THE PHARMACOLOGY OF SUBSTANCES AFFECTING THE THYROID GLAND[1]

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This review is concerned with the pharmacology of substances that affect the structure and function of the thyroid gland. Transport of thyroid hormones and pharmacological agents that affect the peripheral actions have been competently reviewed elsewhere (1–3).

SUBSTANCES WHICH STIMULATE THE THYROID

THYROTROPIN (TSH)

ORIGIN AND CHEMICAL NATURE

The presence in the pituitary gland of a substance that stimulates the thyroid has been known since 1922 (4). The principle has been purified and characterized. Human TSH has a sedimentation coefficient of 2.9 and a calculated molecular weight of 26,000 based on density gradient centrifugation. Disc electrophoresis of highly purified human TSH has shown multiple components, whereas double diffusion in Ouchterlony gels of both human and bovine TSH gives only one precipitin line. The various components seen on gel electrophoresis appear to have the same amino acid composition. Cystine content is high and there are seven or eight disulfide bonds per molecule. Serine, valine, glutamic acid, proline, and lysine are present in relatively large amounts. Threonine and phenylalanine are said to occupy N-terminal positions. TSH is a mucoprotein containing mannose, glucose, galactose, sucrose, glucosamine, and galactosamine. The apparent heterogeneity of TSH has been attributed to minor differences arising in a number of amide groups, or to differences in secondary or in tertiary structure. For bibliographical documentation of these findings, see the 1967 review of Condliffe & Robbins (4).

MOLECULAR COMPONENTS

TSH is synthesized and stored as cytoplasmic granules in basophilic Schiff-positive cells of the anterior pituitary. These granules may be solubilized by desoxycholate to yield a monomer of TSH, which at pH 2.5 aggregates to a dimer (5). The monomer may be digested with papain to yield a

[1] This work supported by USPH Grant No. AM-10092.

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biologically active dialyzable component that has a molecular weight of approximately 7,000. Crude TSH is inactivated slowly by papain. The highly purified hormone is more susceptible to proteolysis (6). Thus, it appears that TSH contains a core which is biologically active and is surrounded by a nonessential papain-digestible peptide. The peptide, however, appears to be unstable in purified preparations.

Three different glycopeptides have been obtained from bovine TSH by tryptic digestion. Amino acid sequences were found to have considerable homology with one of the two subunits of luteinizing hormone (LH). A third peptide did not appear to be related to the third tryptic peptide of LH and was more difficult to purify. Each of the three glycopeptides contained glucosamine, galactosamine, and mannose (7). Countercurrent distribution has been used to purify TSH. No evidence was found for separation into subunits such as occurs with LH (8). Electrophoresis of the reduced and S-carboxyamidomethylated derivatives revealed a polymorphism which did not appear to represent dissociation into subunits.

CONTROL OF SYNTHESIS AND SECRETION

Thyroxine and other small molecules.—The plasma concentration of thyroxine is a major factor in control of anterior pituitary activity. Destruction of the thyroid induces a 15-fold increase in the labeling frequency of pituitary cells with tritiated thymidine within 12 months; this frequency returns to normal within 5–10 days after the start of thyroxine therapy (9). Incorporation of ^{3}H-uridine into RNA is also enhanced by thyroidectomy and depressed by administration of triiodothyronine (10). Changes in the opposite direction occur in the liver. In rat liver, pituitary TSH depleted by treatment with propylthiouracil cannot be repleted unless there is a small amount of thyroid hormone present (11).

The pituitary responds slowly to a fall in thyroid hormone content of the blood. When plasma thyroid hormone was rapidly reduced by exchange transfusions no increase in thyroid hormone secretion was observed for several hours, and it was 5 days before the plasma-free thyroxine level returned to normal (12). These findings contrast sharply with those showing an almost immediate fall in plasma TSH upon administration of thyroid hormone; synthesis of TSH is turned on in response to need much more slowly than it is turned off.

Factors governing TSH secretion have been studied in explants. Secretion of TSH was stimulated by theophylline and by exposure to dibutyryl cyclic 3′,5′-adenosine monophosphate (DB_cAMP). This stimulation was inhibited by thyroxine and enhanced by epinephrine and phentolamine; it was partially blocked by propranolol (13). Synthesis of TSH has been studied in rat pituitary glands in vitro by incubation with ^{14}C-labeled glucosamine and isolation of isotopically labeled TSH by immunoprecipitation. Incorporation was enhanced by addition of propylthiouracil to the diet and was depressed to well below control levels when the rats were given thyroxine. No evi-

dence was found that administration of TSH-releasing factor (TRF, see below) increased the incorporation of labeled glucosamine although TRF promoted release of TSH into the medium. These experiments did not exclude a possible role of endogenous TRF in the increased TSH synthesis in the hypothyroid state (14).

There is a marked functional heterogeneity within the normal pituitary gland with respect to secretion of TSH. Sinusoidal blood collected from various regions of the pituitary during surgery varied in concentration from less than 0.4 to more than 2,600 ng per ml (15).

Normal human pituitary cells in explant culture or in dispersed cell subculture produce immunoreactive TSH which falls rapidly and may be undetectable after 4 months of subculture. Cultures of adenoma tissue maintain TSH secretion (16).

Vasoactive substances.—Vasopressin, oxytocin, and epinephrine release TSH from anterior pituitary tissue in vitro (17) and stimulate ^{14}C-glucose oxidation by explants. Vasopressin does not stimulate the release of hormone from exteriorized sheep thyroid preparations (18) and is not active in vivo in man (19). Phenoxybenzamine blocks the stimulatory effect of epinephrine but not of vasopressin on TSH release. Interestingly, oxidation of glucose is unaffected by all three hormones in concentrations which were maximally effective in releasing TSH (17).

Release of TSH may be mediated by cyclic AMP. The dibutyryl derivative increases release of pituitary TSH in vitro; release is blocked by thyroxine and augmented by theophylline and by epinephrine. The epinephrine effect is partially blocked by propranolol (13).

TSH in the newborn.—The TSH concentration of cord blood at the time of delivery is appreciably higher than in maternal blood taken at the same time (20). The serum concentration of TSH rises rapidly within the first few minutes after birth and falls again by 48 hr. This rapid rise, due partially to a discharge of stored pituitary TSH immediately after birth, is also partially due to cold exposure attendent upon birth and is largely eliminated if the infant is warmed during the first 3 hours of life. Even with warming, however, there is a significant rise in serum TSH which persists through the first 24 to 48 hr of extrauterine life.

Thyrotropin-releasing factor (TRF).—It has long been suspected that the rate of TSH secretion is at least partially under the control of the hypothalamus. A spectacular advance of recent endocrinology has been the identification of the thyrotropin-releasing factor secreted by the hypothalamus. This factor is a tripeptide, pyroglutamyl-histidyl-prolinamide (21–27). It is effective when administered directly into the pituitary (28), when injected intravenously, or when ingested (29). It is resistant to proteolytic activity (29).

Neither puromycin nor cycloheximide, inhibitors of protein synthesis, alter the immediate release of TSH which follows administration of TRF, but both inhibitors prevent triiodothyronine from blocking TSH released by TRF. Thus, it appears that TRF causes a discharge of preformed TSH from the pituitary, and in order for triiodothyronine to block this effect protein synthesis is required (*23*). Actinomycin D also blocks the inhibitory effect of triiodothyronine but does not affect the release of TSH triggered by TRF (*23*). TRF was found to increase both synthesis and release of TSH in rat pituitary tissue in organ culture (30). It has been detected in the peripheral blood of thyroidectomized-hypophysectomized rats stimulated by cold (31).

