



CHAPTER I

INTRODUCTION

Thyroglobulin is an important source of reserve thyroid hormone of mammalian species, (Fig.1) (1)..

Thyroglobulin is a glycoprotein, existing as a colloid in the lumen of thyroid gland whose principle (80 %) component has a sedimentation coefficient (Svedburg units = S) of about 19S and a mol. wt. ~ 660,000. Two heavier components (27 S and 31-34 S) and three lighter components are also present. Heat, alkali and denaturing agents will cause dissociation of some 19 S thyroglobulin into 12 S subunits with mol.Wt. ~ 330,000. When the thyroglobulin is deficient in iodine, dissociation of the 19 S molecule occurs readily and it was suggested that 19 S thyroglobulin exists in two forms : in the labile form the two 12 S. molecules are joined by non-covalent bonds, whereas in the stable 19 S. form the 12 S subunits are joined by one or more disulphide bridges. (1,2).

Thyroglobulin (19 S) contains 202 half-cystein residues, most of which are present as disulphides (2). Homogeneity of human

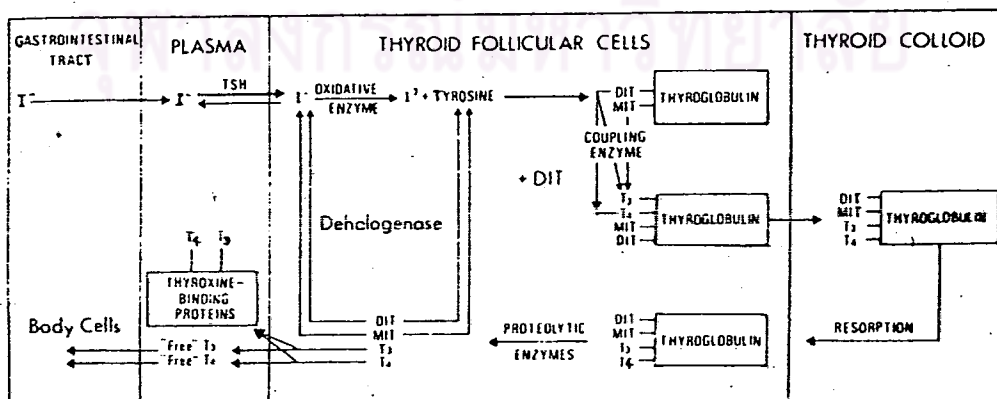


Fig. 1 Thyroid hormone synthesis

thyroglobulin solution was established by cellulose acetate electrophoresis (single band in the interalpha region) and absorbanted at 280 nm. (3,4).

Normal thyroglobulin is produced by thyroid cells but several studies have shown that thyroid cancer cells are capable of producing thyroglobulin.

In the past cumbersome and semiquantative methods based on tanned red cell hemagglutination inhibition or electrophoretic immunorention were used to measure the thyroglobulin in blood of human beings. Roitt and Torrigiani were the first to describe radioimmunoassay for the thyroglobulin. Van Herle and Uller have shown a simplified, sensitive and specific radioimmunoassay for human serum thyroglobulin. Bodlaender et.al. described a reliable and convenient double antibody radioimmunoassay for human thyroglobulin. (3,5)

The normal range concentration of thyroglobulin in serum is 10-150 ng/ml (RIA, Torrigiani et.al. 1969). Elevated concentrations could be detected in pregnant women and newborn, Distinctly elevated values are present mainly in three types of thyroid disorder : those associated with goitre and thyroid hyperfunction (Graves' disease) ; those associated with inflammatory or physical injury to thyroid ; and differentiated thyroid tumors. Serum thyroglobulin concentrations are increased in patients with both benign and differentiated malignant tumor of the thyroid in situ. Consequently, there are data concerning the concentration of free thyroglobulin in the serum of patients with Hashimoto's disease (6)

## ELECTROPHORESIS

Electrophoresis, the process of separating molecules by migration in an electrical field, could be conducted in many ways. A tiny sample of protein solution, perhaps of blood serum, is placed in a thin line on a piece of paper or cellulose acetate. The sheet is moistened with a buffer and electric current is passed through it. An applied voltage of a few hundred volts suffices to separate serum proteins in about an hour.

