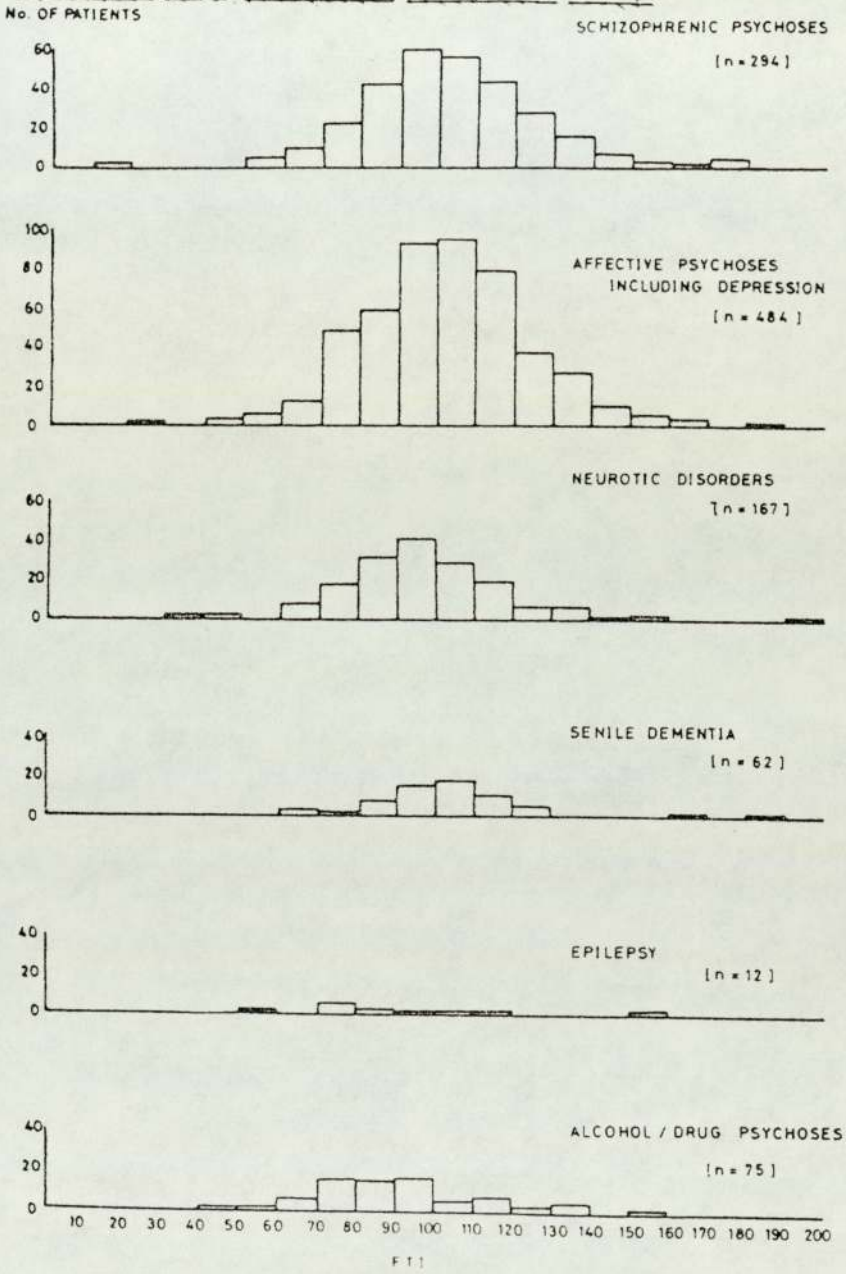
Table 6

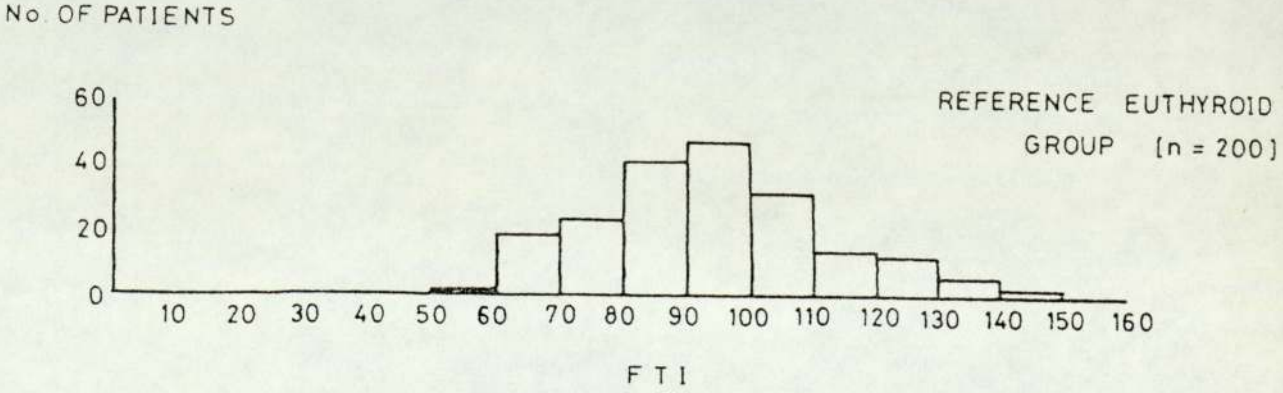
Comparison of the mean (± 1SD) concentration for serum T^4, T^3U and FTI in male/female patients with various psychiatric disorders (n = 1094)

Diagnosis	Schizophrenic Psychoses	Affective Disorders	Neurotic Disorders	Senile Dementia	Epilepsy	Alcoholism/ Drug Abuse	Euthyroid Group	
							Male	Female
No. of patients (Male and Female)	294	484	167	62	12	75	200	
Age (years)	41 (± 16)	53 (± 15)	32 (± 11)	75 (± 11)	43 (± 14)	44 (± 14)	37 (± 13)	35 (± 10)
Serum T^4(nmol/l)	109.8 (± 24.0)	110.1 (± 23.1)	105.6 (± 23.4)	108.2 (± 21.7)	95.3 (± 23.4)	96.2 (± 22.1)	97.5 (± 20.7)	101.8 (± 21.7)
T^3U (%)	106.9 (± 7.4)	107.2 (± 7.6)	108.6 (± 7.8)	104.9 (± 11.1)	107.1 (± 7.6)	105.2 (± 7.9)	104.8 (± 7.2)	107.3 (± 7.2)
FTI	102.0 (± 21.3)	101.9 (± 20.9)	96.9 (± 20.4)	101.9 (± 19.9)	89 (± 25.2)	90.2 (± 22.5)	92.5 (± 18.6)	94.8 (± 17.7)

Figure 28

The Distribution of Serum FTI in Patients with Various Psychiatric Disorders, Compared to a Reference Euthyroid Group.





5:4 Discussion

The results obtained in this study of an acute psychiatric population have indicated that a clinically significant number of patients with thyroid disorders, especially females with hypothyroidism, may pass unrecognised and be therefore deprived of optimum treatment. In the Highcroft population, the total incidence of thyroid dysfunction represented 2.65% and the prevalence of thyroid abnormality in females was 4.14% and in males, 0.34%.

In a similar study of an adult community in Whickham, County Durham (Tunbridge et al., 1977), the overall incidence of thyroid dysfunction was 2.7% and therefore the prevalence of thyroid disease in the Highcroft group was not significantly different from the general population. The small reference group of hospital patients which were selected in the present survey provided an overall incidence of thyroid abnormality of 2.86% which again was similar to that quoted by Tunbridge et al. (1977).

Research carried out by McLarty et al., (1978) on a psychiatric population of in-patients resulted in an overall prevalence for thyroid dysfunction of 1.2% (or 2.0% in females). The authors concluded that since these results were similar to those found for the general population, this argued against there being a reservoir of clinically significant, undiagnosed thyroid disease in psychiatric in-patients. This finding suggests that the overall prevalence rate of thyroid disorders is lower in the chronic psychiatric population, compared to the acute out-patient group currently under study.

In the Highcroft patients, the overall incidence of previously diagnosed hypothyroidism was 0.58%; this figure being rather lower than

that reported by Tunbridge et al., (1977) who quoted a figure of 0.8% in established cases rising to 1.1% when all non-confirmed cases of hypothyroidism were included. Several other researchers have quoted even higher figures, but many of these have been carried out on elderly (Bahemuka and Hodgkinson,(1975) or psychogeriatric patients (Henschke and Pain, 1977),in whom hypothyroidism is more commonly found. The lower incidence figure obtained for the present study may in part be explained on this basis, since the Highcroft population was a predominantly younger group.

The incidence of hypothyroidism for new cases detected during the survey was 0.65% and this figure is approximately twice that reported by Tunbridge et al., (1977) for the corresponding group in the general population, i.e. 0.33%. All ten patients identified as hypothyroid were female and the prevalence of unrecognised hypothyroidism in the female Highcroft population was therefore 1.06%. This represents a considerable increase over that quoted by Tunbridge et al., (1977) especially since all the subjects diagnosed as overtly hypothyroid in their survey were female. Other workers have also revealed lower prevalence rates for untreated hypothyroid females and in a recent screening programme of 3,885 women for thyroid disease, Kagedal et al., (1981) found a prevalence rate of 0.64%.

McLarty et al., (1978) found hypothyroidism was not a major problem in the psychiatric in-patient population that he investigated; he reported an overall prevalence of 0.5% (0.78% in females, 0.18% in males).

The reasons for the high incidence of unrecognised hypothyroidism in the Highcroft group are unclear. It has been known for a long time that myxoedema may present as one of several psychiatric illnesses, but that

paranoid or depressive psychoses are the most common (Easson, 1966). Psychiatric symptoms often dominate the clinical picture and the current diagnosis may be difficult to obtain. The classic symptoms of overt hypothyroidism are often not present in a particular patient and depression may be the only early manifestation of the disease (Evered et al., 1973; Brown, 1980). Patients often complain of tiredness, loss of weight, decreased appetite and apathy and these symptoms may be misinterpreted. Previous workers have stated that the psychiatrist must always be aware that hypothyroid patients may appear clinically depressed or delirious with few, if any, of the physical signs of thyroid disease (Pitts and Guze, 1961). For these reasons, patients with atypical symptoms of myxoedema may pass unrecognised, particularly when the insidious nature of the disease is considered. The high incidence of new cases of hypothyroidism detected in this study may be explained by these diagnostic difficulties. Also Gold et al., (1981) in an investigation of 250 patients admitted to a psychiatric hospital for treatment of depression, reported that only 10% of hypothyroid patients could have been identified by serum T^4 and T^3U measurement alone. Further, they stated that this low yield may help to clarify the low incidence reported in the literature for hypothyroidism in psychiatric patients, given the higher incidence of myxoedema presenting as depression reported in several clinical trials. This evidence therefore gives further support for the finding of a relatively high incidence of unrecognised hypothyroidism in the present survey.

In the Highcroft population the prevalence of patients with previously diagnosed hyperthyroidism was 1.04% and this figure represented the highest incidence for any group. Sixteen patients were identified (15 female and 1 male) and the incidence within the respective population

was 1.59% for females and 0.17% for males. Previous work has shown that the prevalence of thyrotoxicosis varies between 0.3% and 2.3%; the variation being due to both the methods used for the assessment of thyroid function and the nature of the population studied (Thomson et al., 1972; Jeffreys, 1972). Tunbridge et al.,(1977) reported that the prevalence of hyperthyroidism in established cases was 1.1% rising to 1.6% when all non-confirmed cases were included. It is possibly significant that in both the Highcroft and Tunbridge populations, the previously diagnosed hyperthyroids were the largest groups.

Finally, the prevalence of patients with unrecognised hyperthyroidism was 0.19% (all female). This figure is considerably lower that that quoted by Tunbridge in which a figure of 0.45% is given; Kagedal et al., (1981) similiary reported an incidence of untreated hyperthyroidism of 0.57% in the female group of patients they investigated. The low incidence found in the Highcroft population, may be explained by the fact that general practitioners are acting as a less effective screen for hypothyroids than thyrotoxics, where symptoms are more easily recognisable than those of myxoedema.

As previously discussed, 13 patients with unrecognised thyroid disorder were identified in the survey and all of these were female. Ten patients were hypothyroid and of these 5 were suffering from depressive illness and 5 from schizophrenia. Hyperthyroidism occured in 3 patients, with coexisting depression as the major psychiatric abnormality. The effect of certain drugs in suppressing thyroid function was noted in two hypothyroid patients (Case No 3 and 4), although subsequently a diagnosis of mild myxoedema was confirmed in both subjects. Several chemotherapeutic agents in common usage within psychiatric hospitals interfere with thyroid metabolism (See Appendix 2) and the psychiatrist

must therefore be aware that misleading biochemical results may be obtained in patients receiving these medications.

The present study indicates the contribution of thyroid dysfunction to morbidity in acute psychiatric patients and in particular how both hypo- and hyperthyroidism may be overlooked, especially in females who lack classical signs and symptoms of the disease. Of the 13 patients with unsuspected thyroid disorder, 11 received definite treatment with either T^4 or carbimazole, together with appropriate therapy for their psychiatric disorder. At follow up, two years or more after initial admission, 7 patients were sufficiently well and symptom free, to be discharged from psychiatric surveillance. A further 4 patients showed an improvement in their mental state but retained some psychiatric disability. Of the two remaining patients, one died shortly after discharge from psychiatric care and one was lost from follow up, shortly after commencing treatment for her thyroid disorder.

At the inception of this work it was intended to investigate the affectiveness of thyroid therapy per se, in effecting a reduction in psychiatric symptoms, but unfortunately this was not possible because of ethical reasons. Optimal treatment of individual patients could only be provided by the use of both psychiatric and thyroid therapy and therefore this line of investigation was not pursued. However in several cases, the addition of thyroid therapy to pre-treatment with psychiatric agents resulted in a marked improvement in the patients clinical state.

The association between thyroid disorders and psychiatric abnormalities lacks satisfactory explanation of the fundamental mechanisms involved, although considerable research has been carried out. The importance of thyroid hormones in maintaining the structural and

biochemical integrity of the central nervous system has been known for many years and both serum T^3 and T^4 are associated with both neurophysiological and behavioural processes in the adults (Nicholson and Altman, 1972). In rats, thyroid hormones have been shown to stimulate protein metabolism and synthesis of nucleic acids leading to increased synthesis of brain proteins (Weichsel, 1974). In contrast, neonatal thyroidectomy has been shown to decrease the metabolism of protein and nucleic acids (Geel and Timiras, 1967). Decreased incorporation of amino acids into proteins in the cerebral cortex of thyroidectomised rats was demonstrated by these workers and was dependent upon magnesium, potassium and sodium ion concentrations. Alterations in electrolyte concentrations within brain cells has been shown in patients with affective disorders, particularly depression (Frizel et al., 1967), and other metabolic and psychic disturbances have been associated with both thyroid deficiency and affective illness (Whybrow et al., 1969). Biochemical studies have shown that patients with hypothyroidism and depression, both demonstrate a diminished response to infused noradrenalin (Prange et al., 1967) and also both are characterised by a high urinary excretion of catecholamines and their metabolites. Whybrow and Ferrell (1974) reported that many of the symptoms commonly seen in myxoedema are also found in depressed patients, for example somnolence, slowness of speech, reduced sensory capacity, lack of energy, social withdrawal and altered sleep pattern.

Several researchers have indicated that depressed patients have reduced thyroid hormone levels and Wilson et al.,(1973) found that depressed patients also showed a reduced TSH response to the administration of TRH; similar results have been reported by Pühringer et al., (1975) and Gold et al., (1979). This effect has been observed

particularly in females, and at present the reason for the apparent defect in the hypothalamic - pituitary - thyroid axis of depressed patients remains unclear. Early workers reported an antidepressant action of TRH and this effect was stated to be powerful, but transient (Prange et al., 1972; Kastin et al., 1972). However, the beneficial effect of TRH in patients with depression is controversial and more recent studies have failed to substantiate these early observations (Prange et al., 1979). TRH administration does lead to mild euphoria in normal subjects and to an increased sense of well-being in the alcohol - withdrawal syndrome (Prange et al., 1979) and these responses may reflect direct central effects of TRH on the autonomic nervous system. Further work is necessary to fully elucidate the mechanisms involved in the neuropharmacological effects of TRH on the central nervous system.

The association between hypothyroidism and depression has been evaluated by Gold et al., (1981) in a survey of 250 patients referred to a psychiatric hospital. It was foundthat hypothyroidism coexisted and was aetiologically related to depression, although these workers concluded that without prospective study of individual patients, the question of whether the primary disturbance producing the depression or anergia was related to thyroid dysfunction, could not be answered. These findings are consistent with the present results, where 9 patients diagnosed as hypothyroid had coexisting depression. However, depressive illness is not inevitably associated with myxoedema, since 14 subjects in this category were hyperthyroid, an unexplained finding when the biological effects of thyroid hormone upon the central nervous system are considered.

The link between the thyroid and alterations in behaviour may also

be indirect and involve the adrenal (Prange et al., 1979). Thus the thyroid can stimulate the clearance of various steroids, including cortisol from the liver; decreased clearance of cortisol being observed in hypothyroidism, due to the direct effect of lowered thyroid hormone levels reducing enzyme activity in the liver. Singhal and Rastogi (1978) studied the relationship between behaviour and altered biogenic amine metabolism in the brain, induced by changes in cortico steroid levels. They found that reduced corticosteroid levels resulted in a general rise in brain catecholamine turnover. Hyperthyroidism results in lowered serum cortisol levels and an increased turnover of noradrenalin in brains of manic patients has been observed (Messiha et al., 1970). Singhal and Rastogi, (1978) concluded that on the basis of their findings, the depressed behaviour of hypothyroid animals may be associated with decreased synthesis and turnover of brain monamines including noradrenalin, dopamine and 5 - hydroxytryptamine. Conversely, the behavioural excitability of hyperthyroid animals may be associated with enhanced activity of noradrenergic, dopaminergic and 5 -hydroxytryptaminergic neurons in the brain. However much of the work of Singhal and Rastogi (1978) was performed in neonatal rats and these workers stated that caution must be exercised in extrapolating these results to adult animals. Whether the association between the thyroid and behaviour does involve the adrenal is not clear, especially as there are inherent dangers in postulating from animal experiments the possible mechanisms involved in humans. This is further emphasised by the current study, where the majority of patients with depression were hyperthyroid rather than hypothyroid, this contradicting the work of Singhal and Rastogi.

Traditionally, researchers have demonstrated that hyperthyroidism produces psychological symptoms including emotional lability, restlessness

irritability, over-reactiveness with predominant anxiety and tension (Whybrow and Ferrell, 1974) and more severe disorders may be encountered if such patients are left untreated. Clower et al., (1969) reported that both thyrotoxicosis and myxoedema can masquerade under a variety of clinical psychiatric conditions, especially that of severe depression. One case was identified where a patient with severe depression was both thyrotoxic and later mildly myxoedematous. As previously mentioned, depression has been associated with a relative deficiency of catecholamines due to a decreased synthesis of noradrenalin. However other workers have shown that adrenocortical activity is elevated in some depressive disorders, resulting in elevated levels of catecholamines (Woodbury, 1958) due to the involvement of steroid hormones in their biosynthesis.

The question as to whether abnormal thyroid function is the result or the cause of mental disorders is still unanswered, although recent evidence favours the alteration of thyroid hormone levels as the causative agent. However the mechanisms involved are far from clear and although depression is classically associated with hypothyroidism and anxiety symptoms with hyperthyroidism, the present study illustrates that this is not an invariable finding. Several biochemical investigations have, in part, explained the mechanisms involved, yet little work has been carried out into the underlying cause of depression when associated with hyperthyroidism.

The current survey examined differences found in the thyroid function tests, serum T^4, T^3U and FTI, between various psychiatric categories and a reference euthyroid population (Table 6). Serum T^4 and corresponding FTI mean levels in the psychiatric groups, which included schizophrenic psychoses, affective disorders, and senile dementia, were

similar, although significantly higher than the reference euthyroid population. McLarty et al., (1978) also investigated the differences occuring in thyroid hormone levels between various psychiatric groups and found serum T^4 levels to be significantly reduced in patients with senile dementia compared to those found in schizophrenia. However, these workers attributed this effect to age differences, although recent work by Evered et al., (1978) indicated that serum T^4 levels increased with age thus confirming previous work by Britton et al., (1975). In the present work, the lower values observed for serum T^4 in the euthyroid group, compared to those seen in the three previous psychiatric categories, may in part be explained on the basis of age difference. Furthermore, subjects receiving oral contraceptives were excluded from the reference population but not from the groups of psychiatric patients suffering from schizophrenia, affective disorders and senile dementia. The reduced level of serum T^4 in the neurotic group may also be explained by age differences, especially as this population represented the youngest group studied.

Serum T^4 concentrations were lowest in the epileptic and alcohol/drug abuse groups and this was possibly due, in part, to drug interference. With particular reference to the epileptic group, the majority of patients were receiving phenytoin medication and this drug is known to lower serum T^4 levels. (see Appendix 2). Recent evidence by Yeo et al., (1978) concluded that the major effect of anticonvulsants upon thyroid function was due to an increased rate of clearance and catabolism of thyroid hormones. This applies only to serum T^4 levels and no reductions in serum T^3 levels were observed. Yeo et al., (1978) postulated that this effect was due to enhanced peripheral conversion of T^4 to T^3 brought about by exposure to phenytoin and this has also been described

by Liewendahl and Majuri (1976).

Patients within the alcohol/drug abuse group were receiving a diverse range of "inappropriate" medication and several drugs known to affect thyroid function, including phenobarbitone and salicylates were being administered. These agents have the effect of lowering serum T^4 concentrations and it is suggested therefore that this offers an explanation for the reduced serum T^4 levels found in these patients.

In summary, one must conclude that the effect of psychiatric illness upon the thyroid gland cannot be clearly established by this study, since there are several factors involved which affect the overall results and these may conceal any underlying differences. The absence of any marked differences in the thyroid parameters studied, both in the mean concentration and overall spread of results (see Figure 28 page 146) indicates that no specific alteration in thyroid hormone metabolism occurs in any single psychiatric patient group. However further work is obviously necessary to fully elucidate this complex problem, and particular attention should be applied to exclusion of the various complicating factors,the effects of drugs etc., encountered during this survey.

