THE THYROID AND ITS CONTROL

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THYROID HORMONE HOMEOSTASIS

In considering thyroid hormone regulation and homeostasis, it is important to examine some characteristics of this hormone in relation to the others. The hormones may readily be divided into two distinct physicochemical and biological classes. (Table 1). The protein and peptide hormones are water soluble and evidently exist in the plasma without demonstrable interaction with other serum proteins. These hormones have a rapid metabolic turnover with a half-time in minutes. Therefore it is not surprising to observe rather striking fluctuations in concentration in the blood within a few minutes in the case of all these peptide hormones, including parathyroid hormone, thyrocalcitonin, growth hormone, FSH, LH, prolactin, ACTH, MSH, TSH, vasopressin, insulin, secretin, angiotensin II, and glucagon (Table 1). Indeed, the amino acid hormones, epinephrine and norepinephrine, may fluctuate markedly within a few seconds.

In contrast, the “target” hormones—cortisol, progesterone, estradiol, testosterone, and thyroxine—are relatively hydrophobic and exist in aqueous solution in the blood by virtue of their firm binding by one or more serum protein carriers (Table 1). The biological half-times vary with the magnitude of the “free” or nonprotein-bound moiety. Thus cortisol, which is about 95% protein bound and 5% unbound, has approximately a one-hr half-time, and thyroxine, more than 99.96% protein bound and less than 0.04% unbound, has a biological half-time of turnover approx-

1Abbreviations used: T₃, Triiodothyronine; T₄, Thyroxine; TSH, Thyroid Stimulating Hormone; FSH, Follicle Stimulating Hormone; LH, Luteinizing Hormone; ACTH, Adrenocorticotropic hormone; MSH, Melanocyte Stimulating Hormone; PTU, Propylthiouracil; cAMP, cyclic adenosine monophosphate; MIT, Monoiodotyrosine; DIT, Diiodotyrosine; hCG, human Chorionic Gonadotropin; hCT, human Chorionic Thyrotropin; GH, Growth Hormone.

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Table 1  Soluble and hydrophobic hormones

<table>
<thead>
<tr>
<th>Soluble</th>
<th>Hydrophobic</th>
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<tr>
<td><strong>Protein and peptide hormones</strong></td>
<td><strong>Small-molecule “target” hormones</strong></td>
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<tr>
<td>Parathyroid</td>
<td>Cortisol</td>
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<td>Thyrocalcitonin</td>
<td>Progesterone</td>
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<td>Growth hormone</td>
<td>Estradiol</td>
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<td>FSH, LH, prolactin</td>
<td>Testosterone</td>
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<td>ACTH, MSH</td>
<td>Thyroxine</td>
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<td>TSH</td>
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<td>Vasopressin</td>
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<td>Insulin</td>
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1 week. The concentration of thyroxine, therefore, ordinarily remains constant for long periods in health and in disease, although it may vary somewhat within a day or two in the postoperative period after major surgery. Cortisol can fluctuate markedly within hours and shows pronounced diurnal variation. Cortisol and thyroxine may be considered the extreme examples within the category of the protein-bound hormones of small molecular size; however, the turnover of even the fastest on the list, cortisol, is sluggish compared to the turnover of the peptide hormones. Aldosterone, which is appreciably less firmly bound than cortisol, has, as expected, a faster turnover than cortisol.

The biologic half-time of turnover of thyroxine (T₄), approximating one week in normal man, is much more prolonged than the biologic half-time of turnover of triiodothyronine (T₃), which is approximately one day. The difference can be largely explained by the much firmer binding of T₄ by the three serum protein carriers. Appropriately, the onset of physiological action of T₃ when given to a myxedematous subject, is more rapid but less sustained than that of T₄. Despite the more rapid effect of T₃ compared to T₄, the effects of thyroid hormone are generally much more sustained than those of the other hormones.

In marked contrast to the emergency functions of the catecholamines and of cortisol, the thyroid hormones generally provide a homeostatic background which is maintained at a constant level for physiological requirements in health.

THYROID AUTOREGULATION

The concept of thyroid autoregulation, whereby levels of certain metabolic activities in the gland are modulated not only by pituitary thyrotropin (TSH) but also by the iodide supply, is apparently unique among the endocrine organs under pituitary control. Experimentally, autoregulatory responses may be examined in hypophysec-
tomized animals either left untreated or given TSH in constant doses. In this situation iodine levels can be made to vary over wide ranges. In vitro experiments using dispersed thyroid cells have also proved valuable. These and other methods have determined that while TSH certainly influences every step in thyroid hormone synthesis and secretion, evidence exists that iodide does so as well (70).

