MINIREVIEW

Iodine Metabolism and Thyroid-Related Functions in Organisms Lacking Thyroid Follicles: Are Thyroid Hormones also Vitamins? (44098)

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Abstract. **Thyroid-related functions in organisms devoid of follicular thyroid tissue have been reviewed. In the lamprey, a primitive vertebrate, the larva concentrates iodide and synthesizes thyroid hormones (TH) by iodoperoxidase (IP)-mediated iodination of a thyroglobulin (TG)-like molecule in a subpharyngeal afollicular endostyle. The endostyle is the thyroid homolog, and it reorganizes into a follicular thyroid at metamorphosis to the adult. Ascidians and amphioxus, invertebrate protochordate relatives of vertebrates, also concentrate iodide and synthesize TH in a subpharyngeal afollicular endostyle, but the endostyle never transforms to follicles. Ascidian plasma contains L-thyroxine and its more biologically active derivative 3,5,3′-triiodo-*L*-thyronine, and TH receptors exist, but TH effects are poorly understood. No other invertebrates possess an endostyle. Several invertebrates concentrate iodide at other sites and form protein-incorporated iodohistidines and iodotyrosines; however, *de novo* iodothyronine biosynthesis through IP-mediated TG iodination has not been established. Nevertheless, TH occur in invertebrates, and exogenous iodotyrosines or iodothyronines have effects on jellyfish, insects, and sea urchins. Furthermore, gut bacteria metabolize TH, and plants may synthesize TH by nonenzymatic oxidative iodination. Thus, TH occur in many organisms and, after ingestion and enteric absorption, can enter the food chain. Indeed, sea urchin larvae obtain TH required to induce metamorphosis from plant diatoms. Thyroid hormones can therefore have vitamin-like effects and, in conjunction with vitamin D_3 and possibly with other steroids, may be more aptly termed vitamones. Availability of exogenous TH has implications for models of invertebrate and vertebrate TH metabolism and iodine salvaging, and it may explain the prominent and probable ancestral role of peripheral mechanisms in regulating thyroidal status.** [P.S.E.B.M. 1997, Vol 214]

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Aspects of this study were supported by a grant-in-aid of research to the author from the Natural Sciences and Engineering Council of Canada.

[P.S.E.B.M. 1997, Vol. 214]

0037-9727/97/2144-0302$10.50/0
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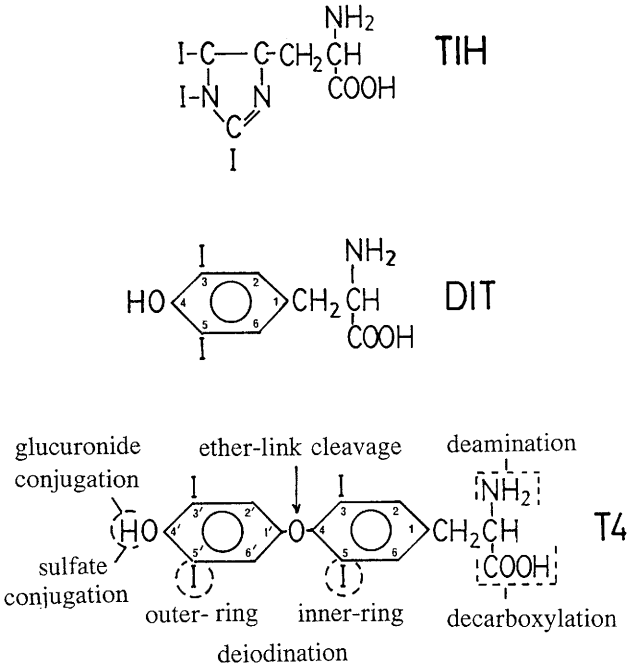

Figure 1. Structures of selected iodoamino acids. TIH, 1,2,5-triiodohistidine; DIT, 3,5-diiodotyrosine; T4, *L*-thyroxine. Various degradative pathways are shown for T_4. Two other commonly encountered iodoamino acids not shown above are MIT (monoiodotyrosine, which lacks the 5-iodine of DIT) and T_3 (3,5,3′-triiodo-*L*-thyronine, which is formed from T_4 by removing the 5′ iodine).

Thyroid tissue has been identified in all vertebrate species examined to date. It is characterized by highly vascularized "spheroidal" follicles consisting of a layer of epithelial cells surrounding a lumen filled with proteinaceous colloid. The collective function of the follicles is to ensure secretion of thyroid hormones (TH), which regulate metabolism. The primary TH are the iodothyronines, *L*-thyroxine (T_4) (Fig. 1), and 3,5,3′-triiodo-*L*-thyronine (T_3) which lacks one outer-ring iodine. Effects of TH differ among vertebrates, but the follicular appearance is remarkably uniform, and TH of fish are identical in structure to those of mammals (1). Thyroid follicles are found only in vertebrates, but it has been recognized since the late 19th century that thyroidal function is not confined to vertebrates with follicular thyroid tissue (reviewed in Refs. 2–10). Furthermore, the TH and their actions have now been reported in invertebrate animals and plants, and bacteria metabolize TH. The purpose of this review is to describe thyroid-related functions in invertebrate animals (Table I) and other organisms lacking a follicular thyroid. This survey shows that the occurrence and actions of TH and related iodocompounds extend to many organisms, and that TH pass through the food chain. Thus the definition of a hormone as an endogenously produced specific chemical messenger may not strictly apply to the TH. This has implications for interpreting TH metabolic pathways and their regulation in both invertebrate and vertebrate animals.

Criteria for Thyroid Function

What evidence for thyroid-related function might one anticipate in organisms lacking follicular thyroid tissue? Based on the vertebrate model (reviewed in Ref. 11), there may be evidence of TH synthesis, TH metabolism, TH actions, and regulation of thyroidal status.

TH Synthesis. Iodide (I^-) is required for TH synthesis but is scarce in many environments. Thus, if an organism is forming TH it may need to concentrate iodide, probably by a specific energy-dependent carrier-mediated pump comparable to that in the vertebrate thyroid (Fig. 2). There will also be a need to iodinate and couple tyrosines to form iodothyronines (TH). In the thyroid, these processes are achieved through synthesis of two key proteins, thyroglobulin (TG) and iodoperoxidase (IP). Thyroglobulin provides the appropriate stereochemistry for tyrosine (tyrosyl) iodination and iodotyrosyl coupling; IP activates iodine to accomplish iodination and couples the iodotyrosyls to form iodinated TG (TGI). One might therefore anticipate a TG/IP system, or some equivalent, in an organism synthesizing TH. However, nonenzymatic mechanisms of TH formation have been demonstrated *in vitro* (12), which could occur *in vivo* (13). Thus TG/IP may not be obligatory for TH synthesis.