Plasma TSH rises within 3–6 min in patients injected with porcine TRF and reaches a peak at about 30 min; the level falls to baseline within 2 hr. These findings indicate the short half-life of both TRF and TSH (*22*).

Synthesis of TSH is not stimulated acutely by TRF (14); although release of TSH by the rat pituitary in vitro is stimulated by a crude hypothalamic extract and by partially purified TRF, incorporation of labeled glucosamine into TSH was unchanged. Pituitary glands removed from hypothyroid rats show increased incorporation of labeled glucosamine into TSH, and, conversely, incorporation is reduced in the rat pretreated with thyroxine.

The precise physiological role of TRF remains to be determined, and little information is presently available regarding the control of the secretion rate of the releasing factor. Thyroxine in small doses inhibits the pituitary response to TRF (*32*). Although glucocorticoids depress secretion of TSH they do not impair TRF-mediated secretion of TSH. These findings suggest that the glucocorticoid control of TSH secretion is at a suprahypophyseal level (33). One may surmise that both higher neural centers and plasma thyroid hormone control release of TSH. It seems clear that releasing factor both stimulates discharge of preformed TSH from the pituitary and, after a latent period, induces a burst of synthesis of TSH.

THE EFFECTS OF TSH ON THE THYROID GLAND

Protein synthesis.—TSH exerts a multitude of effects on the thyroid, as exhaustively reviewed by Dumont, Neve & Otten (34). Some effects are immediate and some are delayed. There is an almost immediate increase in glucose metabolism, but the effects on protein synthesis and cell division appear only many hours later. TSH increases the ability of suspended bovine thyroid cells to incorporate amino acids into protein, even after preincubation with actinomycin D. Thus, this action of TSH does not depend primarily on increased synthesis of template RNA. Since impairment of glucose metabolism abolishes protein synthesis resulting from TSH stimulation, it may be that the increase in protein synthesis depends on TSH stimulation of glucose metabolism (35). Administration of actinomycin D, cyclo-

heximide, or puromycin inhibits TSH release of thyroid radioiodine, but a time lapse of 8 or more hours is required for this to become apparent. Thus, new protein synthesis is not required for the acute effects of TSH, but evidently is required for the late effects (36). Actinomycin D increases thyroid iodide trapping, presumably because the rate of iodide efflux from the thyroid is lowered (37).

Nucleotide synthesis.—TSH stimulates RNA synthesis, as does the long-acting thyroid stimulator (LATS—see below) (38). This includes synthesis of both ribosomal and messenger RNA (39). TSH also stimulates RNA synthesis in cultures of rat fetal thyroids (40) and incorporation of orotic acid into pyrimidine nucleotides of bovine thyroid slices (41). TSH causes no noteworthy change in total pyridine nucleotides but increases the oxidation of NADPH and NADH (42).

TSH also accelerates incorporation of ^{3}H-thymidine into thyroid cells in culture, an effect impaired by propylthiouracil. Propythiouracil and TSH together accelerate the loss of ^{3}H-thymidine from cultured thyroid cells (43). In the chick embryo at about the 11th day, when endogenous TSH secretion begins, there is a sharp drop in labeling of the nuclei with tritiated thymidine. This was interpreted to mean that TSH is a stimulus under which the embryonic thyroid gland switches over to gland function from rapid autonomous cell reproduction (44).

Lipid metabolism.—TSH increases the incorporation of glucose, glycerol, palmatate, and oleate into phosphatidylinositol, and increases the proportion of these compounds incorporated into 1,2-diglycerides. TSH also increases the incorporation of palmatate into phosphatidylinositol and 1,2-diglycerides, but not its incorporation into phosphatidylcholine, phosphatidylethanolamine, or 1,3-diglycerides (45). Kerkof & Tata (46) found that TSH caused a coordinated increase in thyroid phospholipid, RNA, and iodoprotein associated with the endoplasmic reticulum. TSH increases the incorporation of ^{32}P into phospholipid at a step beyond ATP (38, 39, 47).

Lysosomal stability.—The lysosomal stabilizing substance, chlorpromazine, inhibits TSH stimulation of colloid droplet formation in the thyroid and also blocks TSH-induced stimulation of 1-^{14}C-glucose to $^{14}CO_2$. These results suggest that TSH may exert an effect directly or indirectly on lysosomal stability, and indicate a link between colloid droplet formation induced by TSH and oxidation of glucose (48). Stimulation of endocytosis by TSH is inhibited by actinomycin (49).

Thyroid hormones.—As part of the response to trophic hormone stimulation, the thyroid increases the ratio of triiodothyronine to thyroxine, but this effect is dependent also on the amount of iodine in the thyroid and is

more related to iodine than to TSH; TSH seems to have a permissive role (50, 51). In the absence of TSH, a thyroglobulin is formed which has a slightly lower sedimentation constant than normal and which, in solutions of low ionic strength or high pH, is unusually sensitive to degradation into half molecules. This thyroglobulin is low in iodine (52, 53).

Blood flow.—TSH enhances blood flow in the thyroid within minutes after injection into intact rats. The effect is accompanied by a fall in serotonin content of the gland (54). Within 5 min after TSH administration, serotonin starts to be mobilized from storage sites in the perivascular mast cells; it is not mobilized from mast cells of extrathyroidal tissues (55). One may presume that mobilization of this vasoactive substance is related to the vascular response. The number of mast cells in the thyroid is increased by TSH (56).

The thyroid cell surface and TSH.—It has been established that TSH interacts with the thyroid cell membrane and can stimulate metabolic activity in the thyroid cell without actually entering the cell itself. Presumably there are specific binding sites on the plasma membrane of the parenchymal cells, and specificity may reside in this interaction. Tissue binding of TSH is affected directly by thyroxine; possibly in this way thyroxine directly regulates the biological activity of TSH (57). Treatment of thyroid slices with lecithinase blocks the effect of TSH on glucose oxidation and phospholipid synthesis, but does not block the effect of dibutyryl cyclic AMP (DB_cAMP) on glucose oxidation or phospholipid synthesis. Sphingomyelinase had no such effect (58, 59). Exposure of thyroid cells to phospholipase C abolishes the response of the cell to TSH and LATS, but not to acetylcholine, nor is basal phospholipogenesis impaired by phospholipase C (58, 59).

Although Na^+ is required for TSH action, Ca^{++} and Mg^{++} ions are not essential. Ouabain in 10^{-4} *M* concentration abolished the effect of both LATS and TSH on phospholipogenesis (58). Absence of Ca^{++} was found to impair thyroid response in glucose oxidation and phospholipid synthesis to submaximal doses of TSH and DB_cAMP (60).

TSH does not affect the thyroid of the patient with a high plasma titer of LATS (61). It has also been shown that administration of TSH to mice impairs subsequent response to LATS and vice versa. Competitive binding experiments with rat thyroid microsomes show that binding occurs consistently at the same site (62). While these findings suggest competitive interaction between LATS and TSH at the thyroid cell surface, alternate possibilities are not excluded (62).

Other effects.—The growth response of the thyroid to TSH is blocked by dinitrophenol but glandular iodine metabolism is not blocked (63). TSH increases the thyroid level of tyrosine and its concentration relative to tis-

sue RNA, and this increase is enhanced by administration of propylthiouracil (64). TSH also increases the uptake of ^{35}S sulfate by the harderian gland of the mouse. [Interestingly, high titer human serum containing LATS has no such effect on the mouse (65)].

CYCLIC AMP AND OTHER SMALL MOLECULES

Mediation of TSH through cyclic AMP.—Much literature has accumulated concerning the role of cyclic 3', 5'-AMP as the mediator of the effects of TSH and LATS. Metabolism of 1-^{14}C-glucose is increased by cyclic AMP within 15 min. Theophylline, which impairs diesterase degradation of cyclic AMP, also stimulates glucose metabolism and potentiates cyclic AMP. Cyclic AMP can increase glucose oxidation in the presence of theophylline in a concentration as low as 8×10^{-4} *M* (66).

