CHAPTER I

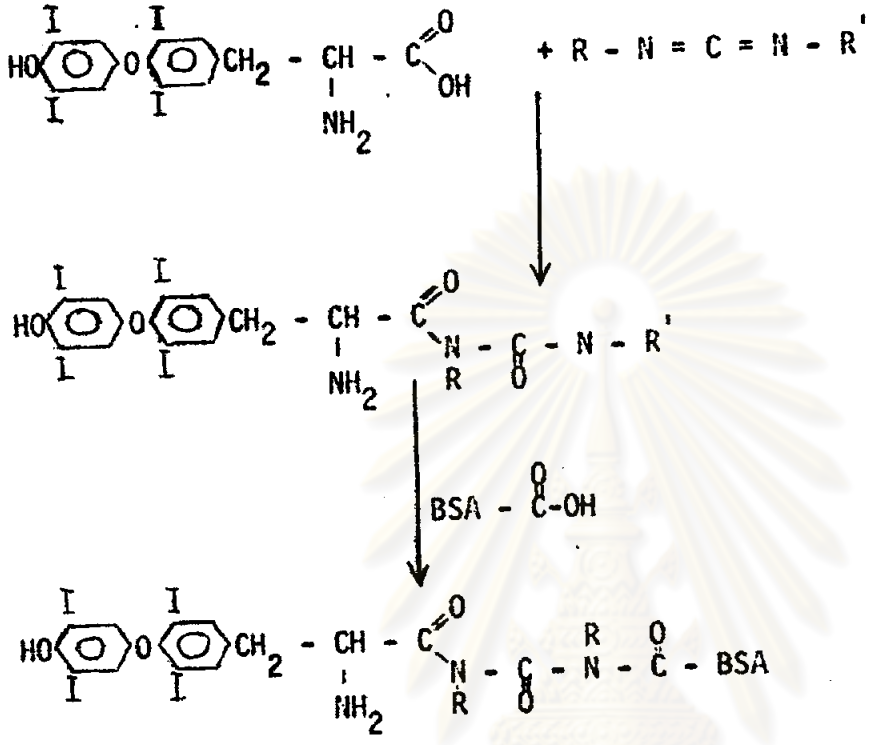
INTRODUCTION

I. The Antithyroxine sera

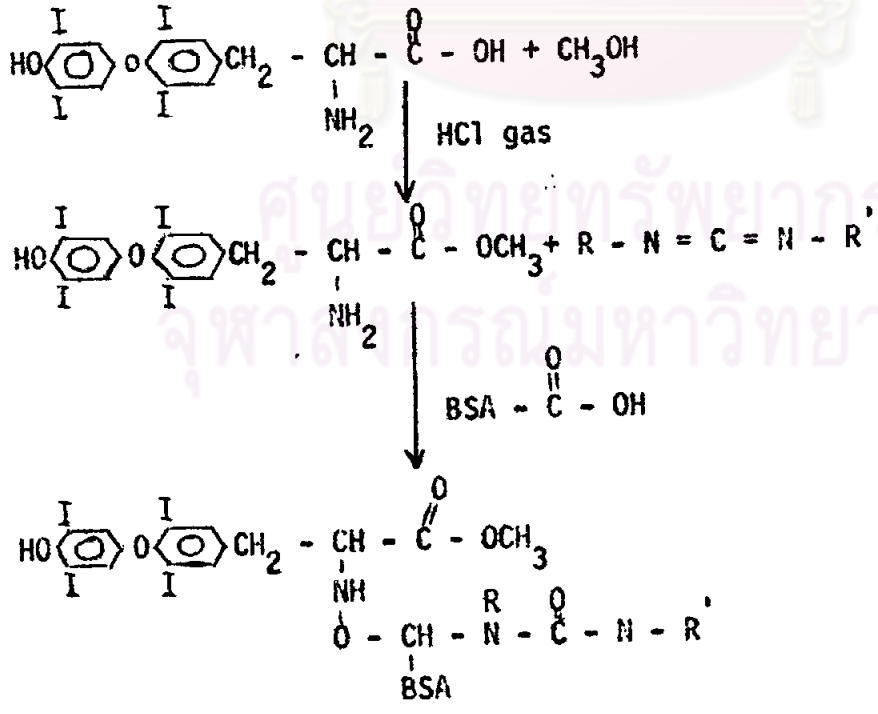
Antibodies have been prepared against many compounds of low molecular weight since Landsteiner (1) demonstrated that such compounds can elicit antibody formations when conjugated as haptens to carrier proteins.

