CHAPTER I

INTRODUCTION

Because of low education standards and poor earnings, the majority of Thai people living in the remote areas scattering all over the kingdom has to face with poor sanitation and scanty health services caused by shortage of doctors and trained public health personnel, therefore, to find that most Thai people have to buy their own drugs from drugstore. There is no doubt at all that Thai people are now exposing themselves to a high danger through unrestrain use of such drug as analgesic, sedative, antibiotic, contraceptive, ect. These drugs can be freely acquired without the need of doctor's prescription.

Temporary use of such drug in proportional dosage or under doctor's order is quite advantageous, since it can relieve the symptoms and make the patients feel comfortable. On the other hand, it may deleteriously affect the homeostasis of the body. The physical effects of drugs on the human body are variated. Some drugs bring about the change in general, while some other on some particular organs. Several reports[1-6,61] from other countries revealed their experiments on the effects of drugs on thyroid function. Most studies of these drugs were concerned with experimental animals[5,8,40] but

the purpose of this investigation is to study the short-term effects of therapeutic doses of acetaminophen, diazepam and nordiol on the circulating levels of total T_4 and free thyroxine index (FTI) in patients and in healthy volunteers compared to the controls. Thus, a sensitive and specific radioimmunoassay of serum T_4 by using polyethylene glycol (PEG) for separating the bound from the free fractions is modified and improved to establish the optimum methods. The concentration of total T_4 in serum is divided by the value of T_3-uptake test or the number of unoccupied thyrobinding sites on the serum proteins giving a free thyroxine index, which is directly proportional to the concentration of the unbound or "free" thyroxine. Thus, it is expected that this FTI should give a very good assessment of thyroid function in treated patients with the drugs mentioned above.

Review of Literatures.

Thyroid gland is an endocrine gland which is located below the larynx on either side of anterior to the trachea. It is composed of large numbers of encysted follicles filled with a substance called colloid and line with cuboidal epitheloid cells that secrete into the interior of the follicles. Once the secretion has entered the follicles, it must be absorped back through the follicular epithelium into the circulating blood when it is metabolized in the body.(9)

A. Synthesis of thyroid hormones.

Thyroid gland produces two hormones which are thyroxine (T_4) and triiodothyronine (T_3). The gland is composed of iodine and thyroglobulin.(10) The epitheloid cells synthesize thyroglobulin from amino acids entered the thyroid cell from the blood and are assembled into protein chain on the rough endoplasmic recticulum. Iodine enters the basal membrane from the capillary through diffusion and through an iodine pump. It will be oxidized by a peroxidase in the microvilli at the apical cell membrane. The oxidized form of iodine immediately becomes organically bound to peptide linked tyrosyl group in the thyroglobulin molecule adjacent to it to form monoiodotyrosine (MIT) and diiodotyrosine (DIT).(11) Two molecules of DIT then conjugate by phenolic group of one DIT attached to the phenolic group of another DIT molecule, forming a new molecule of T_4 and leaving the alanine side chain of one DIT still attached to the