The effect of iodide on the thyroid iodide transport system has been studied in detail ever since the identification of this process which actively transports iodide at the basal membrane of the thyroid cell (5, 158). TSH is the most important factor regulating I- transport, causing an early increase in thyroid I- efflux followed by a late increase in unidirectional clearance. The biphasic effect of TSH on I- transport is mediated by cAMP. The ability of the thyroid to concentrate I- measured following hypophysectomy was, as predicted, reduced; but if total thyroidal iodide was then decreased either by iodine restriction or by treatment with PTU, then the iodide concentrating mechanism was stimulated. The striking inverse correlation between glandular content of organic iodine and T:S[I-]3 ratio (148) led to the postulate that the loss of concentrating ability was mediated by a hypothetical intrathyroidal organic inhibitor of iodide transport (61). This inhibitor has not been isolated. Autoregulatory control remains after hypophysectomy. For example, the T:S[I-] remained substantial after the operation if it was high before (60). Rats fed a low iodine diet before operation for three generations maintained a high T:S[I-] after surgery despite thyroid involution (52). When rats maintained on a low or high iodine regimen were hypophysectomized and the thyroids depleted of inorganic I- in vitro, the T:M[I-]4 of the iodine-poor glands was higher than that of the iodine-rich glands (82). Further studies have shown that relatively small doses of I- depressed the T:S[I-] of hypophysectomized iodine-deficient rats (129). This inhibitory effect could not be correlated with total organic iodine formation or with iodothyrosine or iodothyronine formation, making it hard to accept the view that the inhibitory compound referred to above is an iodothyrosine or thyronine.

Recent extensive studies of changes in thyroid function in response to iodine deficiency in the rat have been carried out by Greer and co-workers (43–45, 54). It is known that the adaptive changes of thyroidal iodine metabolism to iodine deficiency, primarily induced by increased pituitary TSH secretion in response to lowered circulating thyroid hormone levels, include thyroid hypertrophy, high radioiodine uptake, and an increase in intrathyroidal labeled MIT:DIT and T3:T4 ratios (53, 136). In iodine-deficient infant rats, a high T3:T4 ratio was not observed until the third month (30), probably because of a coupling deficiency in newborn rats resulting in a proportionately greater formation of iodothyrosines than of iodothyronines compared to older animals. The adaptive changes in thyroid hormone levels are largely mediated by an increase in TSH secretion; plasma TSH may be increased as early as the third day of a low iodine diet. Plasma T4 falls more rapidly than T3, suggesting that the rise in TSH may be responsive to a fall in the former

3The ratio T:S[I-] refers to the concentration gradient of tissue iodide to that of serum iodide.
4The ratio T:M[I-] refers to the concentration gradient of tissue iodide to that of iodide in the medium.
hormone. A major fraction of plasma T₃ in the iodine-deficient state apparently derives from a preferential thyroid secretion rather than from peripheral conversion from T₄. When iodide is administered to iodine-deficient rats, TSH levels fall in proportion to the dose of iodide administered. However, after six weeks of supplementation, radiiodine uptake was inversely correlated with dietary iodine intake despite normal concentrations of TSH, T₄, and T₃. This confirmed the original suggestions that intrathyroidal iodine content is a primary determinant of the iodide concentrating activity of the gland (59, 148). Although thyroid autoregulation is a factor in adapting iodide transport to iodine supply, TSH is still important in the early response to experimental iodine deficiency. Thus autoregulation is a rather sluggish response, indicating perhaps two levels of thyroid control in this situation, a rapid TSH response modulating a slower autoregulatory intrinsic control.

Large doses of iodide will reduce the T:SI⁻ ratio acutely due to saturation of the iodide pump by its substrate (158). There is an associated reduction of the unidirectional I⁻ clearance and an increased exit rate constant (122, 138). Studying these effects on the pump alone is difficult because of the organic binding reactions that occur almost simultaneously with iodide transport. Studies are therefore carried out on glands blocked with thionamides. As the human salivary glands concentrate iodide to many times the plasma level but do not organify it (5), studies of salivary iodide in response to excess iodide loads in human subjects are valuable. For example, one study showed that higher serum concentrations of exogenously administered iodide were required to inhibit the salivary iodide trap in patients with iodide-induced goiter compared to controls (81). This would allow excess iodide to enter the thyroid and cause damage as described by Wolff (159).

The oxidation and subsequent organic binding of thyroidal iodide have been reviewed by Taurog (141). Briefly, in a model system the incubation requirements for iodination include thyroid peroxidase, iodide, acceptor (protein or free tyrosine), and H₂O₂. In such a system, oxidized iodide is able to iodinate tyrosyl residues in thyroglobulin to produce monoiridotyrosine (MIT) and diiodotyrosine (DIT). The subsequent reactions resulting in the production of T₃ and T₄ are influenced by the availability of iodide, best exemplified by the classic investigations of response of thyroid hormone synthesis to acute alterations in the availability of iodide. In rats injected with 10–500 μg¹²⁷I– labeled with ¹³¹I–, organic binding of injected iodide was decreased so long as plasma iodide remained above 20–35 μg/100 ml (the Wolff-Chaikoff effect) (162). During this decrease in the formation of total organic iodine, qualitative change in the distribution of the organic iodine compounds also occurs, i.e. an increase in the MIT:DIT ratio and a decrease in iodothyronine (T₄ and T₃) formation (160). Increasing doses of iodide administered to rats causes a progressive decrease in ¹³¹I– uptake, in organization of thyroid iodide, in the proportion of thyroid DIT and iodothyronines among the iodinated amino acids, and in the absolute rate of organic iodinations and iodothyronine synthesis (98). However, the thyroid is apparently able to prevent an acute increase in the formation of active hormones in response to moderate doses of iodide only after a small transient increase of hormone release has depressed TSH secretion (135). The Wolff-Chaikoff effect should probably be defined in terms of the entire phase of iodine metabolism in which iodination decreases in response to increasing doses of
iodide rather than in terms of the quantity of organic iodine formed (97). It is apparent that the iodide transport activity and TSH concentrations affect the Wolff-Chaikoff mechanism. For example, increased sensitivity to iodide inhibition observed in rats on a low iodine diet (72) and in rats pretreated with TSH (109) may be explained by the higher T:S[I-I] established in such conditions. The work of Rosenfeld & Rosenberg (117) has shown when TSH was given to rats prior to administration of large doses of iodide, glandular organification was enhanced compared to rats similarly treated with iodide but without TSH in which organification was severely depressed. The precise mechanism of the acute inhibitory action of excess iodide is not known, but several theories are prevalent: hypoiодous acid, which may be the reactive form of iodine that enters into organic combination in the gland, could be decreased by the addition of excess iodide. Alternatively, the triiodide ion, which is incapable of carrying out iodinations, could be produced from oxidized iodine in the presence of high concentrations of inorganic iodide. The effect of varying concentrations of iodide on the model system described above (139, 140) showed that the rate of I₂ formation appeared to be reciprocally related to the rate of iodination of protein. This suggests that I₂ formation competes with iodination and that the formation of I₂ is favored by a high concentration of I⁻. However, none of these postulated mechanisms has been proved in vivo.

