Reflections on the thyroidology of fishes:  
from molecules to humankind

by

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CONTENTS

1. Introduction .................................................................................................................. 3
   i. The emergence of comparative endocrinology as a discipline
   ii. Fish thyroidology: origins and concepts
2. Phylogeny, ontogeny, morphology and evolution of thyroid tissue ............................ 6
3. The hypothalamus-pituitary gland-thyroid tissue axis in fishes ................................. 11
   i. The changing scene: from 1969-1993
   ii. The hypothalamus-thyrotropic cell axis in fishes
   iii. TSH in fishes
   iv. Thyroid hormone synthesis and secretion
      a. Iodide uptake
      b. Incorporation of iodide into thyroglobulin
      c. Secretion of $T_4$ and $T_3$
      d. Production of $T_3$ from the thyroid hormone precursor, $T_4$
   v. Thyroid hormone transport
   vi. Periodicity (rhythmicity) of thyroid hormone secretion in fishes: entrainment by experimental treatments (caveat emptor)
4. Thyroid hormone actions in fishes ............................................................................. 24
5. Thyroid hormones and ontogeny and development of lampreys and fishes .............. 26
   i. Transformation of lamprey ammocoetes
   ii. Transformation of teleost fishes
   iii. The thyroid hormones and early ontogeny
   iv. Special cases
      a. Transformation of pelagic bilaterally-symmetrical flounder larvae to asymmetrical benthic juveniles
      b. Smoltification of salmonid fishes
6. Thyroid hormones and reproduction in fishes ............................................................ 32
7. Thyroid hormones, growth and energy partitioning in fishes .................................... 38
   i. Growth
   ii. Intermediary metabolism
      a. Lipid metabolism
      b. Carbohydrate metabolism
      c. Protein metabolism
   iii. Tissue respiration (thermogenesis)
   iv. Thyroid hormone economy during reduced dietary intake
8. Thyroid gland function in fishes: environmental considerations ............................. 45
   i. Iodide deficiency and "simple goitres"
   ii. Goitres that do not have an enviromental aetiology
   iii. Human health implications of the thyroid lesions in Great Lakes salmon
9. Finalé ........................................................................................................................ 59
10. References cited ......................................................................................................... 61
1. Introduction

Lest constant respiration and great difficulty of speaking and shouting should dry up too much of the trachea and the lungs, nature has provided those glands continually to prepare moisture for these.

Boehlittus (1585) (on the function of the thyroid gland)

The thyroid gland may be defined in a fairly simple way as the tissue which is capable of accumulating iodide in great excess and combining it into an organic compound, thyroxine.

A. Gorbman & H.A. Bern (1962)  
(A Textbook of Comparative Endocrinology)

Begin at the beginning... go on till you come to the end: then stop.”  
L. Carroll (C.L. Dodgson) (1832-1898)  
(Alice Through the Looking Glass)

In the beginning...

Genesis 1:1

This essay is not intended to be a review of the field of fish thyroidology. Rather, it is a personal view of the scope of the field (molecular to environmental) and of landmark developments that have taken place in the field during the last 50 years. I also attempt to put the fish thyroidology field (a small aspect of the much larger “endocrinology” discipline), into some sort of historical perspective. This latter undertaking is fraught with potential pitfalls. One cannot but be impressed by Henry Ford’s view of history (“History is more or less bunk.”, Chicago Tribune, 1916). Moreover, history is viewed through the wrong end of a telescope. As succinctly put by Ferris (1988), “history... is comprehended backward though it must be lived forward, and when we examine our predecessors we bring our own lamp”. In this essay, not only do I bring my own lamp, but my telescope lens is both unfocused and rose-tinted. Furthermore, the axiom that one should never believe any version of cultural history that is written by the culture that is under review, is worthy of note. Propaganda and truth (if indeed truth exists), although theoretically at opposite poles of the same spectrum, are more often than not common bedfellows in our remembrances and perceptions of past events. For all of these reasons, and more besides, it is with trepidation that I begin to write.

i. The emergence of comparative endocrinology as a discipline

Although the discipline of “endocrinology” is now widely recognised as an important sub-discipline of animal physiology, the concept of chemical messengers being carried in the blood from one cell to another is relatively new. Bayliss & Starling (1901/1902, 1902) are usually credited with establishing the field of endocrinology, because it was these authors who coined the term “endocrine” to describe those glands that secrete biologically active substances into the blood. In a series of elegant experiments carried out on dogs, Bayliss & Starling concluded that a biologically active factor (which they termed secretin) was released into the blood from the duodenal mucosa when food was introduced into the duodenum. Secretin, carried from the duodenum to the pancreas via the vascular system, elicited the release of pancreatic enzymes and fluids. Secretin and similar “chemical messengers” were termed “hormones” (from the Greek hormao = to excite, or to put into effect quickly) by Bayliss & Starling (1901/1902), to differentiate them from the more general class of materials (referred to by Claude Bernard as “internal secretions”) such as blood metabolites and excretory products. Bayliss & Starling recognised that the factors that they called hormones, unlike metabolites, exerted specific regulatory actions.

For many years, the traditional definition of a hormone was centred on the secretion of the factor into the blood, and its transfer to a site of action that was distant from the site of secretion. In this sense, it differed from factors such as neurotransmitter substances, which are released at synaptic junctions and exert their effect(s) on the postsynaptic membrane. Paradoxically, the same chemical substance (e.g., epinephrine) could be considered either as a neurotransmitter substance or a hormone depending on whether it appears in the blood following their release from the secretory tissues. Clearly, this strict adherence to the classical definition has little biological value, and as our understanding of the range of biologically active secretory products has evolved endocrinologists have come to view the spectrum of chemical messengers as a continuum. One end of the spectrum is occupied by neurotransmitter substances and locally acting factors (termed “autocrine” if they exert an effect on the cell that secretes them, or “paracrine” if they exert an effect on cells adjacent to the
site of secretion) which do not involve transport of the chemical messenger in the blood. The blood-transported hormones occupy the centre of the spectrum, and chemical messengers that are carried within the lumen of the gastrointestinal tract, or pheromones that transmit chemical signals between organisms, occupy the opposite pole of the spectrum.

While it is certainly true that the definitions "endocrine" and "hormone" were first proposed in the early part of this century, it must be emphasized that the concept of "ductless glands", secreting their product into the blood, has been a familiar theme in oriental and occidental science and medical practice since the 18th century (Medvei 1982). Moreover, humankind has had an intimate familiarity with several aspects of endocrine physiology and endocrine pathology for thousands of years of documented civilization. Perhaps the best example is the widespread practice of castration of domesticated animals that was developed by many agriculturally-based cultures. The practice is (and presumably was) carried out, not to prevent gamete production and transfer, but to modify the behaviour of domesticated animals, and make them easier to control. Castration of human beings, either as a form of punishment, or to produce eunuchs and castrati has also been practiced for several thousand years, and records of the altered behaviour and characteristics of the castrated subjects appear in early writings from Western and Eastern civilizations (Medvei 1982). As was the case with domesticated animals, castration was not necessarily carried out to prevent the subject from breeding (although this probably was the case for eunuchs, the "keepers of the harem"), but was usually undertaken to elicit desired behavioural, metabolic or morphological changes in the subject. This is prima facie evidence that these cultures had an intimate knowledge of the endocrine functions of the testis, as distinct from the gametogenic function of the testis.