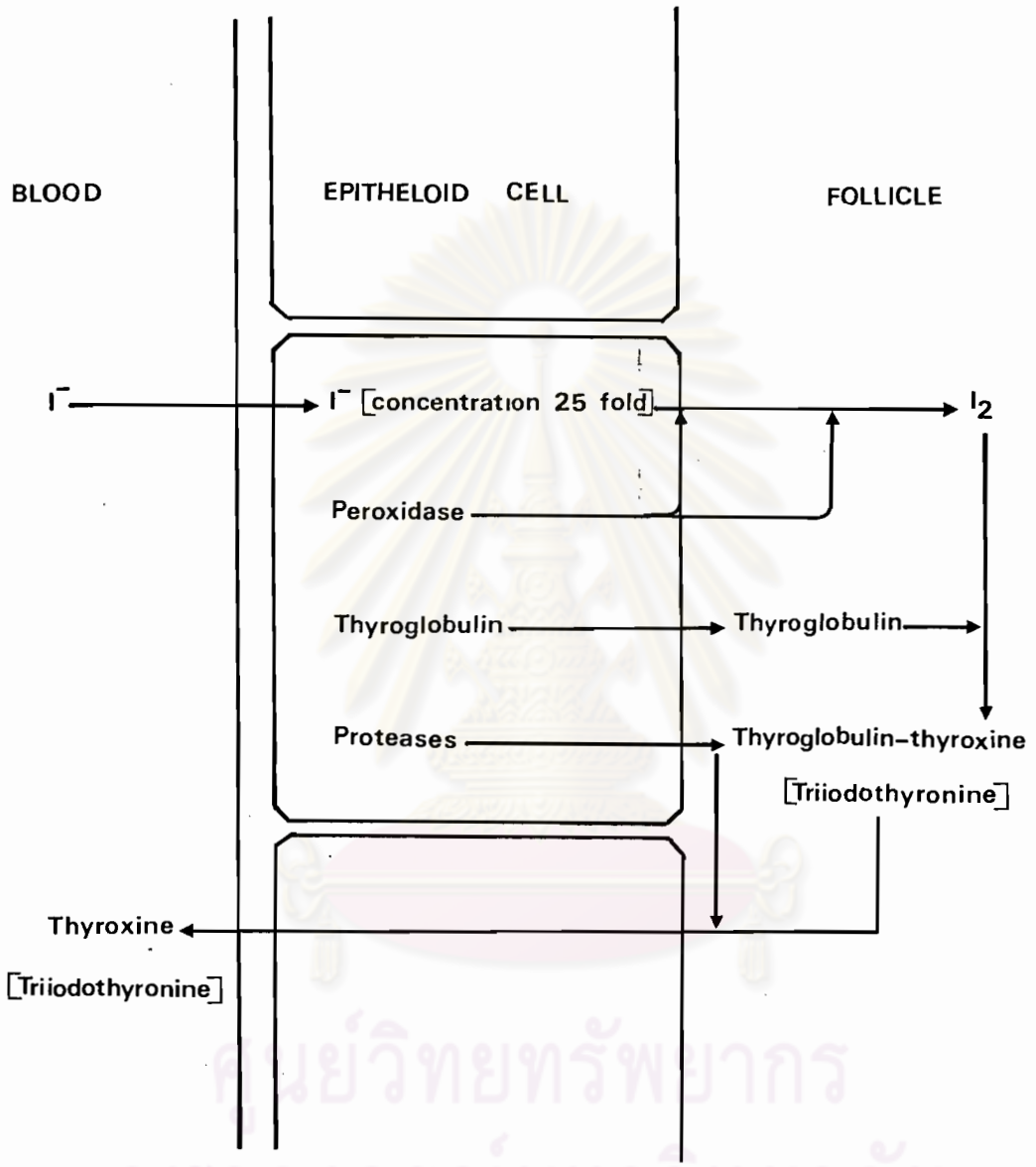


Fig.1. Mechanisms of iodine transport, thyroxine formation and thyroxine release in to the blood.[Triiodothyronine formation and release parallels that of thyroxine.][9]

