REVIEW OF LITERATURE

Chemical characteristics of thyrotropin

Thyrotropin (TSH) is a glycoprotein having a molecular weight of about 25,000 to 30,000.[7,48,50] This hormone regulates the level of thyroid activity. It is produced in the anterior pituitary gland and can be isolated from pituitary extracts by a combination of solvent fraction and column chromatography.[7,36] The most active fractions obtained by these means have potencies of about 40 I.U./mg, which is approximately 1,000 times that of bovine lyophilized pituitary tissue. When examined in the ultracentrifuge, the sedimentation constant of the material is between 2.5 and 2.7 S.[7,47,36] When studied by starch gel electrophoresis,[36] there appear to be several biologically active components, which do not seem to differ in their amino acid composition. It has been suggested that the multiple components may result from complex formation of an unspecified sort.[33] When examined by immunochemical procedures,[7] a preparation which had two gel bands gave only 1 or 2 lines in a gel diffusion experiment in which the antibody used was prepared with crude thyrotropin as the antigen.[51] In addition to the common amino acids found in most proteins, thyrotropin contains perhaps as much as 20% carbohydrate components, including glucosamine, galactosamine and some hexoses. It isoelectric point lies between pH 6 and pH 8, over which range the electrophoretic mobility is almost zero in free electrophoresis.[48,50]

Normal physiology

Thyrotropin can be detected in concentration between 1.5 and 15 mug/ml in the plasma of approximately 50% of euthyroid individual. The half-time of TSH in human plasma is approximately 1 hour.[50] The normal secretion rate is estimated to be 125 mug/day, an amount roughly equivalent to the normal pituitary content. From bioassay studies, the turnover rate of TSH in plasma is accelerated in thyrotoxic state and slowed in hypothyroidism. A large fraction of TSH removal is attributed to the kidney and small amount of TSH is found in urine and hence is likely that the kidney degrades the hormone.[5,50] In patients with primary hypothyroidism, concentration of TSH in plasma has been found to be 18 to 180 mug/ml.[50] In contrast, TSH has not been detected in the plasma of patients with secondary hypothyroidism. Although the major--ity of patients with untreated hyperthyroidism displayed no detectable TSH in the plasma, normal concentrations were found in some, a finding which is not readily explained. Large dose of T_3 administered intravenously or oral--ly to myxedematous subjects decreases the plasma concentration of TSH within a few hours. The response to T_4 is slower. The effect of TSH on intrathyroidal iodine metabolism is to enhance essentially all processes leading to the synthesis and secretion of hormones. Abolition of TSH secret--ion by hypophysectomy or suppression is followed by decreased activity of the thyroidal iodine transport mechanism. In addition, organic binding is inhibited, as indicated both by kinetic analysis and by an increase in the

proportion of newly accumulated intrathyroidal iodine present in the organ--ic form. A decreased fraction of organified iodine is present as iodothy--ronines, indicating a decrease in the rate of coupling of iodotyrosines.(50) In the "intact" animal, the fractional release of glandular ^{131}I is retarded, indicating a decrease in proteolysis of thyroglobulin.

In view of suggested evidence that the several stages in thyroid hormone synthesis may be closely linked to or dependent upon thyroidal energy metabolism, considerable interest has centered upon the effects of TSH on glandular intermediary metabolism, a primary site of action of the hormone being sought. In brief, TSH stimulates thyroid oxygen consumption, glucose assimilation, glucose oxidation via the hexose monophosphate shunt and the glycolytic and tricarboxylic acid cycle. As a consequence, produc--tion of carbon dioxide and lactate from exogenous glucose is increased. Oxygen consumption and carbon dioxide production are increased by TSH in the absence of exogenous glucose, indicating increased oxidation of endo--genous substrate. The mechanism whereby TSH enhances glucose metabolism is unknown. TSH also has a pronounced and rapid effect on phospholipid metabolism. The glandular concentration of inorganic phosphate is increased by TSH, reflecting hydrolysis of organic phosphates. This may serve as a stimulus to oxidation metabolism. TSH stimulates incorporation of purine precursors into thyroidal RNA, possibly by providing more ribose derived from activity of the hexose monophosphate shunt. Uptake of alpha-amino-isobutylate by thyroid cells is enhanced by TSH; leucine incorporation is accelerated in the thyroid of rats given TSH. Thus, TSH stimulates both catabolic and

anabolic processes in the thyroid, the former presumably supplying energy requisite for the latter.