Iodoperoxidase is associated with the thyroid cell membrane and catalyzes TG iodination extracellularly at the colloid-cell membrane interface. Since IP and TG are co-packaged by the Golgi into vesicles prior to their secretion into the colloid, intracellular TGI formation could also occur in these vesicles (11) (Fig. 2). Such a "short-circuit" biosynthesis (14), although observed in cultured isolated thyrocytes, is not considered important in mammals *in vivo* (15). However, it indicates the feasibility of TH formation without follicles. In the thyroid, the iodothyronines are formed in the TGI molecules as covalently incorporated amino acids. The iodothyronines are stored as TGI in the luminal colloid whose borders continually undergo endocytosis. Endocytotic vesicles fuse with lysosomes, and proteolytic digestion within resulting phagolysosome releases TH, which pass to the blood (Fig. 2). Therefore, an animal lacking follicles but synthesizing TH might possess extra- or intracellular proteases capable of hydrolyzing TGI.

TH Metabolism. If an organism is synthesizing TH, then TH should occur in body fluids. Because of the low TH solubility in aqueous media, most TH will likely be bound reversibly to circulating proteins. In vertebrates, TH undergo several enzymatic conversions, including deiodination (removal of iodines from outer or inner iodothyronine rings), conjugations with glucuronic acid and sulfate, and quantitatively minor pathways involving deamination, decarboxylation, and diphenylether cleavage (Fig. 1). These various conversions are

Table I. Taxonomic List of the Invertebrate Animals Included in the Present Survey of Iodine Metabolism and Thyroid Function

Phylum	Subphylum	Class	Common name
Chordata[a]	Urochordata	Ascidia	Tunicates
		Larvacea	Sea squirts
		Thaliacea	
	Cephalochordata	Branchiostomata	Amphioxus
Hemichordata		Enteropneusta	Acorn worms
Echinodermata		Echinoidea	Sea urchins
Bryozoa		Gymnolaemata	
Arthropoda		Insecta	Insects
		Malacostraca	Shrimps
			Crayfish
			Amphipods
		Branchiopoda	Daphnia
Annelida		Polychaeta	Sea worms
		Oligochaeta	Earth worms
Mollusca		Gastropoda	Abalone, snails
		Pelecypoda	Clams, bivalves
Nemertea		Enopla	Nemertine worms
Cnidaria		Scyphozoa	Jellyfish
		Hydrozoa	Hydra
		Anthozoa	Corals
Porifera		Calcarea	Sponges
		Demospongia	
		Sclerospongia	
		Hexactinellida	

[a] The Cephalochordata and the Urochordata are often referred to as protochordates, since they represent the most primitive form of chordate organization which probably evolved into the vertebrates.

involved in the extrathyroidally regulated activation and inactivation of TH, and are anticipated in organisms using TH as chemical regulators.

TH Actions. Some rapid TH effects may be due to TH interaction with membrane and mitochondrial receptors, independent of regulation of protein synthesis. However, in vertebrates, TH bind to specific nuclear receptors that are part of a large superfamily of proteins which also serve as receptors for steroids, vitamin D_3, and retinoic acid. The hormone-occupied receptors bind in pairs to adjacent short DNA sequences (thyroid response elements) and constitute transcription factors that regulate specific gene expression. If TH exert biological actions in an organism, these actions are likely mediated by TH-specific nuclear or membrane receptors.

Regulation of Thyroidal Status. Thyroidal status in vertebrates is regulated both centrally and peripherally. Central regulation involves hypothalamic control of pituitary secretion of thyroid-stimulating hormone (TSH), which in turn stimulates TH synthesis and secretion. There is also negative feedback of free or unbound plasma TH to inhibit TSH secretion. Thus, with central control, plasma TH is determined primarily by the setpoint of the hypothalamic-pituitary axis to free TH. In contrast, peripheral regulation adjusts activities of various extrathyroidal enzymatic pathways involved in metabolic conversions of TH. Of particular significance is outer-ring deiodination of T_4 to biologically active T_3, but conjugation and inner-ring deiodination pathways also play roles in inactivating and degrading TH. If TH exert effects in an organism, then central and/or peripheral regulation of the biologically active form of TH (probably but not necessarily T_3) is anticipated.

Survey of Thyroid-Related Functions in Organisms Lacking Thyroid Follicles

Agnatha (Larval Lampreys). The most primitive extant vertebrates are the agnathans, the jawless cyclostomes (lampreys and hagfishes). Parasitic adult lampreys have thyroid tissue resembling that of higher vertebrates, but follicles are absent in their nonparasitic larvae (ammocoetes). However, ammocoetes have an elongated subpharyngeal bilobed glandular sac (subpharyngeal gland or endostyle) originating from the pharyngeal floor between the first and fifth branchial clefts (Fig. 3A). The subpharyngeal gland connects with the pharynx through a narrow hypobranchial duct. Stemming from observations of Schneider in 1879 (16), the endostyle is now established as the thyroid homolog. At metamorphosis the hypobranchial duct closes, part of the endostyle undergoes histolysis or involutes, and the remainder differentiates into thyroid follicles (17–20). Several cell types occur in the endostyle (17, 21–24), and, despite difficulties in following their individual fates during metamorphosis, the consensus is that the

type 3 and possibly type 2 and 5 cells differentiate into follicles (23, 25–28). Functions of other more ventral cell types are unresolved. They may assist in digestion by secreting proteins and mucus, which pass to the digestive tract through the hypobranchial duct.

Anatomical homology with the thyroid does not necessarily mean that the endostyle synthesizes TH. Early studies showed no endostylar stable iodine accumulation (29, 30), but there was uptake of radioiodide (31), and autoradiography showed that mainly type 2 and 3 cells concentrated radioiodide (22, 32, 33). Most endostylar radioiodine was protein-incorporated, and its location correlated with presumed TG (22). The presence of TG has since been confirmed using light and electron microscopic immunocytochemistry in type 2c and 3 cells and some type 5 cells (34–36). Iodoperoxidase has also been identified histochemically in the endostyle (37). Certain other ammocoete tissues, particularly notochord (38–40), concentrate radioiodide. However, *in vitro* enzymatic hydrolysis indicates that only the endostyle contains labeled MIT, DIT, T_3, and T_4 (9, 32, 41–44). Radiolabeled T_4 and some T_3 occur in ammocoete plasma following radioiodide injection (9, 32). High concentrations of TH, particularly T_3, have been measured by radioimmunoassay (RIA) in ammocoete plasma (45, 46).