It has been recognized that the Wolff-Chaikoff effect is transient. Attempts to prolong inhibition by maintaining a high serum iodide level either by nephrectomy after a single iodide dose (161) or by repeated injections were unsuccessful beyond 26-40 hr despite continued high serum iodide concentrations (163). The mechanism of "escape" from iodide inhibition was termed an "adaptation" by Braverman & Ingbar (12), who demonstrated that glands that had escaped lost much of their normal ability to concentrate iodide. A pituitary or TSH mechanism for the escape was shown to be unlikely and it was proposed that escape may occur through "intrinsic control mechanisms."

The effects of reduction or increase in the iodine supply on various other parameters of thyroid function have been studied by different methods. Experiments using dispersed thyroid cells in vitro have shown that prior incubation with iodide led to decreased iodide concentrating activity and reduced the stimulation of the pump that could be induced in response to TSH (123). This latter finding indicates that the influence of iodide is also exerted on the cellular mechanisms mediating the response to TSH. For example, excess iodide injection into normal rats significantly decreases thyroidal adenosine triphosphate (ATP) and pyridine nucleotides as early as 5 min after injection (85). Recently, Sherwin & Tong (124) have shown in experiments with dispersed thyroid cells that preincubation with excess iodide led to decreased stimulation of cAMP production, of iodide pump activity, and of iodinating activity in response to added TSH. These investigations suggest therefore that autoregulation of the thyroid gland does include influences of iodide on these processes; however, two other effects of TSH, the stimulation of [¹⁴C] leucine incorporation into protein and of iodide efflux, were not affected by excess iodide and hence were not included in the autoregulatory influence of iodide. Confirmation of the reduced cAMP response to TSH in iodine-enriched compared to iodine-deficient animals had come from a study of hypophysectomized rats on these dietary regi-
mens (110). In the rat, it seems clear that thyroid autoregulation can cope with moderate increases in iodide administration, but excess iodide decreases the release rate of $^{131}$I from the labeled thyroid and also decreases the blood hormone concentration more in hyperactive thyroids of rats treated with antithyroid drugs than in untreated rats. TSH levels are not crucial to these changes, which are similar to those described in man (104, 105, 157, 164). Excess iodide blocks thyroid hormone release in patients with Graves' disease (99). The thyroid in this condition, in contrast to the normal gland, is abnormally sensitive to iodide. Recently, iodide-induced hypothyroidism has been observed following inorganic iodide administration to patients rendered euthyroid following treatment for hyperthyroidism (15) and patients with Hashimoto's thyroiditis (14). The occurrence of hyperthyroidism following increased iodide supply in some goitrous subjects (145, 149) could well be due to the failure of one or several of the intrathyroidal control mechanisms which enable the normal thyroid to maintain its hormone secretion within narrow limits in the face of a widely varying iodide supply.

**THYROTROPIN (TSH) CONTROL**

The introduction of methods for measuring human thyrotropin in serum (65, 89, 103, 106) has made it possible to define further the thyroid pituitary relationships in normal human subjects as well as those with thyroid disorders. Studies of metabolic clearance and production rates of human thyrotropin (115) have demonstrated that changes in the serum concentration of TSH are mainly due to altered pituitary TSH secretion with only a minor contribution from the change in the metabolic clearance rate of the hormone. Like the other glycoprotein hormones (follicle stimulating hormone, luteinizing hormone, and chorionic gonadotropin), TSH possesses two peptide chains designated alpha and beta. The former is practically the same in all four glycoprotein hormones. The development of radioimmunoassays for the two subunits of TSH (7, 79) has shown that normal pituitary glands contain a predominance of free alpha subunit relative to beta TSH in addition to TSH itself. In hypothyroidism secretion of both free subunits occurs. However, this may represent only a quantitative difference from the normal state; the subunits of TSH appear to respond to the same control mechanism as complete TSH (80).