### THE PROTEINS OF BLOOD PLASMA

Among the most studied of all proteins are those present in blood plasma. Their ready availability together with the clinical significance of their study led to the early development of electrophoretic separations of these proteins. Electrophoresis at a pH of 8.6 (in barbital buffer or veronal buffer) indicates six main components. The major, and one of the fastest moving proteins, is serum albumin. Trailing behind it in the electrophoretic pattern are the  $\alpha_1$ - $\alpha_2$ - and  $\beta$ -globulins, fibrinogen and  $\gamma$ -globulin (7)

### GEL IMMUNODIFFUSION TEST

Gel immunodiffusion tests are most commonly performed in purified agar gels. Agarose and agar are only two of the many gels tested for immunodiffusion studies. In immunodiffusion tests a single antigen and its antibody will form only a single precipitin line. A mixture of antigen-antibody systems will present multiple bands, theoretically one band for each system.

Double diffusion - double dimension.

To perform the double diffusion - double dimension test, better known as the Ouchterlony test, reservoirs are cut or molded into agar on a flat surface. The wells are filled with appropriate solutions of antigens and antiserum, covered to prevent evaporation, and observed periodically for several days. Precipitin bands may become visible in a few hours if concentrated reagents and/or a micromethod are used.

(Fig. 2)

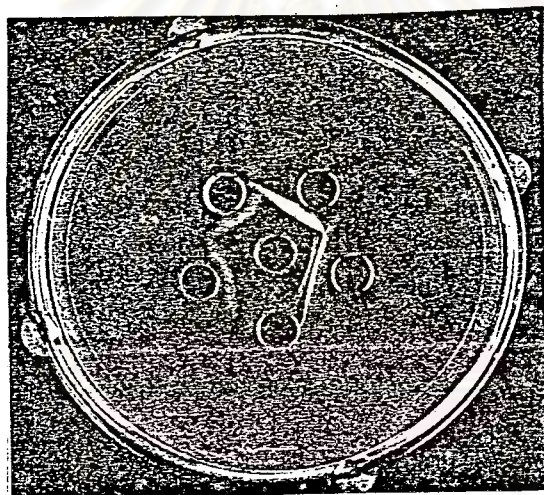


Fig. 2 A double diffusion - double dimension  
(Ouchterlony) test. (8)

RADIOIMMUNOASSAY

Principles of Radioimmunoassay

The principle of radioimmunoassay is expressed in the competitive reaction shown in Fig. 3.1 Unlabelled antigen in unknown samples or known standard competes against labelled antigen (tracer) for binding to antibody and there by diminishes the binding of labelled antigen (9).

The degree of competitive inhibition observed in unknown sample is compared with that obtained in known standard solutions for determination of concentration of antigen in the unknown. (Fig.3.2)

Rosalyn S Yalow originally used radioimmunoassay for the measurement of plasma insulin (10-13) and has since been applied to many other peptide hormone and other substances.