One question that should be considered is the potential value of screening for thyroid disease, both in apparently healthy adults and within general and psychiatric hospitals, as this has yet to be established. Although biochemical evidence, using currently employed routine tests of thyroid function, is of particular importance in the diagnosis of mild or early thyroid disease, no studies have yet demonstrated that treatment is beneficial for either minor subclinical hypothyroidism or hyperthyroidism. In a survey conducted by White and Walmsley, (1978), 500 new patients without any history of thyroid

disease were studied, with reference to the value of requesting *in vitro* thyroid function tests as a routine screen. Of this total, 58 patients were diagnosed with a high/intermediate degree of clinical suspicion of which 33% required treatment. However for those patients with only a low degree of clinical suspicion for thyroid disease (442 patients), less than 0.5% required treatment. The authors suggested that this was a poor clinical yield and concluded that thyroid function tests on patients with only one or two of the signs of thyroid dysfunction were unproductive. This study therefore lends support to clinical rather than biochemical screening of patients, providing they present with classical signs and symptoms of thyroid disease. In considering further subclinical disease, it is clear that treatment of early signs and symptoms of hyperthyroidism cannot be justified for this may induce hypothyroidism or other complications. It may therefore be concluded that general population screening for thyroid disorders, especially in adults, is of limited value and may even be contraindicated.

Whitby, (1974) claimed that the screening of large population groups was justified for a disease with a high morbidity and mortality, where an effective treatment is available and the costs justified by the anticipated benefits. Kagedal *et al.*, (1981) in a screening programme for thyroid disease in middle aged women, found that this group of patients had a high morbidity for thyroid disorders (approximately 1.2%) which was easily treated, although thyroid disorder mortality was low in such patients. This latter factor operates against setting up a screening programme, but the authors stated that the costs of thyroid screening were moderate and if a suitable target group were selected, i.e. middle aged or elderly women, screening for thyroid dysfunction was worthwhile. Hypothyroidism and hyperthyroidism

are common in these age groups (Bahemuka and Hodgkinson,1975; Rønnov-Jessen and Kirkegaard, 1973) and therefore this approach is justified particularly as such groups of patients often present without symptoms of thyroid disease. Further evidence for screening females in the early or middle 50's has also been provided by Nystrom et al., (1981) in a study of 1283 women. These workers found that the serum TSH value was valuable in predicting clinical and subclinical hypothyroidism and considered their results strong support for screening for hypothyroidism in middle-aged and older women.

With reference to screening psychiatric populations for physical illness using laboratory tests, Peet, (1981) stated that the most popular screening tests used in general psychiatry were the same as those commonly used in general medicine, rather than any specific needs of clinical psychiatry. He further recommended that in order to overcome this current unsatisfactory situation, well-conducted prospective surveys of screening tests in psychiatric patients were necessary. Weinberg and Katzell, (1977) conducted a routine endocrine screen for thyroid and adrenal dysfunction amongst psychiatric patients not on drugs and found three patients out of a total of 50 with hyperthyroidism and one patient with adrenal dysfunction; this gave a total incidence of patients with endocrine abnormalities of 8%. In a study of thyroid dysfunction in 104 female psychiatric patients, conducted by Nicholson et al., (1976) in the same psychiatric hospital as that of the present survey, the yield of significantly abnormal results in women over the age of 40, was postulated to be 8%. The authors recommended that all females in this age group should be screened for thyroid dysfunction, providing care was taken to exclude abnormal values due to pregnancy and drugs.

The overall prevalence of thyroid disorders in the present study was 2.65% of the whole population and 4.14% when only females were considered. As discussed previously, these figures are similar to those found by Tunbridge et al., (1977) for the Whickham community and must therefore question the validity of screening psychiatric populations for thyroid disease. The major value of a thyroid screening programme in psychiatric hospitals is for the detection of previously unrecognised cases of thyroid disorders, as often the patients psychotic state masks the underlying thyroid abnormality. The prevalence of unrecognised thyroid dysfunction in the current survey was 0.84% in the whole population and 1.38% of the female population; this representing a significant proportion of the total number of cases of thyroid disorder diagnosed in the survey. With reference to hypothyroidism, all new patients identified were female and the incidence in this group was 1.06%, which represents a considerable increase over that detected by Tunbridge et al., (1977) , for a similar group in the general population.

As indicated by the case histories presented, the effect of treatment on this group was remarkable with, in certain cases, a complete disappearance of psychotic symptoms and subsequent discharge of the patient from hospital management. In an analysis of a screening programme for thyroid disease by Epstein et al., (1981) where physician response, outcome, cost and health effectiveness were examined, the authors concluded that given a biochemical screening pattern is conducted for other reasons, the marginal or incremental cost of including a serum T^4 assay was more than justified by the marginal or incremental increase in health output. On this basis, screening for unsuspected thyroid disease in a psychiatric population is important, especially where the high cost of long term hospitalisation and the

value of early recognition of underlying thyroid disease, both to the patient and community are considered..

Although 41 cases of unrecognised and recognised thyroid disease were detected over a wide range in the Highcroft population, a significant number of patients in this group (34), were over 40 years of age and of these 33 were female. Clearly, the case for screening middle aged and elderly women admitted to psychiatric hospitals is therefore justified, for it is in this age group that thyroid disease often passes unrecognised. Evidence obtained from the psychiatric and biochemical surveillance of the 13 patients with unsuspected thyroid disorder, demonstrated that thyroid dysfunction may cause or aggravate psychiatric symptoms and possibly deprive patients of optimal treatment.

CHAPTER SIX

OVERALL CONCLUSIONS

6. Overall Conclusions

Thyroid dysfunction represents one of the most prevalent forms of endocrine disease and therefore the comprehensive evaluation of thyroid status provides an important role for the clinical chemistry department. This has been further emphasised by studies which have indicated that the disease may present asymptomatically and hence its diagnosis, if based solely on clinical criteria, is often unreliable and inevitably must be confirmed by laboratory investigations (Decaux and Unger, 1978). Over the past two decades there has been a rapid proliferation of *in vitro* techniques for the investigation of suspected thyroid disorders and using these, several different strategies of thyroid function assessment, have been developed (Britton *et al.*, 1975; Rootwelt and Solberg, 1978; 1981).

In this study the FTI test, together with TSH for confirmation of hypothyroidism and T^3 for confirmation of hyperthyroidism, was found to offer the best overall diagnostic discrimination of thyroid dysfunction. This involved the use of the T^3U, which proved to be a superior test to the TBG assay, except in cases where the T^3U was greater than 130%. However the improved performance of the T^3U may simply be a reflection of the extremely high precision of the assay, as methods for TBG determination have only recently been developed and are more complex than those for determination of T^3U. With the improvement in assay techniques for TBG it is probable that its diagnostic efficiency will reflect thyroid status more accurately. Furthermore TBG has an important role in the investigation of inherited abnormalities of thyroid binding proteins (Burr *et al.*, 1980). Nineteen families were studied with abnormal levels of TBG and for those patients with TBG excess, the T^4:TBG ratio, although significantly lower than in normal persons, still gave results within the

euthyroid range and was reported to be diagnostically helpful. In subjects with an absence or deficiency of TBG both the T^4:TBG ratio and the FTI gave misleading results. The value of the T^4:TBG ratio in patients with an inherited TBG excess is therefore demonstrated by this work and provides further support for its use as part of the test strategy, for thyroid assessment, of a comprehensive laboratory service.

The procedure, using FTI adopted by Britton et al., (1975) remains the initial diagnostic profile, although the serum T^3 has proved to be of little value for distinguishing suspected hyperthyroidism from euthyroidism. The T^4:TBG ratio may prove to have greater potential for resolving equivocal results within this area, although further work is obviously necessary to evaluate its relative importance.

The analysis of TBPA is considered of little value as a routine test of thyroid function, although apart from the present study little work has been carried out in connection with this protein. However familial euthyroid hyperthyroxinemia has recently been described as resulting from increased T^4 binding to TBPA (Moses et al., 1982). Therefore it would appear that TBPA measurement, similar to that of TBG, does have a clinical application but probably only at regional or sub-regional centres.

The role of dynamic tests of thyroid function will have less importance as the in vitro test procedures continue to improve (Rootwelt and Solberg, 1978). These workers concluded that even if the results of serum T^4, T^3, T^3U and TSH leave the question of thyroid dysfunction unsettled, often the TRH and TSH stimulation and T^3 supression tests fail to resolve the diagnostic difficulty.

The estimation of free T^4 has a number of theoretical advantages over

that of total T^4, especially as the level of free hormone is reported to be unaffected by the level of binding proteins. However, there is a wide range of methods in use at present and considerable variation in the levels of free hormones have been reported by different laboratories. Also no reference method exists for free T^4 and certain commercial kits are often not direct methods but are calibrated against indirect equilibrium dialysis techniques. Therefore no absolute values can be obtained; furthermore high levels of TBG and also non-thyroidal illness can both give rise to falsely elevated free T^4 levels and thus many workers consider that the FTI is the most superior index of thyroid function currently available (Rootwelt and Solberg, 1981). More extensive clinical evaluation is therefore required before the full potential of the free T^4 can be realised.

This current research, apart from its analytical work has also provided further insight into the identification of thyroid abnormalities in an acute psychiatric population. The prevalence of thyroid disease was found to be 2.65% of the total psychiatric population and the incidence of thyroid disorders in the Highcroft group was thus not significantly different from the figure of 2.7%, quoted by Tunbridge et al., (1977) for their Wickham Survey. Clearly if arguments exist indicating that general population screening for thyroid disease is not worthwhile, then it is also not justified to screen a psychiatric group of patients where the overall incidence is similar. However, 4.14% of the female patients investigated were found to have thyroid dysfunction and of these only 0.63% were under the age of 40 years. Also the number of unsuspected patients with thyroid disease, particularly female hypothyroids, was considerably higher than that quoted by Tunbridge et al., (1977) for a similar group. This evidence provides strong

support for the assessment of thyroid function in selected categories of patients. Epstein et al., (1981) stated that screening for thyroid disorders represented one of the most potentially useful components of a biochemical test panel. Both hypo- and hyperthyroidism can be subtle in their clinical presentation and both are potentially serious disorders which can be prevented or corrected by timely treatment. The value of thyroid therapy in the treatment of psychiatric patients with thyroid disease has also been clearly demonstrated in the present study and continued biochemical monitoring is recommended. The cost of health effectiveness of such screening programmes are difficult to assess but this research has indicated that such assessments of thyroid function are justified in view of the improvement in the quality of life and possibly life expectancy, for several patients.

McKeown (1968) uses the term "prescriptive screening" to describe the provision of a service for detecting presymptomatic or established disease with the intention of making a direct contribution to the health of the individual. This provision implies that prior research has shown that screening will confer some benefit on those with the condition in question and this criterion has been followed by this present work. Furthermore the cost to society of maintaining the health of an individual as an in- or out-patient of a general of psychiatric hospital, is very high and the value of screening must balance the cost of early diagnosis and treatment in relation to total expenditure on medical care. For a routine biochemistry laboratory offering a comprehensive thyroid profiling service in a district general hospital, the inclusion of a small number of extra blood samples from a psychiatric hospital would represent only modest increase in cost relative to the health output achieved. However, the introduction of a screening programme should only

be made as a deliberate policy step, preferably launched as part of a comprehensive laboratory service decided upon by a Health or Regional Authority as a result of appropriate professional advice and the fullest planning. These facts have recently been emphasised by the difficulties that have arisen from the screening for neural tube defects using alpha-fetoprotein (AFP) (Standing et al., 1981), and it is therefore important that a screening programme is well planned before its introduction.

Thyroid dysfunction is an important clinical problem which can be successfully treated in the majority of patients. This study has shown that a clinical chemistry service can make an important contribution to the investigation of thyroid dysfunction, by selective screening with modern in vitro assay techniques.

APPENDIX ONE

DISEASES OF THE THYROID GLAND

Hyperthyroidism

Hypothyroidism

DISEASES OF THE THYROID GLAND

Overaction of the thyroid gland produces the syndrome of hyperthyroidism; undersecretion causes hypothyroidism. Enlargement (goitre) of the gland may be associated with either of these conditions or may occur in a euthyroid (normal) state. A variety of clinical and biochemical features are associated with both abnormal states and these will be discussed in detail.

A 1:1 Hyperthyroidism

Hyperthyroidism as stated above is due to overproduction of the thyroid hormones L-thyroxine (T4) and/or L-tri iodothyronine (T3) by the thyroid or to excessive intake of the hormones. The disorder is most commonly due to Graves' disease but can also occur in cases of multinodular goitre or solitary toxic nodule adenoma.

The term Graves' disease refers to the diffuse hyperplasia of the thyroid and is often accompanied by exophthalmos, especially in young patients. The aetiology of the disease remains obscure and it has been established that thyroid-stimulating hormone (TSH) is not responsible for the excessive hormone production. In the serum of patients with Graves' disease there occurs several thyroid stimulating proteins which are immunologically different from thyroid stimulating hormone of the pituitary. The site of origin of these factors is not yet known, but it is apparently not the pituitary. When the protein is given intravenously, it disappears from circulation more slowly than does TSH. However its actions are similar to TSH but it acts more slowly and therefore has been designated long-acting thyroid stimulator (LATS). Current evidence indicates that LATS is an antibody, possibly γG, developed as an autoimmune reaction against thyroid protein. It

is postulated that many of the clinical features of Graves' disease may be related to LATS, especially enlargement of the thyroid gland and exopthalmos.

Recently, a condition has been described where patients with hyperthyroid symptoms have normal levels of T^4 but elevated levels of T^3, for which the term T^3 - thyrotoxicosis has been used. This disease can occur with diffuse or nodular goitre (including solitary nodules) and since standard tests of thyroid function are normal, diagnosis may be difficult. Criteria for diagnosis are:-

(a) Clinical hyperthyroidism

(b) Elevated serum T^3

(c) Normal serum T^4 , T^3 Uptake (T^3U) and Free Thyroxine Index (FTI)

(d) Normal thyroxine binding globulin (TBG) concentration.

(e) Other evidence of thyroid hyperfunction.

Hyperthyroidism due to solitary toxic nodule is rather uncommon and may only give rise to mild hyperthyroidism. Diagnosis can be confirmed by standard tests and by thyroid scanning after administration of radioiodine.

Hashimoto's disease, associated with elevated levels of thyroid antibodies, is sometimes found to give rise to hyperthyroidism, although more commonly hypothyroidism occurs.

Other causes of hyperthyroidism are extremely rare e.g. TSH secreting tumour of the pituitary, and since they are unlikely to be encountered during the course of the study will be excluded from this discussion.

A 1:1:1 Diagnosis

The clinical features are related to three main factors namely excess

thyroid hormones, the thyroid gland and eye signs. Increased circulating hormones give rise to a raised BMR, irritability, emotional lability,tremor, sweating, intolerance of hot weather and palpitations. There is usually loss of weight and sometimes diarrhoea. The thyroid gland is usually diffusely enlarged, but is sometimes nodular and may be associated with redness of the overlying skin. Much can also be learned by examining the eyes: they may be staring, with infrequent blinking, or protruding with dilated pupils. The upper lid may lag behind the globe as the gaze is lowered, momentarily unmasking a rim of sclera above the cornea - so called "lid-lag".

To improve the accuracy of clinical assessment a diagnostic index (Waynes' index) has been designed in which a score is given for the presence or absence of various features. This may be either positive or negative depending upon the incidence of occurrence of a particular feature. Using this index it is possible to obtain a success rate in diagnosis not dissimilar to that obtained with early laboratory tests, although with the advent of the more sophisticated assays the index may slowly prove to be of less value in the forthcoming years.

A 1:1:2 Laboratory Investigation

Often in a patient with clinically obvious, or florid thyrotoxicosis a single T^4 assay would be adequate for confirmation. Further tests are reserved for those patients in whom doubt still remains after adequate clinical assessment or where additional information is required to select the best form of treatment.

The serum protein bound iodine (PBI) measures iodinated protein compounds of which T^4 normally forms the major part. Normal levels

are 4 - 8 ug/100 ml and include small amounts of other iodoproteins. Levels found in hyperthyroidism are frequently between 8 - 12ug % although this may reach levels of 20 - 30ug %. With the advent of the T^4 assay the PBI estimation has now become obsolete - the main disadvantages of the assay being lack of specificity and the ease with which contamination can occur from other iodine containing compounds. T^4 assays are now performed routinely in a large majority of clinical laboratories.

'Resin uptake' tests measure the unoccupied binding sites on TBG and allow an indirect assessment of T^4 levels, but are also dependent on TBG levels. Normal levels for the Amersham T^3U test are 92 - 117% (expressed as a percentage of a "normal" serum pool) and in hyperthyroidism levels are reduced due to the decreased uptake of radiolabelled T^3 by the saturated patient's TBG. Levels of TBG influence the assay and to eliminate any effect of this protein on the serum T^4, the free T^4 index (FTI) was devised which measures the ratio of T^4 and T^3U, thus:-

$$\frac{T^4 \text{ level}}{T^3U} = \text{F.T.I}$$

Any changes due to altered TBG concentration are eliminated and the normal range for FTI approximates to that of serum T^4 i.e. 58 - 148 nmol/l, irrespective of TBG concentration. The above can be best illustrated by the following table:-

		(a)	(b)	(c)	(d)	(e)
Serum T^4	:	↑	↓	↑	↓	↓
Free Binding Sites	:	↓	↑	↑	↓	↓
Resin Uptake	:	↓	↑	↑	↓	↓
F.T.I.	:	↑	↓	N	N	N

(a) In hyperthyroidism the circulating T^4 is increased. Because more binding sites on TBG are occupied by the excess T^4, the 'resin uptake' is decreased and the resulting FTI is high.

(b) In hypothyroidism circulating T_4 is decreased. Because fewer binding sites on TBG are occupied, the 'resin uptake' is high and the resulting FTI is low.

(c) If increased levels of TBG are present more T^4 than normal is taken up by it from the plasma. This tendency to reduced free T^4 results in increased T^4 synthesis until TBG is again normally saturated and free T^4 is normal. The T^4 is therefore raised and because of the increased number of unsaturated TBG binding sites, 'resin uptake' is high. This occurs in pregnancy, oestrogen and oral contraceptive therapy. The resulting FTI is however normal.

(d) With reduced levels of TBG the reverse occurs - the T^4 is low and the 'resin uptake' is low, due to the decreased number of binding sites. This occurs in nephrosis and the resulting FTI is again normal.

(e) Certain drugs e.g. salicylates and phenytoin occupy binding sites on TBG and displace T^4. The resultant low T^4 and 'resin uptake', again in combination give a normal FTI result.

In hyperthyroid patients both serum T^4 and T^3 are usually increased and the ratio of T^3 to T^4 is higher, although the concentration of T^3 does not equal that of T^4. Whenever a rise in T^4 concentration is encountered the T^3 shows an equivalent rise; however clinical thyrotoxicosis does occur with normal T^4 concentrations and an increase in that of T_3. This as mentioned earlier is termed T^3 - thyrotoxicosis,

and although many individuals have toxic nodular goitres some patients may present with Graves' disease. The T^3 assay has an obvious place in the diagnosis of this disorder.

TSH levels in most cases of hyperthyroidism are usually low due to feedback from raised T^4 levels, and the common feature in these cases of hyperthyroidism is that the stimulus to excess secretion is not TSH.

A 1:1:3 Typical Biochemical Results in Hyperthyroidism

Serum T^4	:	230 nmol/l	(Normal range: 58-148 nmol/l)
T^3U	:	70%	(Normal range: 92-117%)
FTI	:	328	(Normal range: as for T^4)
Serum T^3	:	5.0 nmol/l	(Normal range: 1.1-3.2 nmol/l)
T.S.H.	:	2μIu/ml	(Normal range: less than 8μIu/ml)
Cholesterol	:	2.5 nmol/l	(Normal range: 3.0-7.3 nmol/l)

Treatment involves either surgical removal of the entire gland or a part of it, the use of drugs which interfere with the synthesis of thyroid hormones, e.g. carbimazole, or by using ^{131}I which is selectively concentrated in the gland and destroys it by local radiation.

A 1:2 Hypothyroidism

The clinical syndrome of hypothyroidism is defined as those conditions which result from suboptimal circulating levels of one of both thyroid hormones. Hypothyroidism is a graded phenomenon and patients with thyroid failure may be conveniently classified on the basis of the clinical and laboratory findings.

A 1:2:1 Overt hypothyroidism

The clinical features of this state are well known and include lethargy, cold intolerance, weight gain and hoarseness. Memory is poor and there is progressive mental dulling. The skin is dry, rough and cold and the hair coarse and sparse and falls out progressively; untreated patients becoming bald. Body temperature is lowered and the pulse may be slow due to a reduced heart rate. The absorption of food from the gut is delayed and peristalsis is reduced causing constipation. Spontaneous hypoglycaemia may also occur, possibly from failure to mobilise glucose from liver glycogen.

A 1:2:2 Mild hypothyroidism

The recognition of lesser degrees of thyroid failure presents many problems since patients with mild hypothyroidism frequently have minor non-specific symptoms. Complaints of lack of energy and facial puffiness are typical symptoms especially in patients 'at risk', such as those with a family history of thyroid disease.

Conventional tests of thyroid function may give equivocal results, although the serum TSH level is virtually diagnostic, being elevated in mild hypothyroidism.

A 1:2:3 Subclinical hypothyroidism

This is an asymptomatic state in which a reduction in thyroid activity has been compensated for by an increased TSH output to maintain a euthyroid state. Circulating thyroid antibodies may be present but this is variable. The diagnosis is made by demonstrating an elevated basal TSH level.

A 1:2:4 Aetiology of Hypothyroidism

Florid myxoedema can easily be diagnosed but lesser degrees of hypothyroidism are more insidious in onset and present with less

marked symptoms

Examples of the various causes of hypothyroidism follow:-

(1) Primary

(a) Disease of the gland (auto-immune thyroiditis):

(i) "Primary" myxoedema

(ii) Hashimoto's disease

(b) The result of treatment:

(i) Post-thyroidectomy

(ii) Post ^{131}I therapy for hyperthyroidism

(c) Uncommon causes:

(i) Dyshormonogenesis

(ii) Exogenous goitrogens and drugs

(2) Secondary to TSH deficiency in hypopituitarism

In utero, foetal development may be retarded, leading to cretinism and in children, growth may be impaired.