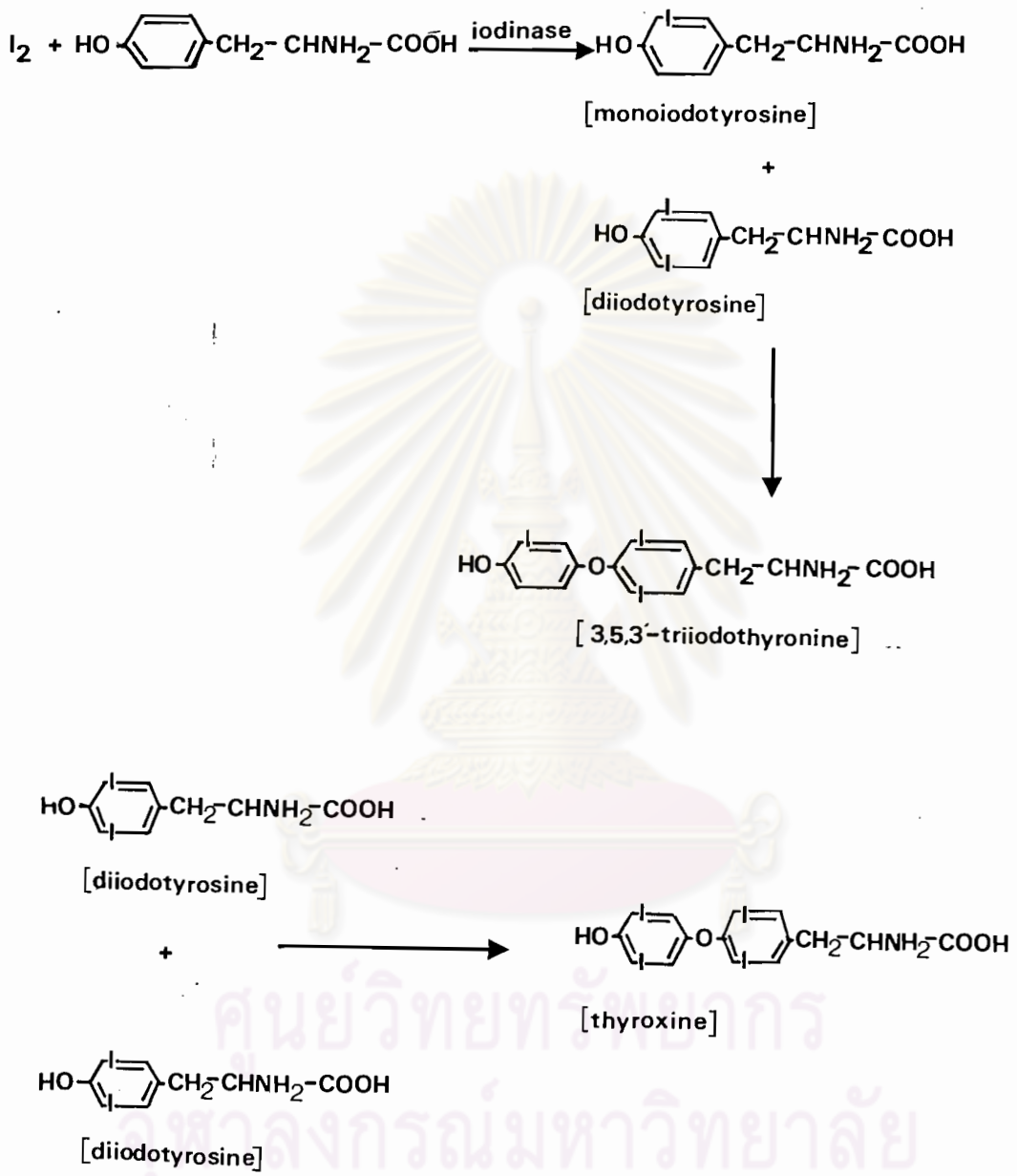


Fig.2. Chemistry of thyroxine and triiodothyronine formation.[9]

peptide chain. T_3 is the more calorigenic form of thyroid hormones. It is formed by coupling one molecule of MIT to one molecule of DIT. This coupling causes some increased bonding between the peptide chains, which increase the stability of the thyroglobulin molecule.[12]

B. Secretion of thyroid hormones.

Thyroglobulin itself is not released into the circulating blood. It is ingested by endothelium cells in a form of colloid droplets which migrate toward the basal cell membrane and simultaneously, lysosomes migrate from the basal part of the cell toward the colloid droplets which are fused to form phagolysosomes. The thyroglobulin is digested by proteolytic enzymes in the phagolysosomes and the free amino acids are released. Free MIT and DIT are almost completely deiodinated in the cell but free T_4 and T_3 are deiodinated to a much lesser extent. Some T_3 is formed by the deiodination of T_4.[12] Both T_4 and T_3 are released into the capillaries, and are immediately and almost entirely bound by specific plasma proteins

C. Control of thyroid secretion.

The regulation of thyroid secretion to maintain a normal basal metabolic rate of the body via a specific feedback mechanism operates through the hypothalamus and adenohypophysis.

1. Adenohypophysis regulation : thyrotropin (TSH) is an

adenohypophyseal hormone. It increases the secretion of T_4 and T_3 by the thyroid gland. The specific effects of thyrotropin on the thyroid gland are; a). increased proteolysis of the thyroglobulin in the follicles with resultant release of T_4 & T_3 into the circulating blood and diminution of the follicular substance itself. b). increased activity of the iodine pump. c). increased size and secretory activity of the thyroid cells. d). increased number of thyroid cells. In addition, recent experiments indicated that thyrotropin increases the quantity of cyclic AMP in the thyroid cells.(13)

2. Hypothalamic regulation: hypothalamus regulates thyrotropin secretion by the adenohypophysis. This control is exerted by hypothalamic secretion of thyrotropin-releasing factor (TRF) into the hypophyseal portal systemic blood. This factor in turn acts on the adenohypophyseal glandular cells to increase the output of thyrotropin.