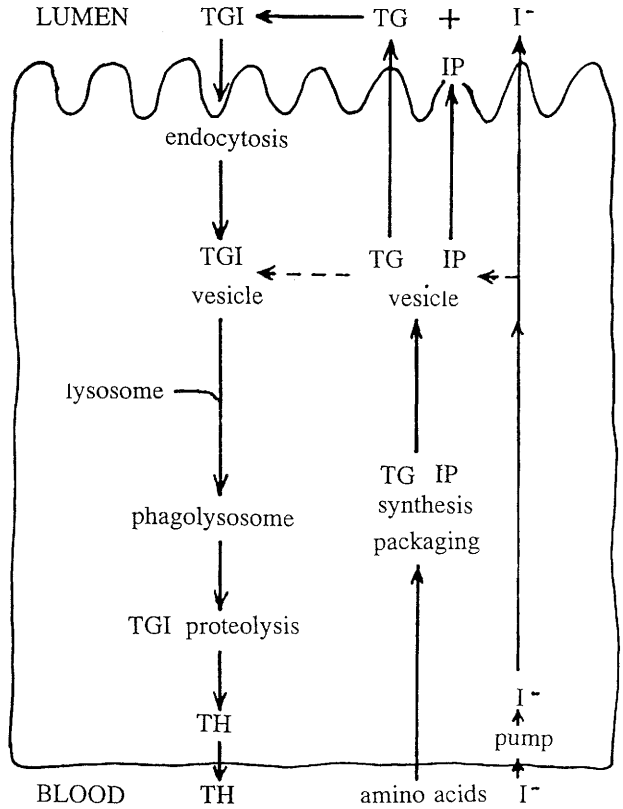

Figure 2. Diagram of a thyroid follicle epithelial cell showing pathways involved in thyroid hormone (TH) synthesis, storage, and secretion I^-, iodide; TG, thyroglobulin; TGI, iodinated thyroglobulin; IP, iodoperoxidase, a membrane-incorporated enzyme responsible for activating iodine to iodinate tyrosyls in the TG and for coupling iodotyrosyls to form iodothyronyls. The broken lines indicate a possible intracellular short-circuit pathway for TGI synthesis.

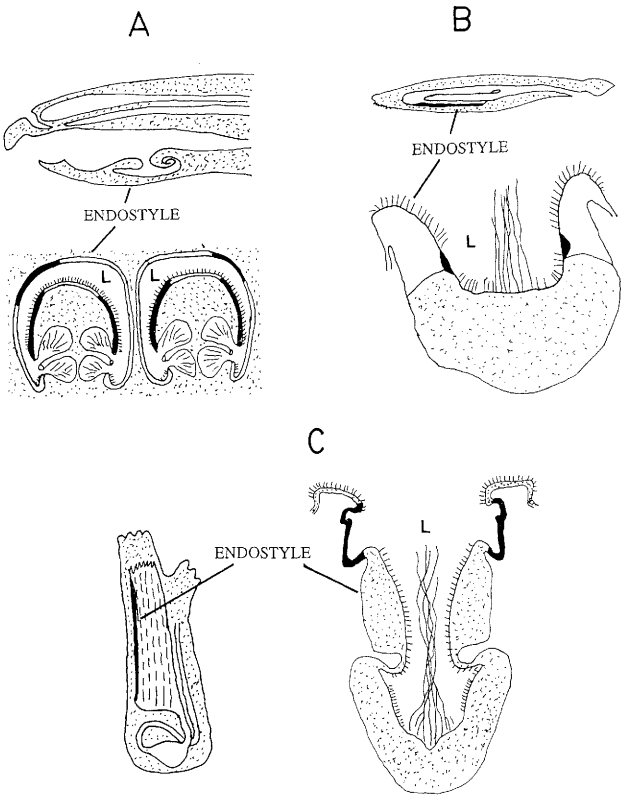

Figure 3. Macro- and micro-structures of the endostyles of an ammocoete larva of the lamprey (A), a cephalochordate, amphioxus (B), and a tunicate (C). The black shaded regions are the primary radioiodide concentrating sites; L, endostyle lumen. (Redrawn after Ref. 6.)

How are TH transferred from endostyle to blood? TG may be secreted into the endostyle lumen and hydrolyzed by a protease to liberate TH (39, 47). Free TH may then be reabsorbed by endostylar cells, or the secretions may pass through the hypobranchial duct into the intestine where further TGI hydrolysis may complete TH release prior to TH absorption from the gut lumen (25). In either case, the endostyle acts as an exocrine tissue. Electron microscope autoradiography indicates most endostylar TG iodination occurs extracellularly at the cell/colloid interface (26). However, intracellular iodination and hydrolysis of TGI may also occur, and the TH so formed may then be released into either the lumen or the blood.

Putative T_3 receptors have been reported in ammocoete liver (48), but TH actions in ammocoetes remain unresolved. Thyroid hormones induce amphibian (49, 50) and piscine (51) metamorphoses and might therefore be expected to induce ammocoete metamorphosis. However, as ammocoete metamorphosis commences, plasma T_4 and T_3 levels decrease (45, 46, 52), and do not increase to a climax as in amphibians and fish (49, 53). Furthermore, ammocoete metamorphosis cannot be induced by iodine, anterior pituitary preparations,

or TH treatments (29, 30, 54, 55), suggesting no role for elevated TH levels in promoting ammocoete metamorphosis.

Hoheisel and Sterba (56) found that potassium perchlorate ($KClO_4$) induced ammocoete metamorphosis. Suzuki (57) also showed that, at least in the larger ammocoetes, $KClO_4$ and other goitrogens induced a normal metamorphosis accompanied by a decrease in thyroidal function. The phenomenon has been documented in greater detail in Youson's laboratory (58, 59). One interpretation is that high plasma TH levels suppress metamorphosis. Thus, when plasma TH levels are lowered by $KClO_4$ metamorphosis can proceed. However, independent (extrathyroidal) actions of $KClO_4$ on metamorphosis cannot be excluded (59).

The endostyle is not pituitary regulated. Neither its histological appearance (55, 60) nor its radioiodide uptake (44) are altered by hypophysectomy. The endostyle is also insensitive to mammalian TSH preparations (40, 55, 61). Some endostylar cells hypertrophy in the presence of goitrogens (62–65), but the goitrogen response is not pituitary mediated, as it occurs after hypophysectomy (60), and is not abolished by T_4 treatment (64, 65). Central pituitary control over the thyroid is also absent in adult lampreys (66). Thus, regulation of thyroidal status in both larval and adult lampreys may occur instead in peripheral tissues through extrathyroidal adjustments of TH metabolism (1, 66).

In summary, the ammocoete endostyle carries out the functions of follicular thyroid tissue most likely by exocrine release of TGI into the gut lumen. The TH are liberated by either intra- or extracellular protease action, and TH probably enter the circulation from the gut lumen. The TH roles are incompletely understood. Regulation of thyroidal status does not seem to involve a central hypothalamo-hypophysial-thyroid/endostyle axis and is probably by peripheral mechanisms.

Cephalochordata. The cephalochordata (amphioxus, Branchiostoma) are marine chordate invertebrates with a notochord homologous to that of vertebrates. Together with the urochordata (see below) they are often termed protochordates. Both larvae and adults have an endostyle (Fig. 3B). In 1914, Van Wijhe (67) termed the amphioxus endostyle the "glandale thyroidea." Despite early debate, the amphioxus and ammocoete endostyles are now considered homologous (3, 6). The amphioxus endostyle exhibits thyroid-like function. It contains certain cell types that accumulate radioiodide (68–71); it contains a TG material with iodination properties remarkably similar to those of mammalian TG (72, 73); IP activity has been established (37, 74) and TH synthesis demonstrated (9, 75, 76).