The essential difference between primary and secondary hypothyroidism is that TSH levels are raised in the former and lowered in the latter.

Hashimoto's disease and 'primary' myxoedema are now considered to be different manifestations of the same disorder, characterised by progressive destruction of thyroid tissue and the presence of circulating thyroid autoantibodies. The term 'dyshormonogenesis' includes the congenital deficiencies of the enzymes involved in T^4 synthesis

T^4 synthesis may be impaired by iodine deficiency, drugs such as para-aminosalycyclic acid or by enzyme deficiency. The consequent slight reduction of circulating T^4 levels results in increased TSH secretion. This stimulates T^4 synthesis and levels are therefore

maintained in the normal range. As a result the thyroid becomes enlarged (goitre) but hypothyroidism is avoided. In areas with low iodine content of the soil, iodine deficiency used to be common (Endemic goitre - "Derbyshire neck", etc.).

A 1:2:5 Laboratory Investigation

The tests discussed under hyperthyroidism are of value in the diagnosis of hypothyroidism. The patient with overt hypothyroidism requires no more than a single test to confirm the diagnosis, although those patients with lesser degrees of thyroid failure may need a battery of tests performing.

A 1:2:6 Typical Biochemical Results in Hypothyroidism (Overt)

Serum T^4	: 25 nmol/l	(Normal Range	: 58-148 nmol/l)
T^3U	: 140%	(Normal Range	: 92-117%)
FTI	: 18	(Normal Range as for T^4)	
Serum T^3	: 0.5 nmol/l	(Normal Range	: 1.1-3.2 nmol/l)
TSH	: 60 μIu/ml	(Normal Range	: less than 8 μIu/ml)
Cholesterol	: 10 nmol/l	(Normal Range	: 3.0 - 7.3 nmol/l)

In secondary hypothyroidism the above results may also be found except for the TSH level which would be undetectable or greatly reduced.

Treatment involves the administration of L-T^4 and most patients respond excellently.

BIBLIOGRAPHY

ZILVA, J.F. and PANNALL, P.R. eds. (1979). Thyroid Function. In : Clinical Chemistry in Diagnosis and Treatment. 3rd Edition. Lloyd-Luke, London, 159 - 172.

GRODSKY, G.M. (1971). The Chemistry and Functions of the Hormones. In : Harper, H.A. ed. Review of Physiological Chemistry, Lange Medical Publications, California, 416 - 422.

HALL, R., ANDERSON, J., SMART, G.A. and BESSER, M. eds. (1974). Thyroid In : Fundamentals of Clinical Endocrinology. 2nd Edition. Pitman Medical, 68 - 137.

APPENDIX TWO

THE EFFECTS OF CHEMOTHERAPEUTIC AGENTS ON VARIOUS ASPECTS OF THYROID METABOLISM

Drugs interfering with thyroid metabolism used in psychiatric treatment

Drugs interfering with thyroid metabolism not used in psychiatric treatment

THE EFFECTS OF CHEMOTHERAPEUTIC AGENTS ON VARIOUS ASPECTS OF THYROID METABOLISM

Many drugs and hormones used in medical treatment are known to effect thyroid function either as a result of their physiological effect upon thyroid hormone metabolism, or their interference with the assays used in the assessment of thyroid function. Therefore, it is important to fully understand how these substances might interfere with the thyroid function of specific patients or with its clinical assessment.

The drugs used in the treatment of psychiatric disorders, being most relevant to the present study, will be discussed initially, followed by the drugs and other compounds used in the treatment of other disorders.

A 2:1 Lithium Carbonate

A considerable amount of work has been published concerning the effects of lithium carbonate therapy on thyroid metabolism. (Sedval et al., 1969; Shopsin, 1970; Singer and Rotenberg, 1973; McLarty et al., 1975; Forrest, 1975). The reversible goitrogenic effect of chronic lithium administration was first described in 1967; goitres appeared in about 4% of lithium treated patients (Emerson et al., 1973) and scattered cases of hypothyroidism have also been reported. Prospective analyses of thyroid function in patients treated long term with lithium have demonstrated slightly decreased levels of thyroxine (T^4) and at least transiently elevated levels of thyroid stimulating hormone, (TSH) in nearly one third of these patients.

Thyroid hypofunction could result from a defect at any site in the hypothalamic-pituitary-thyroidal axis. It may be that lithium acts primarily at the thyroid gland to block TSH induced release of T^4,

Since TSH activates thyroidal adenyl cyclase to produce c-AMP, and since c-AMP then mediates the release of T^4, there are several steps at which lithium can interfere with the action of TSH. Acute lithium administration may directly inhibit TSH-stimulated adenyl cyclase (the hormone receptor site microenvironment) by substitution for an important enzyme co-factor (e.g. sodium, potassium, calcium or magnesium) or by blocking the effects of c-AMP at any step in the bio-synthetic pathway. However, Forrest (1975) stated that the hypothesis of lithium being a general inhibitor of hormones mediated via the adenyl cyclase-c-AMP system has not been substantiated. Indeed, an inhibitory effect of lithium at a step beyond the formation of c-AMP is common to TSH and also vasopressin. Other actions of lithium such as impaired peripheral utilisation of T4 are not yet understood.

After prolonged use in man, lithium decreases production and serum levels of T^4, and by negative feedback secondarily increases TSH production and/or release. The secondarily elevated TSH levels could stimulate thyroid growth, produce the observed goitre in both humans and animals, and return the serum T^4 level to normal. Later, the elevated TSH levels would return to normal due to the negative feedback of the elevated T^4 levels on the pituitary.

McLarty et al., (1975) demonstrated an exaggerated TSH response to intravenous thyrotrophin releasing hormone (TRH) in most patients receiving lithium for affective disorders. A similar response in two patients treated with lithium was also reported by Shopsin et al., (1974). They considered the effect was transitory, but McLarty's findings indicate that the elevated response persisted throughout treatment. Typically, such a response to TRH is found in primary or sub-clinical hypothyroidism together with raised basal TSH levels

Evered et al., (1973), but only a few patients had elevated basal TSH levels in the above study. They concluded that the enhanced response to TRH in the patients with normal basal TSH levels could have arisen in several ways. It may have been the lithium induced small decreases in circulating thyroid hormone levels, although they found no significant difference in thyroid hormone levels between euthyroid lithium - treated patients and a group of patients with affective disorders not receiving lithium, matched for age and sex. Nor was there any trend in thyroid hormone levels in four patients studied at monthly intervals. Thus if a reduction in thyroid hormone levels was the explanation of the enhanced TSH response to TRH, then the hypothalamic-pituitary-thyroid axis must be extremely sensitive to small changes in circulating thyroid hormone levels. Lithium may also have a central affect on the hypothalamus or pituitary or both, or may alter the distribution space of TSH or its rate of degradation.

McLarty, (1975) also observed that only two out of the twenty-one patients studied progressed to frank hypothyroidism and he agreed with the suggestion of Crowe et al., (1973) that hypothyroidism may develop in patients with no apparent evidence of an underlying thyroid disorder. This is further supported by Kirkegaard et al., (1973) who found that lithium not only affected thyroid function in patients predisposed to hypothyroidism but also those patients who were completely normal.

In conclusion, lithium has been shown to have a useful role in the treatment of thyrotoxicosis and could be preferable to other therapy when rapid control of hormone secretion is required (Lazarus et al., (1974).

A 2:2 Diphenylhydantoin (Phenytoin)

Diphenylhydantoin (DPH) competes with T^4 for binding on thyroxine

binding globulin (TBG), resulting in a decreased serum protein bound iodine (PBI) and an increased resin uptake, (Oppenheimer and Tavernetti, (1962). DPH was shown *in vitro* to displace radiolabelled T^3 from TBG to erythrocytes, and it was initially suggested that this drug may act similarly *in vivo*. However the concentrations of the drug used in these studies exceeded the therapeutic concentrations of the drug, and in work carried out by Stjernholm *et al.*, (1975),they found normal T^3 uptakes in patients treated with DPH and these findings are in agreement with those of Mölholm - Hansen *et al.*, (1974). Stjernholm found that serum T^4 concentrations were reduced by approximately 25% in patients receiving DPH and that calculation of the free thyroxine index was thus not entirely valid. Patients who were clinically euthyroid were found to have low free thyroxine index values due to the lowering of their serum T^4. The mechanisms by which they remained euthyroid, despite lowered serum T^4 concentrations however remained unclear. Stjernholm, *et al.*, (1975) postulated that the action of DPH therapy was probably the result of accelerated metabolism of T^4 by the liver and other tissues. He cited other studies supporting his view, which showed increased intracellular sequestration and fractional turnover rate along with increased T^4 clearance in DPH treated subjects (Larsen *et al.*, 1970).

Mölholm-Hansen *et al.*, (1974), reported a decrease in serum T^3 of the same degree as T^4 in patients receiving long term DPH therapy. However Liewendahl and Majuri (1976) found results at variance with this finding, in that a parallel decrease in serum T^3 and T^4 was not observed; T^3 was less depressed than T^4.

Previous studies indicated that DPH increased T^4 catabolism via a stimulation of the hepatic microsomal system (Larsen *et al.*, 1970). No comparable data on T^3 catabolism during medication with DPH is

available, but an increased conversion of T^4 to T^3 during exposure to DPH, has been reported by Cullen at al., (1973). Liewendahl and Majuri (1976) concluded from the above findings that it was possible for an increased production of T^3 from T^4 to counteract an otherwise apparent decrease in T^3, and thus explains why alterations in serum T^3 and T^4 were not quite parallel. Also, the conversion of T^4 to T^3 might also be related to the phenomenon that patients receiving DPH remain euthyroid, despite reduced thyroid hormone concentrations in the blood.

A 2:3 Diazepam (Valium) and Chlordiazepoxide (Librium)

Caprino, (1963) using large doses of Librium, reported depression of thyroid radioiodine release and uptake. In man there has been some evidence that both Librium and Valium may affect the thyroid states (Wahner, 1974). However not all workers agree on this. Librium and Valium were reported to have no appreciable effects on thyroid function in thyrotoxicosis as well as on the euthyroid state (Clark and Hall, 1970); Saldanha et al., (1971) concluded that Valium at a therapeutic level did not alter T^4 binding to serum proteins.

Those who believed there was an effect, thought that it could be due to either mixed drug therapy or mediated through depression of TSH release from the pituitary (Caprino, 1963), although Librium was found to have no effect on TSH. It was also thought to be due to an alteration in the thyroid hormone binding to serum proteins. Saldanha et al., (1971) and Schussler, (1971) both showed in vitro a competition between Valium and T^4 for the protein binding sites, in a manner similar to that of a large number of other ions and drugs.

In a study aimed at clarifying the above conflicting reports, El-Hazmi, (1975) confirmed the interference of these drugs with thyroid

function tests he utilised; these being a competitive protein binding assay for serum T^4 and an ion exchange method for T^3 binding capacity. He also demonstrated their competition for the binding sites on TBG in human sera. Valium was about three-fold more effective than Librium in binding to TBG.

In the studies of Oberman et al., (1963) and Barnes et al., (1972) Librium in vivo was reported to have no significant affect on thyroid function in either euthyroid or thyrotoxic patients and other studies showed similar results. El-Hazmi, (1975) concluded that Librium and Valium compete for the thyroxine binding sites on TBG in vitro, but not in vivo. He stated however that his experiments were performed over a three month period and a long term effect of the two drugs could thus be possible.

A 2:4 Phenothiazines (Chlorpromazine)

There is some evidence to indicate that these drugs have an antithyroid affect which cause a decrease in serum T^4 levels after treatment. This may be due to increased metabolism by hepatic microsomes (Blumberg and Klein, 1969). Gwinup and Rapp, (1975) noted that serum T^4 levels were low in a group of psychiatric patients being treated with phenothiazines, although there were no other clinical or biochemical findings to suggest hypothyroidism. In all the cases, the levels of TBG capacity, T^3 Uptake and free T^4 were normal. In view of the low serum T^4 value, it was surprising to find that serum T^3 levels were also normal, suggesting that these patients either produced excess T^3 or had accelerated conversion of T^4 to T^3.

A 2:5 Amylobarbitone

In a study carried out by Slater, (1972) no effect of amylobarbitone

on thyroid function tests was found, although only a limited range of parameters were investigated.

A 2:6 Clozapine

Simpson and Varga, (1974) noted a slight lowering of thyroid function tests in most patients being treated with Clozapine although no explanation was given as to the nature of this effect.

A 2:7 Phenobarbitone

Phenobarbitone has been shown to enhance both the binding and metabolism of iodotyrosines under *in vivo* conditions. Microsomes harvested from phenobarbitone - treated animals also have been shown to increase the rate of deiodination of T^4 and T^3 *in vitro*. An accelerated fractional disposition of T^4 has been found to be due to enhanced biliary secretion of T^4 and an increased rate of hepatic deiodination (Oppenheimer *et al.*, 1969). Similarly there is an enhanced fractional turnover of T^3 and as a result, a diminished pituitary T^3 level produces increasing amounts of serum T^4 and restores the level of circulating T^4 to normal.

The above work has been carried out in rats, but it may be that phenobarbitone has an important role to play in the management of patients with Graves' disease. Studies performed by Cavalieri and Becker, (1971) have shown a consistent lowering of serum T^4 in such patients, due to the capacity of the drug to accelerate peripheral deposition of the hormone, by induction of metabolically linked sites on the hepatic endoplasmic reticulum.

A 2:8 Drugs interfering with thyroid metabolism not used in psychiatric treatment.

A 2:8:1 Salicylates Salicylates have been shown to lower serum T^4

concentrations in both animals and man (Osorio, 1962). This decrease has been explained by the fact that salicylates displace T^4 and T^3 from TBG with a concomitant increase in the free T^4 and T^3 fraction (Larsen, 1972). Large doses of salicylates also compete with T^4 for binding on thyroxine - binding pre albumin resulting in a decreased T^4 concentration (Musa et al., 1968; Larsen, 1971)

Ramey et al., (1975) found that aspirin treatment significantly decreased the serum TSH response to TRH and significantly decreased the mean total serum T^3 and T^4. The above mentioned increase in the free T^4 and T^3 fraction may have inhibited the TSH response to TRH, although the possibility that aspirin blocked the TSH response to TRH, by some other mechanism than an effect on thyroid hormone binding, is possible. Inhibition of prostaglandin synthesis was a possible explanation of the decreased TSH response to TRH, since prostaglandins are known to affect pituitary and thyroid function. However Ramey at al., (1975) failed to prove this and concluded that the TSH response to TRH he observed was explained simply by the displacement of T^4 and T^3 from TBG.

A 2:8:2 Bishydroxycoumarin and Heparin

Bishydroxycoumarin may compete with T^4 for protein binding as reported by Baverman and Foster, (1968) although this appears to be clinically insignificant.

Schwartz et al., (1973) noted that the administration of heparin to patients prior to haemodialysis and during the treatment of myocardial infarction resulted in an increase in the plasma concentration of free T^4, due primarily to a decrease in plasma protein binding. The mechanism and significance of these heparin induced changes remains unclear, especially since these alterations cannot be stimulated by

in vitro addition of heparin to serum (Schatz et al., 1969).

Hershman et al., (1972) reported a decrease in TSH in euthyroid subjects given an injection of heparin and they suggested that a decrease in circulating TSH resulted from a reduction in plasma protein binding of thyroid hormone. This implies a shift of hormone from plasma protein to cellular pools within the pituitary. Alternatively the authors recognised the possibility that heparin might have an independent effect on cellular mechanisms responsible for the production and release of TSH, Schwartz et al., (1973) found that acute heparin administration does not lead to a detectable shift of T^4 from plasma to cells, and therefore supports the alternative hypothesis proposed by Hershman et al., (1972)

A 2:8:3 Thiazide Derivatives

Thiazide diruetics have been shown to decrease serum PBI levels to only a minor degree (Mehbod et al., 1967).

A 2:8:4 Anabolic Steroids (Androgens)

Administration of androgens and anabolic steroids may decrease TBG levels, resulting in a decreased T^4 level (Fisher and Oddie, 1968).

A 2:8:5 Oestrogens (Including oral contraceptives)

Oestrogens tend to elevate TBG, with a resultant increase in circulating T^4 levels, and there is evidence to suggest that this effect may last for two to four weeks, (Haden, 1966). As expected the T^3U is increased due to the increase in TBG.

A 2:8:6 Sulphonylurea Hypoglycaemics

Laboratory evidence for hypothyroidism and clinical hypothyroidism have been reported to be more common in patients receiving sulphonylureas (Hunton et al., 1965). However other investigators have been unable

to confirm an increased incidence of hypothyroidism (Burk et al., 1967). It is thought that these drugs compete with T^3 and T^4 for binding on TBG and this effect could be enhanced by other drugs known to displace thyroid hormones from protein binding: e.g. Phenytoin, salicylates, etc.

A 2:8:7 Clofibrate (Atromid-S)

It has been proposed that Clofibrate competes with T^4 for the acidic binding sites on serum albumin (Thorpe, 1963). Clofibrate affects oxygen consumption and the mechanisms involved may be elicited through the actions of endogenous T^4 (Mackener, 1977). In this regard, Clofibrate causes some T^4 - like effects such as enhanced levels of mitochondrial α - glycerol phosphate dehydrogenase and this plus other factors suggests that Clofibrate causes a hyperthyroid condition in the liver. The accumulation of T^4 in the liver leads to a reduction in the level of circulating T^4. The low level of circulating T^4 may also be due to the effect that Clofibrate has on depressing T^4 binding by TBPA, without effect on binding by TBG. The artifically increased free T^4 levels cause a reduction in circulating T^4 due to negative feedback on the pituitary.

A 2:8:8 Catecholamines

Epinephrine and norepinephrine have been shown to increase iodine organification and biosynthesis of thyroid hormones in cells of calf thyroid isolated by trypsinisation (Maayan and Ingbar, 1968). Catecholamines have also been reported to stimulate, through an action on both α and β adrenergic receptors, the secretion of thyroid hormones in mice (Ericson, et al., 1970). However Maayan et al., (1977) concluded a dual control mechanism of thyroid hormone secretion, after performing several experiments on mice. They state that TSH stimulated secretion, whereas catecholamines inhibited the action of TSH, possibly exerting a continuous inhibitory effect under physiological conditions.

A 2:8:9 Phenylbutazone

Several workers have demonstrated a fall in serum levels of PBI and a marked reduction in thyroidal uptake of radioactive iodine, following oral administration of phenylbutazone (Linsk et al., 1957)

The mode of action of phenylbutazone may be exerted directly on the thyroid gland or be secondary to an effect on the pituitary (Linsk et al., 1957). It has also been shown that phenylbutazone can displace T^4 from its binding sites on protein (Hansen, 1962). He also suggested that the low serum levels he found were due to suppressed TSH production secondary to an assumed rise in free T^4 levels. Dussault et al., (1973) found no suppression of TSH production in phenylbutazone-treated patients, although they noted a fall in total, but not free T^4 levels. They concluded that the fall in total serum T^4 in the presence of normal free T^4 levels, suggested than in addition to T^4 displacement from serum proteins, phenylbutazone may have a direct inhibitory effect on the thyroid gland.

A 2:8:10 Antithyroid drugs (Propylthiouracil, Carbimazole, Thiourea)

Certain compounds act as anti-thyroid agents, inhibiting the production of T^4 by preventing the gland from incorporating inorganic iodide into the organic form. The antithyroid compounds have an almost immediate effect, since they act during the early stages after iodide uptake by the gland. Under their influence, thyroid synthesis may also be inhibited by the drug preferentially combining with the active iodide (organic iodine) and thereby preventing its incorporation into the tyrosine moieties. A direct action to decrease maturation of thyroglobulin to its final polymeric form may also occur.

A 2:8:11 Corticosteroids

There is conflicting evidence concerning the effect of these agents on serum T^4. Decreases may occur following large doses, due to an effect causing TBG concentration to be reduced (Kendall-Taylor, 1972). In human subjects, a dose of 60mg prednisone acutely suppresses TSH release, and rebound hypersecretion occurs the day after cortisone is withdrawn (Nicholoff et al., 1970). Since cortisone has no effect on pituitary secretion of TSH in vivo (Otsuki et al., 1973), these actions may occur through suppression of TRH secretion or action. It has been suggested that TRH secretion is normally modulated by the circulating blood cortisol level, and that this explains why the diurnal rhythm of cortisol has a reciprocal relation with TSH secretion.

Corticosteroids thus have an overall effect of depressing thyroid function. Cortisone depresses uptake and clearance of iodide by the thyroid, increases renal clearance of iodide (Berson and Yalow, 1952; Ingbar, 1953)., decreases thyroid secretion rate (Brown-Grant et al., 1954), and produces clinical hypothyroidism. Part of the decrease in T^4 other than the effect on TRH and TSH as stated previously, can be ascribed to increased catabolism or decreased synthesis of protein, with a subsequent decrease in TBG.

Pharmacological doses of cortisone have been reported to induce a fall in LATS concentration (Benoit and Greenspan, 1967) which may ameliorate Graves' disease in certain cases.

APPENDIX THREE

LONG ACTING THYROID STIMULATOR AND LONG ACTING THYROID STIMULATOR PROTECTOR

LONG ACTING THYROID STIMULATOR AND LONG ACTING THYROID STIMULATOR PROTECTOR

Twenty-five years ago, Adams and Purves (1956) in New Zealand using a guineapig bioassay system to measure TSH, reported the presence of an abnormal thyroid stimulator (LATS) in the serum of some patients with Graves' disease. This substance contained thyroid-stimulating activity quite distinct from pituitary TSH.

Fractionation of the sera by various methods show that the activity is associated with a diverse population of immunoglobulins of the IgG class (Smith, 1971). Biochemical studies show that thyroid-stimulating sites are formed by combination of IgG heavy and light chains in the Fab part of the molecule as are the antigen binding sites of classical IgG antibodies. This suggests that thyroid-stimulating immunoglobulins (TSI) are thyroid-stimulating antibodies (Smith et al., 1969).

TSI are bound by and inactivated by human thyroid material. This substance is probably a cell surface protein which, for some reason, becomes antigenic and initiates the synthesis of thyroid-stimulating antibody molecules and the cell surface protein presumably initiates cell stimulation.