Knowledge of endocrine physiology was not limited to reproductive endocrinology. Descriptions of dysfunctional conditions (and the associated signs and symptoms) of the thyroid and adrenal glands are to be found in scholarly works dating back over 3,500 years (Medvei 1982). In the field of thyroidology, not only were the signs and symptoms of thyroid hormone deficiency well-documented in ancient scripts, but the use of (iodiderich) seaweed as a treatment for the condition was also recognized (Medvei 1982).

In more recent times, and particularly in this century, most of the advances in the field of endocrinology have followed on the tail of technological advances in four related disciplines, namely biochemistry, immunology, microscopy and molecular genetics. In biochemistry, advances in steroid, peptide and protein chemistry facilitated the isolation and chemical characterization of most of the major classes of hormones. Advances in the field of immunology, particularly in the production of antibodies, provided the means by which we could measure the incredibly low levels of hormones that are present in the blood of most vertebrates. The development of appropriate embedding materials to support biological materials in the inhospitable low pressure and high temperature conditions that are implicit in electron microscopy technique facilitated major shifts in our perception of the role of membranes in the compartmentalization of cells, and the nature and the secretory mechanisms of some endocrine glands. Last, but certainly not least, during the last two decades the emergence of the discipline of molecular genetics, and the introduction of molecular technologies to endocrine studies has revolutionized our concepts of hormone structure, receptor structures, hormone-receptor interactions and signal transduction (hormone stimulation to target cell response).

Because of the clinical interest in hormone physiology, much of the work that has been undertaken in the field of endocrinology to date has emphasized mammalian, and specifically, human endocrine systems. A specific interest in hormone structure and function of non-mammalian vertebrates and invertebrates animals was late to emerge, and even today, this field of study is usually considered under the separate heading of "comparative endocrinology".

The term "comparative", in the endocrinological context, was coined in the early 1950s to identify those studies that focused on non-mammalian animals, usually comparing the system in the "lower vertebrate" with that of the better known mammalian "model". Early proponents and advocates of this approach include H. A. Bern, I. Chester Jones, J. M. Dodd, W. S. Hoar, A. Gorbman and the Scharrer's (E. & B.); these eminent scientists had the foresight to understand the value of such comparative works, and they set the stage for the rapid growth in the interest in comparative endocrinology that occurred between the 1970s to the present.

In the last 40-45 years, there has been an increasing awareness by comparative endocrinologists of the fallacy and inherent danger of attempting to study comparative aspects of physiology in this inverted evolutionary framework. It is axiomatic that the endocrine systems extant in contemporary "lower" vertebrates have not been derived from homologous mammalian systems; in fact, the reverse may be true. The endocrine physiology of mammals has adapted by selective evolutionary pressures to meet the specific physiological needs of a specific class of terrestrial endotherms. Therefore, there is limited value in the adoption of a "comparative" endocrinological approach to lower vertebrates. Unfortunately, the comparative endocrine literature is replete with examples of studies that have attempted to extrapolate from the mammalian model (e.g., the thermogenic actions of the thyroid hormones) to other classes of vertebrates. The so-called "lower vertebrates"
are faced with entirely different physiological challenges from those of mammals, particularly aquatic and terrestrial ectotherms. Consequently, comparisons of the endocrine control of processes such as metabolism and growth in, for example, fish and mammals are at best difficult to interpret, at worst, biologically meaningless. That is not to deny the surprising commonality throughout vertebrates of the endocrine control of some processes. One particularly fine example of that extreme conservatism is the endocrine control of key events in reproductive endocrinology, despite the remarkable diversity of reproductive styles that have been adopted by different vertebrate species.

Most comparative endocrinologists no longer emphasize the “comparison with the mammalian model.” Rather, they seek commonalities in the endocrine physiology of different vertebrate and invertebrate organisms with a view to establishing underlying principles and evolutionary relationships of the groups. This approach is particularly appropriate for reductionist studies of the physiology of hormone synthesis and hormone receptors and has contributed to major advances in the endocrine field. Another interesting phenomenon that has arisen in the last two decades is the emergence of comparative endocrinologists who focus on the endocrine physiology of a specific taxon, such as fish. For these endocrinologists, the term “comparative” usually relates to a differences among species within the taxon.

Because of the increasing world-wide commercial importance of fish as a food source, for sportsfishing and for the aquarium hobbyist industry, the interest in all aspects of fish biology has grown remarkably within this century. Moreover, because of the centrality of the endocrine system to the regulation of fundamental processes of growth, development, metabolism and reproduction in fish, the study of fish endocrinology, as a discipline in its own right, has developed rapidly both in terms of the number of investigators, and in advances made in the field. In the 1960s the inchoate fish endocrine field was still fundamentally centred on histophysiological studies, and contributions to the physiology of the hormones themselves, and to the chemical nature of the hormones were relatively rare. That approach is in stark contrast to the emphasis placed on receptor biology and molecular genetic studies that typify the 1990s.

In this essay I explore some of these developments in fish endocrinology as they relate specifically to fish thyroidology (i.e., the study of the structure and function of the thyroid tissue and of the function of thyroid hormones in fishes). The focus of the paper is the thyroid gland (tissue). However, since one endocrine gland is influenced by any number of hormones (or autocrine or paracrine factors (see below)), and since the action of one hormone may be (usually is) dependent, directly or indirectly, on the actions of other hormones, it is no longer possible to consider any particular part of the endocrine “system” in isolation. Thus, the significant relationship of hormones of the hypothalamus, pituitary gland, adrenal gland (interrenal tissue), gonads and gastrointestinal tract with the thyroid gland will be discussed.

ii. Fish thyroidology: origins and concepts

The presence of the thyroid gland in mammals (specifically human beings) has been recognised for several thousand years. The recognition of comparable tissue in fishes was not made until the mid-late 19th century. According to Hoar (1939), the first reports of the presence of thyroid tissue in fishes were made by Simon (1844) and Maurer (1886), but it was only with the latter paper and additional studies in the early 20th century (e.g., Guernersch 1911) that the ubiquitous presence of thyroid tissue in fishes (and indeed in all vertebrates) became widely accepted.

Reports of thyroid dysfunction in teleost fishes appeared in papers published late in the 19th century (see below). These initial diagnoses of the thyroid diseases in several species of fishes were confounded by the fact that, unlike the situation in tetrapods, the thyroid tissue of most teleostean fish is not encapsulated by connective tissue. Thyroid follicles are scattered throughout the aerolar connective tissue of the lower jaw (pharyngeal region), usually in the midline, and usually in the vicinity of the ventral aorta. Pathological descriptions of thyroid carcinoma (a malignant cancer) in mammals are based, in part, on cellular characteristics (nuclear appearance, etc.), and also on the presence of thyroid cells outside the thyroid capsule (including foci within blood vessels). In the earliest reports of thyroid enlargement in teleost fish, mammalian diagnostic signs were applied and the lesions were termed “carcinomas” (Bonnet 1883, Scott 1891, cited in Radulesch et al. 1968). However, in the early part of this century, Marine and Lenhart (1910a, b, 1911, 1914) published a series of papers in which they documented in abundant detail the histological appearance of thyroid tissue in goitred brook char, Salvelinus fontinalis; these papers were the first to correctly diagnose the thyroid lesions as simple diffuse goitres. Numerous subsequent publications (e.g., Guernersch 1911, Hoar 1939) have described the distribution of thyroid tissue in many species of fishes, and have described the labile characteristic of thyroid tissue in fishes, and its propensity to occur in some species in so-called “ectopic” (i.e., non-pharyngeal) sites (see Leatherland et al. 1994 for review).
2. Phylogeny, ontogeny, morphology and evolution of thyroid tissue

Let there be light . . .