D. Transport of thyroid hormones.

When radioiodine-labelled T_4 and paper electrophoresis become available, detailed analysis of the binding protein was possible and a specific T_4-binding protein was clearly established in serum. About 85-90% of T_4 migrated in a zone intermediate between the α_1-α_2-globulins during electrophoresis in barbital buffer at pH 8.6 of serum labelled with tracer radio-T_4. This T_4-binding protein has been designated thyroxine-binding globulin (TBG), the major transport protein, which has the greatest affinity for T_4 and TBG carries a

half to two-thirds of the circulating T_4 despite its low concentration in plasma. Serum albumin is a secondary carrier of T_4 which has only a minor role under normal circumstances, despite its much greater concentration in plasma. In addition, a small quantity of radioactivity has been found to migrate just in front of albumin and this carrier has been designated thyroxine-binding pre-albumin (TBPA).

T_3 also is transported in blood in association with plasma proteins and again is bound primarily to TBG, although with a binding affinity 2-6 times less than that for T_4.[14] Thus, the main carrier for both T_4 and T_3 in the circulation is TBG, and these thyroid hormones are each bound to TBG by a reversible interaction which occurs rapidly.[15] TBG binds T_3 with a much lower affinity than T_4. Reports suggest that in plasma about 0.04% of T_4, but 0.4% of T_3, is in the free state at equilibrium with the bound forms.[16] There is a rapid redistribution between the free and the protein-bound pools as the cells use hormone, maintaining a constant supply of free hormones for the cells. It is now widely believed that free T_4 and T_3 from serum exert their biological activity, while it is presumed that the protein-bound moieties are biological inactive as long as they remain bound. Therefore, the level of unbound hormone (free hormone) is the most important factor in determining the extent of the hormones' peripheral action.[17] However, the importance of the protein-bound forms in the economic use of the hormones may be to retain them in plasma and regulate their rates of penetration into cells.

E. Metabolism and excretion of thyroid hormones.

Physiological effects of thyroid hormones are diverse and the effects are biphasic, with low concentrations of thyroid hormones tending to stimulate anabolism but high concentrations tending to promote catabolism. The effects on growth and cellular differentiation appear to be the more basic function of thyroid hormones, while the regulation of basal metabolic rate (BMR) is a secondary or tertiary effect involving the production of many enzymes.[18] The normal physiological level of thyroid hormones are necessary for growth and development, nerve function, bone development, energy metabolism, temperature regulation and water, electrolytes, chloresterol and lipid metabolism. In addition, to all these effects, thyroid hormones are also involved in the control of heart rate, reproduction, muscular contraction, vitamin metabolism and anion and cation transport, as well as of numerous other systems in the body and many of these effects have been reviewed by Pitt-Rivers and Tata (1959).

Thyroid hormones are metabolised into inactive forms, then their metabolites are mainly excreted into faeces and urine by the liver and kidney, although some reabsorption does occur in the small intestine and kidney. There are three main types of metabolic pathway for the transformation and inactivation of thyroid hormones:
a). deiodination, b). phenolic conjugation with glucuronic acid or sulphate

and c). alteration of the alanine side-chain through deamination or decarboxylation. Deiodination appears to be the most important of these pathways and it occurs in all tissues. Conjugation is also an important mechanism for inactivation or detoxification of thyroid hormones and this pathway is predominant in the liver although the other pathways also operate in this tissue. Reaction involving alteration of the alanine side-chain are generally considered least important, but deamination is prominent in kidney. In conclusion, excretion of thyroid hormones and their metabolites occurs by two major pathways through the liver and kidneys into faeces and urine, respectively. The thyroid hormones and their metabolites appear mainly in the faeces, while iodine is predominantly in the urine, where only small amounts of T_4, T_3 and their analogues are found. Thus, urinary excretion seems to reflect a deiodinating metabolism of thyroid hormones while faecal excretion is the end-point of their enterohepatic metabolism.