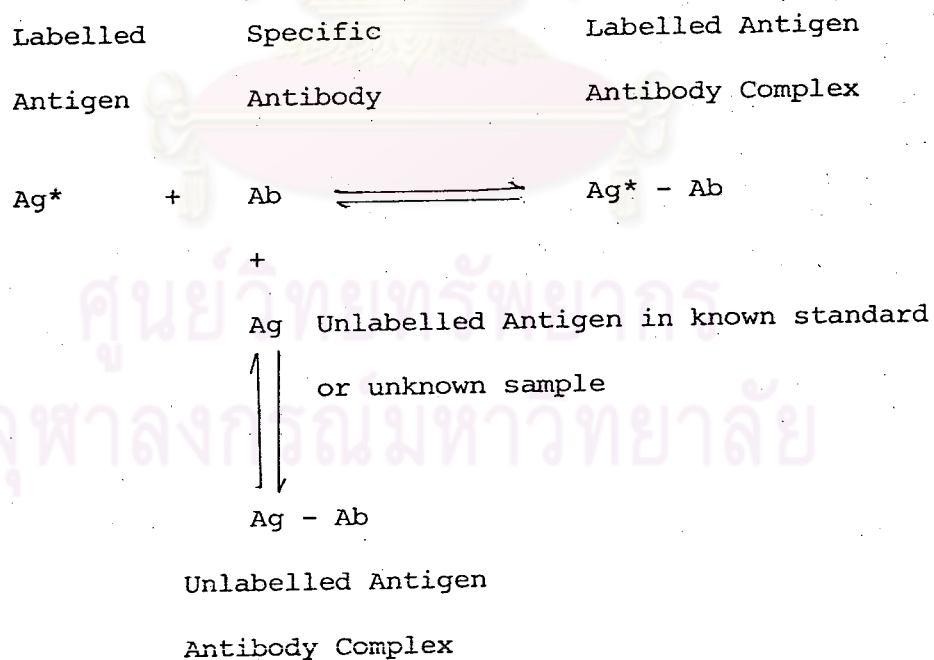
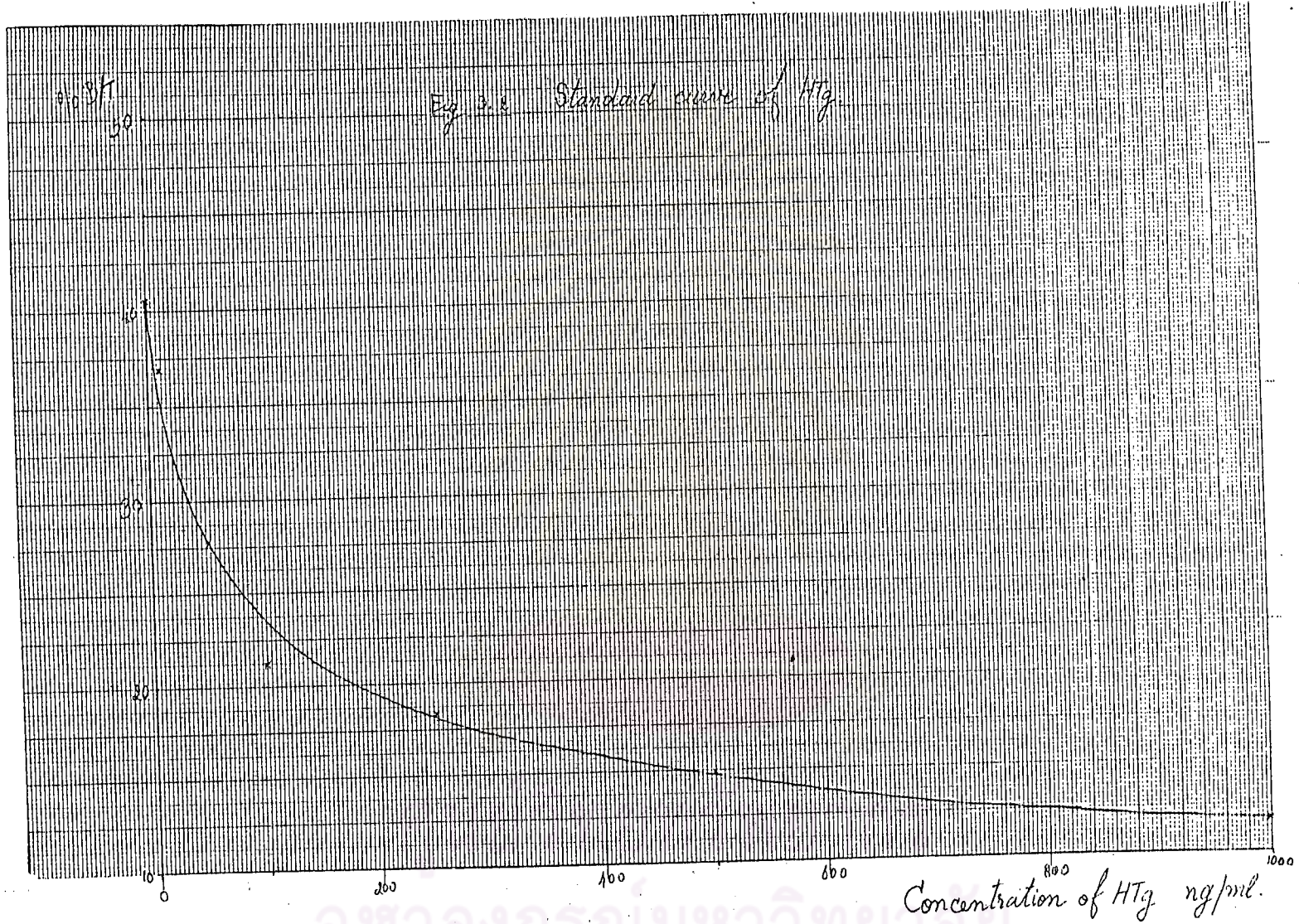


Fig.3.1 Competitive antigen - antibody reactions on which radioimmunoassay is based. (9)



จุฬาลงกรณ์มหาวิทยาลัย