Genesis 1:1

All juvenile and adult vertebrates possess a distinctive thyroid gland (tissue), comprising thyroid follicle units. Follicles are not found in cyclostome annamcoetes, but endostylylar cells having iodide uptake capabilities that resemble those of the thyroid follicular cells, are present (see below).

In tetrapods, the thyroid follicles are gathered together into discrete, highly vascularized, glands that are either paired (amphibia, birds, mammals) or present as a single midline tissue mass (most reptiles). The cartilaginous fishes also have a single thyroid gland, located in the midline of the lower jaw. In all cases, the glands are contained within a capsule of connective tissue, although some species (e.g., some urodele amphibians) commonly have “accessory” thyroid follicles in other sites.

Most orders of teleost fishes (exceptions include the tuna fishes and parrot fishes), and juvenile and adult cyclostomes lack the “glandular” form of thyroid tissue. In these groups, the thyroid follicles are unencapsulated and are scattered through the connective tissue of the lower jaw from the tip of the heart to the tip of the jaw (Fig. 1a,c, e-f); they are usually aggregated around the ventral aorta, occasionally, as in the pink salmon, Oncorhynchus gorbuscha, arranged in the form of a pseudoglândular (i.e., unencapsulated) cluster (Fig. 1a). However, because there is no discrete gland, and no connective tissue barrier to the migration of the follicles, it is not unusual (at least in teleost fishes) to find follicles in ectopic (unusual) sites. This non-pharyngeal ectopic or heterotrophic thyroid tissue was first described in

Figure 1.

The collection of low power photomicrographs in this figure are of sections of the thyroid tissue of several fish species.

Figure 1a shows pharyngeal thyroid tissue of a sexually mature pink salmon collected from British Columbia. The follicles in this species are gathered together in the form of a highly vascularised “gland” which resembles that of mammals. However, unlike the situation in mammals, the follicles are not contained within connective tissue. This photomicrograph shows “normal” thyroid tissue, but in many ways it resembles that of other species; this emphasizes the importance of establishing the norm for a particular species.

Figures 1b and 1c show similar tissue of a sexually mature coho salmon collected from British Columbia; both micrographs show the basic structure of the follicle, with a squamatous or cuboidal epithelial cell layer encompassing the colloid-filled lumen; vesiculation of the colloid is evident in some follicles. Figure 1b shows a small bunch of follicles adjacent to a large vascular space; these follicles are partially depleted of colloid. Figure 1c shows follicles scattered throughout a highly vascularized region of the lower jaw, adjacent to the ventral aorta.
*Xiphophorus* spp. by Baker in the 1950s (see review by Baker-Cohen 1959), and later seen to occur widely in cyprinid, xiphophorid and poeciliid species (Leatherland et al. 1994). The most common site of ectopic thyroid is the head kidney (Fig. 1d), although other non-pharyngeal sites (eye, brain, spleen) have been identified, usually in animals afflicted by goitres. Evidence based on iodide uptake measurements indicate a higher activity of the head kidney thyroid compared with the pharyngeal thyroid tissue of some species (Chavin & Bouwman 1965).

Except for lamprey ammocoetes, the histological appearance of thyroid tissue is similar in all vertebrates (Fig. 1). The tissue comprises follicles and is usually highly vascular with blood capillaries lying in close prox-
Figure 1d shows a section of the head kidney of a goldfish containing “ectopic” thyroid follicles among the haematopoetic tissue of the anterior kidney. In the same section can be seen interrenal tissue bordering one of the major blood vessels.

Figures 1e and 1f show pharyngeal thyroid tissue in a rainbow trout, in Figure 1e a cluster of follicles is seen adjacent to the ventral aorta. Note the relatively uniform appearance of the epithelial cells of the follicles in this animal, also the vesiculated nature of the peripheral region of the lumen. The clear spaces immediately adjacent to the apices of the follicle cells is probably an artefact caused by the shrinkage of the colloid during tissue preparation. However, the vesicles that are evident in the colloid are the result of pinocytic activity of the follicle epithelial cells. Figure 1f shows a section taken from the same region of thyroid tissue as in Figure 1e above. This section was incubated in a solution of a monoclonal antibody that specifically binds to thyroxine ($T_4$). The sites of antibody binding were located using the avidin-biotin method which results in a brown precipitate forming over the areas that were “immunostained” with the antibody. [Similar approaches have been taken in studies of thyroid gland structure in higher vertebrates (Kameda et al. 1986).] These preparations provide a means of identifying the sites of immunoreactive $T_4$ in the tissue. Note that there is little “immunostaining” in the central regions of the colloid in the follicular lumina, and that the immunostaining is restricted to the periphery where pinocytic activity is taking place. It is hypothesized that the absence of antibody binding to the colloid in the central regions of the lumina indicates that the $T_4$ when incorporated as part of the thyroglobulin matrix does not have available epitope sites for antibody fusion. An unexpected finding was the appearance of immunoreactive sites in some (but not all) of the follicle cells of some (but not all) follicles. The significance of this observation is still not clear.

Figures 1d and 1f also illustrate the relationship of the follicles to the base of the follicle cells. Each follicle is formed of epithelial cells surrounding a fluid-filled lumen; tight junctions, located toward the apices of the follicle cells ensure the relative isolation of the contents of the lumen from the extracellular compartment. The lumen contains a colloidal suspension of an iodide-rich protein, called thyroglobulin. The appearance of the follicles can vary markedly between individuals of a given species, depending on the level of pituitary gland stimulation of the thyroid gland to secrete thyroid hormones. Even within a gland, there is marked variation in appearance suggesting a variable response of the different follicles to the level of pituitary stimulation. In the unstimulated thyroid tissue, the follicles are usually large, their epithelial cells are squamous or cuboidal in appearance, the
nucleus:cytoplasm ratio of the epithelial cells is high, and the colloid within the lumen is usually homogeneous. Following thyrotrophic stimulation of the thyroid tissue, the follicles tend to be smaller, the epithelial cells columnar, the nucleus:cytoplasm ratio smaller, and the colloid partly or wholly depleted. This loss of luminal colloid from the lumen begins at the periphery of the lumen, with the appearance of clear "vesicles", and progresses centripetally.

The ontogeny of the thyroid tissue in lampreys probably provides the best indication of the evolutionary origin of the thyroid gland in fishes and other vertebrates. The form of the thyroid in adult lampreys resembles that of most teleost fishes, with thyroid follicles scattered through the aerolar connective tissue of the lower pharyngeal region. In the ammocoete, however, there are no thyroid follicles per se. Iodide is actively absorbed from the ambient water by specific ciliated epithelial cells of the endostyle (subpharyngeal gland), and these cells synthesize and secrete iodinate amino compounds. Several extensive descriptions of endostyle structure and function have been published (Gorbman & Bern 1962, Barrington & Sage 1972), and a brief overview of the structure will suffice for the purposes of this essay. The endostyle of lamprey ammocoetes (Fig. 2) is situated in the floor of the pharyngeal region of the gastrointestinal tract. It is a complex hollow tubular structure, comprising adjacent paired lateral chambers and a posterior medial chamber; the cavity of the endostyle is connected to the pharynx via a duct. Part of the epithelial layer of the lateral chamber is "glandular" and projects into the cavity of the chamber as a ciliated ridge. Several distinct cell types comprise this ridge-like formation. Type I cells are large columnar mucous-secreting cells embedded in the glandular ridge; their secretory products enter the lateral chamber and are moved toward the intestinal tract by the action of the ciliated cells covering the surface of the glandular ridge. Microorganisms from the ambient water are trapped in the mucous stream and the microorganism-rich mucous thence enters the intestinal tract.