Of particular interest is the endostylar site of TH iodination (69). Electron microscopic autoradiography indicates that, shortly after radioiodide administration, the silver grains, representing newly synthesized labeled material, occur in the lumen close to type 5 cells (70, 74). Later, the grains are associated with cellular structures of type 5 and 6 cells, which may have taken up iodinated materials from the lumen in a manner similar to vertebrate TGI endocytosis (70).

The metabolism, regulation, and action of TH have not yet been studied.

Urochordata. The ascidians (tunicates or seasquirts) are marine invertebrate chordates with a notochord, but lacking any cranial formation. They are mainly sessile suspension feeders with an endostyle producing a mucous filter as part of their feeding mechanism (Fig. 3C). The endostyle concentrates radioiodide from seawater (77–88) and contains proteins related immunologically to TG (89, 90) and to IP (87, 91, 92). Endostylar iodine is utilized to form MIT, DIT, and T_4 (79, 81, 83, 84, 91). Iodination and TH biosynthesis are restricted mainly to type 7 cells (87). Indeed digestive and thyroidal functions are distinct, and tissues associated with these two functions are spatially separated (93). Most of the TH synthesized by the endostyle undergo exocrine secretion into the gut lumen. Secretions are then carried by mucus through the pharyngeal cavity and may be absorbed by caudal cells in the alimentary tract (74).

Radioiodine is also incorporated into MIT and DIT in the tunic, the outer covering of the tunicate. Some workers report that iodothyronines are made there (90, 94, 95), but others contend that tunic iodinations are "nonthyroidal" in nature, resembling scleroprotein iodination found at the surface of skeletal tissues in some other invertebrates (81, 82). Several unusual tunic iodinated *L*-tyrosine alkaloids have been identified using nuclear magnetic resonance in a colonial ascidian (96); being cytotoxic, the alkaloids may defend against predation.

Fredriksson *et al.* (97, 98) recorded immunoreactive T_4 in low levels in plasma (0.2 ng/ml) and in extracts of the pharynx, alimentary canal, and tunic (2.7–8.4 ng/g). Tissue and plasma immunoreactive T_3 correlated with the distribution of administered [^{125}I]T_3, but the T_3 concentrations were much lower than those for T_4. However, the relatively high T_3 levels and T_3/T_4 ratio in the alimentary tissue suggested T_4-to-T_3 conversion at that site; deiodination of T_4 to T_3 has been demonstrated *in vivo* (99). Putative nuclear receptors for T_3 were identified in the pharynx and alimentary canal ($K_d = 1 \times 10^{-10}$ *M*) (98), but the receptor abundance was low (maximal binding capacity: 2.0–4.2 fmole T_3/mg protein).

Information on biological actions of TH in ascidians is sparse and based on potentially pharmacological TH levels (10^{-5}–10^{-7} *M*) (98). In the compound ascidian, Periphora, stolen elongation was enhanced by T_4 or T_3 treatment (100); in Phallusia, treatment with TH increased activity of polyphenol oxidase, an enzyme associated with growth and development (101); in

Ascidia, metamorphosis to the adult was accelerated by T_4 (102). This latter effect is of interest since TH also induce development and metamorphosis in vertebrates (49, 51). However, free-swimming larvae do not concentrate radioiodide (102), and if TH do influence ascidian development the source and regulation of TH production at the larval stage need to be determined.

In summary, although the biological actions of TH and their mechanism of control have not been established, the ascidians do produce and metabolize TH. Furthermore, despite considerable anatomical differences in the endostyles of lamprey ammocoetes, amphioxus, and ascidians, the site of iodine organification in each case occurs in cells on the lateral and dorsal endostylar regions. Thus, there is a common protothyroid region (70). This is also true in the most primitive ascidians, the Larvacea (appendicularians) (103, 104), which retain several larval features and are free swimming as adults. They are of evolutionary interest since, as swimming ascidians, they may represent a form ancestral to vertebrates.

Hemichordata. The enteropneusts, or acorn worms, were once allied to chordates based on presence of gill slits and a notochord. However, their notochord is not homologous to that of chordates, and they are no longer considered close chordate relatives. Early workers found autoradiographic evidence of radioiodide binding by surface epidermal and glandular tissues (105). Later radiochromatographic studies involving prolonged immersion in seawater containing radioiodide revealed MIT but no DIT or iodothyronines (106, 107). Thus, there is no evidence that hemichordates produce iodothyronines.

Echinodermata. Using three species of sea urchin, Chino *et al.* (108) established that TH promote, in a dose-dependent manner, the metamorphosis of the pluteus larva. Thyroxine was effective at 10^{-10}–10^{-9} *M*; T_3 at 10^{-9}–10^{-8} *M*; 3,3′,5′-triiodo-*L*-thyronine (reverse T_3 = rT_3) at 10^{-8}–10^{-7} *M*; T_3 propionate at 10^{-7}–10^{-6} *M*. In contrast to TH effects on vertebrates, T_4 potency exceeded that of T_3, and rT_3 was modestly effective.

High-performance liquid chromatography (HPLC) and radioimmunoassay (RIA) techniques showed that larval TH contents increased during metamorphosis. The T_4 content at the four-arm stage was 0.05 pg/10^4 larvae, increasing to 1.5 pg/10^4 larvae at the six-armed stage and 2.9 pg/10^4 larvae at the eight-armed stage. The changes in T_3 were much greater, increasing from 5 pg/10^4 larvae at the six-armed stage to 143 pg/10^4 larvae at the eight-armed stage. Surprisingly, the larval TH content depended on the planktonic unicellular algal food that was required for metamorphosis to proceed. Algal T_4 content was 0.8 pg/10^9 cells; T_3 content was 18 pg/10^9 cells.

Thiourea and PTU did not block metamorphosis, suggesting that larval TH synthesis is not required for metamorphosis. However, both $KClO_4$ and KSCN, competitors of iodide transport, retarded development at concentrations of 1–10 m*M*. This inhibition was not completely offset by T_4 (10 n*M*) treatment, indicating toxic effects of the inhibitors or a co-requirement for iodide in development.

Developmental progress of the crown-of-thorns starfish, *Acanthaster planci,* through its larval stages is also accelerated by T_4 in a dose-dependent manner (109).

In summary, TH are important in echinoderm development, and there is strong evidence in sea urchins that ingested unicellular algae are the primary TH source. Receptors related to TH receptors may occur in sea urchins (110).

Bryozoa. Radioiodide was concentrated from seawater in the whole body of *Bugula neritina* and *Shizoporella errata* by ouabain- and dinitrophenol-sensitive transport (111–113). Uptake was blocked by potassium thiocyanate (KSCN), a probable competitor of iodide transport, but was unaffected by perchlorate or thiourea. The presence of organic iodine derivatives has not been studied.

Arthropoda (Insects and Crustaceans). Following an early observation of radioiodide incorporation into the *Drosophila* cuticle during tanning (114), Limpel and Caseda (115) injected radioiodide into representative insects from several orders and reported labeled MIT in the hemolymph, labeled DIT and T_4 in the muscle, and unidentified organic radioiodine derivatives in other tissues. Monoiodohistidine was the main excreted iodocompound. They examined the tissue distribution of the labeled materials in the cockroach, *Periplaneta americana. L*-thyroxine, MIT, and DIT were identified in muscle, brain, nerve cord, and fat body; T_3 was not identified conclusively by their single-dimension paper chromatography. Tong and Chaikoff (116) essentially repeated the above study on *Periplaneta,* but used three different paper chromatographic systems. They identified MIT and DIT, and reported other labeled organic derivatives, but found no labeled T_3 or T_4. They concluded that the T_4 reported earlier (115) was probably a radioiodinated scleroprotein derivative.