As yet, no unifocal site of TSH action thyroidal intermediary metabolism has been strongly supported by experimental evidence, although several possible sites have been suggested. The similarities between the metabolic effect of TSH in the thyroid and metabolic changes in macrophages during phagocytic activity raise the possibility that enhanced phagocytosis of colloid may initiate at least some of the foregoing metabolic effect of TSH. A closely related postulate is that TSH may affect activation of lysosomal activity in the gland as initiating metabolic event. In keeping with current interest in the possibility that hormones may exert their effects through stimulation of specific protein synthesis have been a number of studies of the influence of inhibitors of nucleic acid and protein synthesis of thyroidal iodine and intermediary metabolism.

Action of TSH on the thyroid

TSH influence many aspects of thyroid structure and function: the sites and the vascularity of the gland, the height and activity of the follicular epithelium and the amount of colloid are all controlled by TSH, Fig.2. Every step of the thyroid hormone biosynthetic pathway is stimulated by TSH as are numerous aspects of all metabolism e.g., glucose utilization, oxygen consumption, phospholipid synthesis and RNA synthesis. These actions begin within a few minutes of administration of TSH had recently been

attributed to activation of adenyl cyclase after combination with a receptor site on the cell surface.[13] The resulting formation of cyclic AMP leads, via an effect on m-RNA, to synthesis of protein concerned in the individual steps of thyroid hormone synthesis i.e., the iodide trap, iodine incorpor--ation into thyroglobulin, uptake and proteolysis of thyroglobulin, and releasing of thyroid hormones into the circulation.

Unlike other endocrine organs, the thyroid hormones are not stored in the gland cell but in colloid containing vesicles enclosed by thyroid epi--thelium as a precursor protein, thyroglobulin. Within a thyroglobulin molecule, tyrosine residues are iodinated to form monoiodotyrosine (MIT) and diiodotyrosine (DIT), which then combine to form the iodothyronine.

The thyroid hormones affect oxygen consumption and heat production in the whole animal and stimulate the metabolism of isolated tissue, particu--larly liver and muscle. The mechanism of this metabolism is not yet clear, but the hormones seem to act finally on the energy producing electron-trans--fer processes in the respiratory enzyme system of the mitochondria. Thyroid hormone administration alters the number of physical structure of the mito--chondria and reduces the efficiency of oxidative phosphorylation. These changes may be manifestations of excess hormone action; the effect of thyroid hormones at the physiological leval involves increased transcription of m-RNA, probably via cyclic AMP. The resulting increase of protein synthesis in mitochondria and microsomes is necessary for subsequent stimulation of

cell respiration caused by thyroid hormones. Thyroid hormone also assimilates many other processes, protein breakdown is increased in collagen and other tissues, carbohydrate and lipid turnover are increased, calcium is metabo--lised from bone, and heart rate is accelerated. Several of the actions of thyroid hormones are consisted with increased sensitivity of beta-receptor to catecholamines. In addition, and important action on body growth and maturation results from the direct effect of tyrosine on tissue growth and a permission effect on growth hormone secretion by the anterior pituitary gland.