Cyclic AMP and DB_cAMP increase the release of mouse thyroid hormone. The effect is augmented by simultaneous administration of theophylline. Release is also effected in vivo (but not in vitro) by 5'-AMP, 5'-ADP, and 5'-ATP (67).

Cyclic AMP increases the release of ^{131}I bound during life and also increases ^{131}I labeling of thyronines in mouse thyroid glands in culture medium; these effects are enhanced by theophylline (68). Infusion of DB_cAMP into dogs stimulated secretion of hormonal ^{131}I from the thyroid within 20 to 30 min. It also increased the release of inorganic iodide from the thyroid. Secretion was tenfold greater than during the control period (69). Administration of DB_cAMP stimulates formation of colloid droplets in the thyroid parenchymal cells of the dog but not of the rat (70). DB_cAMP stimulates the release of preformed triiodothyronine and thyroxine from the thyroid in vitro, similarly to TSH (71). Proteolysis of thyroglobulin is stimulated by DB_cAMP and prostaglandin E_1, as well as by TSH; a common pathway of action appears to be involved, since the stimulating effects at maximally effective concentrations are not additive (72).

TSH and dibutyryl cyclic AMP have essentially identical stimulatory effects on iodine incorporation into the thyroglobulin of suspended bovine thyroid cells. Incorporation of labeled leucine into protein was also stimulated (73). Theophylline also enhanced the effects of both TSH and LATS on ^{131}I metabolism (74). The cyclic AMP concentration in the thyroid is increased within 1 min by TSH and reaches a maximum within 3–6 min (75). Adenyl cyclase activity is also increased within 1 min (76). The increase in cyclic AMP concentration parallels the concentration of TSH over a wide range. Prostaglandin E_1 increases cyclic AMP concentration in the thyroid and also increases glucose oxidation.

Lissitzky et al. (77) found that cyclic AMP increases protein synthesis in a cell-free system of thyroid polyribosomes. The system was not stimulated by TSH, a finding consistent with the presumed mediation of the TSH effect through activation of the cyclase of the plasma membrane.

The effects of TSH on glucose oxidation and on colloid droplet formation are mimicked by low concentrations of prostaglandin E_1 and are additive at submaximally stimulating doses. The actions of prostaglandin E_1 are inhibited by the lysosomal stabilizer, chlorpromazine (78).

Ahn & Rosenberg (79) observed that organic binding of iodine and formation of thyroxine were stimulated by TSH, DB_cAMP, fluoride, and prostaglandin E_1. TSH and prostaglandin E_1, but not fluoride, enhanced synthesis of cyclic AMP from adenine in canine thyroid slices. These results suggested that stimulation of organic binding of iodine by TSH and prostaglandin E_1 is mediated through increased formation of cyclic AMP, but that fluoride acts through a different mechanism. Sodium fluoride, which stimulates adenyl cyclase activity in thyroid homogenates, failed to increase the concentration of cyclic AMP in dog thyroid slices. Two other substances, carbamylcholine and menadiol sodium diphosphate, increase glucose oxidation in thyroid slices but do not change the concentration of cyclic AMP (76). The increase in cyclase activity is transient, but increased glucose metabolism persists for as long as 1 hr. As little as 1 mu of TSH is stimulatory. TSH does not stimulate adenyl cyclase activity in other glandular structures, and it does not modify phosphodiesterase activity. Prostaglandin E_2 has the same effect as TSH on glucose oxidation and cyclic AMP concentration, whereas prostaglandin F_{1_A} stimulates glucose oxidation but does not increase cyclic AMP levels. Prostaglandin B_1 does not modify either cyclic AMP concentrations or glucose oxidation (80). These findings are consistent with the concept that the effects of TSH are channeled through cyclic AMP, but they also indicate that fluoride and other substances may influence thyroid gland metabolism by different mechanisms (76).

Alternative routes of the TSH effect.—While there is abundant evidence that many of the responses of the intact thyroid to TSH, and perhaps also to LATS, are mediated through the adenyl cyclase system and cyclic AMP, the possibility cannot be excluded that TSH has effects channeled through other pathways. This possibility has been carefully considered by Field and his colleagues (81). They advise caution, as does Burke (82), in interpretation of indirect pharmacological evidence that may be influenced by dosage effects and the nonspecificity and ambiguity sometimes associated with use of many agents, such as α- and β-adrenergic blocking drugs. Studies with α- and β-adrenergic blocking agents and catecholamines indicate that TSH stimulation of adenyl cyclase does not involve an adrenergic receptor mechanism (83). Wolff, Berens & Jones (84) found that low concentrations of lithium ion inhibited the TSH stimulation of adenyl cyclase activity in a preparation of beef thyroid. Lithium inhibits several aspects of thyroid metabolism (85). It is possible that inhibition results from an effect on adenyl cyclase.

TSH and fluoride both activate the adenyl cyclase system in thyroid

slices, and cyclase activity can be increased by either in the presence of an abundance of the other, indicating that the compounds act at different sites. Adrenergic blocking drugs inhibit TSH effects on cyclase without blocking fluoride-induced enzyme activation. Thus, the adenyl cyclase system in the thyroid responds to TSH and to fluoride, but the two stimulators may act at different sites to evoke their effects (86).

Burke (87) has found that there are low concentrations of TSH which greatly increase phospholipogenesis but have no effect on adenyl cyclase activity, glucose metabolism, or colloid droplet formation. On the other hand, prostaglandin E_1 stimulates adenyl cyclase, endocytosis, and glucose oxidation without affecting phospholipogenesis. These findings suggest that cyclase activation and stimulation of endocytosis may result from stimulation of glucose oxidation and that TSH stimulation of phospholipogenesis may be mediated otherwise.

There are other observations which are difficult to explain with the cyclic AMP mediation hypothesis. For example, prostaglandin E_1 increases the intracellular levels of cyclic AMP and stimulates glucose oxidation and iodide binding to protein. However, neither fluoride, which activates adenyl cyclase in cell-free systems, nor prostaglandin E_1, which in some tissues increases intracellular cyclic AMP, has any effect on intracellular colloid droplet formation, an effect long associated with early TSH action (88). On the other hand, as Burke (87) has noted, prostaglandin E_1 affects endocytosis. Also, Dumont and his colleagues (89) observed iodide organification, endocytosis, and glucose metabolism in dog thyroid slices in the presence of TSH or cyclic AMP; the effect was increased by caffeine. Kerkof & Tata (46) found that the rapid effects of TSH on transport and metabolic functions were mimicked by DB_cAMP but that the latter failed to influence the slower biosynthetic responses.

Gilman & Rall's studies with bovine thyroid slices present evidence that TSH and cyclic AMP have somewhat different effects on thyroid function (90). It appeared that cyclic AMP could not mediate TSH-stimulated oxidation of 1-^{14}C-glucose unless the concentrations of TSH were low.

Burke (91) has compared the effects of TSH and LATS upon 1-^{14}C-glucose metabolism and upon the ratios of oxidized to reduced pyridine nucleotides. No differences were apparent in the effects of TSH and LATS in these respects, and there was no correlation between the changes in the ratios of pyridine nucleotides on the one hand and stimulation of glucose oxidation and phospholipogenesis on the other, but cyclic AMP regularly stimulated glucose oxidation and phospholipogenesis, decreasing the ratios of oxidized to reduced pyridine nucleotides. Thus, there appeared to be a difference between the effects of the peptide stimulators and the stimulation derived from cyclic AMP.