The material which binds thyroid stimulating immunoglobulins is disrupted by freezing and thawing thyroid homogenates. Most of the binding protein is associated with cell surface fragments in the 4S fraction and has a molecular weight of about 30,000. It forms a soluble complex with TSI consisting of one antibody molecule with two binding protein molecules each attached to one of the Fab parts of the immunoglobulin (Smith, 1971).

Thus with the finding of LATS in the serum of patients with Graves' disease, the aetiology of this disease, it seemed, was soon to be explained. But as the years have passed current knowledge of the role of LATS in hyperthyroidism has become increasingly confused.

The first doubts about LATS arose from inability to demonstrate it in all cases of Graves' disease (McKenzie, 1972). Insensitivity of the assay system was originally blamed but assays of concentrated IgG fractions of hyperthyroid sera produced only about 80% positive results (Carneiro et al., 1966); about 20% of hyperthyroid patients seemed to have no detectable circulating LATS. New theories were then advanced for the aetiology of the disease. The most popular being based on the concept of disturbed cell-mediated immune responses (Volpe et al., 1974), or on proposed primary abnormalities of the thyroid cell receptor site for TSH resulting in continuous activation of the c-AMP protein-kinase complex (Solomon and Chopra, 1972). However these theories do not explain the fact that LATS is almost invariably found in the serum of hyperthyroid neonates born to mothers with Graves' disease and also LATS has only been found in patients who have or have had Graves' disease, or occasionally in euthyroid relatives (Wall et al., 1969)

In 1967 Adams and Kennedy reported the presence of a substance in some hyperthyroid sera which could block the effect of LATS on human thyroid tissue and a follow up paper in 1971 suggested that this acted specifically on human thyroid gland and was, unlike LATS, ineffective in the stimulation, or binding to, mouse thyroid glands. They applied the rather unfortunately chosen LATS-protector (LATS-P). It was suggested that sera from most if not all hyperthyroid patients contained abnormal thyroid stimulating immunoglobulins, either human specific LATS-P or non human specific LATS (Adams and Kennedy, 1971; Shishiba et al., 1973).

Mukhtar et al., (1975) detected thyroid stimulating immunoglobulins in the serum of all patients with untreated Graves' disease, and in these patients the levels of immunoglobulins correlated significantly with the early uptake of ^{131}I by the gland. They also found that the frequency of TSI in patients treated solely by antithyroid drugs, by radioiodine or by patent thyroidectomy was 53%, 50% and 17% respectively. They attributed this high detection rate of TSI to the more sensitive and precise radio-receptor assay they developed. Previously thyroid stimulating activity was estimated by a relatively insensitive bioassay which depended on the stimulation of release of radioiodine from thyroid glands of mice in vivo (McKenzie, 1958). Mukhtar et al., (1975) stated that the relationship between the TSI measurements in his receptor assay and LATS and LATS-P was not clear. Smith and Hall, (1974) suggested that LATS-P was closely related to the TSI activity measured in the receptor assay, and the significant relation between TSI activity measured in the receptor assay and early ^{131}I uptakes is similar to that reported by Adams et al., (1974) between early ^{131}I uptakes and LATS-P. Mukhtar et al., (1975) concluded that since thyroid-cell stimulation has been shown to be mediated by the adenyl cyclase/c-AMP system (Kendall-Taylor, 1970), this suggested that the effects of Graves' disease immunoglobulins on the binding of TSH to thyroid membranes reflected their thyroid stimulating activity. He also thought it likely that hyperthyroidism in Graves' disease was likely to be caused by serum TSI, due to their observations on the relationship between TSI levels and ^{131}I uptakes.

Kendall-Taylor et al., (1975) reported that LATS-P can be detected in serum from the majority of patients with Graves' disease and was found in a much higher proportion that is LATS - it was not found in serum from 32 normal subjects., in 14 patients with non toxic nodular

goitre or 12 patients with Hashimoto's disease, nor in 4 patients whose disease appeared to have remitted. It is sometimes possible to demonstrate LATS-P in serum which also contains LATS.

Recent work has been directed to determining whether LATS-P stimulates the human thyroid. Thyrotoxic LATS - negative Ig does not activate human thyroid adenylcyclase, whereas LATS does (Kendall-Taylor, 1973) but, in contrast to this Onaya et al., (1973) found that LATS-negative serum stimulated c-AMP accumulation in thyroid slices in vitro. However the most definite evidence is the personal observation of Adams et al., (1974), that injection of LATS-P increased thyroid function in man in vivo. Kendall-Taylor et al., (1975) suggested that hyperthyroid sera contains an IgG distinct from, and perhaps additional to LATS, which stimulates the human thyroid. This was probably the same as LATS-P but might be better termed human-specific thyroid stimulator.

Clague et al., (1976) using TRH and T^3 suppression tests in patients with Graves' disease and hyperthyroidism, showed that thyroid function in these cases was not controlled by TSH. Fifty patients were studied with thyroid disease and the results of the above tests were compared with the levels of serum TSH measured by a radio-receptor assay. They found in euthyroid and hyperthyroid patients, the presence of TSI corresponded with the absence of TSH control of thyroid function. However, in two hypothyroid patients with detectable TSI levels, T^3 suppression and TRH tests indicated that thyroid function was under TSH control

McKenzie and Zakarija (1976) tried to clarify the current theories implicating TSI as the cause of hyperthyroidism in Graves' disease. Several assays for TSI have been developed and depending on the method, different names have been used and distinct entities thus implied.

McKenzie (1976) using an increase in c-AMP in the human thyroid slice after 2 hours of incubation, as an index of thyroid stimulation, identified thyroid-stimulating activity in all of an unselected series of sera from 11 patients with Graves' disease, but LATS by mouse bio-assay, in only 3. He proposed the theory that TSI is probably present in all such patients; it may be seen as a polyclonal antibody to a single human antigen that has a variable cross-reaction with a corresponding thyroid antigen in the mouse and in the other species.

PSYCHIATRIC DISORDERS (PSYCHOPATHOLOGY)

A 4:1 Introduction

Psychopathology deals with "abnormal"behaviour, although the definition of this is not easy. Generally the diagnosis of abnormal behaviour is based on several criteria as no single definition is satisfactory. One such criteria is statistical frequency; "abnormal behaviour" is that which is statistically infrequent or deviant from the norm. Social standards also help to define behaviour as normal or abnormal and usually such behaviour is statistically infrequent in that society in which one lives. A third definition is based on adaptiveness of behaviour; abnormal behaviour, being maladaptive, has adverse effects for either the individual or society. Using this criterion an individual, who due to an intense fear of crowds, could not travel to work would be classified as abnormal. Another criterion deals with the individuals personal feelings - most people diagnosed as "mentally ill" feel very miserable and are anxious,depressed and may suffer from loss of appetite, sleep and from numerous aches and pains. However none of the preceeding definitions provides a complete answer for distinguishing between normal and abnormal behaviour and certain characteristics possessed by an individual are also valuable in demonstrating good mental health. These include an efficient perception of reality, an ability to excercise voluntary control over behaviour, self esteem, an ability to form affectionate relationships and productivity.

Psychopathology involves a broad spectrum of mental disorders and several classification systems have been devised to encompass abnormal behaviour. The most widely accepted classification system groups people according to the behavioural symptoms they display. The major groups under this classification are neuroses, psychoses, psychophysiological disorders and personality disorders and these can be further subdivided.

APPENDIX FOUR

PSYCHIATRIC DISORDERS (PSYCHOPATHOLOGY)

Introduction

Neuroses

Psychoses

Psychophysiological Disorders

Personality Disorders

The major categories will now be briefly described followed by an examination of the subcategories.

A 4:1:1 Neuroses A major distinction must be made between neuroses and psychoses. The neuroses are a less severe form of psychological disorder and do not involve personality disintegration or loss of contact with reality. The primary symptom of neurosis is anxiety and neurotic individuals can usually cope with society even though their anxiety prevents them from functioning at full capacity.

A 4:1:2 Psychoses The psychoses are characterised by an impairment in mental functioning that seriously interfered with the individuals ability to meet the demands of daily life. There is gross distortion of reality, so that the person can no longer distinguish between fantasy and reality. These distortions may take the form of delusions or hallucinations.

A 4:1:3 Psychophysiological disorders These are also referred to as psychosomatic illness and are physical illnesses in which psychological factors play a major role. The illness is real : e.g. migraine headaches, ulcers - but psychological stress is presumed to be an important precipitating factor.

A 4:1:4 Personality disorders These are usually long standing patterns of socially maladaptive behaviour. Antisocial or sexually deviant behaviour, alcoholism and drug addiction are some of the disorders included in this category. There is no gross distortion of reality or intellectual impairment unless due to secondary factors e.g. brain damage following drug addiction - when the patient may well be labelled psychotic and be classified accordingly.

None of the above four categories are ideal and no single person may fall exactly into one of these, as each persons set of symptoms,

emotional background etc is so unique. However such a classification is essential for those working with disturbed patients as a diagnostic label helps communicate information and leads to more efficient treatment. A fuller discussion of these four categories now follows:-

A 4:2 Neuroses (Neurotic disorders)

The term neuroses refers to a group of disorders in which the person has developed certain behaviour patterns that avoid, rather than cope with problems. This situation invariably results in anxiety, tension and restlessness unless a realistic solution cannot be achieved. In the neurotic the latter rarely occurs and usually alleviates only a small part of the total anxiety which ultimately creates further problems. Anxiety therefore represents the core of all neuroses and the patient appears strained and tense, insomnia, indigestion, diarrhoea, inability to concentrate or sexual impotence may be present. Often a vicious cycle is set up - the individual feels inadequate to cope with everyday problems and so avoids them by defensive manoeuvres; he then feels guilty, unhappy and even more inadequate because of his failure to deal directly with situations that others handle with ease. Many types of neurotic reactions have been observed of which the following occur more frequently- obsessive-compulsive reactions, phobias, conversion reactions and neurotic depression.

A 4:2:1 Anxiety

This represents the most common neurotic complaint. Although this is a typical feature of neuroses, in many neurotic reactions it is concealed by other symptoms. However in anxiety reactions, this represents the main feature of the disorder. The typical anxiety neurotic lives each day with a level of tension much greater than that of the normal individual. This chronic state of apprehension is often punctuated by acute anxiety attacks when the individual has an

overwhelming feeling that something dreadful is about to happen and this is often accompanied by rapid breathing, palpitations, perspiration etc. These psychological symptoms result from excitation of the sympathetic nervous system and it is important to realise that several organic conditions such as hyperthyroidism can produce the same symptoms as an anxiety attack! The anxiety neurotic usually has no clear idea of why he is frightened and this fear is not related directly to external stimuli but often instead to feelings and conflicts within the individual. The normal individual may feel anxious and tense in the face of threatening or stressful situations but these feelings are only considered neurotic when they become habitual ways of responding to situations that most people can easily cope with.

A:4:2:2 Obsessive - compulsive reactions

In obsessive - compulsive reactions the individual is compelled to think about things be would rather not think about or to perform acts that he does not wish to carry out. Obsessions are persistent intrusions of unwelcome thoughts. Compulsions are irresistible ways to execute certain acts - for example, thoughts of lurking disease bacteria combined with the compulsion of excessive hand washing. Normal individuals have persistently recurring thoughts and ways towards ritualistic behaviour but the neurotic finds these obsessive thoughts and compulsive ways occupying so much time that they seriously interfere with his daily life. He often recognises the irrationality of his thoughts and behaviour but is unable to control them. These elaborate, time consuming rituals seem to serve two main functions - they establish order and control in a confusing and threatening world; a carefully organised, rigid pattern of bahaviour may prevent anything from going wrong. Also they defend against anxiety by keeping threatening impulses out of awareness; a continually

busy person has less opportunity for improper thoughts or actions.

A:4:2:3 Phobia Reactions

These are excessive fears of certain kinds of situations in the absence of real danger, or fears that are totally out of proportion to the amount of danger a situation may involve. The person usually realises that the fear is irrational but still feels anxiety which is relieved only by avoiding the phobic situation. The list of objects or situations that can evoke phobic reactions is endless; some of the more common ones are : fear of closed spaces (claustrophobia), fear of high places (acrophobia), fear of crowds (ocholophobia), fear of animals (zoophobia), and fear of the dark (nyctophobia). Normal individuals have some minor irrational fears, but in phobic reactions the fears are so intense that they interfere with the persons daily living.

A:4:2:4 Conversion Reactions

Conversion reactions or hysteria are physical symptoms which appear without any underlying organic cause. The symptoms may be a) Sensory - loss of sensation in some part of the body, blindness or deafness; b) Motor - paralysis of a limb or entire side of the body, speech disturbances, muscle tremors or tics ; c) Visceral - including such symptoms as coughing or sneezing, hiccuping, choking and a variety of aches or pains.

Freud believed that reactions of this type represented the "conversion" of anxiety into physical symptoms. However the physical symptoms are now usually interpreted as providing an unconscious means of avoiding a stressful situation. Although no organic cause can be found, the individual with a conversion reaction is not faking; his disorder is quite real to him and it usually is easy to distinguish

him from a malingerer.

Almost every conversion reaction may be traced to an attempt to avoid or solve a problem by means of an illness. However the more dramatic types of reactions, such as sudden paralysis, are becoming increasingly rare in civilian life, although they are still relatively common among servicemen during wartime. Patients seem to be developing vague aches and pains instead possibly due to the increasing medical sophistication of our population, since dramatic afflictions are no longer viewed as medically feasible.

A:4:2:5 Neurotic Depression

In neurotic depression the individual reacts to a distressing event with more than the usual sadness and fails to recover within a reasonable length of time. The main symptoms of depression are passivity and dejection. The individual lacks motivation - he feels unable to make decisions, to initiate activity or to take an interest in anything or anyone. He broods over his inadequacies, has crying spells and may contemplate suicide. Many theories have been proposed as to the cause of this disorder - depression appearing spontaneously is often called endogenous; when it follows external events which one regards as having precipitated it, it is exogenous or reactive.

In summary all neurotic reactions are exaggerated forms of normal defense mechanisms (such as denial of reality or rationalisation); neurotic symptoms are responses that the individual uses to defend against anxiety and to increase feelings of security. Feelings of inadequacy and anxiety underlie all neurotic reactions but exactly what determines the type of symptoms a particular individual develops is not known. It is thought that the neurotic symptoms are extreme forms of the response the individual learnt in early childhood to cope with stress,

which are reused in the incorrect situation.

A:4:3 Psychoses

A psychotic individual is more severely disturbed than one suffering from a neurotic reaction. The psychotic personality is disorganised and normal social functioning is greatly impaired. It has been thought that a psychosis represents an extreme form of neurosis, although this remains unproven. Some experts believe that there is a continuity from normality through neuroses and psychoses, the differences being largely a matter of severity of symptoms. Others believe that the psychoses are qualitatively different from the neuroses, involving physiological changes in the nervous system that are possibly genetically based.

The most apparent distinction is that the neurotic is trying desperately to function in the world, whereas the psychotic has to some extent given up the struggle and lost contact with reality. Frequently the thought processes are disturbed to the extent that the psychotic individual experiences delusions or hallucinations. For these reasons the psychotic is more likely to require hospitalisation and protective care than the neurotic.

It is customary to distinguish between two general categories of psychoses : organic and functional. Organic psychoses occur as a result of damage to the central nervous system caused by head injuries, brain tumours, hardening of the arteries and toxic poisoning from heavy metals and drugs (e.g. LSD). This group contains patients suffering from epilepsy and senile dementia. Functional psychoses are disorders that are presumed to be primarily psychological in origin, although genetic and other biological factors may play a significant role.

The distinction between organic and functional psychoses is not clear cut, yet it is practical to distinguish between those psychoses in which nervous system disturbances have been identified (the organic psychoses) and those in which physiological factors are unknown and environmental conditions are assumed to play a major role (the functional psychoses). Functional psychoses are subdivided into two main categories.

(i) The Affective Psychoses - in which a major disorder of mood appears to be the primary disturbance, with resulting secondary delusional ideas and sometimes hallucinatory experiences.

(ii) The Schizophrenic Psychoses - in which delusional thinking may be marked though not explicable by mood disturbance. There may also be a disorder of thinking and hallucinations, and these too do not appear to be related either to intellectual impairment or mood disorder.

A major affective psychosis is manic depressive psychosis which will now be described.

A4:3:1 Manic-depressive Psychoses

Manic-depressive psychoses are characterised by recurrent shifts of mood from normal to either a manic state (strong excitement and elation) or a depressed state (extreme fatigue, despondency and sadness). Some patients exhibit the whole cycle, but most vary between the normal mood state and one of the extreme phases, depression being the most common.

A:4:3:2 Manic States

In the milder forms of manic (hypomania) the patient shows great energy and enthusiasm. He talks continually, had unbounded confidence in his ability, rushes from one activity to another with little need of sleep, and makes grandiose plans with little attention to their practicability. His behaviour is similar in some respects to an individual who is mildly intoxicated.

In the more severe form of manic the person may be continually pacing about,singing, shouting obscene phrases and screaming. He is confused and disorientated and may experience hallucinations and delusions. Some hypermanic individuals abandon all moral inhibitions and my exhibit unrestrained sexual behaviour or violent assaultive behaviour.

:3:3 Depressed States

The depressed individual's behaviour is essentially the opposite of that of someone in the manic phase. Instead of being overactive, his mental and physical activity is much slower than normal. Instead of feeling overconfident and boastful, his self-esteem is at its lowest ebb. He feels rejected and discouraged and life seems hopeless and not worth living. Feelings of worthlessness and guilt predominate and it is not infrequent for patients to attempt suicide. In the most intense state of depression the patient is bedridden and indifferent to all that goes on around him. He refuses to eat and often has to be fed intravenously and completely cared for by others.

The depressed state of the manic-depressive psychosis differs from other depressions in that (i) there is no apparent precipitating cause (ii) the depression usually lifts spontaneously after a time and (iii) subsequent periods of depression almost invariably occur. The cyclical nature of the illness suggests some sort of disturbance or defect in the neurohormonal mechanisms that control emotion. The relationship between the thyroid gland and its effect upon behaviour is well known and thus an organic cause can sometimes be found, not only for manic-depressive psychoses but also for the other main group of functional psychoses - schizophrenia.

A4:3:4 Schizophrenic Psychoses

Schizophrenia is by far the most common of all the psychotic disorders and it has been estimated that approximately 50% of all psychiatric beds are occupied by such patients. The word schizophrenic is derived from the greek words Schizein ("to split") and phren ("mind") The split however does not refer to multiple personalities but rather to splitting of the thought processes from the emotions. Schizophrenia is actually a label for a group of psychotic disorders. The symptoms are many and varied but the main features can be summarised under the following six headings:-

(i) Disturbance of affect : The schizophrenic does not show emotion in a normal way. He usually appears dull and apathetic, or he may display inappropriate emotions (e.g. speaking of tragic events without any display of emotion or while actually smiling).

(ii) Withdrawal from realism : The schizophrenic loses interest in the people and events around him.

(iii) Autism : Withdrawal from reality is usually accompanied by absorbtion of an inner fantasy life. This state of self-absorbtion is known as autism. The schizophrenic may be so enmeshed in his fantasy world that he is disorientated in time and space and thus may not know what day or month it is or where he is.

(iv) Delusions and Hallucinations : The most common delusions of the schizophrenic are the beliefs that external forces are trying to control his thoughts and actions or that certain people or groups are persecuting him. The schizophrenic frequently hears voices and when the persecutory delusions or hallucinations are predominent, the person is called "paranoid". He may become suspicious of friends and relations, fear that they are poisoning him, complain that he is being watched,

followed and talked about. It is thought that these paranoid delusions are an extreme form of defense mechanism where rather than face the anxiety generated by recognition of his own hostile impulses, the paranoid schizophrenic projects his hostility into others.

(v) Bizarre behaviour : The schizophrenics behaviour may include peculiar gestures, movements and repetitive acts that make no sense to the observer but are usually closely associated to the schizophrenics fantasy world.

(vi) Disturbance of thought : Disturbed thought processes contribute the most fundamental symptom of schizophrenia and some of the other symptoms are related to this. Whereas the manic-depressive psychoses are characterised by disturbances of mood, in schizophrenia thought disorders predominate.

The thought disorder in schizophrenia appears to reflect a general difficulty in "filtering out" irrelevant stimuli unlike the normal person who selectively focuses his attention on one specific stimulus. The schizophrenic is continually receptive to many stimuli at one time and has trouble making sense out of the profusion of input bombarding him.

Schizophrenia usually occurs during early childhood, the peak of incidence being between the ages 25 - 35. In some cases the symptoms appear suddenly, following a period of stress. But more often they are the result of a gradual process of increasingly unsatisfactory interpersonal relationships, inability to cope with the world and withdrawal from social contacts. Attempts have been made to classify schizophrenia on the basis of the predominent clinical symptoms. The four categories most often used are :-

(a) Simple : This type shows gradual development of social withdrawal, loss of interest and emotional apathy that usually begins at adolescence.

The individual exhibits less bizarre behaviour than other types and hallucinations and delusions are rare. Many 'simple' schizophrenics maintain marginal adjustment without hospitalisation, working in solitary occupations.

(b) Hebephrenic : With this type reactions resemble the common stereotype of the psychotic with sudden fits of laughing or crying, silly grins or grotesque facial expressions, hallucinations and bizarre delusions. The individual often talks to himself or to fantisied companions. In advanced stages he may regress to infantile behaviour including soiling and wetting, rocking to and fro, and head banging.

(c) Catatonic : In addition to some of the other symptoms of schizophrenia, the catatonic individual has extreme mood fluctuations from stuporous, immobile states to wild excitement.

(d) Paranoid : In this category the individual suffers from delusions of persecution or grandeur and often hears 'voices'. He is highly sensitive and suspicious and often hostile and belligerent. Generally there is less severe personality disorganisation than other types, presumably because the patient is projecting onto others qualities he cannot accept in himself.

The cause of schizophrenia is not known although many theories have been proposed. Several studies have been developed to try and ascertain if there are genetic, biochemical or pysiological differences between schizophrenics and normal individuals, although no conclusive evidence has yet been obtained as to the aetiology of the disorder. The present study, relating abnormal thyroid function with various psychoses, may provide further insight into this controversy.