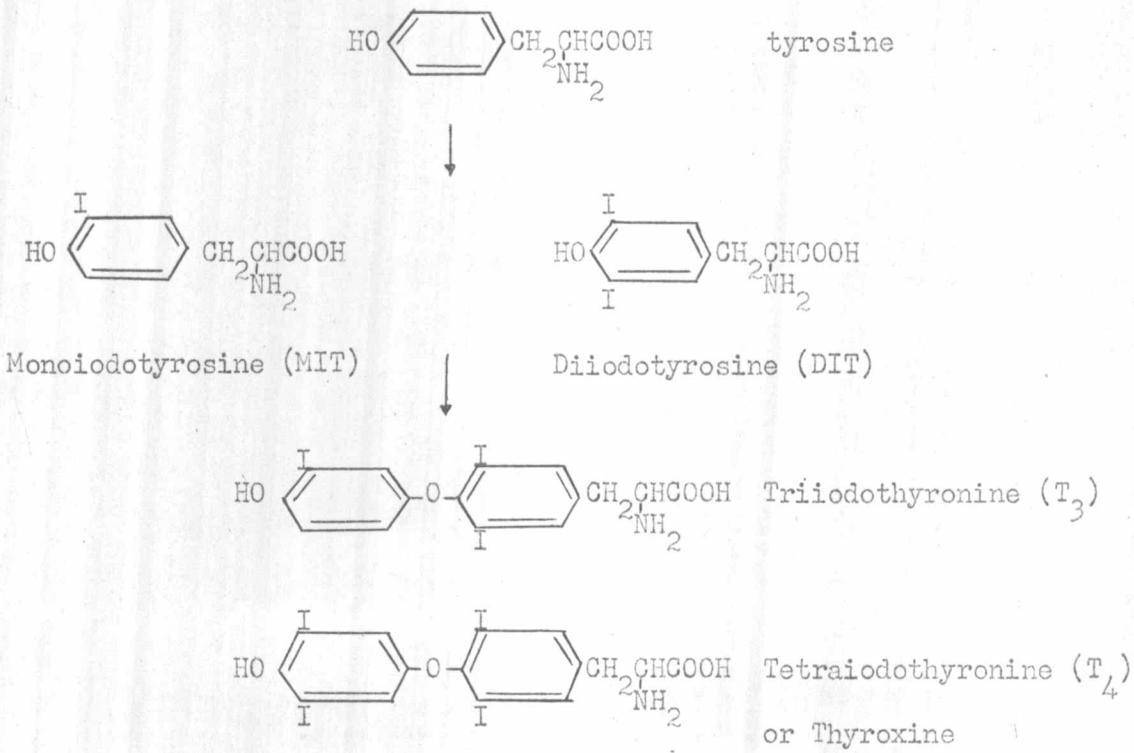

Figure 1 - Intrathyroidal synthesis of organic hormonal iodine

Properties of thyrotropin

1. Increases the size and number of acilar cells.
2. Increases the thyroid plasma iodide gradient.
3. Increases the rate of synthesis of thyroxine.
4. Stimulates proteolysis of thyroglobulin.
5. Increases the rate of transfer of thyroxine from the thyroid gland to the blood stream.

Thyroid stimulating hormone releasing factor (TSHRF or TRF)

Thyrotropin which is secreted by specific pituitary cells under the influence of a tripeptide releasing factor in the hypothalamus and transported to the anterior pituitary via the portal vessel. Normally, thyroid hormones inhibit excessive TRF action by a direct effect on the pituitary. TRF has been isolated and characterized as a tripeptide with the stucture (pyr) Glu-His-Pro-(NH_2), by Schally.[17] TRF has been synthesized and shown to have full activity in men and other animals. It causes a striking release of TSH in vivo within seconds and is rapidly inactivated by plasma enzymes. The releasing effect of TRF requires energy but does not involve protein synthesis. The effect of TRF on the pituitary is inhibited by treatment with T_3 or T_4, which is consistent with the inhibit--ory effect of thyroxine on TRF action is mediated by a step involving protein synthesis, it is blocked by cyclohexamine. The regulation of TSH

secretion by TRF and T_4 is shown in Figure 2. The hypothalamic release of TRF may be relatively constant, except during acute exposure to cold. Otherwise, regulation occurs by interaction, at the thyrotroph cell, between TRF, thyroid hormones and enzymes destroying TRF.(13)