Burke has also questioned the role of cyclic AMP in TSH-mediated effects on the thyroid (82, 92). He observed no potentiation by cyclic AMP of TSH or of LATS effects upon either glucose oxidation or phospholipo-

genesis; he also found no interaction between theophylline and submaximal doses of TSH or of LATS upon either glucose oxidation or ^{32}P incorporation. He contended that the effect of TSH and LATS on thyroid intermediary metabolism is not mediated completely by cyclic AMP.

TSH and autonomic drugs.—Activation of adenyl cyclase by TSH is impaired by three adrenergic blocking agents, DL-propranolol, D-propranolol, and phentolamine in concentrations approximately 100 times larger than those required to produce α- and β-blockade. DL-propranolol and phentolamine impaired TSH stimulation of glucose oxidation in dog thyroid slices, but at the same concentration the drugs did not impair stimulation induced by DB_cAMP. Glucose oxidation was stimulated in bovine thyroids by TSH at a concentration of propranolol which abolished stimulation by adenyl cyclase (93).

Onaya & Solomon (78) found that chlorpromazine, a drug which stabilizes lysosomes, and propranolol, which blocks adenyl cyclase activity, block the effects of TSH and LATS on colloid droplet formation. These drugs also block stimulation of glucose oxidation by TSH and LATS. Contrary to Levey, Roth & Pastan (93), they found that propranolol also inhibits the effect of DB_cAMP. This indicated that propranolol blocks beyond its effect on adenyl cyclase. Both drugs appear to stabilize the lysosomal membranes.

SCHEME OF ACTION

Onaya, Solomon & Davidson (94) have outlined the following scheme: TSH activates adenyl cyclase in the plasma membrane; the resulting cyclic AMP travels to the luminal side of the thyroid cell and activates the apical cell membrane to undertake endocytosis of the colloid; lysosomes labelized by cyclic AMP fuse with the colloid droplets, whereupon the thyroglobulin undergoes hydrolysis and releases thyroid hormones for distribution into the blood.

ROLE OF TSH IN DISEASE STATES

Two excellent TSH assay methods, the McKenzie mouse bioassay and radioimmunoassay, have provided much information about changes under varied physiological conditions and in disease states (95). Lemarchand-Beraud & Vannotti (96) found a reciprocal circadian rhythm in free plasma thyroxine and plasma TSH concentrations, although TSH variations did not reach statistically significant levels. TSH increased during pregnancy. Hennen & Freychet (97) have also found elevated thyroid stimulating activity in the plasma during pregnancy, and suggest that it may be of chorionic origin.

There is a concensus now that TSH is low or undetectable in thyrotoxicosis (98–100), although plasma values may be somewhat above normal in nontoxic nodular goiter and diffuse enlargement of the thyroid. Values are high in thyrotoxic patients treated with antithyroid drugs when the plasma

PBI falls below 2 μg/100 ml (101). TSH is not found in the blood of patients with pituitary hypothyroidism, but it may reach 15 times normal in myxedema. Elevations also may be found in thyroid carcinoma, and thyroiditis (99, 102, 103).

Patients with endemic goiter may have normal or elevated TSH values. High levels have been recorded in members of three generations in a hyperendemic region: The grandparents had nodular goiters, and the parent and child were both cretins (104). Levels approximately 100 times normal were found in the sera of apparently cretinous persons in highland New Guinea (105). Gaitan et al (106) obtained indirect evidence that their patients with endemic goiter were not under maximal TSH stimulation. High values in an endemic region fell following administration of iodized oil as a prophylaxis (107).

TSH level is normal in Down's syndrome (108). Using the McKenzie assay, El Kabir et al (109) found plasma concentrations of TSH significantly elevated in paranoid schizophrenic patients and in a substantial fraction of those with affective disorders. It is not elevated by convulsive electroshock (110).

The Long-Acting Thyroid Stimulator (LATS)

This substance, first detected in the plasma of patients with Graves' disease, characteristically reaches its maximal stimulating effect at approximately 8 hr, whereas TSH reaches its peak effect within 1 or 2 hr. It is found only in the plasma of patients who have or who have had Graves' disease and it is by no means detectable in all of these. It reaches exceedingly high titer in some patients, more commonly those with pretibial myxedema but pretibial myxedema can occur without a high titer. LATS is a 7S gamma globulin of the I_gG class (111). It is secreted into the suspending fluid of phytohemaglutinin-stimulated cultured lymphocytes from patients with Graves' disease (112, 113).

TSH and LATS

TSH can be extracted from serum both by ethanol-salt percolation and by fractional precipitation with acetone, whereas LATS is not extracted by these procedures (114). TSH is more resistant than LATS to heat inactivation, and has a much shorter biological half-life when injected [in rats, 10–20 min for TSH, and 7.5 hr for LATS (115)]. Furthermore, the two stimulators have different sedimentation coefficients; TSH is recovered with the 4S fraction, LATS with the 7S fraction. Finally, TSH activity is inhibited by incubation with anti-TSH antiserum but LATS activity is not (116).

Extensive studies have failed to establish any essential difference between the effects of TSH and of LATS on the thyroid gland. Injection of LATS over several days causes increased weight of the gland, increased protein content, increased incorporation of tritiated leucine, and increased

specific activity of protein-bound iodine. LATS in chronic administration does not appear to damage the thyroid cell in any way (117). LATS stimulates release of thyroid radioiodine. The release is blocked by actinomycin D, cycloheximide, and puromycin; eight or more hours are required for this effect (36). The effect of LATS on ^{131}I uptake by the thyroid is enhanced by theophylline (74). TSH and LATS have parallel effects on glucose oxidation and phospholipogenesis, but these changes do not correlate with changes of pyridine nucleotide ratios; nor do the effects of the two peptide stimulators correlate (82) with the changes induced by cyclic AMP on pyridine nucleotide levels. LATS and TSH also cause a parallel and comparable rise in RNA synthesis and phospholipid formation (38). There is evidence that LATS interacts with the thyroid cell more slowly than does TSH (118). Studies suggest, however, that LATS and TSH resemble one another qualitatively in their effects on glucose oxidation and endocytosis, and that the action of LATS is as rapid as, or perhaps slightly more rapid than, that of TSH (119).

LATS AS IMMUNOGLOBULIN

It is well established that LATS is an immunoglobulin. Kriss (120) has found that in several different sera containing LATS, the activity was inhibited partially by anti-kappa and partially by anti-lambda sera and that complete inactivation was achieved when both antisera were employed. This finding indicates that LATS is not derived from a monoclonal line of cells. Chromatography of sera containing LATS on DEAE Sephadex has resolved LATS into two zones in the I_gG globulin region (121). LATS is partially purified by adsorption to and elution from thyroid microsomes, and has a high degree of affinity and specificity for the microsomes. These studies show that LATS is a rather small part of the total I_gG population (122).

LATS activity is neutralized or absorbed by whole homogenates of thyroid. Most of this absorbing capacity appears to be in the 4S fraction, which is a soluble fraction that contains thyroglobulin, hemoglobin, and other serum proteins (123). Beall et al (124) have found also that LATS is inhibited by incubation with both particulate and soluble fractions derived from thyroid. They found that the inhibitor was widely distributed along the sucrose gradient of the soluble fraction of the homogenate. Phospholipase C abolishes the response of thyroid slices to both TSH and to LATS but not to acetylcholine. This observation indicates that phospholipid must be present on the cell membranes if there is to be a response to the peptide hormones. Omission of Na^+ from the buffer as well as the presence of ouabain reduced the response to both peptide stimulators. It is worth noting that although these two peptide hormones have strikingly different physical, chemical, and immunological properties, they both appear to mediate stimulatory action on the thyroid cell by interacting with a component or compo-

nents of the thyroid cell membrane (58). It is not known which thyroid protein acts as antigen. Beall et al (125) immunized baboons with the microsomal fraction from human thyroids. They developed a rise in plasma TSH-like activity, whereas rabbits similarly immunized developed a LATS-like thyroid-stimulating substance. Injury to the thyroid, as with radioiodine therapy, fails to induce a rise in LATS titer, as might be expected if a specific antigen were leaked into the blood (126).