A4:4 Psychophysiological Disorders

Sometimes the effects of emotional stress manifest themselves in impaired physical health. A psychosomatic illness is a physical illness that has psychological causes and stress invariably plays an important role as a precipitating factor. The anatomical and physiological basis for the psychosomatic reaction are to be found in the central nervous system. The autonomic nervous system, and the neuro-endocrine system, whose pivotal centre can be regarded as the hypothalamus and pituitary gland, represent three inter-related systems of communication whereby the individual maintains equilibrium respectively between himself and his external environment, and within his own organism. Interaction, therefore, between these systems is a constant and invariable aspect of human health and sickness. Thus a breakdown in one part of this system will cause an inbalance between the other two leading to a physical illness.

A:4:5 Personality Disorders

Personality disorders include a group of behavioural patterns that are pathological from society's viewpoint rather than in terms of the individual's own discomfort or unhappiness. The person fails to behave in socially approved ways because he lacks either the motivation or the skills necessary to do so. Personality disorders are distinguished from neuroses and psychoses in that they are more often long-standing patterns of maladoptive behaviour rather than reactions to conflict or stress. But this distinction is largely a matter of degree as is true of most attempts to separate individuals into categories.

Included among the personality disorders are alcoholism, drug dependence, sexual deviations, "immature" personalities and psychopathetic personalities.

BIBLIOGRAPHY

BROOKE, E.M., COOPER, J.E., JABLENSKY, A., KRAMER, M. and SARTORIUS, N., eds. (1978). Mental Disorders : Glossary and guide to their classification in accordance with the Ninth Revision of the International Classification of Diseases. World Health Organisation, Geneva, 9 - 60.

MORGAN, H.G. (1979). Mental Health. In : Read, A.E., Barritt, D.W. and Hewer, R.L. eds. Modern Medicine., A Textbook for Students, 2nd Edition, Pitman Medical, 424 - 480.

TRETHOWAN, W.H. ed. (1979). Psychiatry. 4th Edition, Balliere Tindall, London.

RUSSELL DAVIS, D. ed. (1972). An Introduction to Psychopathology. 3rd Edition. London, Oxford University Press.

APPENDIX FIVE

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THYROXINE BINDING GLOBULIN (TBG) AND THYROXINE BINDING PREALBUMIN (TBPA) MEASUREMENT, COMPARED WITH THE CONVENTIONAL T^3 UPTAKE (T^3U) IN THE DIAGNOSIS OF THYROID DISEASE

A5:1 Summary

An electroimmunoassay for the determination of thyroxine binding prealbumin is described. The diagnostic efficiency of the assay when used in conjunction with the serum thyroxine as a thyroxine : thyroxine binding prealbumin ratio, is compared with the conventional free thyroxine index and the more recently developed thyroxine : thyroxine binding globulin ratio. The population studied included euthyroid, hypothyroid and hyperthyroid patients and also those who were either pregnant or receiving oral contraceptive therapy.

Despite recent evidence establishing the theoretical/practical advantages of using a direct measurement for thyroid binding proteins rather than an indirect method (tri-iodothyronine uptake), results obtained from this study suggest that for the majority of patients requiring biochemical assessment, the free thyroxine index is still the superior discriminator of thyroid abnormality.

A5:2 Introduction

At present the free thyroxine index (FTI) is the most widely employed method of thyroid assessment. This relies upon the measurement of the serum thyroxine (T^4) combined with the tri-iodothyronine uptake (T^3U) as an indirect measure of binding protein concentration. This assay was originally introduced by Hamolsky et al., (1) and represents a measure of binding protein capacity, rather than concentration and therefore has limitations as described by Souma (2) and Goolden et al., (3). However, the assay is capable of good precision (4,5) and has been shown to correlate well with the free thyroxine concentration (6).

Recently the value of a direct assay of thyroxine binding globulin (TBG) has been shown by several workers (7,8) and also the ability of

the T^4 :TBG ratio to correct the serum T^4 for widely varying concentrations of TBG. Reasonable discrimination between thyrotoxic, myxoedematous and euthyroid states was also demonstrated and compared with the FTI.

Little work has been published relating to the value of thyroxine binding prealbumin (TBPA) in the assessment of thyroid function, as TBG is the major transport protein of thyroid hormones (9). TBG has also been shown to exhibit a higher affinity for T^4/T^3 than TBPA (10). However, Robbins (11), concluded that both TBG and TBPA contribute equally to the turnover of the metabolically active free thyroxine pool, and current views suggest that TBPA represents a more readily accessible source of T^4 than TBG, due to its greater concentration and lower binding affinity.

In view of this, a method for the assay of TBPA is described and its value for correction of the serum T^4 in the evaluation of thyroid status, compared with the recently developed TBG assay and the more conventional T^3U.

A5:3 Materials and Methods

The following groups were studied : 200 healthy euthyroid subjects (100 male, 100 female); 71 hyperthyroid patients (13 male, 58 female); 44 hypothyroid patients (6 male, 38 female); 18 female patients who were receiving oral contraceptive therapy; and 50 women who were in their second and third trimesters of pregnancy.

The euthyroid group comprised patients whose blood samples had been received by this department for thyroid function tests. Subjects with values falling outside our normal range for the routine thyroid function parameters (in brackets) namely serum T^4 (58-148 nmol/l), serum T^3U (90-118%) and the derived index, FTI (58-148) were automatically excluded, as were females who were either pregnant or on oral contraceptives. Borderline hypo- and hyperthyroids, i.e. patients with values between two and three standard deviations from the mean, were further analysed by measurement of triiodothyronine (T^3) and thyroid stimulating hormone (TSH) assays respectively, as recommended by Britton et al., (12), and the patients with abnormal values again excluded.

In all the groups serum T^4, T^3 and TSH were assayed using the Corning Medical (IMMUNOPHASE T.M.) radioimmunoassay test system and serum T^3U measured using the Radiochemical Centre (Thyopac - 3) competitive protein binding assay.

Both TBG and TBPA were assayed by the electroimmunoassay method developed originally by Laurell (13) and often referred to as "Rocket Immuno-electrophoresis". The TBG assay was based on that of Bradwell et al., (14), with minor modifications including the use of a 0.05 ionic strength barbital buffer, an antiserum concentration of 1.25%

and a 16 hour electrophoresis at 25-30 volts.

The TBPA assay was modified as follows: Rocket immunoelectrophoresis was carried out using a 1% agarose gel with a sodium barbitone/barbituric acid buffer, pH 8.6, ionic strength 0.05. Sample wells of 2.5 mm diameter were cut in a 1 mm thick agarose gel containing 2.0% of the TBPA antiserum. Samples, standards and controls were pre-diluted 1 in 5 and applied in 3 µl aliquots to each of the appropriate wells. Electrophoresis was allowed to proceed for 16 hours at 30 volts without cooling. The gel was then press dried and stained with Coomassie Brilliant Blue R-250 and the peak heights measured against the standards. Samples falling outside the range of the standard curve were either further diluted in buffer and re-assayed, or a lower dilution employed.

A5:4 Calibration and Quality Control Materials.

The TBG assay was calibrated using pooled serum obtained from pregnant patients. The assay value of 34 mg/l was derived by standardisation against a purified preparation of human TBG, kindly supplied by Dr. A.R. Bradwell, Department of Medicine, Queen Elizabeth Hospital, Birmingham; who also provided the TBG antiserum.

The TBPA assay utilised a serum protein standard (Protein Standard Serum B) and also specific antiserum, both manufactured by Behringwerke and available through Hoechst Pharmaceuticals.

The TBG assay was controlled using a serum pool prepared in the laboratory and also a serum reference preparation (Seward Laboratory, product number BR.99). Quality control of the TBPA assay was performed using stabilised human serum manufactured by Behringwerke (Standard-Human-Serum, stabil).

A5:5 Results

The precision of the TBPA assay was calculated to give a between batch coefficient of variation of 5.7% at a level of 340 mg/l. This compares favourably with data obtained by other workers.

The mean value, one standard deviation (S.D.) and the range covering two S.D. for serum T^4, T^3 Uptake, TBG and TBPA in all the five groups, are summarised in Table 1. For the euthyroid group, the small sex difference that occurs for all the parameters may be ignored for clinical purposes. The five groups can be seen to be statistically different on the basis of the serum T^4 and T^3U values, this being less marked for both TBG and TBPA. Marginally improved discrimination between hypo- and hyperthyroid groups is shown for TBPA when compared to measurement of TBG.

On plotting the T^3U against TBG, an excellent correlation was demonstrated in all groups for values of TBG up to 20 mg/l, however, a plateau is reached at a T^3U value of between 135 - 140%. T^3U relative to serum T^4 showed equal or improved correlation for the hypothyroid, hyperthyroid and oral contraceptive groups when compared with TBG and TBPA.

The thyroid mapping technique, as discussed by Mardell, (15) was also used to correlate serum T^4 with T^3U, TBG and TBPA. Figure 14 shows a comparison of the calculated parameters FTI, T^4:TBG and T^4:TBPA for all five groups. To facilitate ease of comparison, the mean of the normal range has been equalised in each case by calculation of the "percentage of reference range" for all the FTI, T^4:TBG and T^4:TBPA indices. The hypothyroid group is well differentiated by both the FTI and T^4:TBG from the euthyroid group. However, for the hyperthyroids, the FTI shows improved discrimination from the euthyroid range, compared to the T^4:TBG in which an overlap with the latter group occurs.

Pregnant women or those on oral contraceptive therapy show a slight overlap with the hyperthyroid group, when the FTI is used, compared to a more significant overlap with the hypothyroid group, when the T^4:TBG is employed.

When comparing the FTI with the T^4:TBPA, although the latter is able to distinguish between the various groups, there is poor differentiation from the euthyroid group and only partial correction for abnormal protein levels, this being partly due to absence of rise in TBPA concentration during pregnancy (See Table 1).

TABLE 1

Mean and one standard deviation values with two S.D. range for Serum T^4 T^3Uptake, TBG and TBPA in the groups studied

ASSAY		EUTHYROID		HYPOTHYROID	HYPERTHYROID	ORAL CONTRACEPTIVE	PREGNANT
		Male	Female				
T^4 (nmol/l)	Mean	97.5	101.8	19.2	227.2	106.0	152.3
	S.D.	20.7	21.7	13.6	47.6	16.2	22.3
	Range	56.2-138.8	58.5-145.1	0-46.4	132.1-322.4	73.6-138.4	107.7-196.9
T^3U (%)	Mean	104.8	107.3	124.8	91.0	111.6	137.4
	S.D.	7.2	7.2	10.7	14.2	7.2	5.7
	Range	90.5-119.1	93-121.6	103.3-146.3	62.7-119.3	97.2-126.0	126.0-148.8
TBG (mg/l)	Mean	12.1	13.2	15.9	13.4	14.4	31.9
	S.D.	2.6	2.3	3.1	4.8	2.2	4.2
	Range	7.0-17.2	8.7-17.9	9.6-22.0	3.8-22.9	10.0-18.7	23.5- 40.3
TBPA (mg/l)	Mean	254.0	230.1	264.0	181.2	239.2	225.0
	S.D.	75.5	57.0	55.2	54.3	37.2	29.0
	Range	103.0-405.0	116.1-344.1	153.5-374.3	72.6-289.8	164.8-313.6	167.0-283.0

A5:6 Discussion

The results of this study suggest that the TBPA assay is of limited clinical value as a routine test of thyroid function, due to the observed wide overlap of the normal and both hypo- and hyperthyroid ranges. Poor correlation was shown between TBPA and serum T^4 in all the groups studied. However, good correlation between TBG and serum T^4 was observed in the euthyroid and pregnant population.

TBG levels increased in pregnancy, in contrast to those of TBPA which showed a slight decrease. A similar result was reported by Ingenbleek et al., (16) who suggested that this was due to a stimulation of hepatic synthesis of TBG (17) and a depression of TBPA by oestrogens (18). The T^4:TBPA ratio failed to correctly classify just under 50% of the patients in the pregnant group, demonstrating the limited value of the TBPA to correct the serum T^4 in subjects with abnormal protein binding. Only in the hyperthyroid group was the T^4:TBPA found to give improved discrimination from the euthyroid range when compared to the T^4:TBG ratio.

The T^3 Uptake test has been condemned by several workers (5,7), as being only an indirect measure of TBG and also for its reported inability to measure high concentrations of TBG.

The present study compares the FTI with the T^4:TBG ratio and out of 68 euthyroid patients with elevated levels of TBG (i.e. the pregnant/ oral contraceptive group) 4 were labelled as "thyrotoxic" by the FTI and 13 were diagnosed as myxoedematous by the T^4:TBG.

Attwood et al., (8) drew no direct comparison between his investigations of the T^4:TBG ratio and the FTI. In fact his work showed a poorer discrimination of both hypo- and hyperthyroid sera from

the euthyroid range, than other workers have shown for the FTI. Comparison of the FTI with the T^4:TBG ratio for these two groups in the present study, have shown the FTI to be a more powerful discriminator, especially for hyperthyroidism, where 7 clinically proven thyrotoxics were misclassified by the T^4:TBG ratio as euthyroid.

The mapping technique (15) was also used to correlate serum T^4 with T^3U, TBG and TBPA in all groups. This method demonstrates improved separation of the five groups for the T^4 v T^3U plot compared to T^4 v TBG or T^4 v TBPA. Similar results were recorded by Roosdorp and Joustra (19), using a computerised modification of this technique to compare T^3U values with TBG levels. The authors concluded that the T^4:TBG did not appear to offer any real diagnostic advantage over the FTI.

In a recent paper by Cusick (20), the T^4:TBG ratio was found to be misleading in assessing thyroid status in patients with reduced TBG levels. The T^4:TBG ratio gave very high results in patients with undetectable levels of TBG, and was near the upper limit of normal for those patients with markedly lowered levels. The FTI however, produced results which although were below the reference range, proved of greater clinical value.

In summary the results of this study suggest that the TBPA is of little value in the routine assessment of thyroid disease, especially with regard to interpretation of the serum T^4. The T^4:TBG ratio is of value only in patients with a raised serum T^4 and an elevated T^3 U above 135%. In practice this rarely occurs, the incidence in this laboratory being in the order of 0.59%. Accordingly Mardell (21) observed from a study of 2,400 patients, that only 0.5% of T^3U values gave results of 135% or above and that none of these patients had T^4 levels high enough for misclassification into the thyrotoxic group, on

the basis of an FTI calculation.

Although a number of laboratories now routinely employ direct measurement of TBG as part of their thyroid function test profile it is clear from the findings of this study, that for the majority of patients, calculation of the FTI still offers the best overall diagnostic discrimination.

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Key Words

Thyroid function tests; thyroxine binding globulin; thyroxine binding prealbumin; tri-iodothyronine uptake; thyroid diseases.

References

1. Hamolsky, M.W., Stein, M. and Freedberg, A.S. (1957) The thyroid hormone - plasma protein complex in man. 11. A new *in vitro* method for study of "uptake" of labelled hormonal components by human erythrocytes. J. Clin. Endocrinol.Metab. 17,33-44.

2. Souma, J.A., Niejadlik, D.C., Cottrell, S. and Rankel, S. (1973) Comparison of thyroid function in each trimester of pregnancy with the use of triiodothyronine uptake, thyroxine iodine, free thyroxine and free thyroxine index. Am. J. Obstet. Gynaecol. 116, 905-910.

3. Goolden, A.W.G., Gartside, J.M. and Sanderson, C. (1967) Thyroid status in pregnancy and in women taking oral contraceptives. Lancet 1, 12-15.

4. Bold, A.M. and Browning, D.M. (1975) Quality control of thyroid function tests *in vitro*. J. Clin. Path. 28, 234-238.

5. McDowell, D.R. (1979) An evaluation of serum thyroxine binding globulin as a routine test of thyroid function. Ann. Clin. Biochem. 16, 81-85.

6. Olsen,K.J. and Anderson, C.J. (1979) Determination of free thyroid fractions using a simple T^3 - Uptake test. Scand. J. Clin. Lab. Invest. 39, 119-123.

7. Burr, W.A., Ramsden, D.B., Evans, S.E., Hogan, T., and Hoffenberg, R. (1977) Concentration of thyroxine-binding globulin : value of direct assay. Br. Med. J. 1, 485-488.

8. Attwood, E.C., Seddon, R.M. and Probert, D.E. (1978). The T^4:TBG ratio and the investigation of thyroid function. Clin. Biochem. 5, 218-221.

9. Ingbar, S.H. (1958) Prealbumin : A thyroxine-binding protein of human plasma. Endocrinology 63, 256-259.

10. Cheng, S.Y., Poges, R.A., Saroff, H.A., Edelhoch, H. and Robbins J. (1977) Analysis of thyroid hormone binding to human serum prealbumin by 8-anilinonapthalene-1-sulfonate fluorescence. Biochemistry 16, 3707-3713.

11. Robins, J. (1975) Thyroxine-Transport Proteins. Thyroid Hormone Metabolism (Harland, W.A. and Orr, J.S., eds). 1-22, Academic Press, London, New York, San Francisco.

12. Britton, K.E., Quinn, V., Brown, B.L. and Ekins, R.P. (1975) A strategy for thyroid function tests. Br. Med. J. 3, 350-352.

13. Laurell, C.B. (1966) Quantative estimation of proteins by electrophoresis in agarose gel containing antibodies. Anal. Biochem. 15, 45-52.

14. Bradwell, A.R., Burnett, D., Ramsden, D.B., Burr, W.A., Prince, H.P. and Hoffenberg, R. (1976) Preparation of a monospecific antiserum to thyroxine binding globulin for its quantitation by rocket immunoelectrophoresis. Clin. Chim. Acta 71, 501-510.

15. Mardell, R. (1978) A strategy for in vitro tests of thyroid function. The Radiochemical Centre Limited, Amersham, Buckinghamshire.

16. Ingenbleek,Y ., Luypaert, B. and De Nayer, P.H. (1980) Nutritional status and endemic goitre. Lancet, Feb 23, 388-392.

17. Glinoer, D., Gershengorn, M.C., Dubois, A. and Robbins, J. (1977) Stimulation of thyroxine - binding globulin synthesis by isolated Rhesus monkey hepatocytes after in vivo β - estradiol administration. Endocrinology 100, 807-813.

18. Sakaruda, T., Saito, S., Inagaki, K. and Tayama, S.and Torikai,T. (1967)

Polyacrylamide gel electrophoretic study on the effect of oestrogen on human thyroxine-binding prealbumin. Tohoku, J. Exp. Med. 93, 339-362

19. Roosdorp. N. and Joustra, M. (1979) A numerical comparison of the use of T^3 - Uptake values and of TBG levels for the estimation of free thyroxine in serum. Clin. Chim. Acta 98, 27-33.

20. Cusick, C.F. (1979) Thyroid function tests in thyroxine-binding globulin deficiency. Ann. Clin. Biochem. 16, 313-314.

21. Mardell, R. (1977) Concentration of thyroxine-binding globulin. Br. Med. J. May 14, 1282-1283.
(Letter).

REFERENCES

ABBASSI, V. and McKENZIE, J.M. (1967). Lack of iodide effect on serum and pituitary thyrotropin *in vivo*. Endocrinology, 81, 871-876

ADAMS, D.D., FASTIER, F.N., HOWIE, J.B., KENNEDY, T.H., KILPATRICK, J.A. and STEWART, R.D.H. (1973). Stimulation of the human thyroid by infusions of plasma containing LATS protector. J. Clin. Endocrinol Metab. 39, 826-832.

ADAMS, D.D., and KENNEDY, T.H. (1967). Occurence in thyrotoxicosis of a gamma globulin which protects LATS from neutralisation by an extract of thyroid gland. J. Clin. Endocrinol. Metab. 27, 173-177.

ADAMS, D.D. and KENNEDY, T.H. (1971). Evidence to suggest that LATS protector stimulates the human thyroid gland. J. Clin. Endocrinol Metab. 33, 47-51.

ADAMS, D.D., KENNEDY, T.H. and STEWART, R.D.H. (1974). Correlation between long-acting thyroid stimulator protector level and thyroid I^{131} uptake in thyrotoxicosis. Br. Med. J. 2, 199-201.

ADAMS, D.D. and PURVES, H.D. (1956). Abnormal responses in the assay of thyrotrophin. Proceedings of the University of Otago Medical School, 34, 11-12.

ANNAN, W. and ISHERWOOD, D.M. (1969). An automated method for the direct determination of total serum cholesterol. J. Med. Lab. Technol. 26, 202-211.

ASHER, R. (1949). Myxoedematous madness. Br. Med. J. 2, 555-562.

ATTWOOD, E.C. and PROBERT, D.E. (1978). An assessment under routine conditions of an immunoelectrophoretic assay for thyroxine-binding globulin. Clin. Biochem. 11, 226-229.

ATTWOOD. E.C., SEDDON, R.M. and PROBERT, D.E. (1978). The T^4/TBG ratio and the investigation of thyroid function. Clin. Biochem. 5, 218-221.

BAHEMUKA, M. and HODGKINSON, H.M. (1975). Screening for hypothyroidism in elderly patients. Br. Med. J. 2, 601-603.

BARNEA, A., BEN-JONATHAN, N., COLSTON, C., JOHNSTON, J.M. and PORTER, J.C. (1975). Differential sub-cellular compartmentalization of thyrotropin - releasing hormone (TRH) and gonadotropin releasing hormone (LRH) in hypothalamic tissue. Proc. Natl. Acad. Sci. USA 72, 3153-3157.

BARNES, H.V., GREENBERG, A.H., OWINGS, J. and BLIZZARD, R.M. (1972). The effect of chlordiazepoxide (Librium) on thyroid function in thyrotoxicosis. John Hopkins Med. J. 131, 298-302

BECKERS, C., CORNETTE,C. and THALASSO, M. (1974). Serum L-triiodothyronine radioimmunoassay : Measurement in normal subjects and in thyroid patients. J. Nucl. Med. Biol. 1, 121-129.

BENOIT, F.L. and GREENSPAN, F.S. (1967). Corticoid therapy for pretibial myxoedema. Observations on the long-acting thyroid stimulator. Ann. Intern. Med. 66, 711-720.

BERMUDEZ, F., SURTES, M.I. and OPPENHEIMER, J.H. (1975). High incidence of decreased serum triiodothyronine concentrations in patients with non-thyroidal disease. J. Clin. Endocrinol. Metab. 41, 27-40.

BERNSTEIN, G. and OPPENHEIMER, J.H. (1966). Factors influencing the concentration of free and total thyroxine in patients with non-thyroidal disease. J. Clin. Endocrinol. Metab. 26, 195-201.