The epithelial cells that occupy much of the surface area of the glandular ridge (type III cells) have the ability to accumulate iodide in a "thyroid-like manner" and synthesize the iodoamino compounds (Gorbman & Bern 1962) and are the most likely progenitors for the follicular thyroid tissue of the adult lamprey (see review by Barrington & Sage 1972).

In the lamprey ammocoete, it is likely that the iodoamino compounds synthesized by the type III cells are secreted into the lumen of the endostyle, and find their way into the gastrointestinal tract, i.e., the secretion is exocrine. However, very high concentrations of iodinated tyrosine compounds such as L-thyroxine (T₄) and triiodo-L-thyronine (T₃) are present in the serum of lamprey ammocoetes (Wright & Youson 1977, Lintlop & Youson 1983a, Leatherland et al. 1990a, J. Youson & J. F. Leatherland, unpublished data), suggesting the presence of an efficient uptake of the compounds from the gut. Moreover, the serum levels of T₄ and T₃ in ammocoetes can change rapidly in response to specific stimulation, such as changes in ambient temperature, treatment with antithyroid agents and stressor challenges, suggesting that the endostyle secretion of these compounds is variable, and that the rate of their delivery into the blood compartment is similarly variable. Whether the iodothyronine compounds have an endocrine function in lamprey ammocoetes (or even in the juvenile and adult periods) is still not clear, but it is reasonable to assume that there is some selective advantage to the remarkable metamorphosis of the exocrine type III cells of the endostyle to an endocrine thyroid gland.

There is general agreement that the thyroid tissue of hagfishes, lampreys and fishes has its origin in some form of endostyle-like organ. Moreover, the structural and functional homology of the protochordate and lamprey endostyles suggest an evolutionary relationship. Further, the analogous (and arguably homologous) features of the ascidian endostyle as regards iodide capture and incorporation into iodinated compounds, provide some support for the ascidian endostyle as the protochordate thyroid progenitor. However, there are several non-endostyle iodide uptake sites in ascidians, and there is no consensus as to which of these sites is the most likely ancestor of the endostyle of lampreys (see reviews by Eales 1979, Specker 1988). Moreover, the capacity of various invertebrates (including non-marine species) to bind iodine in protein form has been known for many years (e.g., Gorbman et al. 1954, Davoli et al. 1991). At least some of these iodination events involve peroxidases, and are strikingly similar to the processes of thyroglobulin synthesis by thyroid follicle cells (e.g., in the brain and ventral cord of earthworms, Davoli et al. 1991). Thus, it would appear that the capacity to iodinate proteins is widespread, it may have evolved several times, and it certainly is not restricted to marine organisms living in an iodide-rich milieu.

As interesting as the various hypotheses of thyroid gland evolution are, the eco-physiological-evolutionary questions concerning the affinity of "pre-vertebrate" aquatic organisms and lamprey ammocoetes for iodide are more intriguing. Iodide entrapment would theoretically have adaptive value for organisms that exploit freshwater (low iodide) environments (e.g., lamprey ammocoetes). However, what is the adaptive advantage for aquatic organisms that occupy seawater, of retaining an immensely efficient iodide capturing mechanism? Intuitively, for these organisms, iodide excretory mechanisms would be more likely to have adaptive value, and one could postulate that the protein iodidination processes of marine organisms serve a prophylactic function.
Figure 2.
This series of schematic diagrams illustrates the morphology of the endostylar organ of ammocoete lampreys. The upper figure shows the relationships between the two lateral chambers and the medial chamber. The median groove (MG) leads into the medial chamber via the median duct (D). The posterior extension of the median chamber (PMC) forms a coil extending dorsally.

The central figures show transverse sections of the endostylar organ in the regions of the anterior lateral chamber and duct (left and right, respectively). In the anterior section (left), the lateral chambers (LC) are separated by a median septum. A ridge of tissue extends into the chamber; glandular structures comprised of mucous secreting (type I) cells are embedded into the ridges on each side, and ciliated cells line the surface of the ridge. In the posterior section (right) the lateral (LC) and median chambers (MC) are shown.

The lower figure shows details of the ciliated ridge of tissue extending into the lateral chamber (LC). Cell types II, III, and IV occupy the surface of the ridge. Of these, only the type III cells (shown in black) appear to have the ability to selectively take up iodide, and are the likely progenitors of the thyroid epithelial cells. Adapted from Kraentzel (1933), Gorbman & Bern (1962), Barrington & Sage (1972)
3. The hypothalamus-pituitary gland-thyroid tissue axis in fishes

Science is a process, not an edifice, and sheds old concepts as it grows.

T. Ferris (1988) (Coming of Age in the Milky Way)

Theories are like withered leaves, which drop off after having enabled the organism of science to breathe for a time.

E. Mach (1960) (The Science of Mechanics)

i. The changing scene: from 1969 to 1993

Figure 3a provides a schematic summary of our understanding of the control of thyroid function in fishes as described in the major review by Gorbman (1969). In brief, (1) some form of hypothalamic control of pituitary thyrotropic cell function was suspected, but it was not known whether it was stimulatory or inhibitory; (2) there was general agreement that the teleostean pituitary gland (under the influence of hypothalamic factors) secreted a thyroid stimulating hormone (TSH); (3) the role of T4 in the negative feedback control of TSH secretion (probably acting via the hypothalamus) was understood.

It should be remembered that at that time, the presence of TSH was only suspected; no such factor had been chemically identified in any fish species. Even today, our direct knowledge of the structure of fish TSHs is meagre, and the evidence in support of the presence of TSH which stimulated iodide uptake, the synthesis of the thyroid hormones and the release of T3 and T4 from the thyroid tissue of fishes, although abundant, is all indirect.

By the end of the 1960s, the relative potencies of T4 and T3 in mammals (with T3 having a much higher potency) were well established, although this was not the case for fishes because the functions of the thyroid hormones were still inadequately defined.

While the 1969 general pattern of hypothalamus-pituitary gland-thyroid tissue control still holds true today, the picture has changed in a number of key aspects, particularly the inclusion of extrathyroidal sites of T3 production (Fig. 3b). It is now generally accepted that there is relatively little T3 released from the thyroid tissue of most vertebrates, and that the major source of the hormone is from the enzymatic conversion of T4 to T3 by the removal of iodide from the outer ring of the thyronine molecule (i.e., by 5'-monodeiiodination) by some peripheral tissues (see iii.d). However, recent evidence from studies of selenium-deficient rats suggests that thyroidal T3 output is significantly higher than was formally believed (Chanoine et al. 1993).

ii. The hypothalamus-thyrotropic cell axis in fishes

In the late 1950s attempts to determine the nature of hypothalamic control of thyrotropic cells in fishes were limited to histological studies of pituitary glands cultured in vitro or grafted at a site away from hypothalamus in the same (autographs) or recipient animal (heterographs) (Ball et al. 1965, Oliverave & Ball 1966, Leatherland 1970, 1971, Grau & Stetson 1977). These studies consistently showed hyperactivity of the thyrotrops of the denervated pituitary glands in several species of fishes, providing indirect evidence of a principal inhibitory hypothalamic control of thyrotropic cell activity in fishes. Additional support for the hypothesis came from the hyperplastic and hypertrophic responses of the thyroid tissue and/or elevated blood T4 levels in the recipient animals, consistent with the concept of an increased level of TSH stimulation (see Schreibman et al. 1973 for review, Grau & Stetson 1977).