In three laboratories immunization of rabbits with either thyroid homogenates or thyroid microsomes was followed by the appearance of thyroid-stimulating activity in the serum. No clinical sign of thyroid hyperfunction was detected (127–129), but McKenzie (129) found that the thyroids were not suppressible with triiodothyronine; presumably the stimulator was not TSH. More recently Solomon & Beall (130) have reported their success in inducing a thyroid-stimulating substance in rabbits by repeated immunization with human thyroid microsomes. This substance appears to be of extra hypophyseal origin, is an immunoglobulin of the I_gG class, gives an assay response between those of TSH and LATS, is partially neutralized by anti-bovine TSH, and is not suppressed when plasma thyroxine concentration is above normal. The component was not TSH-bound to plasma globulin. Weight loss suggested that the rabbits might have been thyrotoxic.

LATS AND GRAVES' DISEASE

The role of LATS in Graves' disease is far from clear. A close relationship, but not necessarily a causal one, is suggested by the high percentage of patients with circulating LATS (when concentrated gamma globulins are used for the assay) (131), the correlation between the level of circulating LATS and the ^{131}I turnover rate (132), and the transient presence of circulating LATS in neonatal thyrotoxicosis (a transient disease occurring in children of thyrotoxic mothers) (133, 134).

The role of LATS in the origin of Graves' ophthalmopathy is even less clear. Kriss and associates (135) reported three patients in whom ophthalmopathy followed the appearance of circulating LATS, but this sequence was not found by Burke in his group of patients (136). Furthermore, there is frequently no relationship between symptoms of thyroid activity and ocular involvement.

OTHER PROTEINS WITH THYROID-STIMULATING ACTIVITY

CHORIONIC THYROTROPINS

A thyroid-stimulating substance derived from the placenta and called chorionic thyroid-stimulating factor was first detected by Hennen in 1965 (137). It is somewhat less acidic than is normal pituitary TSH. It has been brought to 3,500 times its original concentration (138). The amount of this factor present in the placenta is approximately the same as the amount of TSH present in the pituitary. Chromatographic evidence suggests that it

has approximately the same molecular weight as normal human TSH. The time course of its effect on the thyroid is similar to that of normal human pituitary TSH. Its immunological cross-reaction is poor with human pituitary TSH but strong with bovine and porcine TSH (138, 139). It is much more electronegative than bovine TSH. Thus, although TSH and chorionic thyrotropin share major immunologic determinants, there are significant molecular differences (Hennen, to be published).

A TSH-like component is detectable in the blood of pregnant women, but it is uncertain whether its origin is pituitary or placental (97). Its appearance may result in the thyroid hypertrophy and hyperfunction of pregnancy (138). The relationship of this factor to a thyroid-stimulating substance found on bioassay of plasma from patients with trophoblastic tumors remains to be determined (139). Seemingly normal human TSH has been obtained from a bronchial carcinoma (140).

A second placental factor with activity similar to, but weaker than, TSH was eluted by carboxymethyl cellulose chromatography of a placental extract (139). Antiserum to human chorionic gonadotropin (HCG) cross-reacts with human TSH. This may be due to sharing of common antigenic determinates or to contamination of the HCG used in preparing the antiserum with TSH of pituitary or placental origin (141).

A thyroid-stimulating substance with unusual properties has been detected in the plasma of a male subject with choriocarcinoma. This factor had the time course of LATS but when diluted twofold the time course of response was similar to that of TSH (142). The substance produced no increased activity in the patient's thyroid.

A variety of other substances have LATS-like activity. Among these are α- and β-MSH, arginine and lysine vasopressins, serotonin, ACTH, kallidin, TSH coupled with basic peptides, and a complex of TSH and specific antibody.

TSH has been isolated from a transplantable pituitary tumor of mice (143). The TSH had a sedimentation constant of 2.9 to 3.0 and a molecular weight of 28,500, as determined by density gradient centrifugation. The amino acid composition was similar to that of bovine TSH, but galactose was the principal hexose, whereas mannose is the predominant hexose in bovine TSH. The murine preparation contained only three residues of glucosamine per mole, as opposed to eight in bovine TSH; there was no galactosamine in the murine TSH, in contrast to three residues per mole in bovine TSH.

OTHER PITUITARY THYROTROPINS

There is a single report of a thyroid-stimulating substance obtained from the pituitary of a patient with Graves' disease. This factor was only partially neutralized by anti-TSH serum under conditions which completely

precipitated normal TSH. The factor had a dose response curve similar to that of normal TSH (144).

BACTERIAL PROTEINS

A thyroid-stimulating substance has been detected in the growth medium of cultures of *Clostridium perfringens* (145). The substance has been purified about 300 times by ammonium sulfate fractionation, filtration through Sephadex G-100, and chromatography on DEAE cellulose. The stimulating substance has been separated into two factors, one of which was identified as a phospholipase relatively specific for sphingomyelin. Sphingomyelinase, when incubated with thyroid slices, increased glucose oxidation and phospholipid synthesis, but when injected it failed to increase the formation of colloid droplets or to deplete the gland of radioiodine.

During incubation with sphingomyelinase 65–90% of the sphingomyelin was lost from the slices (146, 147). The action of sphingomyelinase on the thyroid may not be specific and its usefulness in clarifying the mechanism of the thyroid stimulation is therefore limited.

LOW-MOLECULAR-WEIGHT SUBSTANCES WHICH STIMULATE THE THYROID

Acetylcholine, epinephrine, serotonin, menadione, and prostaglandin.— Some of the effects of epinephrine and prostaglandin E_1 on the thyroid have been described already. Acetylcholine at low concentration stimulates glucose uptake, glucose oxidation, and ^{32}P incorporation into phospholipids in thyroid slices, but the mechanism of action is different from that of TSH since 1-^{14}C-glucose and 6-^{14}C-glucose are oxidized at the same rate, whereas 1-^{14}C-glucose is preferentially oxidized with TSH. Furthermore, atropine and iodine, which do not effectively prevent TSH action, completely or partially abolish stimulation with acetylcholine (148).

Epinephrine (149), serotonin (150), menadione (151), and prostaglandin E_1 (80) all stimulate thyroid glucose oxidation but not phospholipid metabolism (152). The mechanism of action of epinephrine (149) has been carefully studied. Like norepinephrine, it stimulates the oxidation of 1-^{14}C-glucose more than that of 6-^{14}C-glucose. This finding is consistent with activation of the hexose monophosphate pathway, and is confirmed by the fact that epinephrine stimulates oxidation of NADPH to NADP, which is the limiting factor in the oxidation of glucose through this pathway. Acetylcholine and menadione, like TSH, increase the total content of NADP. TSH stimulates new synthesis of NADP from NAD, thereby explaining its effect on the total content of triphosphopyridine nucleotides. Epinephrine and serotonin, on the other hand, increase the NADP content of the gland as a result of increased oxidation of NADPH (153). The effect of phospholipid metabolism appears to be independent from both glucose oxidation and NADP concentration.

Acetylcholine does not activate adenyl cyclase activity in beef thyroid

homogenates (154). Menadione, which increases glucose oxidation in dog thyroid slices, does not affect cyclic AMP (976). Prostaglandin E_1, effective in stimulating both glucose oxidation and cyclic AMP content, does not increase phospholipid metabolism (76). It is not easy to disentangle this melange of effects.