Total iodine was measured in insects from a prairie river in Manitoba, Canada, in the North American goiter belt (117). Levels ranged from 0.3 to 8.57 μg iodine/g dry wt. Highest levels were found in Trichopteran cases, supporting the view that organically incorporated iodine (probably iodoamino acids) may be formed in insect scleroproteins even in areas where iodine is not particularly abundant.

Administered TH influence insect physiology. *In vitro* addition of T_4 (3 μ*M*) to fat bodies of *Hyalophora*

cercropia promoted release of diglycerides and fatty acids within 3 hr (118). *In vivo* injection of T_4 into larvae of the silk moth, *Bombyx mori,* increased protein synthesis, RNA synthesis, silk production, and modified heart function (119). Subsequent studies on the same species confirmed that T_4 alters protein the nucleic acid content of the testis (120), fat body (121), and ovary (122), and alters the glycogen content in the fat body of the female (123). The response was biphasic and maximal at 1 ng T_4/g body wt. Single injections of different doses of T_4 (0.5–5 ng/g) decreased the glycogen content of the ovary during the larval stage and increased it during pupal and adult stages (123, 124). In the same species, T_4 acted in a dose-dependent manner (0.5–2 ng/g) to increase Na^+/K^+ and Mg^{++} ATPases in larval and pupal tissues (gonads and silk gland); doses of 0.25 ng/g were usually ineffective; in adults, only the Na^+/K^+ ATPase in testis was increased (125). Also in the same species, feeding T_4 to larvae increased their growth as well as their silk and egg production, possibly due to an increase in circulating ecdysosteroids (126, 127). In another silk moth, *Antheraea mylitta,* T_4 (0.5–1 ng/g) increased protein and amino acid turnover in ovary, testis, and fat body, and modified hemolymph amino acid profiles (128–131).

In all the above studies, T_4 was the administered TH, and in all but one instance (118) T_4 was either fed or injected. However, TH also exert a rapid (5- to 7-min) *in vitro* action to decrease the volume of the eggs of the locust, *Locustra migratoria* (132). This TH action resembled that of juvenile hormone (JH). The actions of both TH and JH were blocked by the Na^+/K^+ ATPase inhibitor, ouabain, and by ethoxyzolamide, which specifically blocks JH receptors. Furthermore, the rapid effect of both hormones precluded actions through modified protein synthesis and indicated involvement of a membrane receptor. TH may closely resemble the phenoxyphenols, such as fenoxycarb, that act as JH agonists after binding to the JH receptor. T_4 was more effective than T_0 (thyronine with no substituted iodines) but two orders of magnitude less potent than T_3, which exerted a detectable action at 1 n*M* or lower. The nature of the insect JH receptor has not been determined, but the above results suggest that it could belong to the protein superfamily that includes the TH receptors.

Ecdysone is another hormone important in insect development. The ecdysone receptor is also related to the TH receptors and is a member of this superfamily (133–135). Thus, TH effects on ecdysone function are also possible.

Few crustacea have been studied but there is evidence of iodine organification. The total iodine contents in the prairie freshwater amphipod, *Hyalella,* and the decapod crayfish, *Astacus,* were respectively 1.15 and 4.87 μg iodine/g dry wt (117). Scleroproteins in the developing exoskeleton of *Daphnia* incorporate radioiodine (105). Analysis by RIA of a shrimp diet for fish indicated a T_4 content of 10–15 ng/g and a T_3 content of 28 ng/g (136). Both T_4 and T_3, as well as T_4-to-T_3 conversion, have been detected by RIA and HPLC in the brine shrimp, *Artemia* (Brown C, personal communication). There are no reports on the metabolism or actions of TH in crustaceans.

In summary, there is yet no proof that arthropods synthesize TG, IP, or iodothyronines, although they can organify iodine to form protein-incorporated iodotyrosines and iodohistidines. Nevertheless, their tissues may contain immunoreactive T_3 and T_4. Insects can also respond to exogenous TH and possess receptors in the same protein superfamily as TH receptors.

Annelida. Marine polychaete worms bind ambient radioiodide mainly in the scleroproteins of the pharyngeal teeth, cuticle, parapodia, and chaetae (105, 137). In the polchaete, *Nereis diversicolor,* iodine is absorbed from seawater at about 5 ng/worm/day, resulting in a whole-body content of 23–30 μg of iodine/g (138). There is extensive whole-body iodine loss, but 15%–30% binds at the base of chaetae where tanning occurs. The iodotyrosines, MIT and DIT, were identified by paper chromatography; other labeled compounds did not correspond to iodothyronines or their derivatives.

In the oligochaete, *Eisenia foetida,* immunohistochemical analysis of the nervous system revealed TG-like activity which was not co-located with cholinesterase activity (139). This precludes misidentification of immunoreactive TG as cholinesterase, which shows striking homology to a TG subunit (140, and see below). Light microscopic autoradiography of radioiodide-injected worms showed silver grains selectively concentrated in the central nerve cord, particularly in the cerebral ganglion (141). The main concentration was between the neurosecretory cells and the neuropile fibers in the zone of the presumptive plexiform neurohemal complex. Treatment with methylmercaptoimidazole (MMI), an IP inhibitor, did not alter setal radioiodine content, but radioiodination in the nervous tissue was blocked supporting an IP-iodinating system there.

In summary, iodine organification and iodotyrosine synthesis occur in the few annelids studied. However, despite indications of neural TG-like materials and an IP requirement, there is no direct evidence of iodothyronine formation.

Mollusca. Ambient radioiodide is incorporated by scleroproteins of pelecypod molluscs (105, 137). T_3 and T_2, but not T_4, were identified by paper chromatography in the snail, Planorbis (142). Tong and Chaikoff (116) found that radioiodide administered to snails was incorporated into MIT and DIT and other unidentified compounds. These iodocompounds were analyzed in three paper chromatographic systems, but did not correspond to iodothyronines or their derivatives.

Even though gastropod molluscs may not synthesize TH, they may have the potential to metabolize TH. Commercially available β-glucuronidase (Sigma Chemical Co., St. Louis, MO) prepared from abalone (Haliotis) entrails or the snail, *Helix pomatia,* contained contaminent T_4 outer-ring deiodination activity that was inhibited by propylthiouracil and blocked by excess T_4 (DiStefano JJ III, personal communication). This implies functional significance for conversion of T_4 to T_3 in these molluscs.

Based on data available from very few species, at least some molluscs may synthesize iodotyrosines. Synthesis of TH *de novo* has not been established, but some molluscs may be able to metabolize TH. There have been no studies on TH receptors or TH effects in mollusca.