BERSON, S.A. and YALOW, R.S. (1952). Effect of cortisone on iodine accumulating function of thyroid gland in euthyroid subjects. J. Clin. Endocrinol. 12, 407-422.

BLACK, E.G., GRIFFITHS, R.S., FINUCANE, J. and HOFFENBERG, R. (1975). Radioimmunoassay of T^3 and T^4 in serum and urine. In : Harland, W.A. and Orr J.S., eds. Thyroid Hormone Metabolism. Academic Press, 347-365.

BLUMBERG, A.G. and KLEIN, D.F. (1969). Chlorpromazine procyclidene and imipramine : effects on thyroid function in psychiatric patients. Clin. Pharmacol. Ther. 10, 350-354

BOLD, A.M. and BROWNING, D.M. (1975). Quality control of thyroid function tests in vitro. J. Clin. Pathol. 28, 234-238.

BRADWELL, A.R., BURNETT,D., RAMSDEN, D.B., BURR,W.A.,PRINCE, H.P. and HOFFENBURG, R. (1976). Preparation of a monospecific antiserum to thyroxine binding globulin for its quantitation by rocket immunoelectrophoresis. Clin. Clin. Acta. 71, 501-510.

BRAVERMAN, L.E. and FOSTER, A.E. (1969). Effect of chronic administration on thyroid hormone binding in serum. J. Nucl. Med. 10, 511-513.

BRAVERMAN, L.E., VAGENAKIS, A., DOWNS, P., FOSTER, A.E., STERLING, K. and INGBAR, S.H. (1973). Effects of replacement doses of sodium-L-thyroxine on the peripheral metabolism of thyroxine and triiodothyronine in man. J. Clin. Invest. 52, 1010-1017.

BRAVERMAN, L.E., WOEBER, K.A. and INGBAR, S.H. (1969). Induction of myxoedema by iodide in patients euthyroid after radioiodine or surgical treatment of diffuse toxic goitre. N. Engl. J. Med. 281, 816-821.

BRITTON, E.K., QUINN, V., BROWN, B.L. and EKINS., R.P. (1975). A strategy of thyroid function tests. Br. Med. J. 3, 350-352.

BROWN, B.L., EKINS, R.P., ELLIS, S.M and REITH, W.S. (1970). Specific antibodies to triiodothyronine hormone. Nature (Lond.) 226, 359-360.

BROWN, B.L., EKINS, R.P., ELLIS, S.M. and WILLIAMS, E.S. (1971). The radioimmunoassay of triiodothyronine. In : Fellinger, K. and Hofer, R., eds. Further Advances in Thyroid Research (6th International Conference). Academy of Medicine, Vienna, 1107.

BROWN, G.M. (1980). Psychiatric and neurologic aspects of endocrine disease. In : Krieger, D.T. and Hughes, J.C. Eds. Neuroendocrinology. Sunderland, Mass. Sinauer Associates. 185-194.

BROWN, J. and MUNRO, D.S. (1967). A new in vitro assay for thyroid stimulating hormone. J. Endocrinol. 38, 439-449.

BROWN-GRANT, K., HARRIS, G.W. and REICHLIN, S. (1954). The influence of the adrenal cortex on thyroid activity in the rabbit. J. Physiol. 126, 41-46

BROWNLIE, B.E.W., TURNER, J.G., ELLWOOD, M.A., ROGERS, T.G.H. and ARMSTRONG, D. (1977). Thyroid vascularity-documentation of iodide effect in thyrotoxicosis. Acta. Endocrinol. (Kbh) 86, 317-322.

BURKE, C.W. and EASTMAN, C.J. (1974). Thyroid hormones : Radioimmunoassay and saturation analysis. Br. Med. Bull. 30, 93-99.

BURKE, G., SILVERSTEIN, G.E. and SORKIN, A.I. (1967). Effect of long-term sulphonylurea therapy on thyroid function in man. Metabolism 16, 651-657.

BURR, W.A., GRIFFITHS, R.S., RAMSDEN, D.B., HOFFENBERG, R., MEINHOLD, H. and WENZEL, K.W. (1976). Effect of a single dose of dexamethasone on serum concentration of thyroid hormones. Lancet 2, 58-61.

BURR, W.A., RAMSDEN, D.B., EVANS, S.E., HOGAN, T. and HOFFENBERG, R. (1977). Concentration of thyroxine-binding globulin : value of direct assay. Br. Med. J. 1, 485-488.

BURR, W.A., RAMSDEN, D.B. and HOFFENBERG, R. (1980). Hereditary abnormalities of thyroxine-binding globulin concentration. Quant. J. Med. 195, 295-313.

BURSTEN, B. (1961). Psychoses associated with thyrotoxicosis. Arch. Gen. Psychiatry 4, 267-273.

CANNAN, R.K. (1958). Proposal for a certified standard for use in haemoglobinometry : Second and final report. J. Lab. Clin. Med. 52, 471-476.

CAPRINO, L. (1963). Influence of chlordiazepoxide in thyroidal radioiodine uptake. Boll. Soc. Ital. Biol. Sper. 39, 671-673, 674-677.

CARNEIRO, L., DORRINGTON, K.J. and MUNRO, D.S. (1966). Recovery of the long-acting thyroid stimulator from serum of patients with thyrotoxicosis by concentration of immunoglobulin G. Clin. Sci., 31, 215-221.

CARO, J.F., SLOANE, N.G. and GLENNON, J.A. (1980). Hyperthyroidism with normal serum total T^4, T^3 and thyroid binding protein concentration. J. Clin. Pathol. 33, 868-870.

CAVALIERI, R.R. and BECKER, C.E. (1971). Effects of phenobarbital on thyroid hormone kinetics in Graves' Disease. Programme of the American Thyroid Association. 47th Annual Meeting, Birmingham, Alabama, 15.

CAVALIERI, R.R., CASTLE, J.N. and SEARLE, G.L. (1969). A simplified method for estimating free thyroxine fraction in serum. J.Nucl. Med. 10, 565-570.

CHENG, S.Y., PAGES, R.A., SAROFF, H.A., EDELHOCH, H. and ROBBINS, J. (1977). Analysis of thyroid hormone binding to human serum prealbumin by 8-anilonapthalene-1-sulfonate fluorescence. Biochemistry 16, 3707-3713.

CHOPRA, I.J. (1972). A radioimmunoassay for measurement of thyroxine in unextracted serum. J. Clin. Endocrinol. Metab. 34, 938-947.

CHOPRA, I.J. (1976). An assessment of daily production and significance of thyroidal 3, 3',5' - triiodothyronine (reverse T^3) in man. J. Clin. Invest. 58, 32-40.

CHOPRA, I.J., CHOPRA, U., SOUTH, S.R., REZA, M. and SOLOMON,D.H. (1975) Reciprocol changes in serum concentrations of 3, 3',5' - triiodothyronine (reverse T^3) and 3, 5',3'-triiodothyronine (T^3) in systemic illness. J. Clin. Endocrinol. Metab. 41, 1043-1049.

CHOPRA, I.J., HERSHMAN, J.H. and HORNABROOK, R.W. (1975). Serum thyroid hormone and thyrotropin levels in subjects from endemic goitre regions in New Guinea. J. Clin. Endocrinol. Metab. 40, 326-333.

CHOPRA, I.J., HO, R.S. and LAM, R. (1972). An improved radioimmunoassay of triiodothyronine in serum : its application to clinical and physiological studies. J. Lab. Clin. Med. 80, 729.

CHOPRA, I.J., VAN HERLE, A.J., CHUA TECO, G.N. and NGUYEN, A.H. (1980). Serum free thyroxine in thyroidal and non-thyroidal illnesses : A comparison of measurements by Radioimmunoassay, Equilibrium dialysis, and free thyroxine index. J. Clin. Endocrinol. Metab. 51, 135-143.

CHRISTENSEN, L.K. (1959). A method for the determination of free, non-protein bound thyroxine in serum. Scand. J. Clin. Lab. Invest. 11, 326-331.

CLAGUE, R., MUKHTAR, E.D., PYLE, G.A., NUTT, J., CLARK, F., SCOTT, M., EVERED, D., REES SMITH, B. and HALL, R. (1976). Thyroid-stimulating immunoglobulins and the control of thyroid function. J. Clin. Endocrinol. Metab. 43, 550-556.

CLARK, F. (1975). Serum protein bound iodine or total thyroxine. J. Clin. Path. 28, 211-217.

CLARK, F. and BROWN, H.J. (1970). Free thyroxine index. Br. Med. J. 1, 543 and 672.

CLARK, F. and HALL, R. (1970). Chlordiazepoxide (Librium) and tests of thyroid function. Br. Med. J. 2, 266-268.

CLARK, F. and HORN, D.B. (1965). Assessment of thyroid function by the combined use of the serum protein-bound iodine and resin uptake of I^{131} - triiodothyronine. J. Clin. Endocrinol. Metab. 25, 39-45.

CLARK, H.G.M. and FREEMAN, T. (1967). A quantative immunoelectrophoresis method (Laurel electrophoresis). In : Peters, H. ed. Prot. Biol. Fluids, 14, Ebevier, Amsterdam, 503-509.

CLOWER, C.G., YOUNG, A.J and KEPAS, D. (1969). Psychiatric states resulting from disorders of thyroid function. Johns Hopkins Medical Journal, 124, 305-310.

CROSBY, W.H., MUNN, J.I. and FURTH, F.W. (1954). Standardizing a method for clinical haemoglobinometry. U.S. Armed Forces Med. J. 5, 693-703.

CROWE, M.J., LLOYD, G.G., BLOCH, S. and ROSSER, R.M. (1973). Hypothyroidism in patients treated with lithium : a review and two case reports. Psychol. Med. 3, 337-342.

CROXON, M.S., HALL, T.D. and NICOLOFF, J.T. (1977) Failure of triiodothyronine to inhibit TSH-mediated hormone release in man. J. Clin. Endocrinol. Metab. 45, 593-596.

CULLEN, M.J., BURGER, A.G. and INGBAR, S.H. (1973). Effects of diphenylhydantoin on peripheral thyroid hormone economy and the conversion of T^4 to T^3. Israel J. Med. Sci. 8, 1868-1871.

CUSICK, C.F. (1979). Thyroid function tests in thyroxine-binding globulin deficiency. Ann. Clin. Biochem. 16, 313-314.

DECAUX, G. and UNGER, J. (1978) Letter : Thyroid function and laboratory tests. Lancet 2, 1153.

DEWHURST, K.E., ELKABIR, D.J. and HARRIS, G.W. and HANDELBROTE, B.M. (1969). Observations of the blood concentration of thyrotrophic hormone (TSH) in schizophrenia and the affective states. Br. J. Psychiatry 115, 1003-1011.

DISSANAYAKE, S.A. and LEIBERMAN, D.M. (1969). Hypopitiutarism with paranoid psychosis : a description of two cases. J. Neurol. Neurosurg. Psychiatry 32, 233-236.

DRYSDALE, I.P., RAMSDEN, D.B. and HOFFENBERG, R. (1975). A routine method for the estimation of thyroxine binding globulin in human serum. In : Harland, W.A. and Orr, J.S., eds. Thyroid Hormone Metabolism. Academic Press, 261-279.

DUMONT, J.E., VAN SANDE, J., LAMY, F. (1976). The regulation of thyroid cell metabolism. In : Dumont, J.E., Brown, B.L. and Marshall, N.J., Eds. Eukaryotic Cell Function and Growth. Plenum Publishing Corporation, 685-710.

DUSSAULT, J.H., FISHER, D.A., NICOLOFF, J.T., ROW, V.V. and VOLPE, R. (1973). The effect of alteration of thyroxine binding capacity on the dialyzable and absolute fractions of triiodothyronine in circulation. Acta Endocrinol. 72, 265-271.

EASSON, W.M. (1966). Myxoedema with psychosis. Arch. Gen. Psychiat. 14, 277-283.

EASTMAN, C.J., CORCORAN, J.M., JEQUIER, A., EKINS, R.P. and WILLIAMS, E.S. (1973). Triiodothyronine concentration in cord and maternal sera at term. Clin. Sci. 45, 251-255.

EAYRS, J.T. (1960) Influence of the thyroid on the central nervous system. Br. Med. Bull. 16, 122-127.

EILERS, R.J. (1967). Notification of final adoption of an international method and standard solution for haemogobinometry specifications for preparation of standard solution. Am. J. Clin. Path. 47, 212-214.

EKINS, R.P., WILLIAMS, E.S. and ELLIS, S.M. (1969). The sensitive and precise measurement of serum thyroxine by saturation analysis (competitive protein binding assay). Clin. Biochem. 2, 253.

EL-HAZMI, M.A.F. (1975). On the interaction between thyroid hormones and the tranquillisers Librium and Valium. Clin. Chim. Acta 63, 211-221.

EMERSON, C.H., DYSON, W.L. and UTIGER, R.D. (1973). Serum thyrotrophin and thyroxine concentration in patients receiving lithium carbonate. J. Clin. Endocrinol. Metab. 36, 338-346.

ENGLER, D., DONALDSON, E.B., STOCKIGT, J.R. and TAFT, P. (1978). Hyperthyroidism without triiodothyronine excess : an effect of severe non-thyroidal illness. J. Clin. Endocrinol. Metab. 46, 77-82.

EPSTEIN, K.A., SCHNEIDERMAN, L.J., BUSH, J.W. and ZETTNER, A. (1981). The "abnormal" screening serum thyroxine (T^4) : analysis of physician response, outcome, cost and health effectiveness. J. Chron. Dis. 34, 175-190.

ERICSON, L.E., MELANDER, A., OWMAN, C. and SUNDLER, F. (1970). Endocytosis of thyroglobulin and release of thyroid hormone in mice by catecholamines and 5-hydroxytryptamine. Endocrinology 87, 915-923.

EVERED, D.C. (1974). Diseases of the thyroid gland. In : Investigation of Endocrine Disorders. J. Clin. Endocrinol. Metab. 3, 425-450.

EVERED, D.C. and HALL, R. (1972). Hypothyroidism. Br. Med. J. 1, 290-293.

EVERED, D.C., ORMSTON, B.J., SMITH, P.A., HALL, R. AND BIRD, T. (1973). Grades of hypothyroidism. Br. Med. J. 1, 657-662.

EVERED, D.C., TUNBRIDGE, W.M.G., HALL, R., APPLETON, D., BREWIS, M., CLARK, F., MANUEL, P. and YOUNG, E. (1978). Thyroid hormone concentrations in a large scale community survey. Effect of age, sex, illness and medication. Clin. Chim. Acta, 26, 365-368.

EVERED, D.C., VICE, P.A. and CLARK, F. (1976). The investigation of thyroid disease. Radiochemical Centre, Medical Monographs. No.9.

EVERED, D.C., VICE, P.A., GREEN, E. and APPLETON, D. (1976). Assessment of thyroid hormone assays. J. Clin. Path. 29, 1054-1059.

FEDERMAN, D.D., ROBBINS, J. and RALL, J.E. (1958). Effects of methytestosterone on thyroid function, thyroxine metabolism and thyroxine-binding protein. J. Clin. Invest. 37, 1024-1030.

FELDMAN, C.B. and CARTER, A.C. (1960). Endocrinologic and metabolic effects of 17α - methyl - 19 - nortestosterone in women. J. Clin. Metab. 20, 842-857.

FELICETTA, J. and GREEN, W.L. (1980). Value of free thyroxine index. N. Engl. J. Med. 302, 1480-1481.

FISHER, D.A. and ODDIE, T.H. (1968). Effect of methyl testosterone on thyroxine metabolism and on triiodothyronine kinetics. J. Clin. Endocrinol. 28, 1690-1698.

FLORSHEIM, W.H. (1958). The effect of anterior hypothalamic lesions on thyroid function and goitre development in the rat. Endocrinology 62, 783-789.

FLYNN, F.V. and HOBBS, J.R. (1971). The assessment of thyroid function. Ann. Clin. Biochem. 8, 59-72.

FORREST, J.N. Jr. (1975). Lithium inhibition of c-AMP - mediated hormones : a caution. N. Engl. J. Med. 292, 423-424.

FREEDMAN, A.M., KAPLAN, H.I. and SADOCK, B.J. (1975). Comprehensive textbook of Psychiatry : Modern Synopsis. New York.

FREEMAN,T. and PEARSON, J.D. (1969). The use of quantitative immunoelectrophoresis to investigate thyroxine binding human serum proteins. Clin. Chim. Acta. 26, 365-368.

FRIZEL, D., MALLESON, A.and MARKS, V. (1967). Plasma levels of ionised calcium and magnesium in thyroid disease. Lancet 2, 1360-1361.

FYFFE, J.A., AYOUB, L., COHEN, H.N., TURNER, J.G., THOMASON, J.A. and RATCLIFFE, J.G. (1980). Clinical and laboratory evaluation of four methods of assessing free thyroxine status in thyroid clinic patients. Ann. Clin. Biochem. 17, 334-338.

GALE, C.C. (1973). Neuroendocrine aspects of thermoregulation. Ann. Rev. Physiol. 35, 391-430.

GEEL, S.E. and TIMIRAS, P.S. (1967). The influence of neonatal hypothyroidism and of thyroxine on the ribonucleic acid and deoxyribonucleic acid concentrations of rat cerebral cortex. Brain Res. 4, 135-142.

GHARIB, H., RYAN, R.J. and MAYBERRY, W.E. (1972). Triiodothyronine (T^3) radioimmunoassay : A critical evaluation. Mayo. Clin. Proc. 47, 934-937.

GLINOER, D., GERSHENGORN, M.C., DUBOIS, A. and ROBBINS, J. (1977). Stimulation of thyroxine-binding globulin synthesis by isolated Rhesus monkey hepatocytes after in vivo β-oestradiol administration. Endocrinology 100, 807-813.

GOLD, M.S., POTTASH, A.L.C., DAVIES, R.K., RYAN, N., SWEENEY, D.R. and MARTIN, D.M. (1979). Distinguishing unipolar and bipolar depression by thyrotropin release test. Lancet 2, 411-413.

GOLD, M.S., POTTASH, A.L.C. and EXTEIN, I. (1981). Hypothyroidism and depression. J.A.M.A. 245, 1919-1922.

GOOLDEN, A.W.G., GARTSIDE, J.M. and SANDERSON, C. (1967). Thyroid status in pregnancy and in women taking oral contraceptives. Lancet 1, 12-15.

GORDON, A.H., GROSS. J., CONNOR, D. and PITT-RIVERS, R. (1952). Nature of the circulating thyroid hormone-plasma protein complex. Nature 169, 19-20.

GREGERMAN, R.I. (1971). Intrinsic physiological variables and non-thyroidal illness. In : Werner, S.C. and Ingbar, S.H. eds. The Thyroid. New York, Harper and Row. 914.

GROSS, J. and PITT-RIVERS, R. (1952). The identification of 3',5',3'-L-triiodothyronine in human plasma. Lancet 1, 439-441.

GWINUP, G. and RAPP, N. (1975). Low serum thyroxine in phenothiazine - treated psychiatric patients. Am. J. Clin. Pathol. 63, 94-97.

HADEN, H.T. (1966). Thyroid function tests. Physiologic basis and clinical interpretation. Postgrad. Med. 40, 129-137.

HALL, R., AMOS, J. and ORMSTON, B.J. (1971). Radioimmunoassay of human serum thyrotrophin. Br. Med. J. 1, 582-585.

HALL, R., ORMSTON, B.J., BESSER, G.H., FRYER, R.J. and McKENDRICK, M. (1972). The thyrotropin releasing hormone test in diseases of the pituitary and hypothalamus. Lancet 1, 759-762.

HALL, R., SMITH, B.R. and MUKHTAR, E.D. (1975). Thyroid stimulation in health and disease. Clin. Endocrinol. 4, 213-230.

HAM, T.H. and CURTIS, F.C. (1938). Sedimentation rate of erythrocytes. Medicine 17, 447-517.

HAMOLSKY. M.W., STEIN, M. and FREEDBERG, A.S. (1957). The thyroid hormone-plasma protein complex in man II. A new in vitro method for study of "uptake" of labelled hormonal components by human erythrocytes. J. Clin. Endocrinol. Metab. 17, 33-44.

HANSEN, H.H. (1965). Sephadex binding of I^{131} labelled L-triiodothyronine as a test of thyroid function. Scand. J. Clin. Lab. Invest. 18, 240-244.

HANSEN. J.E.M. (1962). Mechanism of the effect of phenylbutazone on the thyroid gland. Acta Endocrinol. (Kbh). Suppl. 67, 162.

HELENIUS, T. and LIEWENDAHL, K. (1979). Abnormal thyroid function tests in severe non-thyroidal illness : diagnostic and pathophysiologic aspects. Scand. J. Clin. Lab. Invest. 39, 389-397.

HENSCHKE, P.J. and PAIN, R.W. (1977). Thyroid disease in a psychogeriatric population. Age and Ageing 6, 151-155.

HERLE, A.J.V., VASSANT, G. and DUMONT, J.E. (1979). Control of thyroglobulin synthesis and secretion. N. Engl. J. Med. 301, 239-249, 307-314.

HERSHMAN, J.M. (1974). Clinical application of thyrotropin-releasing hormone. N. Engl. J. Med. 290, 886-890.

HERSCHMAN, J.M. and HIGGINS, H.P. (1971). Hyatidiform mole - a cause of clinical hyperthyroidism. Report of two cases with evidence that the molar tissue secreted a thyroid stimulator. N. Engl. J. Med. 284, 573-577.

HERSHMAN, J.M., JONES, C.M, and BAILEY, A.L. (1972). Recripocal changes in serum thyrotropin and free thyroxine produced by heparin. J. Clin. Endocrinol. Metab. 34, 574-579.

HERSHMAN, J.H. and STARNES, W.R. (1969). Extraction and characterisation of a thyrotropic material from the human placenta. J. Clin. Invest. 48, 923-929.

HESCH, R.D., GATZ, J., McINTOSH, C.H.S., JANZEN, J. and HEHRMANN, R. (1976). Radioimmunoassay of thyroxine-binding globulin in human plasma. Clin. Chim. Acta 70, 33-42.

HESCH, R.D., HÜFNER, M. and VON ZU MÜHLEN, A. (1972). The radioimmunoassay of triiodothyronine. (Abstr.) In : Proceedings of the IVth International Congress of Endocrinology, 613.