Methylxanthines.—Wolff & Varrone (155) recently reported the goitrogenic effect of methylxanthines. Rats chronically fed a low iodine diet containing 10 μ moles of methylxanthine per gram of diet developed goiters over a period of 3 weeks. Theophylline was more effective than caffeine, which in turn proved more effective than theobromine. Serum PBI was low in the rats treated with theophylline but was normal in the rats which had received caffeine, although the latter showed more than 50% increase in thyroid weight. Methylxanthines were ineffective in hypophysectomized animals. When tested for in vitro antithyroid activity, theophylline was found to cause a 20% reduction of iodine uptake but no change in TCA precipitable fraction; these changes occurred only at a high concentration (1×10^{-3} M), however. Sera from theophylline-treated rats did not affect thyroid activity in vitro. Finally, there is a synergism between theophylline and propylthiouracil at low doses.

Cysteine, as well as cystamine, increases glucose oxidation of thyroid slices (156). The pattern is typical of TSH, since the rate of oxidation of 1-^{14}C-glucose was higher than that of 6-^{14}C-glucose. Tyrosine and arginine, but not other amino acids, are also somewhat stimulatory. Cysteine also modifies iodine metabolism of the thyroid in vitro but not in vivo. When incubated with thyroid slices at a concentration of 10^{-3} to 10^{-2} M, cysteine reduced the incorporation of radioiodine into protein and the synthesis of monoiodotyrosine and diiodotyrosine (157).

Cystamine, which was as effective as cysteine in stimulating glucose oxidation (158), acutely increased the ability of the blocked thyroid to accumulate iodide ion in vivo (158). In in vitro experiments (158) no accumulation of iodide ion could be demonstrated, but the ability of cystamine to prevent organification of iodide was quite evident. Antithyroid activity could also be demonstrated in vivo. Iodide accumulation occurs faster than with TSH and faster than the increase in glucose oxidation, suggesting that glucose oxidation and iodine metabolism are independent results. This conclusion is confirmed by the results of the experiments with cysteine, which were effective in stimulating glucose oxidation (156) while inhibiting iodine metabolism (157).

SUBSTANCES WHICH INHIBIT THE THYROID

Inorganic Ions

IODIDE

It has been known for more than 20 years that large doses of iodide inhibit thyroid hormone synthesis in the rat (159). This phenomenon, called

the Wolff-Chaikoff effect, has been demonstrated also in man. If administration of iodide is continued for more than a few days, the inhibition of synthesis disappears, coincidentally with a fall in iodide transport into the thyroid. Thus, organification of iodine apparently results in production of a factor that inhibits iodide transport. Thyroid iodide concentration then falls below a critical level, and organification of iodine recommences (160). It is interesting that oftentimes myxedema is rapidly and easily induced by administration of therapeutic amounts of iodide to patients previously successfully treated with radioiodine or, less frequently, after subtotal thyroidectomy (161). Experiments employing iodide which were designed to explore the qualitative relationships between thyroid iodide space and acute depression of iodide transport have indicated that the depression is clearly related to organification of some of the iodide, but the response is not directly dependent upon the quantity of iodine synthesized (162).

Little is known about the state of inorganic iodide in the cell. The findings described above indicate the importance of this component of the total intracellular iodide. The intracellular carrier for iodide may be a phospholipid. It has been suggested that two molecules of lecithin and one metal ion serve as an iodide carrying complex with strong affinity for iodide (163); Posner & Ordoñez (164) have found small amounts of iodine covalently linked to several different lipids in the thyroid.

Iodide has several interesting effects on the intermediary metabolism of the thyroid. In thyroid slices it inhibits TSH-induced stimulation of glucose oxidation and phospholipogenesis but it either has no effect or augments glucose oxidation and phosphate incorporation induced by LATS (165). Relatively large doses of iodide cause an immediate fall in the ATP and pyridine nucleotide level in rat thyroid but not in rat liver. These effects are blocked by administration of perchlorate but not by methimazole, and accordingly are presumably related to iodide transport and not to organification of the iodide (166).

A double isotope technique has been used to demonstrate that iodide acts on the toxic thyroid gland primarily by inhibiting degradation of thyroglobulin (167). When iodine is in short supply, monoiodotyrosine increases relative to diiodotyrosine, and iodotyrosine residues increase relative to thyroxine and triiodothyronine. There also tends to be more triiodothyronine relative to thyroxine. Triiodothyronine formation correlates with circulating TSH (168). Mayberry & Hockert have throughly studied the kinetics of iodination of thyroglobulin in the formation of iodinated amino acid residues (169). The amount of iodine in the gland determines the thyroglobulin molecule's conformation and its stability to freezing in the presence of methyl mercaptoimidazole. Thyroglobulin prepared from the iodine-depleted thyroid has a somewhat lower sedimentation constant and is readily dissociable when frozen into 11-12S subunits, whereas glands from iodine-sufficient animals show a downward shift in the sedimentation constant but no comparable dissociation (170). Other investigators have found that the

quantity of iodine makes little difference in the physical properties of thyroglobulin (171). There is evidence that the effectiveness of TSH depends upon the iodine content of the gland; iodine-depleted thyroids are much more sensitive to TSH (172). Kinetic data have indicated that under circumstances of widely varying iodine intake the quantity of thyroxine being turned over remains constant. Thus, thyroid homeostasis seems related to the amount of thyroid hormone being secreted (173).

In an exhaustive review, Wolff (174) has examined the physiological and pharmacological effects of iodide on the thyroid with particular attention to the production of iodide goiter and the mechanism of the Wolff-Chaikoff effect.

Iodized oil in iodine deficiency.—There continues to be interest in the widespread problem of endemic goiter. Most investigators agree that this disease primarily results from iodide deficiency (175), although scattered reports suggest that genetic or additional dietary factors may be responsible or may contribute to the diseases (176–179).

Considerable success has attended the use of injected iodized poppyseed oil for the prevention and treatment of endemic goiter in regions where iodized salt prophylaxis is not practicable (107, 180–182). These programs have generally been accompanied by a sharp reduction in the prevalance and degree of goiter. This form of prophylaxis has produced little, if any, effect on linear growth or skeletal maturation in children (180), but there is mounting evidence that cretinism may be prevented by this measure (183, 184). A few patients with large nodular goiters who were injected with iodized oil have developed thyrotoxicosis; no other untoward effects have been recorded (180).

The efficacy of adding iodide or iodate to table salt has been recognized for so long that reports are no longer appearing with any regularity. The most recent is that of Perinetti from western Argentina (185) where excellent results in goiter prevention have been achieved.

THE EFFECT OF OTHER IONS ON THYROID IODIDE TRANSPORT

A number of anions can displace iodine from the iodine space of the thyroid gland. Among these are ClO_4^- (perchlorate), NO_3^-, TeO_4^-, SCN^-, NnO_4^-, ReO_4^-, and $SeCN^-$; (186, 187); the most potent appears to be perchlorate. Chow, Chang & Yen have recently used ^{36}Cl to show that perchlorate is selectively concentrated in the thyroid gland but is not formed into an organic substance (188). Ameritium is concentrated in the thyroid, but not in the follicular cells (189). Perchlorate has a curious, unexplained effect on the thyroid gland of patients with Penred syndrome (190). In normal persons the effect of perchlorate is largely dispelled within 48 hr. In the two patients studied, however, the inhibiting effect on iodide transport was prolonged for many days (175).

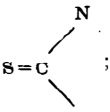
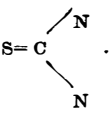
The kinetics of the inhibition by thiocyanate of thyroid iodide accumula-

tion has been re-examined (191) in the rat. The rate constants of iodide entry to and exit from the gland were measured. Low doses of thiocyanate greatly enhanced the exit of iodide but had relatively little effect on entry; apparently thiocyanate affects the permeability of the thyroid cells to iodide, and thereby enhances the exit rate more than it impairs the entry (192).