Nemertea. Two nemertean worms, *Lineus ruber* and *Amphiporus angulatus,* incorporated radioiodide from seawater into the surface mucous coating (143). Paper chromatography of mucous extracts revealed labeled MIT, DIT, and T_3, and at least one other unidentified organic material; T_4 was not identified. *L. ruber* mucus had a higher tyrosine content and a higher content of organically bound radioiodine than *A. angulatus.* The validity of the paper chromatographic analysis has not been checked by other methods.

Cnidaria (Coelenterata). Corals accumulate considerable quantities of iodine, and research primarily on the Gorgonacea, Pennatula, and Antipatharia taxa established the location and identity of the iodocompounds (reviewed in Refs. 144 and 145). Significant quantities of MIT and DIT are synthesized in the structural scleroproteins. Indeed, DIT was originally termed gorgonin, or gorgonoic acid, because it was first identified from the gorgonid corals (146). The tyrosine content correlates with the degree of iodination, and both are greater in younger, more actively growing corals. However, there has been no convincing demonstration of iodothyronine synthesis. Lack of iodotyrosine coupling probably reflects the physical separation of the fibers containing the iodinated scleroproteins. The scleroproteins of two hydroids lacking the extensive skeletal structure of the corals also incorporated iodide from seawater and formed iodotyrosines (147). No biological effects of iodocompounds on corals have been documented.

There has also been interest in iodine metabolism and effects of iodomaterials in Scyphozoan jellyfish. The free-swimming medusae bud by strobilation from scyphystomae of sessile polyps. The strobilation process requires iodide and is induced by iodide and certain iodocompounds (148–154). Iodide concentrates in the polyp (155). This is probably due to trapping by organification, since iodide uptake was not blocked by perchlorate (156). Iodide efficacy in inducing strobilation was reduced by MMI, which blocks iodide organification (156). Thus, contrary to earlier reports, iodide itself is not the active material. Based on radioiodine incorporation, the most common identifiable iodocompounds have been MIT and DIT. Labeled T_4 was reported by Spangenberg (149, 150) and by Black and Webb (152), but not by Silverstone *et al.* (156).

The most consistent induction of strobilation is with MIT and DIT. T_4 effects are equivocal. For example, T_4 may induce strobilation (150), exert no effect (154), or at high doses inhibit statolith development (151). Radioiodomaterials other than MIT and DIT have been identified chromatographically and might also induce strobilation (156). Formation of iodocompounds was greatest under environmental conditions favoring strobilation (29°C, 10^{-7} *M* iodide) (156), suggesting a regulatory function for these materials.

What are the tissue levels of MIT and DIT, do MIT and DIT have receptors, and do MIT and DIT undergo metabolic conversions?

Porifera. The sponges, particularly sclerospongiae, contain a fibrous scleroprotein, spongin, which incorporates iodine. In some species, the iodotyrosine content attains 14% of the total amino acids. but iodothyronines have not been reported in significant amounts (reviewed in Refs. 144, 145, and 157).

Protista. T_3 binds specifically to the membrane of the ciliate protozoan, *Tetrahymena pyriformis,* but binding has not been linked to any TH action and is reported to lead to T_3 endocytosis (158).

Bacteria. Studies on thyroid-related functions in bacteria are confined to *Escherichia coli* and other gastrointestinal bacteria of mammals. There is no evidence that TH are made by bacteria or exert actions on bacteria. However, bacteria participate in TH enteric metabolism.

Bacteria in the large intestine enzymatically hydrolyze TH-glucuronide and TH-sulfate conjugates to liberate unconjugated TH and other metabolites (159–161). As conjugates, TH are biologically less active, are hydrophilic, and are poorly absorbed across the intestinal wall (162). However, unconjugated TH are absorbed and contribute to an enterohepatic cycling of the biliary-excreted TH and their derivatives (163). Thus, the deconjugative bacterial activity increases the enteric TH pool available for enterohepatic cycling (161). However, there is no evidence that deconjugative bacterial activity is regulated in accordance with the physiological needs of the host body.

Nevertheless, T_4 and particularly T_3, but not MIT, DIT, or iodide, are transported into cultured *E. coli* and become bound to intracellular proteins (164). Radioiodide is generated in the presence of radioiodinated T_4 or T_3, indicating iodide removal from the labeled outer iodothyronine ring (161 and references cited, 164). However, labeled T_3 is not a product, indicating subsequent deiodination of the inner unlabeled tyrosyl ring.

Bacteria also decarboxylate and deaminate TH (161 and references cited). The consequences to the bacteria in metabolizing either the conjugated or unconjugated TH are unclear.

Plants. Marine algae concentrate halogens, including iodide, from seawater. Iodide is important for growth, morphogenesis, and reproduction of certain marine algae (165). Marine algae also form iodotyrosines (166). Until recently, there was no convincing evidence to support either formation or presence of iodothyronines by plants. However, with HPLC and RIA, T_4 (0.78 pg/10^9 cells) and T_3 (18.4 pg/10^9 cells) have been measured in unicellular marine algae (108). The mechanism of algal T_3 and T_4 formation was not studied, but it probably differs from that involving TG/IP in the thyroid. Under the conditions of high radiant solar energy available to plants, direct iodotyrosine oxidation and coupling may occur.

Through the use of gas chromatography-mass spectrometry-selective ion monitoring and also time-of-flight mass spectrometry, it was found that seeds of the plant *Sinapis alpa* and the axillary bulbils of the tiger lily, *Lilium tigrinum,* could produce T_4, but not T_3, by a presumed nonenzymatic process (166). In the presence of oxidizing agents, such as ascorbic acid, extracts from these plants could convert DIT to T_4. It is of future interest to examine other plants to determine the prevalence of formation and occurrence of iodocompounds.

The TH roles in plants are poorly understood. Thyroxine exerts an auxin-like activity in cotton seedlings, competing with indole acetic acid (IAA) for receptor sites, which suggests a developmental role (167). Thyroxine was effective at a concentration two to three orders of magnitude below that for IAA.

However, the main actions of iodocompounds may not be on the plants themselves, but serve to protect plants against organisms that infect or consume them. For example, iodotyrosines in marine algae are toxic to bacteria and may defend against such pathogens (166). Plant iodotyrosines may be harmful to vertebrates; if present in significant levels in blood, MIT and DIT depress catecholamine synthesis by blocking tyrosine hydroxylase (168). Plant iodothyronines may also affect the metabolism of the consumer. For example, both T_4 and T_3 mimic JH action on insect ovaries (132) and may interfere with insect metabolism and development. Plant TH may also elevate the thyroidal status of vertebrate consumers. This possibility is unexplored, but conversely some plants produce substances that depress the thyroidal status of their consumers. For example, cabbages and rapeseed contain the progoitrin/myrosinase combination. Unless removed by heat treatment or selective plant breeding, myrosinase converts progoitrin to goitrin, a potent antithyroid agent for humans, livestock, and fish (169, 170).