Surprisingly, relatively few additional details of this inhibitory control of thyrotropic cell function in fishes have emerged since that time, largely due to the fact that homologous TSH assays have yet to become widely used. (One such assay has been developed for salmonid fish, but except for the initial validation, it has not been extensively applied.) Studies of the role of several hypothalamic factors, including somatostatin, thyrotrophin-releasing hormone (TRH) and melanotrophin-inhibiting hormone (MIH) have provided contradictory results (see review by Leatherland 1988), none of which explain the relatively potent inhibitory control that is indicated by the indirect pituitary graft studies that were initiated in the 1960s.

iii. TSH in fishes

The presence of a pituitary factor responsible for the control of thyroid function in fishes has been widely accepted since the 1950s. Early studies showed a reduction in the apparent activity of the thyroid tissue (based on histological assessment) in hypophysectomized fish,
and evidence of an increase in the activity of the thyroid follicle cells in fishes that were administered extracts of pituitary glands (see reviews by Eales 1979, Leatherland 1982). Such studies suggested direct or indirect involvement of the pituitary hormones in the regulation of thyroid gland activity in fishes. However, the question as to which pituitary hormone(s) was involved, was more difficult to address. Histological methods were applied in an attempt to identify the suspected TSH-secreting cells in fish.

During the 1950s and much of the 1960s, the identification of pituitary cell types was based on the application of polychrome staining techniques (originally developed for the demonstration of connective tissues) to the study of pituitary cytology (see Fig. 4a-c, 5a, 6a). By examining the histological changes that take place in pituitary cells of animals that had been subjected to physiological manipulation (the so-called “histophysiological” approach) it was possible to identify those cell types that might be involved, directly or indirectly, in any given compensatory physiological response. Surgical thyroidectomy was used widely in mammals to identify the thyrotropic cell type; removal of the thyroid, and the subsequent reduction in blood thyroid hormone levels, gives rise to an increased secretion by the thyrotrophic cells, and hence a change in their histological appearance (usually an

Figure 3.

Figures 3a and 3b show a schematic flow chart showing the hypothalamus-pituitary gland-thyroid gland axis in teleost fish, based on information available in the 1960s (Fig. 3a) and 1990s (Fig. 3b). In the 1960s the nature of the hypothalamic control over the pituitary gland secretion of thyrotropic hormone (TSH) was unclear, and the thyroid tissue was thought to be the sole source of both thyroxine (T4) and triiodothyronine (T3). By the 1990s, two parts of the picture had changed (at least in those species that had been investigated). First, the hypothalamic control of TSH secretion in the species investigated appears to be inhibitory (although the nature of the hypothalamic factor responsible is still not clear), and second, the primary source of circulating T3 was argued to be peripheral tissues (such as the liver and kidney) and not the thyroid tissue. [Only the liver is included as a site of peripheral monodeiodination in the figure, but other tissues have deiodinase activity. Moreover, in the figure only 5'-monodeiodinase activity (mda) (catalysing the conversion of T4 to T3) is shown; 5'-monodeiodinase activity (catalysing the conversion of T4 to reverse T3) has also been demonstrated in liver and kidney tissue of some teleostan species].

Figure 3c is a schematic diagram illustrating the patterns of hepatic and renal T4 monodeiodination by outer ring deiodination (ORD) (5'-monodeiodinase) or inner ring deiodination (IRD) (5-monodeiodinase), and the possible actions of non-thyroidal hormones on the process (T = testosterone, E2 = 17β-estradiol, GH = growth hormone, IGF = insulin-like growth factor, C = cortisol, SS = somatostatin).
increase in cell size and nuclear size, a decrease in cytoplasmic granulation and an increase in endoplasmic reticulum). However, surgical thyroidectomy is not possible in teleost fishes because of the dispersed nature of the thyroid tissue. Incomplete thyroidectomy can be induced in some species by administering a radioactive isotope of iodide (usually 131I); the 131I is concentrated by thyroid tissue and the thyroid tissue is damaged by radiation. Also, it is possible to inhibit thyroid function by administering certain anti-thyroid chemicals, many of which prevent the synthesis of thyroid hormones. These types of studies provided indirect evidence to indicate that the thyrotrophic control of thyroid function control was centred on specific basophilic pituitary cells (Schreibman et al. 1973).

These putative thyrotropic cells, which are relatively unimpressive, were described as small basophilic cells. They appeared to contain relatively small numbers of cytoplasmic inclusions (granules) that had an affinity for alcin blue, aniline blue and aldehyde fuchsin and they also exhibited a weak PAS response, suggestive of the presence of carbohydrate moieties (Ball & Baker 1969, Schreibman et al. 1973). Application of electron microscopic methods in the late 1960s and 1970s to fish pituitary studies showed that these putative thyrotrophs had small cell bodies, but they had an extensive stellate form with cytoplasmic extensions projecting between adjacent cells (Schreibman et al. 1973). In the late 1970s and 1980s more direct evidence of the nature of these cells was provided by the application of immunohistochemical methods. "Staining" sections with antibodies to mammalian TSH, specifically using anti-human bTSH (hβTSH), enabled a more direct identification of the putative TSH-secreting cells (Fig. 5b, 6b-c, e-g) (see Farbridge & Leatherland 1986, Farbridge et al. 1990 for references).

The accumulated body of evidence suggests that a TSH molecule (or family of related molecules) is present in the anterior pituitary gland (pars distalis) of teleost fishes. Native mammalian TSH has a potent effect on thyroid function in teleosts (see review by Leatherland 1988), although the reverse may not be true. These preparations have been used effectively to assess the degree of receptivity of fish thyroid tissue to exogenous thyrotropic challenge (e.g., Leatherland & Farbridge 1992, among others), and provide evidence of receptors that are specific to TSH-like preparations. The high specificity of anti-hβTSH for TSH-secreting cells in histological sections of fish pituitary glands suggests that the human and piscine molecules have a similar antigenic property. However, these same antibodies do not bind with fish TSH in plasma (unpublished data), thus emphasising the major immunologic differences between the two species of TSH. More recently, it has been possible, with great difficulty, to purify native teleostean TSH as a distinct fraction from the gonadotrophins (Swanson et al. 1987, Bandyopadhyay & Bhattacharya 1993), but this line of work, apart from these two studies, has not progressed significantly.
Figure 4a shows a sagittal section of the whole pituitary gland of an adult carp (Figure 4a); the section has been stained with a trichrome staining method: alcian blue + periodic acid-Schiff + orange G.

As with all vertebrates, the pituitary gland of teleost fishes comprises an anterior region, that originates embryologically from the roof of the mouth ( Rathke's pouch), and a posterior region that is of nervous origin and comprises neurosecretory axons that have their origin in the hypothalamus of the brain. The anterior region comprises the pars distalis (PD) and the pars intermedia (PI), whilst the posterior region consists of a single region called the pars nervosa (PN).

In the carp, and in many other teleost fishes, the PI and PD interdigitate intimately with one another; in this preparation, the PI cells appear as pale regions interspersed among the alcian blue-stained axons of the PD. The PD contains several major categories of cells, based on their staining characteristics. In this section, one class has stained with alcian blue (these cells, collectively termed "basophils", are thought to secrete glycoprotein hormones such as thyroid stimulating hormone (TSH)), and a second class of cells has stained weakly with orange G (these cells, collectively termed "acidophils", secrete protein hormones such as prolactin and growth hormone). A third category of cells, called chromophobic cells, do not stain with any of the dyes used here, but they cannot be seen in this preparation.