HESCH, R.D. and EVERED, D.C. (1973). Radioimmunoassay of triiodothyronine in unextracted human serum. Br. Med. J. 1, 645-648.

HILLIER, A.P. (1971). Human thyroxine-binding globulin and thyroxine-binding prealbumin : dissociation rates. J. Physiol. 217, 625-634.

HIROOKA, Y. (1976). The hypothalamic pituitary thyroid axis : studies on the regulatory role of thyrotropin releasing hormone (TRH). Folia Endocrinol. Jpn. 52, 908-925.

HOBBS, J.R., BAYLISS, R.I.S. and MACLEGAN, N.F. (1963). The routine use of I^{132} in the diagnosis of thyroid disease. Lancet 1, 8-13.

HOFFENBERG, R. (1973). Triiodothyronine. Clin. Endocrinol. 2, 75-87.

HOLLANDER, C.S., MITSUMA, T., NIHEI, N., SHENKMAN, L., BURDAY, S.Z. and BLUM. M. (1972). Clinical and laboratory observations in cases of triiodothyronine toxicosis confirmed by radioimmunoassay. Lancet 1, 609-611.

HOMBURGER, H.A. and HEWAN-LOWE, K. (1979). Predictive values of thyroxine, thyrotropin and triiodothyronine concentrations in serum. Clin. Chem. 25, 669-674.

HORN, D.B. (1975). Available assays for serum thyroxine and for serum uptake tests. J. Chim. Path. 28, 218-224.

HORWITZ, D.L. and REFETOFF, S. (1977). Graves' disease associated with familial deficiency of thyroxine-binding globulin. J. Clin. Endocrinol. Metab. 44, 242-247.

HOWORTH, P.J.N. and MACLAGAN, N.F. (1969). Clinical application of serum total thyroxine estimation, resin uptake and free thyroxine index. Lancet 1, 224-228.

HÜFNER, M. and HESCH, R.D. (1973). A comparison of different compounds for TBG-blocking used in radioimmunoassay for triiodothyronine. Clin. Chim. Acta. 44, 101-107.

HUNTON, R.B., WELLS, M.V. and SKIPPER, E.W. (1965). Hypothyroidism in diabetics treated with sulphonylurea. Lancet 2, 449-451.

INGBAR, S.H. (1953). Effect of cortisone on thyroidal and renal metabolism of iodine. Endocrinology 53, 171-181.

INGBAR, S.H. (1958). Prealbumin : A thyroxine binding protein of human plasma. Endocrinology 63, 256-259.

INGBAR, S.H. (1972). Autoregulation of the thyroid response to iodide excess and depletion. Mayo Clinic Proc. 47, 814-823.

INGBAR, S.H. (1976). Ageing and thyroid hormone economy. J. Am. Geriatr. Soc. 24, 49-53.

INGENBLEEK, Y., LUYPAERT, B. and DE MAYER, P.H. (1980). Nutritional status and endemic goitre. Lancet 1, 388-392.

INGENBLEEK, Y., DE VISSCHER, M. and DE NAYER, P.H. (1972). Measurement of prealbumin as an index of protein - calorie malnutrition. Lancet 2, 106-109.

INGENBLEEK, Y., VAN DAN SCHRIECK, H.G., DE NAYER, P.H. and DE VISSCHER,M. (1975). Albumin, transferrin and thyroxine-binding prealbumin/ retinol-binding protein (TBPA-RBP). Complex in assessment of malnutrition. Clin. Chim. Acta. 63, 61-67.

JACKSON, I.M.D. and REICHLIN, S. (1979). Distribution and biosynthesis of TRH in the nervous system. In : Collu, R., Barbeau, A., Ducharme, J.R. and Rochefort, J.G., eds. Central Nervous System Affects of Hypothalamic Hormones and other Peptides. Raven Press, New York, 3-54.

JEFFREYS, P.M. (1972). The prevalence of thyroid disease in patients admitted to a geriatric department. Age Ageing 1, 33-37.

JIANG, N. and TUE, K. (1977). Determination of free thyroxine in serum by radioimmunoassay. Clin. Chem. 23, 1679-1683.

JOASOO, A. (1975). T^4 thyrotoxicosis with normal or low serum T^3 concentration. Aus. N.Z. J. Med. 5, 432-435.

JOHNSON, W.O. (1928). Psychosis and hyperthyroidism. J. Nerv. Ment. Dis. 67, 558-566.

KAGEDAL, B., MANSON, J.C., NORR, A., SORBO, B. and TEGLER, L. (1981) Screening for thyroid disorders in middle-aged women by computer-assisted evaluation of a thyroid hormone panel. Scand. J. Clin. Lab. Invest. 41, 403-408.

KAJIHARA, A. and KENDALL, J.W. (1967). Evidence for a hypothalamic feedback centre controlling TSH release. Clin. Res. 15, 123-124.

KAJIHARA, A., ONAYA, T., YAMADA, T., TAKEMURA, Y. and KOTANI, M. (1972). Further studies on the acute effect of cold on thyroid activity and its mechanism. Endocrinology 90, 538-544.

KALES, A., HENSER, G., JACOBSEN, A., KALES, J.D., HANLEY, J., ZWEIZIG, J.R., and PAULSON, M.J. (1967). All night sleep studies in hypothyroid patients before and after treatment. J. Clin. Endocrinol. Metab. 27, 1593-1599.

KASTIN, A.J., EHRENSING, R.H., SCHALCH, D.S. and ANDERSON, M.S. (1972). Improvement in mental depression with decreased thyrotropin response after administration of thyrotropin-releasing hormone. Lancet 2, 740-742.

KATZENELBOGEN, S. and LUTON, F.H. (1935). Hyperthyroidism and psychobiological reactions. Am. J. Psychiatry 91, 969-981.

KENDALL-TAYLOR, P. (1970). The action of dibutyrl cyclic 3',5' - adenosinemonophosphate on the mouse thyroid gland in vitro J. Endocrinol. 47, 333-338.

KENDALL-TAYLOR, P. (1972). Hyperthyroidism. Br. Med. J. 2, 337-341.

KENDALL-TAYLOR, P. (1973). Effects of long-acting thyroid stimulator (LATS) and LATS protector on human thyroid adenyl cyclase activity. Br. Med. J. 3, 72-75.

KENDALL-TAYLOR, P., DIRMIKIS, S.M. and MUNRO, C.S. (1975). Long-acting thyroid stimulator and related factors. Proc. Roy. Soc. Med. 68, 252-253.

KIRKEGAARD, C., BIRK LAURIDSEN, V. and NERUP, J. (1973). Letter : Lithium and the thyroid. Lancet 2, 1210.

KIRKHAM, K.E. (1962). A new bioassay technique for the measurement in vitro of thyrotropic hormone in serum and pituitary extracts. J. Endocrinol. 25, 259-269.

KORCEK, L. and TABACHNICK, M. (1976). Interaction of thyroxine and triiodothyronine with human thyroxine-binding globulin. J. Biol. Chem. 251, 3558-3562.

KRANZ, T., TRAUTWEIN, A. and SIEBER, A. (1974). The quantitative determination of thyroxine binding globulin by Laurell electrophoresis. Z. Klin. Chem. Klin. Biochem. 12, 124-127.

KUMAGAI, L.F., JUBIZ, W. and JESSOP, L.D. (1967). A simple and rapid method for determining free thyroxine by charcoal absorption. Clin Res. 15, 124.

LARSEN, P.R. (1971). Inhibition of triiodothyronine (T^3) binding to thyroxine binding globulin by sodium salicylate and its application to immunoassay of T^3 in human serum. Metabolism 20, 976-980.

LARSEN, P.R. (1972). Direct immunoassay of triiodothyronine in human serum. J. Clin. Invest. 51, 1939-1949.

LARSEN, P.R. (1972). Salicylate-induced increase in free triiodothyronine in human serum. J. Clin. Invest. 51, 1125-1134.

LARSEN, P.R. (1972). Triiodothyronine : review of recent studies of its physiology and pathophysiology in man. Metabolism 21, 1073-1092.

LARSEN, P.R., ATKINSON, A.J. Jr., WELLMAN, H.N. and GOLDSMITH R.E. (1970). The effect of diphenylhydantoin on thyroxine metabolism in man. J. Clin. Invest. 49, 1266-1279.

LAURELL, C.B. (1965). Antigen-antibody crossed electropheresis. Anal. Biochem. 10, 358-361.

LAURELL, C.B. (1966). Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. Anal. Biochem. 15, 45-52.

LAURELL, C.B. (1972). Electroimmuno assay. Scand. J. Clin. Lab. Invest. 29, Suppl. 124, 21-23.

LAZARUS, J.H., RICHARDS, A.R., ADDISON, G.M. and OWEN. G.M. (1974). Treatment of thyrotoxicosis with lithium carbonate. Lancet 2, 1160-1163.

LECUREUIL, M., CRONZAT-REYNES, G., BERNARD, J. and CHOFFEL, C. (1978). Correlation of free thyroxine index and thyroxine : thyroxine binding globulin ratio, with the free thyroxine concentration as measured by the thyroxine and thyroxine binding globulin radioimmunoassays. Clin. Chim. Acta. 87, 373-381.

LEE, N.D., HENRY, R.J. and GOLUB, O.J. (1964). Determination of the free thyroxine content of serum. J. Clin. Endocrinol. Metab. 24, 486-495.

LEVY, R.P., MARSHALL, J.S. and VELAYO, N.L. (1971). Radioimmunoassay of human thyroxine binding globulin (TBG). J. Clin. Endocrinol. Metab. 32, 372-381.

LIDZ, T. and WHITEHORN. J.C. (1949). Psychiatric problems in a thyroid clinic. J.A.M.A. 139, 698-701.

LIEBLICH, J.M. and UTIGER, R.D. (1971). Triiodothyronine radioimmunoassay. J. Clin. Invest. 50, 60a.

LIEWENDAHL, K. (1977). On the selection of *in vitro* thyroid function tests. Scand. J. Clin. Lab. Invest. 37, 379-384.

LIEWENDAHL, K. and MAJURI, H. (1976). Thyroxine, triiodothyronine and thyrotropin in serum during long-term diphenylhydantoin therapy. Scand. J. Clin. Lab. Invest. 36, 141-144.

LIEWENDAHL, K., TOTTERMAN, J. and LAMBERG. B.A. (1971). Determination of total and free serum thyroxine in thyroid diseases. Acta Endocrinol. (Kbh), 69, 793-800.

LINSK, J.A., PATON, B.C., PERSKY, M., ISAACS, M. and KUPPERMAN, H.S. (1957). The effect of phenylbutazone and a related analogue (G 25671) upon thyroid function. J. Clin. Endocrinol. Metab. 17, 416-423.

MAAYAN, M.L., DEBONS, A.F., VOLPERT, E.M. and KRIMSKY, I. (1977). Catecholamine inhibition of thyrotropin-induced secretion of thyroxine : Mediation by an α - adrenegic receptor. Metabolism 26, 473-475.

MAAYAN, M.L. and INGBAR, S.H. (1968). Epimephrine : Effect on uptake of iodine by dispersed cells of calf thyroid gland. Science 162, 124-125.

MACKENER, C.R. (1976). Effect of clofibrate administration on several biochemical parameters of normal and thyroidectomized rats. Biochem. Pharmacol. 26, 301-306.

MARDELL, R. (1977). Concentration of thyroxine-binding globulin. Br. Med. J. 1, 1282-1283.

MARDELL, R. (1978). A strategy for *in vitro* tests of thyroid function. The Radiochemical Centre Ltd., Amersham, Buckinghamshire.

MARSDEN, P., HOWORTH, P.J.N., CHALKLEY, S., ACOSTA, M., LEATHERDALE, B. and McKERRON, C.G. (1975). Hormonal pattern of relapse in hyperthyroidism. Lancet 1, 944-947.

MARTINI, L. (1973). Hypothalamic mechanisms controlling anterior pituitary functions. In : Lissak, K. ed. Hormones and Brain Function. New York, Plenum Press, 123-142.

McDOWELL, D.R. (1979). An evaluation of serum thyroxine binding globulin as a routine test of thyroid function. Ann. Clin. Biochem. 16, 81-85.

McKELVY, J.F., SHERIDAN, M., JOSEPH, S., PHELPS, C.H. and PERRIE, S. (1975). Biosynthesis of thyrotropin-releasing hormone in organ cultures of the guinea pig median eminence. Endocrinology. 97, 908-913.

McKENZIE, J.M. (1958). The bioassay of thyrotropin in serum, Endocrinology 63, 372-382.

McKENZIE, J.M. (1972). Does LATS cause hyperthyroidism in Graves' disease ? (A review biased towards the affirmative) Metabolism 21, 883-894.

McKENZIE, J.M. and ZAKARIJA, M. (1976). A reconsideration of a thyroid-stimulating immunoglobulin as the cause of hyperthyroidism in Graves' disease. J. Clin. Endocrinol. Metab. 42, 778-781.

McKEOWN, T. (1968). Validation of screening procedures. In : Lord Cohen of Birkenhead, Williams, E.T. and McLachlan, G. eds. Screening in Medical Care, Oxford University Press, 1.

McLARTY, D.G., O'BOYLE, J.H., SPENCER, C.A. and RATCLIFFE, J.G. (1975). Effect of lithium on hypothalamic-pituitary thyroid function in patients with affective disorders. Br. Med. J. 3, 623-626.

McLARTY, D.G., RATCLIFFE, W.A., RATCLIFFE, J.G., SHIMMINO, J.D. and GOLDBERG, A. (1978). A study of thyroid function in psychiatric in-patients. Br. J. Psychiatry 133, 211-218.

MEDEIROS-NETO, G., NICOLAU, W., TAKEDA, A. and ULHOA CINTRA, A.β. (1975). Effect of iodized oil on iodine content, thyroglobulin maturation and on biochemical constituents of endemic goitre in Brazil. Acta Endocrinol. (kbh) 79, 439-450.

MEHBOD, H., SWARTZ, C.D. and BREST, A.N. (1967). The effect of prolonged thiazide administration on thyroid function. Arch. Intern. Med. 119, 283-286.

MEINHOLD, H. and WENZEL, K.W. (1974). Radioimmunoassay of thyroxine in unextracted serum. Horm. Metab. Res. 6, 169-170.

MESSIHA, F.S., AGALLIANOS, D. and CLOWER, C. (1970). Dopamine excretion in affective states and following Li3 CO_3 therapy. Nature (London) 225, 868-869.

MITCHELL, M.L. (1958). Resin uptake of radiothyroxine in sera from non-pregnant and pregnant women. J. Clin. Endocrinol. Metab. 18, 1437-1438.

MITCHELL, M.L., HARDEN, A.B. and O'ROURKE, M.E. (1960). The *in vitro* resin sponge uptake of triiodothyronine - I^{131} from serum in thyroid disease and in pregnancy. J. Clin. Endocrinol. Metab 20, 1474 - 1483.

MITSUMA, T., COLUCCI, J., SHANKMAN, L. and HOLLANDER, C.S. (1972). Rapid simultaneous radioimmunoassay for triiodothyronine and thyroxine in unextracted serum. Biochem. Biophys. Res. Commun. 46, 2107-2113.

MITSUMA, T., GERSHENGORN, M.C., COLUCCI, J. and HOLLANDER, C.S. (1971). Radioimmunoassay of triiodothyronine in unextracted human serum. J. Clin. Endocrinol. Metab. 33, 364-367.

MITSUMA, T., NIHEI, N., GERSHENGORN, M.C. and HOLLANDER, C.S. (1971). Serum tiiodothyronine : measurement in human serum by radioimmunoassay with corroboration by gas-liquid chromatography. J. Clin. Invest. 50, 2679-2688.

MOLHOLM-HANSEN, J.E., SKOVSTED, L., BIRK LAURIDSEN, V.B., KIRKEGAARD, C. and SIERSBAEK NILSON, K. (1974). The effect of diphenylhydantoin on thyroid function. J. Clin. Endocrinol. Metab. 39, 785-788.

MONTOYA, E., WILBER, J.F. and LORINCZ, M. (1979). Catecholaminergic control of thyrotropin secretion. J. Lab. Clin. Med. 93, 887-894.

MOSES, A.C., LAWLOR, J., HADDOW, J. and JACKSON, I.M.D. (1982). Familial euthyroid hyperthyroxinaemia resulting from increased thyroxine binding to thyroxine-binding prealbumin. N. Engl. J. Med. 306, 966-969.

MUKHTAR, E.D., SMITH, B.R., PYLE, G.A., HALL, R. and VICE, P. (1975). Relation of thyroid-stimulating immunoglobulins to thyroid function and effects of surgery, radioiodine, and antithyroid drugs. Lancet 1, 713-715.

MURPHY, B.P. and PATTEE, C.J. (1964). Determination of thyroxine utilizing the property of protein binding. J. Clin. Endocrinol. Metab. 24, 187-196.

MUSA, B.U., KUMAR, R.S. and DOWLING, J.T. (1968). Effects of salicylates on the distribution and early plasma disappearance of thyroxine in man. J. Clin. Endocrinol. Metab. 28, 1461-1464.

NAUMAN, J.A., NAUMAN, A. and WERNER, S.C. (1967). Total and free triiodothyronine in human serum. J. Clin. Invest. 46, 1346-1355.

NICHOLOFF, J.T., FISHER, D.A. and APPLEMAN, M.D.Jr. (1970). The role of glucocorticoids in the regulation of thyroid function in man. J. Clin. Invest. 49, 1922-1929.

NICOLS, R.E. (1942). A study of the phenomenon of erythrocyte sedimentation. J. Lab. Clin. Med. 27, 1317-1327.

NICHOLSON, G., LEIBLING, L.I. and HALL, R.A. (1976). Thyroid dysfunction in female psychiatric patients. Br. J. Psychiatry. 129, 236-238.

NICHOLSON, J.L. and ATTMAN, J. (1972). The effects of early hypo- and hyperthyroidism on the development of the rat cerebellar cortex. 1. Cell proloferation and differentiation. Brain Res. 44, 13-23.

NICOD, P., BURGER, A., STAEHELI, V. and VALLOTTON, M.B. (1976). A radioimmunoassay of 3,3',5' - triiodo-L-thyronine in unextracted serum : method and clinical results. J. Clin. Endocrinol. Metab. 42, 823-829.

NYSTROM. E., BENGTSSON, C., LINDSTEDT, G. and LUNDBERG, P.A. (1981). Screening for thyroid disease. Lancet, 2, 927-928.

OBSEMAN, H., BUSE, J., HERBERT, E.G., ELLISON, H.S. and BUSE, M.G. (1963). Effect of chlordiazepoxide on thyroid function. J.A.M.A. 184, 342.

ODELL, W.D., WILBER, J.F. and UTIGER, R.D. (1967). Studies of thyrotropin physiology by means of radioimmunoassay. Rec. Progr. Horm. Res. 23. 47-85.

OLSEN, K.J. and ANDERSON, C.J. (1979). Determination of free thyroid hormone fractions using a simple T^3-Uptake test. Scand. J. Clin. Lab. Invest. 39, 119-123.

ONAYA, T., KOTANI, M., YAMADA, T. and OCHI, Y. (1973). A new in vitro test to detect thyroid stimulators in sera from hyperthyroid patients by measuring colloid droplet formation and cyclic AMP in human thyroid slices. J. Clin. Endocrinol. Metab. 36, 859-866.

ONAYA, T., MIYAKAWA, M., MIKIVCHI, M. and FURIHATA, R. (1978). Altered responsiveness to thyrotropin in thyroid slices of Graves' disease patients preoperatively treated with excess iodide. J. Clin. Endocrinol. Metab. 47, 405-408.

OPPENHEIMER, J.H., SCHWARTZ, H.L. and SURBO, M.I. (1972). Propylthiouracil inhibits the conversion of L-thyroxine to L-triiodothyronine. An explanation of the antithyroxine effect of proplythiouracil and evidence supporting the concept that triiodothyronine is the active hormone. J. Clin. Invest. 51, 2493-2497.

OPPENHEIMER, J.H., SURKS, M.I. and SCHWARTZ, H.L. (1969). The metabolic significance of exchangeable cellular thyroxine. Rec. Prog. Horm. Res. 25, 381-422.

OPPENHEIMER, J.H. and TAVERNETTI, R.R. (1962). Studies on the thyroxine-diphenylhydantoin interaction : effect of 5, 5' - diphenylhydantoin on the displacement of L-thyroxine from thyroxine binding globulin (TBG). Endocrinology 71, 496-504.

ORMSTON, B.J., GARRY, R., CRYER, R.J., BESSER, G.M. and HALL, R. (1971). Thyrotropin-releasing hormone : a new test of thyroid function. Lancet 2, 10-14.

OSORIO, C. (1962). Effect of salicylate and dinitrophenol on the binding of thyroid hormones by human and rat serum at pH 7.4. J. Physiol. 163, 151-159.

OTSUKI, M., DAKODA, M. and BABA, S. (1973). Influence of glucocorticoids on TRF induced TSH response in man. J. Clin. Endocrinol. Metab 36, 95-102.

PAIN, R.W. and DUNCAN, B.McL. (1976). Pictorial reporting on multiple thyrcid function results. Clin. Chem. 22, 1562-1566.

PAMENTER, R.W. and HEDGE, G.A. (1980). Inhibition of thyrotropin secretion by physiological levels of corticosterone. Endocrinology 106, 162-166.

PATEL, Y.C. and BURGER, H.G. (1973). A simplified radioimmunoassay for triiodothyronine. J. Clin. Endocrinol. Metab. 36, 187-190.

PATEL, Y.C. and BURGER, H.G. (1973). Serum triiodothyronine in health and disease. Clin. Endocrinol. 2, 339-349.

PEET, M. (1981). Laboratory screening for physical illness in psychiatric patients. Lancet 2, 529-530.

PETERSEN, B.A.,GIESE, R.W., LARSEN, P.R. and JARGER, B.L. (1977). Measurement of free thyroid hormones in serum by dialysis and gas chromatography. Clin. Chem. 23, 1389-1396.

PISAREV, M.A. and KLEIMAN de PISAREV, D.L. (1980). Biochemistry of thyroid regulation under normal and abnormal conditions. J. Endocrinol. Invest. 3, 317-325.

PITTMAN, C.S., CHAMBERS, J.B.Jr. and READ, V.A. (1971). The extrathyroid conversion rate of thyroxine to triiodothyronine in normal man. J. Clir. Invest. 50, 1187-1196.