Although general agreement exists that TSH secretion is under negative feedback control by thyroid hormones, the details of thyroid hormone action at the pituitary cell level and the relative importance of T4 and T3 in suppressing TSH secretion are unclear. In vitro studies of isolated pituitary preparations have examined the effect of inhibitors of protein synthesis, such as puromycin, cycloheximide, and actinomycin D, on TSH production (9a, 9b). If these compounds were administered before the addition of thyroid hormone, they prevented the inhibition of TSH release normally produced by thyroid hormones. Thus the thyroid hormones may cause the intrapituitary formation of an inhibitory protein or polypeptide that interacts competitively with thyrotropin releasing hormone (TRH, vide infra) in regulating TSH metabolism. The nature of this compound has not been identified.

The relative importance of T4 and T3 in feedback inhibition of pituitary TSH release is a very interesting topic, but is far from settled. The finding of high-affinity,
low-capacity receptor sites for T₃ in the anterior pituitary (121) gave rise to the concept of T₃ as a primary suppressor of TSH release. However, specific binding sites for T₄ have also been demonstrated in the adenohypophysis, albeit with a lower affinity than T₃ binding sites (130, 137). The possibility of intrapituitary conversion of T₄ to T₃ has been entertained, but was not supported by the findings of a recent study (49).

Studies on the acute effects of T₄, T₃, and iodide on TSH secretion in rats (43) showed that equivalent physiological doses of T₄ and T₃ exerted immediate and indistinguishable effects on suppressing pituitary TSH secretion.

The problem has been approached in human subjects by assessing the diminution in TSH response to TRH produced by T₃ or T₄ administered at various intervals prior to TRH injection. The recent report by Wenzel et al (153) showed a faster and more pronounced inhibition of TSH release by smaller doses of T₃ when compared to T₄. On the other hand, single intravenous injections of T₃ resulting in marked elevation of serum T₃ concentration may fail to alter TSH response to TRH administration for at least several hours (150). Taken altogether, these findings are compatible with the requirement for synthesis of an inhibiting protein or peptide in the adenohypophysis caused by elevated serum hormone concentrations. Pending further information it seems most reasonable at present to consider both T₄ and T₃ as candidates for feedback regulators of TSH production. Recently a new clinical entity of thyrotropin hyperthyroidism caused by selective pituitary resistance to thyroid hormone has been documented (51). The study of this syndrome of inappropriate secretion of TSH may shed further light on the regulation of the hypothalamic pituitary thyroid axis.

The possibility of a direct feedback effect of the thyroid hormones upon the thyroid gland itself must also be considered. Such inhibition of the intrathyroidal enzyme, ornithine decarboxylase, has been shown by T₄ or T₃ pretreatment, which can counteract the stimulatory effect of administered TSH (165). As these effects were more marked in iodine-deficient animals, the role of autoregulation is also suggested. However, work in progress by Ingbar and co-workers (S. H. Ingbar, personal communication) has failed to substantiate this “short-loop feedback” on the thyroid gland.

**Sympathetic Regulation**

The anatomical and pharmacological studies of Melander and co-workers (91–95) have indicated the importance of the sympathetic adrenergic system in the control of thyroid function. Sympathetic adrenergic nerve fibers are numerous in the human thyroid, and their anatomy suggests an influence of this system on thyroid follicle cells as well as on the thyroid vasculature. Fluorescence histochemistry and electron microscopic autoradiography in mouse thyroids suggest the possibility that the sympathetic innervation may effect prompt short-term alterations in the rate of thyroid hormone secretion (91–95). It appears that aromatic monoamines (norepinephrine and dopamine) can stimulate thyroid hormone synthesis by direct action on alpha-adrenergic receptors in the follicle cells. It also seems probable that the formation of thyroid mast cells, which may participate in the regulation of the
synthesis of thyroid hormone, is controlled by TSH. The TSH-regulated activation of the thyroid may be facilitated and partially mediated by amines released from mast cells by TSH. While most of the studies have been done in animals, and species differences have been observed, there is anatomic and biochemical evidence that similar mechanisms may operate in man.

**Cold Exposure**

Reduction in ambient temperature causes a rise in TSH levels in laboratory and domestic animals. The precise role of changes in thyroid secretion in the homeostatic regulation of metabolism during cold exposure is not clear, although thyroid hormone is essential for survival at lowered environmental temperatures (46). Acute cold exposure in the rat causes a rise in plasma TSH levels within 5–10 min (66, 87). It appears that peripheral thermoreceptors may activate TSH secretion, as hypothalamic core temperatures either show no change or a slight rise in this situation (114). However, that TSH release can also be induced by lowering of core temperature is shown by experiments demonstrating activation of thyroid hormone secretion following hypothalamic cooling in the goat (1), rat (111), and baboon (48).

The effect of chronic cold exposure on TSH levels and subsequent thyroid hormone metabolism is less clear. Most experiments performed in many species have failed to show any change in plasma T₄ levels during chronic cold exposure. However, some workers have noted otherwise. Galton & Nisula (50) found that the rat showed an increased fecal excretion of T₄ secondary to enhanced food intake in response to chronic cold exposure. This may result in a decrease in negative feedback at the pituitary level leading to a rise in TSH and thyroid hormone secretion. Another study of rats exposed to cold, which showed no detectable change in plasma TSH but a 50% reduction in plasma T₄, concluded that, although TSH release is stimulated initially during cold exposure by hypothalamic mechanisms, an increased metabolic clearance of the hormone prevents an elevation of plasma levels (42). A peripheral metabolic response to cold is further suggested by recent studies documenting the rise in plasma T₃ levels and kidney T₃ concentration in rats exposed to cold (74, 99). In addition, T₃ binding proteins in parenchymal tissue are increased, as is the T₄ degradation rate (3). However, whether these changes are due to increased thyroidal T₃ secretion, increased peripheral monodeiodination of T₄ to T₃, or reduced plasma, T₃ clearance is unknown. Increased thyroidal T₃ secretion is probably the most reasonable overall explanation.