Tetraiodothyronine (Thyroxine or T_4) is an endocrine hormone from thyroid gland. It has a low molecular weight of 776.93. Antibodies against T_4 have been raised by several methods but their specificity, affinity and titresare rather low. Imumunization of crude thyroglobulin preparations (2) is simple but gives rather low titres for antibodies to T_4. Since in the case of thyroglobulin, hormones are not covalently bound. This hapten is unstable in vivo and is a poor immunogen. The covalent binding of small hormone molecule to macromolecules generally needs some modifications of the hormone molecule. With exact knowledge of the chemical reactions that take place during the coupling of the modified hormones by carbodiimide the loss of specific antigenicity and inter or intramolecular linkage of amino and carboxyl groups can be avoided. Methyl - ester- HCl of T_4 was coupled to Bovine Serum Albumine (BSA). Protection of the carboxyl group of the hormones by the ester conjugation allows the carbodiimide to bind only at the amino group. There the hapten group contains the intact hormone molecule that in turn is bound to BSA by mean of the amino group (Fig. I).

(1)



(2)



(Fig. I)

(1) Coupling of T_4 with carbodiimide to BSA through carboxyl group.

(2) Carboxyl group is protected by the ester formation so the intact hormone molecule bound to RSA by mean of amino group.

The problems of obtaining suitable antigens that could induce specific antibody production in animal to provide appropriate immune sera suitable for Thyroxine radioimmunoassay have been critically analyze.

In order to study the immunogenic conjugates of Tetraiodothyronine methylhydrochloride to BSA and Tetraiodothyronine to BSA. The antigen (Thyroxine) was prepared by using carbodiimide as the coupling agent. Three rabbits were immunized with each of these conjugates. The immunization technique concists of multiple intradermal injection of approximately 20-30 sites with small doses of antigen emulsified in complete Freund's Adjuvant (3). This technique produced antibodies suitable for T_4 - RIA after six weeks of immunization.

II. Radioimmunoassay

A. Principles of Radioimmunoassay

The principle of radioimmunoassay is expressed in the competing reaction shown in Fig. (2). Unlabelled antigen in unknown samples competes against labelled antigen ("tracer") for binding to antibody and there by diminishes the binding of labelled antigen (4). 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 unknown (Fig. 3).

Rosalyn S. Yalow originally used RIA for the measurement of plasma insulin (5-8) and has since been applied to many other peptide hormone and other substances.

In the absence of a suitable standard preparation plasma (or other biologic fluid) containing a high concentration of antigen may be used as standard for the determination of relative concentration of antigen in other unknown sample (9). The validity of the assay is dependent on the identical behaviour., not of labelled antigen ("tracer") and antigen in unknown sample, but of standard and unknown antigen. An obvious additional reguirement for validity is the control of non-specific effects on the immune reaction. Such effects may depend on salt condentration (10), pH, nature of buffer and protein content of incubation medium. It is therefore always necessary to test for these effects and it is frequently desirable to prepare standard solutions in plasma devoid of hormone to control such effects of the medium when assaying plasma samples.

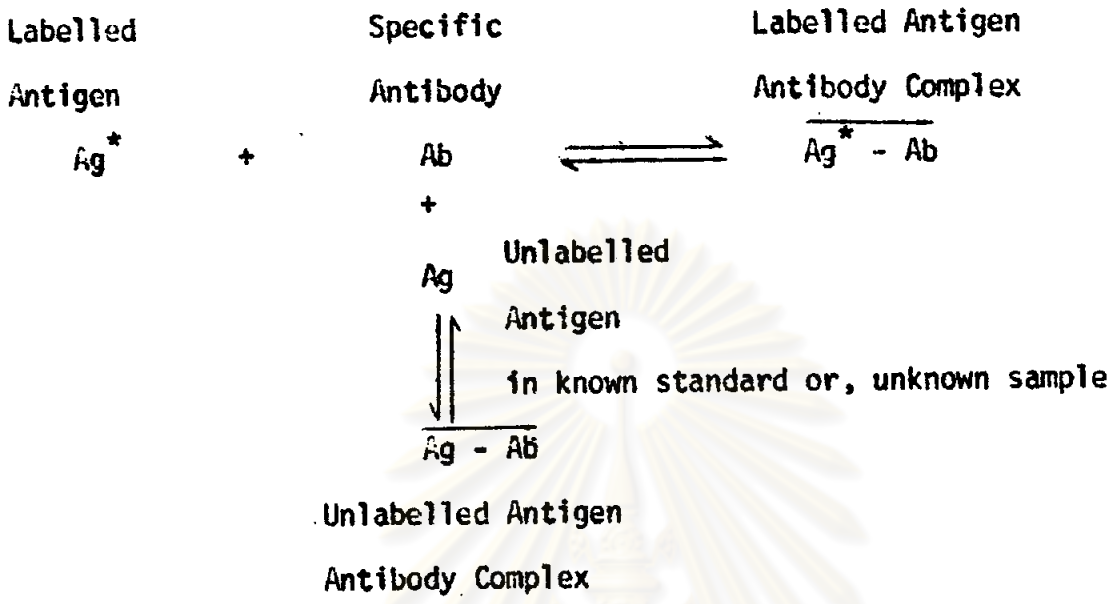


Fig. 2 Competitive antigen - antibody reactions on which radio-immunoassay is based.