PITTMAN, J.A., BROWN, R.W. and REGISTER, H.B.Jr. (1962). Biological activity of 3,3',5' - triiodo-DL-thyronine. Endocrinology 70, 79-83.

PITTS, F.M. Jr. and GUZE, S.B. (1961) Psychiatric disorders and myxoedema. Am. J. Psychiatry 118, 142-147.

PRANGE, A.J. Jr., MCCURDY, R.L. and COCHRANE, C M. (1967). The systolic blood pressure response of depressed patients to infused norepinephrine. J. Psychiat. Res. 5, 1-13.

PRANGE, A.J. Jr. NEMEROFF, C.B., LOOSEN, P.T., BISETTE, G., OSBAHR III, A.J., WILSON, I.C. and LIPTON, M.A. (1979). Behavioural effects of thyrotropin-releasing hormone in animals and man : a review . In : Collu, R., Barbeau, A., Ducharme, J.R. and Rochefort, J.G. eds. Central Nervous System Effects of Hypothalamic Hormones and Other Peptides. New York, Raven Press, 75-96.

PRANGE, A.J.Jr., WILSON, I.C., KNOX, A., McCLONE, T.S. and LIPTON, M.A. (1970). Enhancement of imipramine by thyroid stimulating hormone : Clinical and theoretical implications. Am. J. Psychiatry, 127, 191-199.

PRANGE, A.J. Jr., WILSON, I.C., LARA, P.P., ALLTOP, L.B. and BREESE, G.R. (1972). Effects of thyrotropin-releasing hormone in depression. Lancet 2, 999-1002.

PRANGE, A.J. Jr., WILSON, I.C., RABON, M. and LIPTON, M.A. (1969). Enhancement of Imipramine antidepressant activity by thyroid hormone. Am. J. Psychiatry 126, 457-469.

PREMACHANDRA, B.N., GOSSAIN, V.V. and PERLSTEIN, I.B. (1976). Free thyroxine index versus free thyroxine concentration in inherited partial thyroxine binding globulin defeciency. Clin. Chem. 22, 1408.

PUHRINGER, W., WIRZ-JUSTICE, A. and HOLE, G. (1975). Letter : TRH response as screening for specific antidepressant therapy. Lancet 1, 1344-1345.

RAMEY, J.N., BURROW, G.N., SPAULDING, S.W., DONABEDIAN, R.K., SPEROFF, L. and FRANTZ, A.G. (1976). The effect of aspirin and indomethacin on the TRH response in man. J. Clin. Endocrinol. Metab, 43. 107-114.

RAOUF, A.A.M., GEISOW, M.J., O'GORMAN, P., MARSDEN, P. and HOWORTH P.J.N. (1979). A method for the preparation of human thyroxine binding globulin ; its importance in the establishment of an accurate and specific radioimmunoassay. Clin. Chim. Acta. 104, 25-41.

RAPOPORT, B., WEST, M.N. and INGBAR, S.H. (1976). On the mechanism of inhibition by iodide of the thyroid adenylate cyclase response to thyrotrophic hormone. Endocrinology 99, 11-22.

RASTOGI, G.K. and SAWHNEY, R.S. (1974). Triiodothyronine radioimmunoassay in unexpected serum. Indian J. Med. Res. 62, 225-232.

RATCLIFFE, W.A. RATCLIFFE, J.G., McBRIDE, A.D., HARLAND, W.A. and RANDELL, T.W. (1974). The Radioimmunoassay of thyroxine in unextracted human serum. Clin. Endocrinol. 3, 481-488.

REICHLIN, S., MARTIN, J.B., MITNICK, M., BOSHANS, R.L., GRIMM, Y. BOLLINGER, J., GORDON, J. and MALACARA, J. (1972). The hypothalamus in pituitary-thyroid regulation. Recent Prog. Horm Res. 28, 229-286.

ROBBINS, J. (1956). Reverse-flow zone electrophoresis. A method for determining the thyroxine-binding capacity of serum protein. Arch Biochem. Biophys. 63, 461-469.

ROBBINS, J. (1963). In : Evaluation of Thyroid and Parathyroid Function Sunderman, F.W. and Sunderman, F.W. Jr. Eds. Lipincott, Philadelphia

ROBBINS, J. (1973). Inherited variations in thyroxine transport. Mt. Sinai J. Med. N.Y. 40, 511-519.

ROBBINS, J. (1975). Structure and function of thyroxine-transport proteins In : Harland, W.A. and ORR, J.S. Eds. Thyroid Hormone Metabolism. Academic Press, 1-22.

ROBBINS, J., CHENG, S.Y., GERSHENGORN, M.C., GLINOER,D., CHAPMAN, M.J. and EDELNOCH, H. (1978). Thyroxine transport proteins of plasma. Molecularproperties and biosynthesis. Rec. Prog. Horm. Res. 34, 477-519.

RØNNOV-JESSEN, V. and KIRKEGAARD, C. (1973). Hyperthyroidism - a disease of old age. Br. Med. J. 1, 41-43.

ROOSDORP, N. and JOUSTRA, M. (1979). A numerical comparison of the use of T^3-Uptake values and of TBG levels for the estimation of free thyroxine in serum. Clin. Chim. Acta 98, 27-33.

ROOTWELT, K. and SOLBERG, H.E. (1978). Optimum laboratory test combinations for thyroid function studies, selected by discriminant analysis. Scand, J. Clin. Lab. Invest. 38, 477-485.

ROOTWELT, K. and SOLBERG, H.E. (1981). Free thyroxine, thyroxine/TBG ratio and other in vitro tests of thyroid function evaluated by discriminant analysis. Scand J. Clin. Lab. Invest. 41, 483-491.

ROTI, E., CHRISTIANSON, D., HARRIS, A.R.C., BRAVERMAN, L.E. and VANGENAKIS, A.G. (1978). "Short" loop feedback regulation of hypothalamic and brain thyrotropin-releasing hormone content in the rat and dwarf mouse. Endocrinology 103, 1662-1667.

RUPNOW, J.H., HINKLE, P.M. and DIXON, J.E. (1979). A macromelecule which gives rise to thyrotropin-releasing hormone. Bicohem, Biophys. Res. Commun, 89, 721-728.

RYBAKOWSKI, J. and SOWINSKI, J. (1973). Free thyroxine index and absolute free thyroxine in affective disorders. Lancet 1, 889.

SAKURADA, T., SAITO, S., INAGAKI, K., TAYAMA, S. and TORIKAI, T. (1967). Polyacrylamide gel electrophoretic study on the effect of oestrogen on human thyroxine-binding prealbumin. Tohoku, J. Med. 93, 339-362.

SALDANHA, V.F., BIRD, R. and HAVARD, C.W.H. (1971). Effect of diazepam (Valium) on dialysable thyroxine. Post. Med. J. 47, 326-328.

SCANLON, M.F., WEIGHTMAN, D.R., SHALE, D.J., MORA, B., HEATH, M., SNOW, M.H., LEWIS, M. and HALL, R. (1979). Dopamine is a physiological regulator of thyrotrophin (TSH) secretion in normal man. Clin. Endocrinol. 10, 7-15.

SCHATZ, D.L., SHEPPARD, R.H., STEINER, G., CHONDARLAPATY, C.S. and DE VEBER, G.A. (1969). Influence of heparin on serum free thyroxine. J. Clin. Endocrinol. Metab. 29, 1015-1022.

SCHOLER, J.F. (1962). A simple measure of thyro-binding by plasma : a test of thyroid function. J. Nucl. Med. 3, 41-46.

SCHUSSLER, G.C. (1971). Diazepam competes for thyroxine binding sites. J. Pharmacol. Expt. Ther. 178, 204-209.

SCHUSSLER, G.C. and PLAGER, J.E. (1967). Effect of preliminary purification of I^{131} thyroxine on the determination of the free thyroxine in serum. J. Clin. Endocrinol. Metab. 27, 242-250.

SCHWARTZ, H.L., SCHADLOW, A.R., FAIERMAN, D., SURKS, M.I. and OPPENHEIMER, J.H. (1973). Heparin administration appears to decrease cellular binding of thyroxine. J. Clin. Endocrinol. Metab. 36, 598-600.

SEDVALL,G., JONSSON, B. and PETTERSON, U. (1969). Evidence of an altered thyroid function in man during treatment with lithium carbonate. Acta Psychiat. Scand. Suppl. 207, 59-67.

SEKADDE, C.B., SLAUNWHITE, W.R.Jr. and ACETO, T.Jr. (1973). Rapid radioimmunoassay of triiodothyronine. Clin. Chem. 19, 1016-1021.

SHERIDAN, P., NEWTON, K.E. and PAYNE, R.B. (1978). Interpretation of serum total thyroxine concentration in patients with abnormal thyroxine-binding proteins. Br. Med. J. 2, 477.

SHERWIN, J.E. (1978). Iodide induced suppression of the thyrotropin-stimulated adenosine 3'5' monophosphate production in cat thyroid slices. Horm. Res. 9, 271-278.

SHISHIBA, Y., SHIMIZU, T., YOSHIMURA, S. and SHIZUME, K. (1973). Direct evidence for human thyroid stimulation by LATS-protector. J. Clin. Endocrinol. Metab. 36, 517-521.

SHOPSIN, B. (1970). Effects of lithium on thyroid function:a review. Dis. Nerv. Syst. 31, 237-244.

SHOPSIN, B., SHENKMAN, L., BLUM, M. and HOLLANDER, C.S. (1974). Triiodothyronine and thyroid stimulating hormone response to thyrotropin-releasing hormone : newer aspects of lithium-induced thyroid disturbance in man. In : Prange, J. ed. The Thyroid Axis, Drugs and Behaviour. New York, Raven Press. 177-188.

SIEGEL, S.J.,LINE, W.F., YANG, N.S.T., KWONG, A. and FRANK, C. (1973). Solid phase triiodothyronine radioimmunoassay. J. Clin. Endocrinol. Metab. 37, 526-532.

SIMPSON, G.H. and VARGA, E. (1974). Clozapine - a new antipsychotic agent. Curr. Ther. Res. 16, 679-686.

SINGER, I. and ROTENBERG, D. (1973). Mechanisms of lithium action. N. Engl. J. Med. 289, 254-260.

SINGHAL, R.L. and RASTOGI, R.B. (1978). Neurotransmitter mechanisms during mental illness induced by alterations in thyroid function. In : Garattini, S., Goldin, A., Hawking, F., Kopin, I.J. and Schnitzer, R.J. eds. Pharmacology and Chemotherapy. Academic Press, New York, San Francisco, London. 15, 203-262.

SISSON, J.C. (1965). Principles of, and pitfalls in, thyroid function tests. J. Nucl. Med. 6, 853-901.

SLATER, S.D. (1972). The effects of chlordiazepoxide and amylobarbitone upon thyroid function tests. Br. J. Clin. Pract. 26, 463-465.

SMITH, B.R. (1971). Characterisation of a long acting thyroid stimulator gamma - G binding protein. Biochem. Biophys. Acta 229, 649-662.

SMITH, B.R., DORRINGTON, K.J. and MUNRO, D.S. (1969). The thyroid stimulating properties of long acting thyroid stimulator G subunits. Biochem. Biophys. Acta 192, 277-285.

SMITH, B.R. and HALL, R. (1974). Thyroid stimulating immunoglobulins in Graves' disease. Lancet 2, 427-431.

SNYDER, S.M., CAVALIERI, R.R., GOLDFINE, I.D., INGBAR, S.H. and JORGENSEN, E.D. (1976). Binding of thyroid hormones and their analogues to thyroxine binding globulin in human serum. J. Biol. Chem. 251, 6489-6494.

SNYDER, S.M., CAVALIERI, R.R. and INGBAR, S.H. (1976). Simultaneous measurement of percentage free thyroxine and triiodothyronine : comparison of equilibrium dialysis and Sephadex chromatography. J. Nucl. Med. 17, 660-664.

SOLOMON, D.H. and CHOPRA, I.J. (1972). Graves' disease. Mayo Clin. Proc. 47, 803-813.

SOUMA, J.A., NIEJADLIK, D.C., COTTRELL, S. and RANKEL, S. (1973). Comparison of thyroid function in each trimester of pregnancy with the use of triiodothyronine uptake, thyroxine iodine, free thyroxine, and free thyroxine index. Am. J. Obstet. Gynaecol. 116, 905-910.

STANDING, S.J., BRINDLE, M.J., MACDONALD, A.P. and LACEY, R.W. (1981). Maternal alpha-fetoprotein screening : two years' experience in a low risk district. Br. Med. J. 283, 705-707.

STEFFES, M.W. and OPPENHEIMER, J.H. (1979). The laboratory evaluation of thyroid function. Minnesota Medicine 62, 162-164.

STERLING, K., BELLABARBA, D., NEWMAN, E.S. and BRENNER, M.A. (1969). Determination of triiodothyronine concentration in human serum. J. Clin. Invest. 48, 1150-1158.

STERLING, K. and HEGEDUS, A. (1962) Measurement of free thyroxine concentration in human serum. J. Clin. Invest. 41, 1031-1040.

STERLING, K. and LAZARUS, J.H. (1977). The thyroid and its control. Ann. Rev. Physiol. 39, 349-371.

STERLING, K.and MILCH, P.O. (1974). Thermal inactivation of thyroxine binding globulin for direct radioimmunoassay of triiodothyronine in serum. J. Clin. Endocrinol. Metab. 38, 866-875.

STERLING, K. and MILCH, P.O. (1975). Thyroid hormone binding by a component of mito chondrial membranes. Proc. Natl. Acad. Sci. USA. 72, 3225-3229.

STJERNHOLM, M.R., ALLSEVER, R.N. and RUDOLPH, M.C. (1975). Thyroid function tests in diphenylhydantoin-treated patients. Clin. Chem. 21, 1388-1392.

STUDER, H., BURGI, H., KOHLER, H., GARCIA, M.C. and MORREALE de ESCOBAR, G. (1976). A transient rise of hormone secretion : A response of the stimulated rat thyroid gland to small increments of iodide supply. Acta. Endocrinol. Copenhagen. 81, 507-515.

SYNDER, P.J. and UTIGER, R.D. (1972). Inhibition of thyrotropin response to thyrotropin-releasing hormone by small quantities of thyroid hormones J. Clin. Invest. 51, 2077-2084.

TAKASU, N., SATA, S., TSUKI, T., YAMADA, T., FURIHATA, R. and MARIUCHI, M. (1974) Inhibitory action of thyroid hormone on the activation of adenyl cyclase-cyclic AMP system by the thyroid stimulating hormone in human thyroid tissues from euthyroid subjects and thyrotoxic patients. J. Clin. Endocrinol. Metab. 39, 772-778.

TANJASIRI, P., KOZBUR, X. and FLORSHEIM, W.H. (1976). Somatostatin in the physiological feedback control of thyrotropin secretion. Life Sci. 19, 657-660.

THOMSON , J.A., ANDREWS, G.R., CAIRD, F.I. and WILSON, R. (1972). Serum protein-bound and plasma inorganic iodine in the elderly at home. Age Ageing, 1, 158-616.

THORPE, J.M. (1963). An experimental approach to the problem of disordered lipid metabolism. J. Artheroscler. Res. 3, 351-360.

TUNBRIDGE, W.M.G., EVERED, D.C., HALL, R., APPLETON, D., BREWIS, M., CLARK, F., GRIMLEY EVANS, J., YOUNG, E., BIRD, J. and SMITH, P.A. (1977). The spectrum of thyroid disease in a community ; The Wickham Survey. Clin. Endocrinol. 7, 481.493.

TUTTLEBEE, J.W. and BIRD, R. (1981). A comparison of free thyroxine concentration and the free thyroxine index as diagnostic tests of thyroid function. Ann. Clin. Biochem. 18, 88-92.

UTIGER, R.D. (1974). Serum triiodothyronine in man. Annu. Rev. Med. 25, 289-302.

VAGENAKIS, A.G., RAPOPORT, B., AZIZI, F., PORTRAY, G.I., BRAVERMAN, L.E. and INGBAR, S.H. (1974). Hyperresponse to thyrotropin-releasing hormone accompanying small decreases in serum thyroid hormone concentration. J. Clin. Invest. 54, 913-918.

VALE, W. and GUILLEMIN, R. (1967). Potassium induced stimulation of thyrotropin release in vitro. Requirement for presence of calcium and inhibition by thyroxine. Experientia 23, 855-857.

VALE, W., BURGUS, R. and GUILLEMIN, R. (1968). On the mechanism of action of TRF : Effects of cycloheximide and actinomycin on the release of TSH stimulated in vitro byTRF and its inhibition by thyroxine. Neuroendocrinology 3, 34-46.

VOLPE, R., FARID, N.R., VON WESTARP, C. and ROW, V.V. (1974). The pathogenesis of Graves' disease and Hashimoto's thyroiditis. Clin. Endocrinol. 3, 239-261.

WAHNER, H.W. (1974). Diagnosing thyroid disease by selected laboratory tests. Geriatrics, 29, 83-94.

WALL, D.W. and O'FLANAGAN, P.H. (1978). Unusual presentations of thyroid disease in general practice. J.R. Coll. Gen. Pract. 28, 170-171.

WALL, J.R., GOOD, B.F. and HETZEL, B.S. (1969). Long-acting thyroid stimulators in euthyroid relatives of thyrotoxic patients. Lancet 2, 1024-1026.

WARTOFSKY, L., DIAMOND, R.C., NOEL, G.L., ADLER, R.A. and EARLL, J.M. (1974). Failure of T^3 to block TSH responses to TRH in normal subjects and patients with primary hypothyroidism. Proc. Am. Thyroid Accos. 50th. Meet. (Abstr.).

WEICHSEL, M.E. Jr. (1974). Effect of thyroxine on DNA synthesis and thymidine kinase activity during cerebellar development. Brain Res. 78, 455-465.

WEINBERG, A.D. and KATZELL, T.D. (1977). Thyroid and adrenal function among psychiatric patients. Lancet 1, 1104-1105.

WELLBY, M.L. and O'HALLORAN, M.W. (1966). Measurement of plasma free thyroxine level as a test of thyroid function. Br. Med. J. 2, 668-670.

WELLBY, M.L., O'HALLORAN, M.W. and MARSHALL, J. (1974). A comparison of effective thyroxine ratio, free thyroxine index and free thyroxine concentration in correcting for thyroxine binding abnormalities in serum. J. Clin. Endocrinol. Metab. 3, 63-68.

WENZEL, K.W., MEINHOLD, H. and SCHLEUSENER, H. (1975). Different effects of oral doses of triiodothyronine or thyroxine on the inhibition of thyrotropin-releasing hormone (TRH) mediated thyrotropin (TSH) response in man. Acta, Endocrinol. Copenhagen. 80, 42-48.

WESTERGREN, A. (1921). Studies of the suspension stability of the blood in pulmonary tuberculosis. Acta, Med. Scand. 54, 247-282.

WHITBY, L.G. (1974). Screening for disease. Definitions and criteria. Lancet, 2, 819-822.

WHITE, G.H. and WALMSLEY, R.N. (1978). Can the initial clinical assessment of thyroid function be improved. Lancet, 2, 933-935.

WHYBROW, P.C. and FERRELL, R. (1974). The Thyroid Axis, Drugs and Behaviour. Raven Press. New York.

WHYBROW, P.C., PRANGE, A.J. and TREADWAY, C.R. (1969). Mental changes accompanying thyroid-gland dysfunction. Arch. Gen. Psychiatry 20, 48-63.

WIDE, L. and DAHLBERG, P.A. (1979). Quality requirements for S-TSH assay in discriminating eu- from hyperthyroidism. Scand. J. Clin. Lab. Invest. 39, (suppl. 152), 31.

WILBER, J.F. (1971). Stimulation of C^{14} - glucosamine and C^{14} - alanine incorporation into thyrotropin-releasing hormone. Endocrinology 89, 873-877.

WILBER, J.F. and SEIBEL, M.J. (1973). Thyrotropin-releasing hormone interactions with an anterior pituitary membrane receptor. Endocrinology 92, 888-893.

WILSON, I.C., PRANGE, A.J.Jr., LARA, P.P., LACOE, B., ALLTOP, R.A., STRIKELEATHER, R.N., LIPTON, M.A. and CHAPEL HILL,N.C. (1973). THR (Lopremone) : Pyschobiological responses of normal women. 1. Subjective experiences. Arch. Gen. Psychiat. 29, 15-21.

WILSON, I.C., PRANGE, A.J.Jr., McCLANE, T.K., RABON, A.M. and LIPTON, M.D. (1970). Thyroid-hormone enhancement of imipramine in non-retarded depressions. N. Engl. J. Med. 282, 1063-1067.

WOLFF, J. (1969). Iodide goitre and the pharmocologic effects of excess iodide. Am. J. Med. 47, 101-124.

WOLFF, J. and CHAIKOFF, I.L. (1948). Plasma inorganic iodide as a homeostatic regulator of thyroid function. J. Biol. Chem. 174, 555-564.

WOODBURY, D.M. (1958). Relation between the adrenal cortex and the central nervous system. Pharmacol. Rev. 10, 275-357.

YALOW, R.S. and BERSON, S.A. (1960). Immunoassay of endogenous plasma insulin in man. J. Clin. Invest. 39, 1157-1175.

YEO, P.P.B., BATES, D., HOWE, J.G., RATCLIFFE, W.A., SCHARDT, C.W., HEATH, A. and EVERED, D.C. (1978). Anticonvulsants and thyroid function. Br. Med. J. 1, 1581-1583.

YOUNG, D.S., PESTANER, L.D. and GIBBERMAN, V. (1975). Effect of drugs on clinical laboratory tests. Clin. Chem. 21, 1D 432D

YU, S., FRIEDMAN, Y., RICHMAN, R. and BURKE, G. (1976). Altered thyroid responsitivity to thyrotropin induced by circulating thyroid hormones : a "short-loop" regulatory mechanism ? J. Clin. Invest. 57, 745-755.

ZOR, U., KANEKO, T., SCHNEIDER, H.P.G., McCANN, S.M. and FIELD, J.B. (1970) Further studies of stimulation of anterior pituitary cyclic adenosine 3',5' monophosphate formation by hypothalamic extracts and prostaglandins. J. Biol. Chem. 245, 2883-2888.