Acute cold exposure in the rat has been reported by one group (96) to lead to an increase in plasma TRH levels, but this has not been confirmed (31). Increased hypothalamic TRH levels have been reported in rats exposed to cold (113).

Of major interest are the recent findings of Szabo & Frohman (138a) showing depression of the TSH secretory response in cold-exposed rats after administration of antiserum to TSH; this tends to afford support for the hypothalamic mediation of the cold-induced rise in TSH via TRH.

**Stress**

The study of stress-induced changes in pituitary-thyroid function has produced ambiguous results. Stressful factors may alter pituitary-thyroid function at many
levels of control; such factors include altered TRH secretion, changes in responsiveness of the pituitary to TRH, altered metabolism of TSH, altered metabolism of the thyroid hormones in peripheral tissues, and alterations in the physical state of thyroid hormone binding proteins in blood (113). In general, most studies have indicated that TSH secretion is inhibited during exposure to severe stress (55). A few studies have shown that thyroid hormone may be increased after acute stress in the sheep or man or after aversive conditioning in the rhesus monkey (88). In human subjects exposed to subfreezing cold water, an increase in the urinary excretion of immunoassayable TRH has been reported (47), and insulin-induced hypoglycemia has also been reported to cause release of TSH in human subjects with GH deficiency (57) and in normal rats (83).

**Hormones**

The influence of hormones in modulating TSH secretion has been reviewed by Reichlin and co-workers (113). Acute or chronic exposure to excessively high levels of cortisol will inhibit TSH secretion in man and in the rat (156). The residual pituitary-thyroid activity observed in patients maximally suppressed with exogenous T3 is further reduced by treatment with high doses of corticoids (100). Corticosteroids appear to reduce the sensitivity of the pituitary to TRH and perhaps also reduce the secretion of TRH. Estrogen effects have been difficult to study because of their known effects on peripheral metabolism of thyroid hormone. The findings that pituitary responsiveness to TRH is greatest in that phase of the menstrual cycle associated with the highest levels of estradiol (late follicular phase) (120) and that the pituitary response to TRH in man is enhanced by prior treatment with estrogens (22) suggest that estrogen sensitization causes the greater TSH secretory response to TRH in women than in men (73). It has recently been reported that the administration of therapeutic amounts of growth hormone to patients with hypopituitarism of hypothalamic origin resulted in a decreased pituitary TSH response to TRH (84, 116). Growth hormone release inhibiting factor (somatostatin), a tetradecapeptide isolated from the hypothalamus, has been reported to inhibit the TSH response to TRH in normal men (58, 125, 151). In addition, somatostatin has been shown to have an inhibitory effect on the high nighttime basal levels of serum TSH (152). These data suggest that somatostatin may have a physiological role in the regulation of TSH secretion. In support of this suggestion is the recent demonstration by Ferland and co-workers (35) that, after injection of sheep antiserum to somatostatin into rats, an increase was noted in basal plasma TSH levels and in TSH secretion in response to cold.

**Extremes of Age**

Basal TSH levels have been reported to be both normal and slightly increased in the elderly (28, 71). Studies with TRH in elderly men and women, which showed a lesser increase in T3 concentration after TRH, have not been confirmed and do not indicate a depression of pituitary function (2). Furthermore, since elderly patients can increase their basal serum TSH concentrations strikingly when challenged by frank hypothyroidism, it would follow that the pituitary senses very little, if any, deficiency of thyroid hormone in the normal aging individual (71). Clearly more data
are required before the phenomena of aging can be related to the thyroregulatory mechanisms known to exist at present.

There is much current interest in the effect of pregnancy both on the changes in thyroid gland function in the mother and in the fetus. With the development of rapid radioimmunoassay for thyroid hormones in small blood samples, screening for neonatal thyroid disorders is now practical (37).

Some of the changes in maternal thyroid metabolism during pregnancy may be due to the secretion of placental human chorionic thyrotropin (hCT). Although some human placentas may contain as much as 18,500 mU hCT per placenta (67), most placentas contain less than 10 mU hCT. Aborted placentas from 51 to 133 days of gestation show variable but generally low hCT content (68). In addition, purified human chorionic gonadotropin (hCG) has intrinsic thyrotropic activity in the TSH bioassay (101), but it is only 1/4000 as potent as pituitary TSH on a molecular basis. There is difficulty in establishing plasma levels of these hormones during pregnancy because of differences between bioassay and immunoassay. Thus serum bioassayable TSH has been reported to be high early in pregnancy (62); also, hCT by immunoassay has been shown to rise progressively during pregnancy (142). However, Hershman et al (64) found that hCT was low during pregnancy, and TSH only slightly elevated.

