THE IODOTHYRØNINES:
MEASUREMENT IN SERUM AND APPLICATION OF THESE MEASUREMENTS TO THE DIAGNOSIS OF THYROID DISEASE.

Richard George SYMONS

B.Sc.(Hons)

A thesis submitted for the degree of Master of Science in the Department of Biochemistry of the University of Adelaide, South Australia.

September, 1980

Name : R.G. Symons ..... Course : M.Sc. .....

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Date : 28 Nov 80 ..... Signed : [redacted] ...

CONTENTS

LIST OF FIGURES

LIST OF TABLES

SUMMARY

The development of sensitive and specific techniques for the measurement of the iodothyronines has led to dramatic advances in the areas of thyroid hormone metabolism and action, and their regulation.

The current concepts of the regulation of thyroid hormone concentration and metabolic action by the hypothalamic-pituitary-thyroid gland axis are reviewed. Also reviewed in detail are those studies implicating the role of the peripheral target tissues in altering thyroid hormone concentration and metabolic action by regulating thyroxine (T_4) metabolism to the biologically active 3,3',5-triiodothyronine (T_3) and the biologically inactive 3,3',5'-triiodothyronine (rT_3).

A radioimmunoassay for rT_3 and the equilibrium dialysis-radioimmunoassay (E/D-RIA) technique for measuring the free thyroid hormones were developed in order to evaluate their usefulness in the diagnosis of thyroid disease, particularly where peripheral metabolism of T_4 was abnormal, as in non-thyroidal illness. The assay sensitivity and precision required were attained by empirical manipulation of the tracer and antibody concentrations.

The simplex technique was also used to establish the rT_3 assay. This led to the development of an assay with much lower binding than the empirical assay but similar sensitivity and precision. It was concluded that this technique provided an objective and practical approach to developing radioimmunoassays which relied less on develop-

mental experience than the conventional empirical techniques.

The empirically established serum-free thyroid hormone assays were compared to displacement curves generated using one and two binding site models of hormone binding to antibody. The models and the equations used to determine precision profiles were derived from the law of mass action. The generated precision profiles suggested higher imprecision and poorer sensitivity than was attained in practise. However, it was concluded that the theoretical prediction of precision profiles was a valuable aid to the rapid establishment of radioimmunoassays of a particular required sensitivity and precision.

Free thyroid hormone concentration was within the euthyroid reference range in nearly every subject with abnormal thyronine binding protein (TBP) concentration. A considerable number of these subjects had abnormal free thyroxine index (FTI) or free T_4 (fT_4) concentration as measured by the Corning Free T_4 assay. It was concluded that the Corning assay was prone to binding protein interference.

While all patients with thyroid disease studied had abnormal FTI and fT_4 (Corning), 37% had either fT_4 or fT_3 concentration (E/D-RIA) in the euthyroid reference range. The assay of rT_3 provided no additional information which might be helpful to the diagnosis of thyroid disease in these subjects.

A group of patients with moderate to severe non-thyroidal illness was characterized by the absence of symptoms

of hypothyroidism and low T_3 concentration but free T_3 was not depressed to the same extent suggesting an uncharacterized binding abnormality. The tissue supply of thyroid hormone was considered adequate in view of the normal fT_4 and TSH. Reverse T_3 was variable and provided no useful information in the diagnosis of thyroid disease in this group.

The cholecystographic agent, iopodate (Biloptin), caused a marked increase in total and free rT_3, reduction in total and free T_3, and stimulation of TSH secretion. The interference in T_4 metabolism caused by this agent necessitates the correct timing of thyroid function tests in patients undergoing cholecystography.

The stressful stimuli, acute strenuous exercise, caused an increase in both rT_3 and T_3. These results suggested that there were mechanisms operating in strenuous exercise which caused effects on thyroid pathophysiology different to those seen in other states of stress.

This is to certify that the work embodied in this thesis has not been previously submitted for the award of a degree in any other institution.

September, 1980.

ACKNOWLEDGEMENTS

I wish to thank Dr. Maurice L. Wellby for the provision of facilities in the Department of Clinical Chemistry, The Queen Elizabeth Hospital, South Australia, to carry out the work described in this thesis, and for the guidance and encouragement given as supervisor.

I would like to acknowledge the role of Mr. Stuart Elliot in drawing attention to the Simplex technique for optimising chemical systems, and the theoretical models used for optimising competitive protein binding assays; the help of Mr. Michael O'Halloran in statistical analysis of data; the generosity of Ms. Jan Gooden in providing thyroid function tests and helping with sample collection; and the expert technical assistance of Ms. Julia McEntee and Ms. Barbara McArthur; all of whom were staff members of the Department of Clinical Chemistry, The Queen Elizabeth Hospital, South Australia.

The investigation of peripheral metabolism after ingestion of cholecystographic agents was performed in collaboration with Dr. Clive Beng, Department of Clinical Chemistry, The Queen Elizabeth Hospital, South Australia, and the study of thyroid pathophysiology during acute strenuous exercise in collaboration with Dr. Michael Hooper, Endocrine Unit, Royal Adelaide Hospital, South Australia.