These categories of cells are not randomly distributed in the PD, rather they tend to aggregate into specific regions, giving rise to two more or less distinct zones that are characteristic of the teleostean pituitary gland, a posterior region (the ventral pars distalis (VPD)), and a posterior region (the ventral pars distalis (VPD)). The VPD consists primarily of prolactin secreting cells (seen in Figure 4b as pale orange stained cells) and small basophilic cells which are probably the TSH secreting cells (Figure 4b). The PPD contains growth hormone secreting cells (seen in Figure 4c as pale cells) and large and small basophilic cells; the large cells, many of which can be seen in Figure 4c to contain red stained rod-shaped organelles, are the GtH secreting cells, and the small ones, seen in Figure 4c as spindle-shaped cells are probably TSH-secreting cells.

These figures illustrate the problems experienced by early investigators as regards the identification of cell types, and the attribution of functional roles for the various cells. The "positive" identification of cell types was impossible, although subsequent use of immunostaining methods has essentially validated these earlier studies.
Figure 5.
These photomicrographs of para-adjacent transverse sections of the pituitary gland of a juvenile brook char show a section stained with alcian blue and orange G (Fig. 5a) and a section that was “immunostained” with an antibody specific for human β thyroid stimulating hormone (TSH) to specifically locate the sites of immunoreactive TSH (Fig. 5b). The TSH-secreting cells can be seen to be located specifically among the population of alcian blue stainable cells of the proximal pars distalis.

iv. Thyroid hormone synthesis and secretion

Much of the present state of knowledge concerning thyroid hormone synthesis and release has been derived from mammalian thyroid models, partly because of the clinical interest in the mammalian thyroid, but equally because the glandular mammalian thyroid provides a better model for research than the diffuse thyroid tissue of fishes. As far as can be ascertained, the principles of hormonogenesis and secretion are essentially similar throughout vertebrates, and will only be summarized here. Many of the general details of thyroid hormonogenesis, as we still accept them today (Léssitzky 1990), had been outlined by the early 1960s (Gorbman & Bern 1962). They are illustrated diagramatically in Figure 7, and can be briefly summarized as follows:

a. Iodide uptake

One of the essential characteristics of the thyroid follicle cell (thyrocyte) is its ability to sequester iodide from the extracellular space; such sequestering activity provides the base material for the production of the thyroid hormones which are all iodinated amino acids. In fishes, iodide is taken up from environmental sources via the gills and gut, and is transported in the blood either as free iodide, or in some species bound to plasma proteins (see reviews by Eales 1979, 1985, Eales et al. 1993). Iodide uptake at the basal pole of the thyrocytes is accomplished in mammals (and probably also in fishes) by means of sodium-iodide co-transporter (symporter) proteins that are integral proteins of the basal cell membrane. The symporter proteins couple the energy released by the movement of sodium down its electrochemical gradient with the carrier mediated influx of iodide. The transmembrane sodium gradient that powers the iodide influx is maintained by the activity of sodium + potassium – dependant ATPases that are also integral proteins of the membrane in this part of the cell.
membrane (Golstein et al. 1992, Carrasco 1993). Within the epithelial cells, iodide moves along an electrochemical gradient from the basal to apical pole; the gradient is established by differential potential differences across the membranes at the two cell poles (approximately 45 mV at the base and 0 to -10 mV at the apex).

b. Incorporation of iodide into thyroglobulin

At the apical cell membrane the iodide is oxidized and reacted with the glycoprotein, thyroglobulin; the process is catalyzed by thyroid peroxidase which is probably a component of the apical cell membrane of the epithelial cells. However, this step probably occurs in the lumen since the peroxidases have their active sites directed toward the colloid; thus, iodide must enter the luminal compartment in the non-oxidized form. Iodide channels have been identified on the apical membranes

Figure 6.
The figure shows photomicrographs of sections of the pituitary glands of coho salmon; Figures 6a to 6e are from sexually mature fish, and Figures 6f and 6g from free embryos.

a. This section was stained with alcal blue, periodic acid-Schiff and orange G, and shows part of the proximal pars distalis region. Alcin blue stainable cells of different shades and sizes are in evidence, but it is not possible to confidently place them into distinct categories based on staining characteristics, even less into categories based on function.

b. and c. These sections, from a similar region to Figure 6a, were immunostained with antibodies raised against human β thyroid stimulating hormone (hβTSH) and show the distribution and morphology of cells that contain immunoreactive TSH, the presumptive TSH-secreting cells. They are relatively small cells that tend to be located at the periphery of the cords of cells.

d. and e. These para-adjacent sagittal sections were immunostained with a monoclonal antibody raised against a salmonid growth hormone (GH) (Fig. 6d) or anti-hβTSH (Fig. 6e), as described in Figures 6b and c. Both the large, intensely immunoreactive GH-secreting cells and the weakly immunoreactive TSH-secreting cells share a common distribution through the proximal pars distalis region.

g. and h. These sagittal (upper figure) and transverse (lower figure) sections of the pituitary gland of coho salmon free embryos (approximately 2 weeks after hatching) were immunostained with anti-hβTSH as described in Figures 6b and c, to show the presence of well-developed TSH-secreting cells at this stage.
of thyrocytes (Golstein et al. 1992, Carrasco 1993). The peroxidase probably has two active sites, one that catalyzes the oxidation of iodide to iodine or a free radical of iodine, and one that catalyzes the oxidation of the phenol groups of diiodotyrosine (DIT) residues, thus giving rise to tetraiodothyronine residues.

Iodination of the thyroglobulin molecule occurs independently of the synthesis of thyroglobulin. It occurs at specific tyrosine sites on the highly structured thyroglobulin molecule giving rise to DIT and also moniodotyrosine (MIT) (Fig. 8). Peroxidase activity facilitates the intramolecular coupling of two DIT residues (not necessarily of the same chain) of the thyroglobulin chain to produce tetraiodothyronine, T₄; coupling of MIT with DIT results in the formation of triiodothyronine, T₃ (Fig. 8). At this stage, the iodinated thyronine compounds are an integral part of the thyroglobulin molecule. They represent a considerable reserve of thyroid hormones, but need to be released from the thyroglobulin (by digestion) before they can be secreted into the blood.

c. Secretion of T₄ and T₃

The secretion (release) of thyroid hormones from the follicle is initiated by aliquots of the thyroglobulin being taken into the follicle cell by micro- or macropinocytosis; these aliquots appear as pinocytic vesicles within the cell. Lysosomes, containing proteases, fuse with the pinocytic vesicles. The iodinated thyronine and tyrosine residues are released from the thyroglobulin by proteolysis, and the residues are released into the intracellular compartment. DIT and MIT are largely deiodinated intracellularly, whilst free T₃ and smaller amounts of T₄ are secreted into the blood by mechanisms as yet unknown.

d. Production of T₃ from the thyroid hormone precursor, T₄

Although relatively little T₃ may be released from the thyroid gland of mammals or thyroid tissue of fishes, it is generally considered to be the active form of thyroid hormone. It is proposed that most of the circulating T₃ is produced by non-thyroidal tissues by the enzymatic (5'-monodeiodinase) removal of one of the iodide units of the outer tyrosine ring of T₄. In fishes (and at least one lamprey), the liver is a major site of T₃ production, and the kidney and gills also contribute (see reviews by Eales 1985, Leatherland et al. 1990b, also Leatherland 1981, Byamunga et al. 1992, MacLatchy & Eales 1993, Mol et al. 1993) (Fig. 3). An intriguing recent observa-
This schematic diagram illustrates the processes of thyroid hormone synthesis and release. Hormonogenesis involves (a) uptake of iodide (I\(^{-}\)) from the extracellular space (ECS) by sodium (Na\(^{+}\))–I\(^{-}\) co-transporter proteins (CT) located in the basal cell membrane of the follicle cell (thyrocyte), (b) the intracellular movement of the I\(^{-}\) along an electrochemical gradient in the thyrocyte and the efflux of that I\(^{-}\) into the follicle lumen via I\(^{-}\) channels (IC), (c) the iodination of the tyrosine residues in the thyroglobulin glycoprotein [this process is catalyzed by peroxidases (POx) that are located in the apical membrane and have their active sites directed toward the lumen, and results in the formation of mono- (MIT) and diiodotyrosine (DIT) at specific sites on the thyroglobulin molecule], (d) the condensation of MIT and DIT, or DIT and DIT within the lumen to produce the iodinated thyronine residues, triiodothyronine (T\(_{3}\)) (MIT + DIT) or tetraiodothyronine (thyroxine, T\(_{4}\)) (DIT + DIT) units. [At this stage, these iodinated tyrosine and thyronine units are still part of the thyroglobulin molecule.]