It is conceivable that virtually all significant circulating thyrotropic activity in pregnancy plasma may be ascribed exclusively to hCG, which is always elevated in early pregnancy and may rise to astronomical levels in hydatidiform mole and choriocarcinoma (63). Such a view has the virtue of simplicity as well as the bulk of recent supporting evidence.

The fetal pituitary thyroid system has differentiated in the human fetus and appears capable of function (albeit at a low level) by the end of the first trimester of gestation; it also functions independently of maternal control. It would appear that a general maturation of the neural and neuroendocrine systems controlling the secretion of adenohypophyseal hormones occurs at about 20 weeks; hypothalamic activation results in increased hypophyseal TSH synthesis and secretion, followed by increasing thyroid gland activity (36, 38).

At birth, the main physiological event controlling immediate postnatal thyroid function results from a sharp rise in serum TSH due to increased pituitary TSH release during the early minutes and hours of life (39). The cutting of the umbilical cord rather than body cooling is apparently the primary stimulus to this event (119). However, experiments in which TSH was increased when newborn infants were placed in different ambient temperatures suggest that cooling of the newborn in the extrauterine environment also may be an important stimulus (39–41). In response to the postnatal TSH surge, plasma T4, free T4, T3, and free T3 rise briskly (32), but clinical symptoms of hyperthyroidism do not occur in the newborn because of the transient nature of these rises.

Control of thyroid function during the fetal and neonatal period thus depends on many factors. The development of the hypothalamic-pituitary axis, the stresses experienced by the fetus at delivery, and the developing maturation of fetal enzymes responsible for peripheral metabolism of thyroid hormones are all significant. In
addition, the maternal iodine status is clearly important, at least in the rat (vide infra). Other indirect controlling influences on the thyroid at this time, such as potential variation in peripheral tissue sensitivity to thyroid hormones at the cellular level, have yet to be explored.

**HYPOTHALAMIC THYROTROPIN RELEASING HORMONE (TRH) CONTROL OF TSH**

Ablation of the medial basal hypothalamus of several species has led to diminished TSH secretion and reduced thyroid function (86, 87, 113). In contrast, TSH production may be maintained if a small "hypothalamic island" disconnected from other central nervous system structures is permitted to retain its normal connection with the median eminence and pituitary. Pituitary stalk section or transplantation of the pituitary to a distant site such as the kidney results in diminished TSH production. Conversely, electrical stimulation by carefully placed hypothalamic electrodes produced marked elevation of TSH within ten minutes, a response which was abolished by prior administration of thyroxine to the experimental rat (87).

Such reports along with many others had led to the expectation of the discovery of a "thyrotropin releasing factor" or TRF, a term actually employed by workers prior to the identification of the tripeptide.

The identification of the tripeptide termed "TRF," or "TRH" (thyrotropin releasing hormone) was the outcome of the separate investigations by the laboratories of Guillemin (17–19) and Schally, (10) and must be considered a major advance in neuroendocrinology, since it represents the first proven isolation of a hypothalamic releasing factor. Vast numbers of ovine and porcine hypothalami were extracted, and monumental chemical purification work was required before the tripeptide was isolated and its structure established. Immediately thereafter the tripeptide was synthesized and its biological properties were verified to be the same as the naturally occurring material.

The tripeptide is pyro-Glutamyl-Histidyl-Proline amide (pGlu-His-Pro-NH₂). The cyclization of the N-terminal glutamic acid to the pyro-glutamyl structure is essential, as is the amidation of the proline residue of the carboxy terminus, for full activity. Most minor alterations of structure lead to diminished activity, except for methylation of the 3 position of the histidine residue, which increases potency eightfold, probably by enhancing resistance to degradation in plasma. The plasma half-life of the injected tripeptide in human subjects is quite brief, of the order of a few minutes, with apparent half-time components of 3 and 7 minutes. However, amounts of the order of 12-14% of a bolus injection may be recovered in the urine, mainly within the first hour after injection. The material recovered from the urine has shown physiologic potency and immunologic characteristics indistinguishable from the injected tripeptide.

Merely incubating TRH in plasma or serum results in marked degradation, but this does not occur with serum or plasma previously heated at 65°C for 15 minutes (8). These findings suggest degradation by circulating enzymes such as peptidases, with some intact TRH escaping into the urine. These properties are entirely compat-
ible with a releasing factor secreted from the hypothalamus into the hypophyseal portal vessels with immediate action upon arrival in the anterior pituitary, before the occurrence of the appreciable degradation which would occur in the systemic circulation, with the surviving intact TRH excreted by glomerular filtration and appearing in the urine. It must be conceded, however, that some caution should be exercised in drawing conclusions regarding hypothalamic TRH production from measurement of urinary TRH. Even though the concentration of TRH found in the hypothalamus is by far the highest, it has been found widely distributed throughout all areas of the brain, and the extra-hypothalamic areas may account for as much as 80% of the total brain TRH (33). There is even evidence that TRH may have "neurotransmitter" or other nervous system functions quite apart from its presumed role in control of TSH.

The response of normal human subjects to an intravenous injection of 500 μg TRH is a prompt rise in plasma TSH, peaking within 15–30 minutes at a concentration of 16–26 μU/ml in women (slightly lower in men) from a basal mean value of about 6 μU/ml (127, 128). The peak TSH gradually declines to the basal level over the next 150 minutes. The TSH spike usually evokes a definite elevation in serum T3, but infrequently any elevation of serum T4.