F. Principle of thyroxine radioimmunoassay.

Serum T_4 and radioactive T_4 compete for binding sites on T_4-antibody. After the reaction reach equilibrium, the antibody bound T_4 is separated as a precipitate from the unbound T_4 by polyethylene glycol (PEG). Then take this precipitate to count radioactivity in a gamma counter (postcount), while a few tubes is measured radioactivity during incubation (precount). The postcound/precount

ratios (%B) were plotted against the concentration of T_4 on a semi-log paper to be a standard curve, and the amount of T_4 in unknown samples is quantitated.[19]

G. Principle of T_3-uptake test.

The thyroid gland produces T_4 and T_3 which are transported by the blood, bound primarily to an alpha globulin, thyroxine binding globulin (TBG) and to a lesser degree by thyroxine binding prealbumin (TBPA) and T_4 only to albumin. The affinity of these proteins for T_4 is particularly strong, but it is weaker for T_3, with the result that when the binding sites are saturated, T_3 is particular becomes available for binding to secondary sites. The T_3-uptake procedure is consisted of adding radioactive T_3 into serum to saturate binding sites of plasma protein.[20]

H. Principle of free thyroxine index (FTI).

The determinant of thyroid status is considered to be the concentration of free T_4 present in serum. The T_4-RIA value gives the total T_4 in serum, of which about 99.96% is protein bound. An estimate of the free thyroxine level can be derived from the total T_4 and T_3-uptake test. Thus, T_4 is distributed in serum between the bound and unbound form according to the equilibrium. The equation of FTI is followed the law of mass action, where FT_4 is free T_4, UTBP is the unsaturated capacity of T_4 binding protein, and $TBPT_4$ is the T_4 bound to these proteins.[21]

$$FT_4 + UBPT_4 \longrightarrow TBPT_4$$

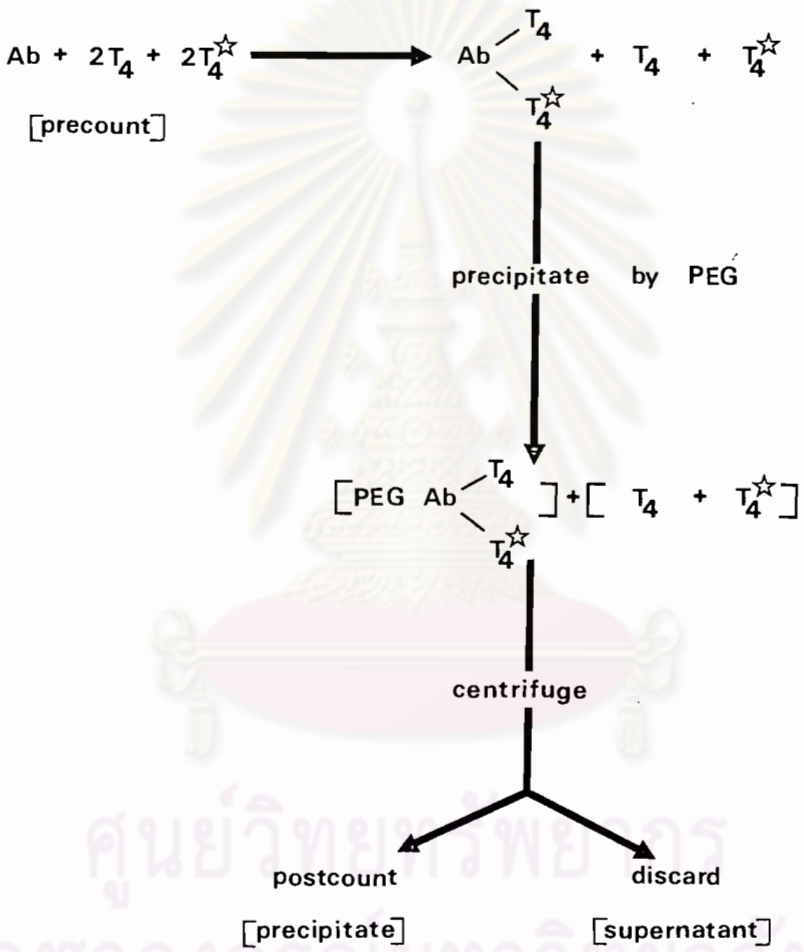
$$\frac{(FT_4)(UBPT_4)}{TBPT_4} = K$$

$$1/K \; FT_4 = \frac{TBPT_4}{UBPT_4}$$

$$= \frac{T_4\text{-RIA}}{T_3\text{-uptake test}}$$

$$\%FTI = \frac{T_4\text{-RIA}}{T_3\text{-uptake test}} \times 100$$

DIAGRAM OF THYROXINE RADIOIMMUNOASSAY.



Ab = Antibody T_4

T_4 = Serum or Standard T_4

$T_4^{☆}$ = ^{125}I-T_4