Other than iodide, the most important application of anions in study of the thyroid is in the use of labeled pertechnitate for the study of iodide transport. A recent review summarized the advantages of pertechnitate in the study of the thyroid (193).

The Thionamide Drugs

STRUCTURE AND FUNCTION

The antithyroid drugs usually employed in clinical medicine have in common the thionamide structure  ; the most effective are those that contain the thiourylene group  . Studies on the relationship between chemical structure and antithyroid activity in rats are not directly applicable to man, but some conclusions seem valid (194). Substitution of up to three hydrogen atoms by methyl groups in the thiourea molecule does not change activity, but replacement of all four hydrogen atoms increases activity. Marked decrease or loss of potency follows substitution, on one or both nitrogen atoms, of polar groups such as amino, imino, or carbonyl groups. Substitution of the sulfur atom is accompanied by loss of antithyroid activity. Dithiobiurea, a compound containing two complete thiourea molecules, and sulfanilthiourea, a compound containing two different active molecules, have no activity. The inclusion of a thiourylene group in a five-membered heterocyclic ring structure increases antithyroid activity. Thus 2-mercaptoimidazole, a substance whose methyl derivative is widely used in therapy, is 15 times as active as thiourea.

As for thiourea, large substituents, additional polar groups, and substitution on the sulfur atoms decrease antithyroid activity. The thiouracil derivatives have been intensively studied because of the effectiveness of the parent compound. Thiouracil is, in fact, as active as thiourea.

Substitution of methyl or ethyl groups on the nitrogen atoms decreases antithyroid activity, particularly if substitution is on nitrogen 3 rather than on nitrogen 5. The activity is highly affected when alkyl groups are attached on carbons at positions 5 and 6.

Methylthiouracil activity equals 0.7 of the activity of the parent compound, but 5-ethylthiouracil is 3.5 times, and 5-n-propylthiouracil 2 times as active as thiouracil.

Among the 6-derivatives, methylthiouracil has the same activity as the parent compound, but ethylthiouracil is 8 times as active. 6-n-Propylthiouracil is the most effective, with activity 11 times that of thiouracil.

The double bond between carbons 5 and 6 is important, since the activity of dihydrothiouracil is only one tenth that of thiouracil. Antithyroid activity is lost after substitution of the sulfur atom, or replacement of hydrogen at positions 5 or 6 by amino, carboxy, carbetoxy, or cyano groups.

METABOLISM

The metabolism of thionamide, particularly thiourea and thiouracil, has been intensively studied. Williams & Kay (195) found that orally administered thiourea was rapidly absorbed from the gastrointestinal tract; it appeared in the blood within 15 min, and attained maximal concentration within 30 min. When injected intravenously in doses of 500 mg, approximately 3% appeared in the urine within 30 min. The rate of urinary excretion was maximal in the first hour after injection and declined progressively during 24 hr. Little urinary elimination was found after 24 hr and none after 48 hr. No thiourea was detected in the stools of subjects receiving 500 mg daily. Similar results were obtained with thiouracil.

More detailed studies were possible when ^{35}S-labeled thiourea became available. Schulman & Keating (196) found that 98% of the activity of 1 mg labeled thiourea was recovered from the urine of the experimental animals within 48 hr after injection. Activity in the stools was slight and probably due to contamination from the urine. Chromatography of the urine showed a single major band identified as undegraded thiourea. Inorganic sulfate accounted for 6.2% of the radioactivity and ethereal sulfate for 5.9%. The highest concentration was in the thyroid and the kidney. Maximal absolute values were found in the thyroid 6 hr after the injection, but the maximal concentration, expressed as a ratio between thyroid ^{35}S concentration and the average ^{35}S concentration in the rest of the animal, was achieved only after 48 hr. The ^{35}S was in the gland primarily as sulfate ions (197). When radioactive sulfate was administered to the animal no concentration by the thyroid was demonstrated, suggesting that the thyroid metabolized thiourea and stored the resulting product.

The absence of a thyroid mechanism for concentrating thiourea was demonstrated by Maloof & Soodak (198). They found 1.95% of the injected radioactivity in the gland 10 hr after injection. The thyroid/serum ratio was 40, but only 3.4% of the label was present as thiourea; 56% was sulfate, and 13% remained at the origin of the chromatograms, presumably bound to protein. Seventy-five % of the label in the serum was thiourea, 15% was sulfate, and 6% remained at the origin. The thyroid/serum ratio for thiourea was found to be close to 1. Maloof & Soodak also demonstrated that the gland actively metabolized the drug. Per gram of tissue, 0.071 moles of thiourea were metabolized during the first 15 min after intraperitoneal injection. A particulate fraction composed of mitochondria and "micro-

somes" was effective in vitro in the presence of thiocyanate and ascorbic acid. The sulfur-containing products were essentially the same as those found in vivo, the major difference being in the relative quantities, since the in vitro system produced principally protein-bound sulfur (199). Hypophysectomy and potassium iodide slowed the rate of thiourea metabolism in vivo, whereas the in vitro system was inhibited by inorganic iodide and aromatic antithyroid compounds, but not by perchlorate (198). Thiouracil was metabolized somewhat differently. Radioactivity in the gland reached its maximal level within 15 to 30 min and then decreased exponentially, with a half-time of about 4.8 hr. There was no concentration gradient for radioactivity between the gland and the serum. Chromatography of the labeled components in the thyroid showed that part of the drug had been converted to sulfate, but the majority had been metabolized to produce several unidentified substances (198).

The drugs most widely used in medicine—propylthiouracil, carbimazole, and methimazole—have been much less studied. Recently, Alexander et al (200) reported preliminary results of metabolic studies with human subjects using ^{35}S-labeled compounds. Although the two imidazoles behaved similarly, propylthiouracil was metabolized differently. Orally administered propylthiouracil was rapidly absorbed and reached a peak in the plasma within about 2 hr. Methimazole was absorbed more slowly, the peak being achieved in about 8 hr. The distribution space of ^{35}S-methimazole approximated the total body water, whereas that of propylthiouracil was considerably less. Excretion was mainly through the urine and was much faster for propylthiouracil than for the imidazoles. The half-life of ^{35}S-methimazole was not affected by the functional state of the thyroid but was prolonged by renal failure. Chromatography of the urine after methimazole administration showed three major products; one was at the origin, and the other two were probably undegraded drug and sulfate. All label in the urine of the propylthiouracil-treated patients remained at the origin. Alexander and his colleagues observed that labeled propylthiouracil was concentrated by the thyroid to approximately the same degree as iodide and was largely present as the administered compound (201). Selective concentrations were blocked by iodide and perchlorate. These results are not in accord with those obtained by Maloof & Soodak with thiourea.

Rat placenta is easily permeated by ^{35}S-thiourea (202). It was found in the amniotic fluid 12 and 24 hr after administration; concentration was lower in the fetal than in the maternal serum. The fetal thyroid started concentrating the label on the 17th day of pregnancy. The iodine-concentrating activity of the fetal thyroid appeared at the same time. In the fetal thyroid ^{35}S was found as sulfate and thiosulfate.

The thionamides interfere with the organic binding of iodine. Formation of diiodotyrosine from monoiodotyrosine and of iodothyronines seems to be blocked by lower doses of propylthiouracil than is organic binding of iodine into monoiodotyrosine (203). Thus, treatment with propylthiouracil results

in an increase of the monoiodotyrosine/diiodotyrosine and the triiodothyronine/thyroxine ratios (204).