This response is markedly exaggerated in myxedematous subjects, with a more pronounced rise above the elevated baseline (56a). Thyrotoxic subjects or normals pretreated with T4 or T3 show abolition of the TSH rise as well as a suppressed baseline (56a). In some studies (113), inhibition by T4 or T3 can be overcome by increased doses of TRH.

Prolactin, as well as TSH, rises on administration of TRH with a similar rapidity, and this response is also suppressed by pretreatment with exogenous thyroid hormones (72a).

The studies of Snyder & Utiger (126), showed that the TSH response to intravenous TRH testing was substantially reduced by administration of small doses of exogenous hormones (15 μg T3 or 60 μg T4 daily for 3–4 weeks), which resulted in no detectable increase in the serum values of the administered hormones. The converse study by Vagenakis and co-workers (144) showed increased sensitivity to TRH after minimal lowering of the plasma T4 by iodide administration. One may interpret these findings to signify the predominating effect of thyroid hormone feedback upon the pituitary, with TRH exerting perhaps a modulating influence, or fine control of the "set-point."

The general picture inferred from all the foregoing is the superimposition of an additional fine control upon the pituitary-thyroid feedback mechanisms, which has been considered to adjust the set-point.

An important illustration of the role of TRH is provided by the disorder called "hypothalamic hypothyroidism" or "tertiary hypothyroidism." It has been found in a number of clinics that hypothyroid subjects without the usual elevation of TSH may indeed have potentially normal pituitary function, as judged by normal or even supranormal TSH rise on administration of TRH. These results led to the inference that the basic defect entailed impaired hypothalamic production of TRH (76, 108).
The mechanism by which TRH stimulates TSH synthesis and release is complex. There is no doubt that TRH is released into the primary capillary tufts of the long portal vessels to arrive at the adenohypophysis. An insight into the control of this mechanism was the observation that, in rats with anterior hypothalamic lesions, much less thyroxine was required to inhibit radioiodine discharge from the thyroid gland than in unlesioned control rats (41a). It would appear that, as the amount of TRH delivered to the pituitary decreases, the sensitivity of the adenohypophysis to feedback inhibition by thyroid hormone is increased. This concept fits with the recent demonstration that chronic exposure of cultured pituitary GH3 cells to TRH leads to a decrease in the number of TRH receptors (69).

The control of TSH release by TRH is also dependent on intracellular cation concentrations. Calcium ions are necessary for the potassium or TRH-stimulated enhancement of TSH release to occur in vitro (146, 147). However, lysis of TSH storage granules isolated from pituitary tissue was unaffected by calcium or magnesium supplementation (9). TRH stimulated adenohypophyseal cAMP (155, 166), even when calcium was removed from the incubation medium. In this latter case, TSH production was inhibited and it is therefore not precisely clear how the effect of TRH, impinging on the cell surface of a thyrotroph, is translated at the membrane into the production of cAMP, which then stimulates the intracellular synthesis of TSH, its accumulation into storage granules, and finally the lysis of the granules and release of the hormone from the cell.

In contrast to the above data on TRH release, it is not yet known which hypothalamic cell type synthesizes TRH. An elegant study of the biosynthesis of TRH in organ culture of guinea pig median eminence tissue showed that this tissue is capable of TRH synthesis before neuronal degeneration occurs, thus implicating neurons as the synthesizing cells (90). Ependymal cells, in the same site, known to be capable of the uptake of substances related to thyroid function and its control, including TRH, are not the cellular site of synthesis of TRH.

Cold exposure of rats increases the activity of TRH synthetase (114). Norepinephrine also increases the synthesis of TRH, as does dopamine, probably through transformation to norepinephrine (56). There is, therefore, monoaminergic control of the synthesis and, possibly, the secretion of TRH, and TRH has been found to be associated with subcellular hypothalamic particles similar in properties to those of synaptosomes containing norepinephrine and dopamine (4). It has been suggested that thyroid hormones act partially at the level of the brain, possibly to modify the secretion of TRH. Experiments by Knigge & Joseph (75, 78) and work reviewed by Reichlin et al (114) suggest a positive rather than negative feedback effect on the hypothalamus. It is clear that static measurements of TRH in neural tissue may mask dynamics of secretion. A more complete account of the factors determining TRH production awaits future work.

PERIPHERAL METABOLIC REGULATION

Previous reviews (131, 132) discussed in detail the role of the three thyroxine transport proteins of human plasma, namely, thyroxine binding alpha-globulin
(TBG), prealbumin (TBPA, or thyroxine binding prealbumin), and albumin. Almost all circulating T₄ and T₃ is protein bound, and only a minute fraction of a percent is dialyzable or ultrafiltrable in vitro, and presumably, freely diffusible across membranes in vivo, thus able to penetrate cells. This unbound moiety is in equilibrium with the bound fraction of each hormone. Although the circulating concentration of T₄ is more than thirty times as great as that of T₃, the latter is much less firmly bound to all 3 protein carriers, and hence has a relatively greater proportion (about ten times as great) in the unbound diffusible state. This is thought to account for the shorter biological half-time of T₃, about one day in normal human subjects, in contrast to the longer half-time of T₄, which approximates one week.