The extracellular stores of thyroglobulin represent the major reserve of the thyroid hormones, but they need to be released from the lumen, into the blood compartment before they can be exert a biological effect. The release (secretion) of the thyroid hormones involves (a') pinocytosis of the thyroglobulin by the apices of the follicle cells, (b') the fusion of the pinocytic vesicles with lysosomes, (c') proteolysis of the thyroglobulin, releasing the iodinated tyrosine and thyronine residues, and (d') the movement of free T\(_{4}\) and to a lesser extent of free T\(_{3}\) out of the follicle cell into the ECS. The MIT and DIT released during proteolysis are largely degraded and the iodide reused.
These diagrams illustrate the molecular structure of tyrosine and some of the major iodoated tyrosine and thyronine compounds. Mono-I (MIT) and diiodotyrosine (DIT) are derived from the tyrosine precursor, tetraiodothyronine (thyroxine, T₄) and 3,5,3' triiodothyronine (T₃) are condensation products of MIT with DIT (T₃), or two DIT residues (T₃). T₃ is also produced by the enzymatic (5'-monodeiodinase) removal of iodide from the outer ring by peripheral tissues, such as liver and kidneys. A biologically inert form of T₃, called reverse T₃ (rT₃), is also produced by the enzymatic (5'-monodeiodinase) removal of iodide from the inner ring.

In mammals, in addition to the removal of iodide from the outer ring (5'-monodeiodination), there is also monodeiodination of the inner tyrosine ring (by 5'-monodeiodination) giving rise to 3,3',5'-T₃ (reverse T₃ (rT₃)) (Fig. 3c, 8). rT₃ has no known biological activity, although the presence of both 5- and 5'-monodeiodination pathways enables the fine-tuning of T₃ production, by converting "excess" T₄ into a non-biologically active product which is further deiodinated to T₂, T₁ and thyronine (T₀). The production of rT₃ in fish may be be much lower than that in mammals, although it does occur under some conditions, particularly when substrate levels are high (Byamungu et al. 1990, Eales et al. 1993a, b, Morin et al. 1993).

In vivo 5'-monodeiodination activity is reduced by elevated by plasma T₃ levels (Scott-Thomas et al. 1992, MacLatchy & Eales 1993), suggesting an autoregulation of T₃ production. In addition, several non-thyroid hormones, including, growth hormone (GH), somatostatin, cortisol, testosterone and 17β-estradiol (Fig. 3c) (see review by Leatherland 1988) also affect hepatic T₃ production, and somatostatin has been found to influence renal T₃ production (Fig. 3c) (Byamungu et al. 1992).
The actions of the steroid hormones on hepatic 5'-monodeiodinase activity appear to be direct, whereas the GH action may be induced by GH-stimulated increases in insulin-like growth factor (IGF) secretion; this conclusion is based on the observation that GH is without effect on 5'-monodeiodinase activity in in vitro hepatocyte preparations (Sweeting & Eales 1992).

The biological value of this dual control of thyroid hormone economy is the partitioning of the regulation of the blood levels of prohormone (T₄) independently of the regulation of the production of the biologically active hormone, T₃. Fluctuations in blood levels of T₄ levels, are thus ameliorated by the independent regulation of T₃. Also, the production of T₃ can be adjusted to meet the metabolic needs of the organism, without necessarily adjusting the “thyrostat” of the negative feedback loop that controls the blood levels of T₄.

v. Thyroid hormone transport

In mammals, a major part of the total plasma T₄ and T₃ (as well as other hormones, such as steroid hormones, prolactin and growth hormone, amongst others) is reversibly bound to blood proteins, with only a small percentage of the total hormone levels being present in the “free” form. Since it is the “free” form of the hormone that constitutes the physiologically active fraction, it is sometimes necessary to measure both “free” and “bound” thyroid hormone levels in order to properly assess thyroidal status. The situation appears to be similar in fishes, although there have been relatively few studies of “free” thyroid hormone concentrations in lower vertebrates. The best data for teleost fishes are for Arctic char (Eales & Shostak 1985) which have nmolar concentrations of “free” thyroid hormones compared with pmolar concentrations of total T₄ and T₃ concentrations. However, in a survey of 16 species of tropical marine fish, Eales & Shostak (1987) showed that the range of free T₄ and T₃ levels within and among species varied by three orders of magnitude. Such observations belie the widespread practice of extrapolating findings made in one species (usually a temperate freshwater species, and often a salmonid) to teleost fishes generally.

In fishes, the carrier protein is most likely a prealbumin-like protein fraction, whereas in mammals globulin, prealbumin and albumin fractions appear to be involved. The adaptive advantages of transporting a high percentage of hormone in a bound (and thus biologically inactive form) are still not fully explored. They would not appear to be essential for normal thyroid hormone use since clinical studies of human beings with congenital absence of thyroid hormone binding proteins reveal normal thyroid hormone function (Lissitzky 1990). The bound hormone can act as a source of readily available “free” hormone as the latter is removed from the circulation by target tissues, or excreted following the formation of glucuronide or sulphate conjugates. This “buffering” effect of the blood proteins enables an effective and rapid homeostatic control of “free” hormone levels without necessitating an “up-” or “down-regulation” of the pituitary-thyroid axis. For some hormones, binding to plasma proteins permits higher levels to be carried than could otherwise be accounted for by simple solution. However, thyroid hormone concentrations are usually low enough that they do not need to be bound to hydrophilic protein. Speculations on the adaptive value of binding proteins include the enhancement of the delivery of thyroid hormones into the blood, the enhancement of hormone uptake by target cells (via the interaction of the binding protein with the target cell membrane), and the prevention of undue loss of hormones via the kidney (because the hormone-binding protein complex is too large too cross the glomerular barrier).

Regardless of their physiological function(s), if any, the presence of thyroid hormone binding proteins has implications for thyroidologists. Factors which alter the blood levels of these binding proteins, or which alter the binding hormone to the proteins (as is the case for some pharmaceutical products) will give rise to changes in total thyroid hormone levels, without necessarily altering the concentrations of “free” hormones. One of the most cited examples of this is the change in total plasma thyroid hormone concentration in pregnant women. When this response was originally identified, it was interpreted to indicate an active role for the thyroid in pregnancy. It was later appreciated that although there was a change in total thyroid hormone level, the “free” hormone levels were not significantly changed. What had changed was the estrogen-induced production of thyroid hormone binding protein, which in turn, had shifted in the “free”:bound hormone ratios, and induced a compensatory increase in the total level of thyroid hormones ensuring the maintenance of the required “free” hormone levels (Ingbar & Woebner 1981).