In summary, there is evidence that plants form iodotyrosines and iodothyronines, but probably by chemical reactions different from the enzymatic steps present in chordate animals. The extent of TH occurrence in plants needs to be explored. Thyroid hormones may have effects on plants; they may also protect plants against either invertebrate or vertebrate herbivores.

Summary of Evidence for Thyroid-Related Functions in Achordate Invertebrates

Neither thyroid follicles nor endostyles are found in invertebrates lacking a notochord (achordata), but is there other evidence of thyroid-related functions? The main quests have been for sites of radioiodide accumulation and radiochromatographic evidence of iodotyrosine and iodothyronine biosynthesis based on *de novo* radioiodide incorporation. Iodotyrosine formation occurs in numerous achordata, from sponges to insects. Iodothyronine formation has also been reported in a few taxa including insects, molluscs, nemertean worms, and jellyfish. However, several of these claims are based on single-dimensional paper chromatography, unsubstantiated by more rigorous immunoreactive, HPLC, or spectroscopy analyses. At present, *de novo* synthesis of iodothyronines in any achordate has not been established with certainty.

TH synthesis in invertebrate protochordates (ascidians and amphioxus) and vertebrates depends on TG and IP. Thus, presence of TG-like molecules and IP activity would also provide evidence of thyroid-like function. Immunoreactive TG has been reported in an annelid brain, which also concentrates radioiodide, but iodothyronine formation was not studied. TG presence in annelid nervous tissue could reflect the presumed common ancestry of TG and acetylcholinesterase (ACE). The carboxyl terminal half of TG shows a striking homology in amino acid sequences with that of ACE (171–174), suggesting a common ancestry for the two peptides. However, the divergence of TG from ACE is reported to stem from genic duplication of ACE close to the time of chordate evolution (175), which would not account for TG presence in the phylogenetically more ancient annelids.

Few attempts have been made to measure TH in achordate invertebrates. Immunoreactive iodothyronines have been reported in tissue extracts of arthropods and echinoderms. Occurrence of TH receptors, pathways for TH metabolism, or TH regulation have not been studied. However, there are several convincing biological effects of administered iodotyrosines and iodothyronines on jellyfish, insects, and larval echinoderms. It is unlikely they all represent nonphysiological or pharmacological TH effects. Consequently, it remains to be explained why in animals that do not appear to synthesize TH one can observe the presence and/or biological actions of TH.

Endogenous and Exogenous (Vitamin) Sources of Thyroid Hormones

In models of thyroid function, the TH source is usually assumed to be exclusively endogenous, but exogenous TH sources also exist. For example, dietary diatoms seem to provide the TH required to promote metamorphosis of larval sea urchins. Thus, exogenous TH of plant or animal origin could account for the occurrence and biological activity of TH in those invertebrates lacking *de novo* TH synthesis. Furthermore, the existence of exogenous TH sources also has important implications for interpretation of vertebrate thyroid function.

Exogenous TH are certainly available to vertebrates. TH occur in milk of eutherian mammals (176, 177) and marsupials (178); thus, both exogenous and endogenous TH sources may be utilized during development. TH also cross the placenta and provide an exogenous TH supply for the mammalian fetus (179). In fish and birds, the eggs may provide a TH source independent of *de novo* synthesis (180, 181). However, the main source of exogenous TH in vertebrates is probably from food. Vertebrates can absorb TH from the gastrointestinal tract (161), permitting the routine oral administration of prescribed TH to human patients. Although many naturally ingested iodocompounds are covalently incorporated (TGI or other iodoproteins), they may be hydrolyzed by intestinal proteases to liberate free TH, which can then be absorbed. Indeed, ascidians, amphioxus, and ammocoetes may secrete TGI directly from the endostyle into the gastrointestinal tract. Thus, the gut is probably the ancestral route for endogenous TH delivery to the circulation (182).

Vertebrates may obtain TH from plant and invertebrate foods and certainly from ingestion of any vascularized vertebrate tissue. Some vertebrates will ingest higher loads of exogenous TH than others. These include parasites such as vampire bats, lampreys, or hagfish subsisting on vertebrate blood or body fluids; predators consuming whole vertebrates (e.g., piscivorous fish or birds); or scavengers that eat carcass remnants. Inevitable ingestion of the thyroid and the gall bladder by some of these animals will provide a particularly rich TH source. Few vertebrate diets have been analyzed for TH content. Recent analysis of fish diets prepared from vertebrate and invertebrate sources revealed appreciable immunoreactive TH levels (T_4, 10–45.2 ng/g diet and T_3 28–114 ng/g diet) (136).

The potential contribution of dietary TH to the plasma pool has received little attention by mammalian researchers. This may reflect the predominance of studies on chow-fed laboratory rats, which in the wild might consume diets with a higher TH content. However, significant thyroid function persists even in laboratory rats after complete and prolonged thyroidectomy (183, 184),

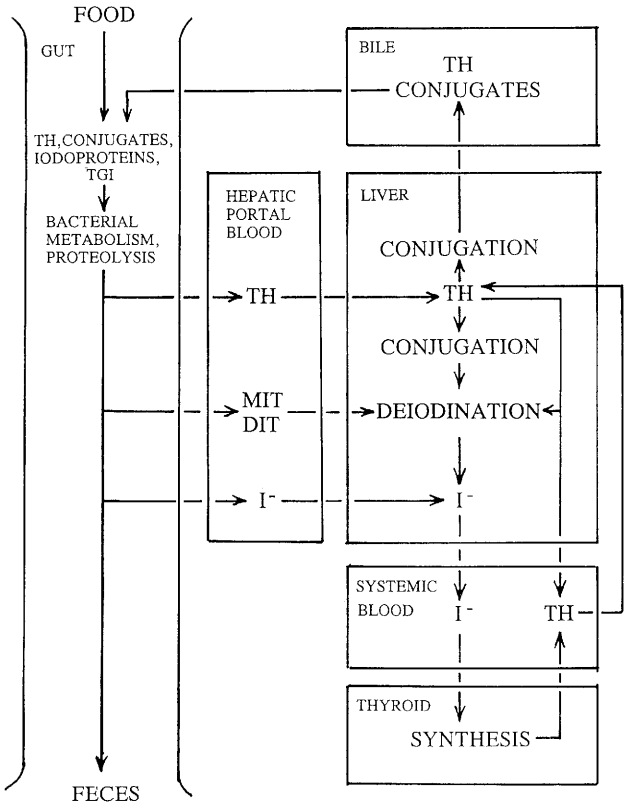

Figure 4. A flow diagram depicting pathways for the metabolism of exogenous and endogenous iodomaterials through the gut, hepatic-portal system, liver, bile, and thyroid. The model depicts the potential contributions of dietary TH and other iodomaterials to either the plasma iodide or TH pools. It emphasizes the roles of the liver both as a peripheral thyrostat and as a salvager of iodine from plasma TH exceeding immediate physiological needs.

and a dietary source of TH has been suggested (185). The human thyroid system certainly responds to dietary TH. A striking example is the outbreak of thyrotoxicosis without true hyperthyroidism, which occurred in 1984 in Minnesota, South Dakota, and Iowa (186), the consequences of which may still be felt (187). This outbreak was traced to ground beef contaminated with thyroid tissue. Thyroid-contaminated pork sausage may also have caused thyrotoxicosis (188).