The mechanism of action of the thionamides has been studied by Maloof and his colleagues (199, 205). They demonstrated that the sulfur moiety of thiourea is transferred to thyroid protein. It is firmly bound to protein, but can be displaced by nucleophilic anions such as sulfite and thiols, both of which are known to cleave disulfide bonds. The same anions also prevent desulfuration of thiourea when the active particulate fraction of the thyroid is preincubated in their presence. On the other hand, preincubation with sulfhydryl group inhibitors is ineffective. Thus, intact disulfide bonds appear to be essential for the desulfuration reaction, but free sulfhydryl groups are not involved. Maloof & Soodak inferred that thiourea acts by cleaving a disulfide bond, which in turn serves as a direct acceptor for the sulfur of thiourea, resulting in formation of a mixed disulfide.

In vitro studies on the iodination reaction with the thyroid cytoplasmic particulate fraction have indicated that an intact disulfide group is also essential to the formation of protein-bound monoiodotyrosine. The action of thiourea could be explained on the basis of competition for this common site.

Cunningham has reported that thiourea and thiouracil reacted very rapidly with β-lactoglobulin sulfenyl iodide, a compound that is relatively stable when reacted with other sulfhydryls like cysteine or mercaptoethanol (206). The reaction led to the formation of the mixed disulfide with β-lactoglobulin. He suggested that a sulfenyl iodide may act as a reactive intermediate in thyroid iodine metabolism.

Substances such as thiouracil and thiourea, which have high rates of reaction with sulfenyl iodide, would interfere with the normal function of the intermediate by reacting with it, liberating I^-, and forming an inactive mixed disulfide. This hypothesis has been supported by the indirect demonstration of sulfenyl iodide groups in the thyroid, and by the finding that ^{14}C-thiouracil binding depends on the presence of these groups (207). Final confirmation will require the isolation and characterization of the sulfenyl iodide compounds.

CLINICAL ASPECTS

Efforts have been made to find a method for selecting patients who can be successfully treated with drugs. Alexander et al (208) found that after 6 months of treatment with carbimazole, patients could be divided into two groups on the basis of their response to the thyroid suppressibility test with triiodothyronine. The patients who showed greatly suppressed ^{132}I uptake had a better chance of avoiding a recurrence; those whose uptake was not suppressed were prone to relapse. Unfortunately, the differentiation was far from perfect. Hales et al (209) have studied ^{131}I uptake and thyroid suppressibility during the first 4 months of therapy and distinguished three

groups of subjects: patients whose uptake was suppressed as an effect of the antithyroid treatment, those with high uptake suppressible with triiodothyronine, and patients with high uptake which was not suppressible. After long-term treatment, only one out of 26 patients of the first group relapsed within the first year after withdrawal of the drugs, while 13 of the 26 subjects of the third group suffered relapse in the same period of time. Patients of the second group had intermediate recurrence rates.

Vanderlaan & Storrie (210) reported that 13% of patients treated with thiouracil experienced untoward reactions, fever and skin rashes being the most common, and 1% developed agranulocytosis. With the drugs currently used, these reactions are much less frequent and severe. For both propylthiouracil and methimazole, skin rash is the most common reaction, followed by migrating joint pain. Vanderlaan & Storrie reported that agranulocytosis developed in 0.44% of the patients treated with propylthiouracil and in 0.12% of those treated with methimazole. Jaundice during methimazole or propylthiouracil therapy is a rare complication (211). Scattered cases of aplastic anemia due both to methimazole and propylthiouracil have been reported (212, 213). Acute psychosis has been reported recently during carbimazole treatment (214), but it is uncertain whether the complication resulted from the acute metabolic change induced by the drug or from the drug itself (215).

The Sulfonamides

The best known antithyroid sulfonamides (or related compounds) are *para*-aminobenzoic acid, *para*-aminosalicylic acid (PAS), sulfanilamide, sulfaguanidine, and sulfadiazine. Their antithyroid activity is between 1 and 30% that of 2-thiouracil.

The amino group on the aromatic ring is essential for antithyroid activity. Blocking of the amino group, or its substitution, leads to loss or reduction of activity. The position of the amino group is important; antithyroidal activity of aminobenzoic acid is greater when the amino group is *para* to the carboxyl group. Similarly, the various isomers of aminobenzene sulfonic acid and aminobenzene sulfonamide do not have equal activities; the ortho isomers are the least effective (194, 216).

These drugs interfere with organification of iodine without inhibiting the trapping of iodide. Milne & Greer (217) found that, like propylthiouracil, sulfadiazine inhibits coupling of iodinated tyrosines more readily than it inhibits iodination of monotyrosine and tyrosine. MacKenzie & MacKenzie (218) suggested that the mechanism of thionamides is different from that of the sulfonamides, since iodine had different effects on the goitrogenic activity of these two groups of drugs. Iodine reduces goiter size in animals treated with thionamides, but increases thyroid hypertrophy in animals treated with sulfonamide.

Milne & Greer (217) described a 1000-fold increase in antithyroid activ-

ity when iodine was given with sulfadiazine. The inhibitory effect of the drug was potentiated by as little as 10 μg of KI daily, and complete inhibition of all organic binding was produced by 100 μg of KI daily.

Pitney & Fraser (219) found differences in the in vitro activity of these two groups of drugs. Studying the inhibition of organic binding of iodine, they described a dose-response slope much steeper for thionamides than for sulfonamides. Taurog et al, on the basis of a correlation that they found between oxidizability of different compounds and their capacity to inhibit in vitro incorporation of inorganic iodide into diiodotyrosine and thyroxine, suggested that these drugs interfere with some oxidative step essential to the organification of iodide (216). This step could be the oxidation of inorganic iodide itself. This conclusion, however, was based on the assumption that formation of diiodotyrosine and thyroxine was inhibited to the same degree, a finding later disproved.

1-5-Vinyl-2-thio-oxazolidone

Among the naturally occurring goitrogens, 1-5-vinyl-2-thio-oxazolidone is of particular interest because of its possible role in the pathogenesis of endemic goiter. The substance has been found both in the seeds and in the green parts of cruciferous weeds that are very common in the fields and meadows of certain goiter areas, and may be consumed in large amounts by cows (220). The substance has also been found in 6 out of 9, and 5 out of 8, milk specimens from farms from two different regions of endemic goiter in Finland (221), but was not found in milk from nongoitrous regions. The concentration of the drug in the milk was between 35 and 100 μg per liter. The drug caused significant enlargement of the thyroid when administered to rats in doses of 0.1 to 0.5 μg per day in long-term experiments. Radioiodine uptake, however, was not affected by oral doses of less than 0.5 μg per day (220).

Six hours after intraperitoneal administration of a single dose of ^{35}S-labeled drug, the concentration of radioactivity in the thyroid was five times that in the liver. Thyroid radioactivity decreased sharply within 2 days, although significant amounts were detectable over a period of 6 days; radioactivity disappeared from liver and kidney in 4 days (222). Doses of 30–400 μg per day administered for 5 months to normal or slightly hyperthyroid patients did not affect the PBI or the radioiodine uptake. On the contrary, the PBI^{131} measured between 24 and 144 hr after ^{131}I administration was significantly decreased by doses of 100 μg per day or more (223). Thus it appears that there may be doses of naturally occuring goitrogens which can reduce the quantity of circulating hormone without lowering the radioiodine uptake. This could be because of a stronger effect on iodothyronine synthesis than on tyrosine iodination. The origin and chemistry of naturally occurring goitrogens has been thoroughly reviewed by Van-Etten (224).

ACKNOWLEDGMENT

We wish to express our gratitude to Drs. Donald Alexander, G. Hennen, and J. M. Hershman for kindly permitting us to examine some of their unpublished studies.

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