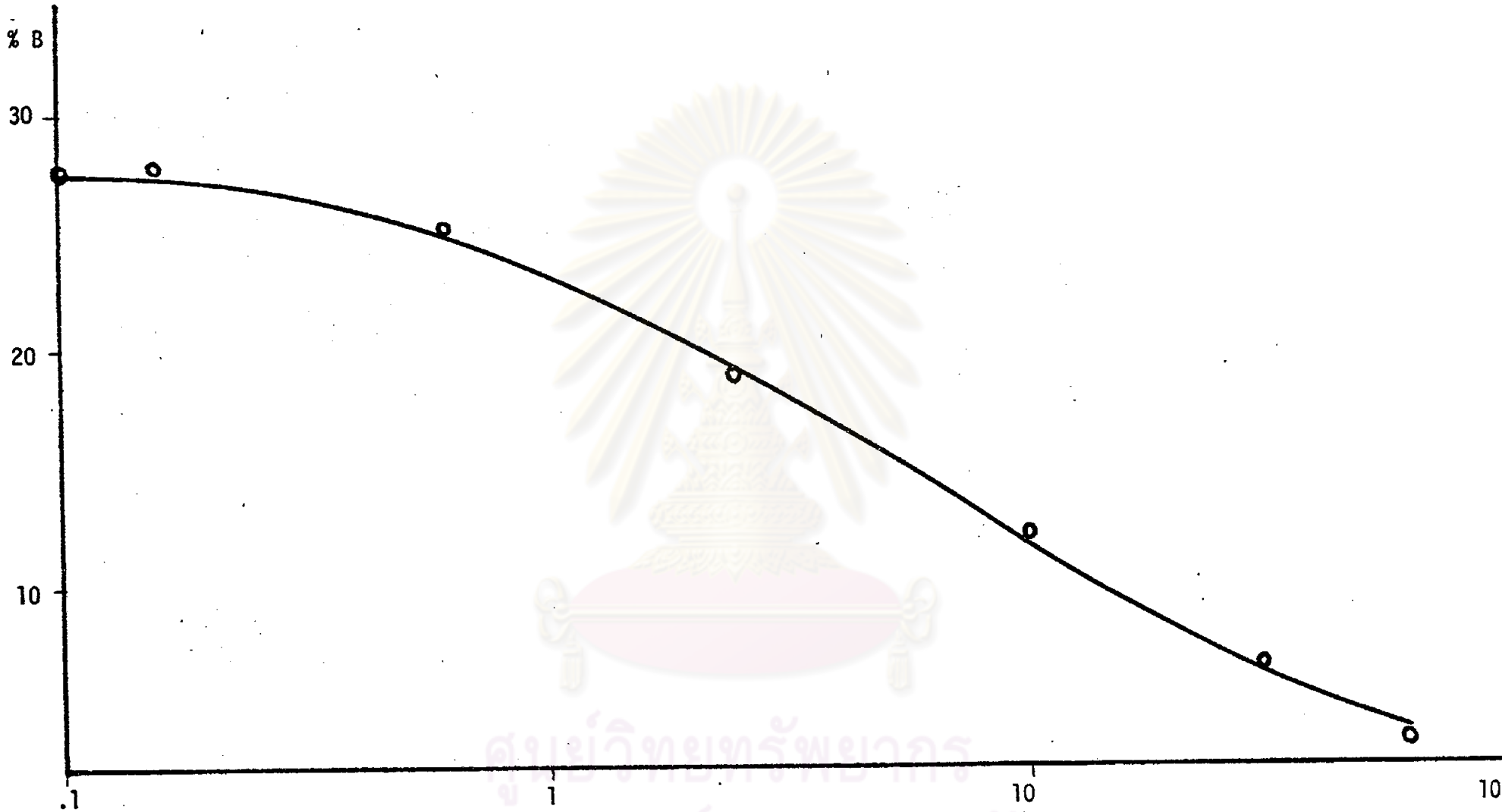


Fig. 3 Standard curve of assay of human thyroxine.

B. T_4 - RIA

The basic approach to measurement of T_4 RIA is similar to that described earlier (11, 12). It adaptation to measurement of the hormone in serum is based on

1. Addition of an excess of 8 - anilino - 1 - napthalene sulfonic acid (ANS) to all standards and unknown sera for two purpose.

a) to displace T_4 bound to thyroxine binding globulin (TBG) and make it available for reaction with T_4 - antibody and thus measurable by RIA.

b) to minimize binding radioactive T_4 to TBG.

2. Setting up assay in barbital buffer pH 8.6 to inhibit binding of T_4 (non - radioactive or radioactive) to thyroxine binding prealbumin (TBPA) (13).