To what extent does the inevitable ingestion of TH *routinely* contribute to the plasma TH pool? Ingestion of significant TH will not necessarily lead to overt hyperthyroidism. Within limits, exogenous TH may contribute to the circulating TH pool and thereby spare use of endogenous TH. Furthermore, autoregulatory deiodinative mechanisms which accommodate unpredictable influxes of exogenous TH to the plasma pool exist in peripheral tissues of both mammals (reviewed in Ref. 11) and fish (1) (Fig. 4).

Invertebrates and vertebrates may therefore derive TH from both exogenous and endogenous sources. In this respect, TH resemble vitamin D_3, a cholesterol derivative obtainable by humans as a vitamin from either plant or animal dietary sources, but also capable of being

formed in the dermis from cholesterol by ultraviolet radiation. Indeed, several other similarities exist between TH and vitamin D_3. They both interact with closely related nuclear receptors, and regulation of blood levels of their active forms (T_3 and $1,25(OH)_2$-vitamin D_3) and inactive forms (reverse T_3 and T_2 or $24,25(OH)_2D_3$ and $1,24,25(OH)_3D_3$) is achieved by balancing remarkably analagous systems of activating and inactivating enzymes in peripheral tissues (189). Furthermore, they both may be formed in plants through the photic energy captured from sunlight. Both vitamin D_3 and T_3 are important regulatory chemicals; just as vitamin D_3 does not fit perfectly the definition of a vitamin (since it may be made in the dermis), neither may T_3 fit the exact definition of a hormone (since it may be obtained from the diet).

Exogenous Thyroid Hormones and Sources of Iodide for Salvaging

Thyroid function depends on iodine sources, which may be limiting in certain environments. Iodine insufficiency (endemic goiter) occurs in humans, but rarely in freshwater or terrestrial animals in their natural habitats. Research on dietary iodine availability has focused primarily on total dietary iodine intake or on inorganic (iodide) supplementation. However, as discussed above, a variable and significant proportion of the dietary iodine may include iodine organically incorporated as plant, invertebrate, or vertebrate substances, such as iodinated proteins (scleroproteins and TGI) and conjugated and nonconjugated iodothyronines and iodotyrosines. There may be a selective advantageous for the consumer to access iodine from these varied organic sources. Selection pressure may have favored evolution of enzymes that liberate iodine in a form and at an anatomical site maximizing eventual iodine use by the thyroid (Fig. 4).

Iodine salvaging from iodothyronines involves iodothyronine-specific deiodinases. The mammalian type I deiodinase, which is active in liver, strips iodines from both rings (Fig. 1) and favors iodothyronine substrates after at least partial inactivation by sulfate conjugation (190). Therefore the liver is strategically placed to salvage iodine from excess TH present in portal blood flowing from the gut, and prior to blood passage to systemic vessels (Fig. 4). To date, enteric absorption of TH has been assumed to involve primarily endogenously produced TH, but ingested exogenous TH will be equally prone to uptake from the gut lumen. Thus, enteric absorption of TH not only permits reuse of a large extracorporal store of recyclable TH (163) but also contributes to iodide salvaging of TH from both endogenous and exogenous sources. Whether absorbed TH are recycled as such or degraded to recapture iodide will depend on the exogenous TH load relative to the demands of the body for TH.

As emphasized above, ingesta may also contain iodotyrosines, iodohistidines, and other iodocompounds. It will be advantageous to deiodinate them as well, either by intracellular iodotyrosine/iodohistidine deiodinases or by enteric bacteria. Iodotyrosines are cleared rapidly from rat plasma (191), and potent iodotyrosine deiodinases occur in the liver and other extrathyroidal tissues of several mammals (192–194). It is of future interest to explore extrathyroidal enzyme systems for salvaging iodine from organic sources other than iodotyrosines or iodothyronines.

Evolution of the Thyroid System and the Regulation of Thyroidal Status

The earliest roles of TH may have been as plant protectant substances or as vitamins. With endostyle evolution in protochordate ancestors, there was presumably a selective advantage in supplementing exogenous dietary TH with endogenous TH synthesis. This presumably led to selection of a TG-like molecule derived from a redundant cholinesterase genic duplication and to selection of an IP system promoting TG iodination. When marine chordate ancestors colonized relatively iodine-poor freshwater habitats there may have been strong selection pressures favoring the organization of thyroid tissue into follicles, with their high TH and iodine storage capacities. However, despite the evolution of a thyroid system for endogenous TH production, use of exogenous TH may persist in extant vertebrates, and indeed this is commensurate with TH and iodine economy. Such an open model (Fig. 4) has implications for the evolution of the regulation of thyroidal status.

The main regulation of thyroidal status in higher vertebrates involves the central hypothalamo-hypophysial-thyroidal axis. In conjunction with negative feedback, this axis ensures appropriate thyroidal secretion of T_4 (and T_3). Central control is supplemented by peripheral mechanisms regulating the balance between the formation and degradation of biologically active T_3. Despite the focus of much recent research, the peripheral control mechanisms in mammals and higher vertebrates tend to be considered secondary in importance to central control mechanisms. However, in lampreys, the most primitive extant vertebrates, no central control through a hypothalamo-hypophysial axis has been found, indicating that vertebrate thyroid function preceded its control by the brain and pituitary. In chordate ancestors relying on exogenous TH sources, peripheral mechanisms may have represented, as they currently do for vitamin D_3, the main means of regulating TH availability to target cells. This may reflect the evolutionary appearance of peripheral mechanisms for controlling thyroidal status in lower vertebrates, such as lampreys, prior to appearance of central control mechanisms (1, 195). It may also explain the persistence in higher vertebrates of peripheral mechanisms that con-

tribute to the regulation of thyroidal status—mechanisms lacking in most other vertebrate endocrine systems.

Conclusions

Among invertebrates, only those with notochords (protochordates) possess an endostyle homologous to the vertebrate thyroid. Other invertebrates concentrate iodide, but the iodocompounds synthesized are primarily iodotyrosines. Despite a lack of convincing evidence for iodothyronine synthesis, some invertebrates contain iodothyronines and respond to exogenous iodothyronines. However, at least some plants can synthesize iodothyronines and may be a source of TH to their consumers. Thus, TH may pass through the food chain, and TH should be viewed as vitamins as well as hormones. Indeed, TH, vitamin D_3, and possibly other steroids comprise a class of regulatory chemicals that are available from both exogenous and exogenous sources, and which might be more aptly termed vitamones. Availability of exogenous TH has implications for interpreting TH metabolism, iodine salvaging, and TH regulation. It may also explain the prominent and probably ancestral role of peripheral mechanisms in regulating thyroidal status.

I with to thank Drs. C. Brown, K. G. Davey, J. J. DiStefano, III, J. M. McCleese, D. S. MacKenzie, and T. J. Wiens, as well as K. R. Finnson, K. Kohel, T. Narayansingh, and an anonymous referee for their help.

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