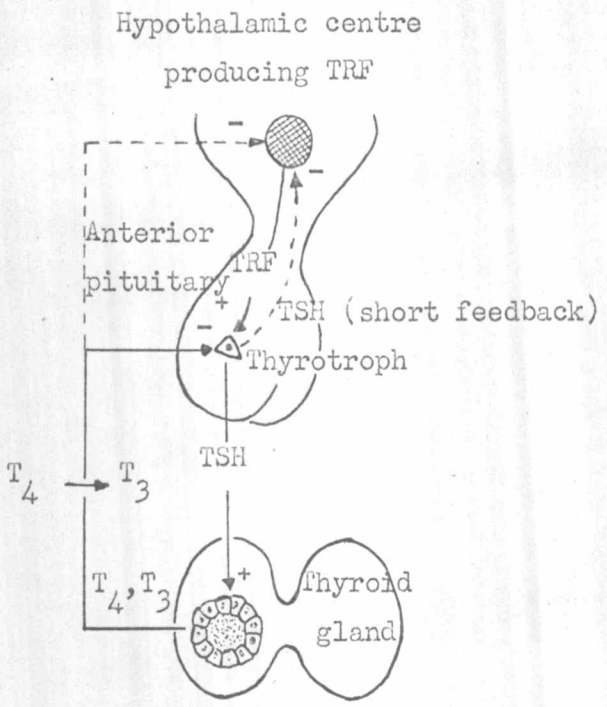

Figure 2 - Regulation of thyroid hormone secretion

Circulating thyroid hormones

The total amount of circulating hormonal iodine is 6-8 ug/100 ml. This is contained in thyroxine, with about 0.3 ug/100 ml in triiodothy-

-ronine. Circulating thyroxine is strongly associated with plasma binding protein and the free plasma hormone is less than 1/2,000 of the total circulating level. The major binding protein are thyroxine binding globulin (TBG), thyroxine binding prealbumin (TBPA) and albumin, accounting for 60%, 30% and 10% respectively of the thyroxine binding capacity of plasma. The level of TBG is influenced by estrogen and the binding capacity is increased during pregnancy and oral contraceptive therapy. The triiodothyronine content of plasma is about 200 ng/100 ml; T_3 binds rather weakly to TBG and is readily replaced by T_4. Thyroxine was formerly believed to be the most important thyroid hormone, and many tests of thyroid function have been based on its measurement. Total serum thyroxine can be measured by displacement binding assay in a system including TBG and labelled T_4.(34)

The biological activity of T_3 is several times greater than that of T_4, and the metabolic effects are more rapid. The metabolism of T_3 is also more rapid, the turnover rate being 5 times of T_4. The daily secretion and util--ization of T_4 is about 80 ug, and that of T_3 is about 50 ug. There is in--creasing evidence that T_3 may be as important as or more important than thyroxine in determining hormonal status.(46)

Radioimmunoassay of thyrotropin

The lack of the pure sample of TSH makes the design of an immunoassay very difficult although possible. The greatest difficulty in establishing this method has been that of determining the optimal dilutions of the anti-

-bodies and of the precipitating antiserum. There is also the problem of the specificity of the TSH used and of the antibodies.[30]

The bioassay of thyrotropin

Junkmann and Schoeller[29] developed the first quantitative assay of thyrotropin in 1932, then there have been many others described and many clinical studies reported where the concentration of TSH in human blood or urine has been studied. a bioassay, however, is still to be described which measures unequivocally normal or subnormal concentrations of this hormone in human blood. A bioassay technique for thyrotropin in serum was developed by McKenzie.[32] Mice were injected with ^{131}I and then had endogenous thyrotropin suppressed by the administration of thyroxine followed by thyroid powder. The effect of an intravenous injection of TSH or the test substance was measured by percentage increase in blood ^{131}I. This had a linear relationship to the logarithm of the dose. Sensitivity of 0.025 mU, with an injection volume of 0.5 ml and an average index of precision (λ) of 0.24 were achieved.

Recently, Sakiz and Guillemin,[40] 1969, described and improved mathe-matical treatment of the data derived from the McKenzie assay. Transforma-tion of the original count rate data into logarithm ($\log_{10}$) for both control and treatment blood samples was made. Covariance analysis was then carried out on the transformed data, the second blood values (dependent variable) being adjusted to the values of the first blood sample (independent variable). This treatment resulted in increased precision and reliability.