In comparisons of the amounts of replacement therapy required to maintain euthyroidism in athyreotic human subjects, T₃ is approximately three times as potent as T₄. In many studies in other species, such as the rat, T₃ appears approximately five times as potent as T₄.

There is some minor residual controversy regarding the relative importance of the two hormones in normal metabolism, but it is now clear that T₃ has a very significant, indeed a major, role in normal physiology. Since T₄ may undergo peripheral conversion to T₃ (13, 107, 133, 134), some have argued that all action of T₄ may be due to peripheral conversion to T₃. Nevertheless at the present time it would seem injudicious to conclude that T₄ cannot have any action as such without deiodination to T₃, even though this pathway may be quantitatively the most significant.

Of the circulating T₃, probably one third or less is of thyroidal origin, while the majority or at least two thirds, arises by peripheral deiodination, with the liver and kidneys having an important role in this transformation. From the standpoint of the metabolism of circulating thyroxine, it may be estimated that perhaps 33-40% is monodeiodinated to T₃, perhaps 15-20% is changed to tetraiodothyroacetic acid (TA₄ or "Tetrac") or conjugated and lost in urine or bile, and probably about 50% to so-called reverse T₃ (rT₃), 3', 5',3-triiodo-1-thyronine, which differs from "orthodox T₃" in having an iodine lacking from the A ring rather than the B ring. The degradation of rT₃ is probably 3 times as fast as the rapid rate of T₃ turnover, hence the very low serum concentration of rT₃ (24). As developed below, the partition of T₄ metabolism among these pathways has a significant effect upon circulating T₃ levels. One of these metabolic products, T₃, has physiological activity greater than that of its precursor, T₄. In contrast, TA₄ and rT₃ have been shown to have no significant physiological potency.

With awareness of the importance of T₃ in hormone physiology, the question naturally arose whether conditions exist where T₃ formation from T₄ is diminished. At present, the clinical circumstances where this occurs are quite numerous and are likely to increase.

In view of the important role of the liver and kidney in monodeiodination of T₄ to T₃ (134), it was not unexpected that T₃ formation should be impaired in severe hepatic disease (cirrhosis and hepatitis) (26, 102) as well as in renal insufficiency (34), despite maintenance by hemodialysis. Only successful renal homotransplantation has been shown to reverse the diminished T₃ of advanced renal insufficiency (34).
Studies of cadaver tissues (112) have shown diminution of T3 in tissues from patients with chronic illnesses as opposed to those who had died after a brief illness. On the other hand, it has been alleged that any major systemic illness, acute or chronic, may lead to diminished T3 attributable to reduced conversion from T4 (6).

Advanced old age is characterized by lower T3 concentrations than are observed in the first six decades of life (118, 127, 128). Even more striking findings are observed in the neonatal state. In the first day of life, the T3 concentration is negligibly low, but there is a vast excess of rT3 (25). Immediately on clamping the umbilical cord there is a burst of TSH secretion (see above), which results in thyroidal production of T3, which begins to rise within hours after birth.

In Cushing’s syndrome or in high-dose steroid therapy (27), the T3 concentration is low, and that of rT3 elevated; these changes are also noted in a variety of systemic illnesses (154) and the postoperative state (20). Indeed, it is noteworthy that T3 and rT3 have been found to vary reciprocally wherever both have been measured (16). Similarly, total starvation for weight reduction results in prompt fall of serum T3 within 3 days with a reciprocal rise of rT3 (143). The reduction of serum T3 has likewise been observed in the chronic food deprivation of anorexia nervosa (11). Recent work related the changes closely to carbohydrate intake, with T3 falling on low or zero carbohydrate diets, and the reverse changes on restoration of carbohydrates to the diet (29).

A mechanism for the reciprocal relationship between T3 and rT3 concentrations has been suggested by the recent findings of Chopra (23). Using a liver homogenate capable of deiodinating T4 to T3, Chopra observed that this transformation was markedly retarded by the addition of rT3 in vitro. This would suggest that elevation of rT3 may play a causal role in the clinical states in which T3 formation from T4 is reduced.

The foregoing conditions with diminished serum T3 and elevated rT3 may or may not have “hypometabolism” as a concomitant clinical feature. Since the virtual disappearance of so-called a indirect methods, it becomes difficult to evaluate the status of these patients, from the standpoint of their peripheral tissue performance in terms of calorigenesis or other thyroidal parameters other than serum hormone concentrations. Not only the basal metabolism (BMR), but even the Achilles reflex time is little used in the current era of supersophistication. The present reviewers would be hard put to define the optimal serum T3 concentration appropriate for someone afflicted with terminal hepatic insufficiency, carcinomatosis, or anorexia nervosa. The term “T4 euthyroidism” (21) may not be entirely unjustified, regardless of facetious implications that might be drawn from it.

The hormone thyroxine has been known since its crystallization by Edward C. Kendall (77), who found the crystals in his laboratory on Christmas Day of 1916—six decades ago. The advent of T3 and the exciting work in the area of T4 to T3 transformation have perhaps unduly adumbrated T4. Finally, it should be reemphasized that T4 must not be overlooked, and that it can evidently support metabolism in health and disease.
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