The significance of hormone binding for the researcher or clinician is that it raises the question as to whether measurements (and changes in concentration of) total thyroid hormone concentrations have any meaning. That question is still unresolved, although in salmonid fishes there is some evidence to suggest that total and “free” thyroid hormone levels parallel one another, so that total values can be used as an indicator of “free” hormone levels (Eales & Shostak 1985). However, it should be emphasized that this relationship has been demonstrated only for a limited set of conditions, and it should not be assumed that the relationship holds under all conditions.
vi. Periodicity (rhythmicity) of thyroid hormone secretion in fishes: entrainment by experimental treatments (caveat emptor)

It is a capital mistake to theorize before one has data.

A. C. Doyle (1859-1930)
(The Memoirs of Sherlock Holmes)

Nam et ipsa scientia potestas est.
F. Bacon (1561-1626)
(Religious Meditations. Of Heresies)

Hormones are not secreted in a continuous manner. Some hormones (e.g., the hypothalamic hormones and some of the pituitary hormones) are secreted in a pulsatile manner, with a predictable ultradian rhythm of release into the the blood. Other hormones are secreted in response to changes in blood chemistry, which are in turn affected by altered activity and/or changes in the feeding activity of the organism. Because activity (including feeding activity) is cyclical, the secretion of these hormones (including the thyroid hormones) that are involved in the regulation of metabolite partitioning processes is similarly cyclical in almost every species that has been investigated to date.

Most comparative endocrinologists freely accept the possibility (indeed the probability) of daily and seasonal changes in thyroid hormone secretion in most vertebrates, including fish. Such circadian (daily), circasyzygic (semi-lunar) and circannual (yearly) rhythms in thyroid hormone secretion are well documented in a selected number of fish species (see reviews by Eales 1979, Leatherland 1982, Boujard & Leatherland 1992, Leatherland et al. 1993a, Spieler 1993). What is remarkable, however, is that despite this tacit recognition of the phenomenon, most comparative endocrinologists are either naively unaware of the significance of such rhythms to their experimental design, or they choose to ignore them. The literature is replete with examples of experiments in which data are collected (blood and other tissue samples taken) from animals at only one time of the day. Ironically, the most commonly expressed justification for this practice is a statement to the effect that sampling at a set time of the day eliminates circadian influences from the data set. Nothing is further from the truth! Time of day of treatment with any procedure (be it feeding, handling or drug-treatment) has an implicit entraining effect on the circadian rhythms of most physiological parameters, and as discussed so succinctly by Meier (1993) and Spieler (1993). As is illustrated in a simplistic manner in Figure 9, if a given treatment phase-shifts the rhythm, even though there may be no net effect of the treatment if data are collected over a 24 hour period, once-only measurements would lead one to conclude that a particular treatment is stimulatory, inhibitory or without effect, depending solely on when the sample is made. Such experimental design obfuscates rather than illuminates, but, sadly, it continues to be widespread.

Many hormonal rhythms in fishes, as in other vertebrates, are influenced (entrained) by the act of feeding and/or the cascade of metabolic events that follow feeding (see reviews by Boujard & Leatherland 1992, Spieler 1993). This is particularly true if the fish are administered food at a set time of day, rather than being permitted to feed ad libitum. For physiologists, consideration of all aspects of feeding, both the feeding procedure (i.e., time of day of feeding, and whether it is administered "on demand" or at set times of the day) and the type and quantity of food made available, is paramount in experimental design. If a particular treatment influ-

![Figure 9](image_url)

The schematic diagram illustrates the problems of interpretation of data when circadian rhythms of a given physiological parameter (such as blood hormone level) are phase-shifted by the treatment. The Y-axis represents a measured physiological parameter, such as blood thyroid hormone levels, as measured over the period of time (the X-axis). The two waves, a and b, illustrate changes in the measured physiological event in response to different treatments (e.g., control and experimental group). If we take a mean measurement of the parameter over the entire time period, then a and b have identical values. However, if measurements are made selectively within the same time period, the observer would make one of several possible deductions, depending on when the observation was made: a is greater than b (at times indicated by 1), a = b (at times indicated by 2), or b > a (at times indicated by 3).
ences feeding activity, then it is also likely to change the patterns of the hormone rhythms, and any recorded responses to the treatment will also be influenced accordingly. Paradoxically, although most fish physiologists accept, as given, that certain treatments (handling, injection, etc.) influence the feeding activity of their experimental animals, they often compensate for such effects by removing food for the duration of the experiment (on this count, I also have to plead guilty)! The consequences of such a decision for interpretation of the results are self-evident. What is perhaps even worse, is the fact that many investigators fail to report whether or not their experimental animals were fed at all (or were feeding) during the critical periods of their experiment (Boujard & Leatherland 1992). Consequently, the literature dealing with hormonal rhythms in fishes, as in most vertebrates, is confused and confusing. There is still much to be learned about the physiological significance of these rhythms, and most of what we think we know is likely to be wrong.
4. Thyroid hormone actions in fishes

...and there was light.

(i. Thyroid hormone actions at a molecular level)

The thyroid hormone, T₃, is generally considered to be the biologically active form of the hormone, with T₄ acting as a precursor or prohormone. Saturable specific T₃ binding sites were first reported in the nuclei of rat liver and kidney cells in 1972 (Oppenheimer et al. 1972). More recently, molecular cloning technology has enabled the characterization of this T₃ nuclear receptor in mammals, and of great interest was the concomitant finding of tissue specific differences in the T₃ receptor molecule (Lissitzky 1990).

Far less is known of the characteristics of the putative nuclear T₃ receptors in aquatic ectotherms. Saturable nuclear binding sites have been demonstrated in the hepatocytes of several salmonid species and lampreys (Van Der Kraak & Eales 1980, Darling et al. 1982, Lintlop & Youson 1983b, Lebel & Leloup 1989, Ichikawa et al. 1989, among others). Although the molecular form of the T₃ receptor of fish has yet to be elucidated, studies in amphibians provide convincing evidence for and β forms of thyroid hormone receptors (e.g., Brooks et al. 1989, Old et al. 1992, Shi & Brown 1993) that operate in a manner that is identical to those of mammals. All evidence to date suggests considerable conservation of the characteristics of the T₃ receptors in vertebrates.

In mammals, most (if not all) of the actions of thyroid hormones that can be demonstrated to occur at the cellular level in response to physiological levels of hormones have a latent period of several hours to several days. This is taken to indicate that the responses depend upon transcription processes and that the responses are the result of the stimulation of the synthesis of specific proteins. We have too few examples of such responses in fishes to be sure that the same is true for that group, although it is likely that a similar modus operandi is present.

One of the best known responses of mammalian cells and tissues to T₃ stimulation is that of increased metabolic rate and increased heat production (thermogenesis). Since the mitochondria are the major organelles involved in heat generation, there was reasonable ground to explore whether there are T₃ receptors in the mitochondria, in addition to nuclear receptors. Putative saturable receptors have been demonstrated in mammals, although the findings are still controversial (Ingbab & Woeber 1981). Several earlier papers also reported effects of T₃ on oxygen consumption of mitochondrial preparations (Ingbab & Woeber 1981) which would support an action of the hormone at a non-nuclear site. However, the T₃ concentrations used for such studies were extremely high, most certainly in the pharmacological range. (Most studies employed T₃ concentrations that exceeded plasma hormone levels, and it must be remembered that cytosolic in vivo free T₃ concentrations will be considerably lower than this, perhaps by an order of magnitude.) In fact, evidence for responses of mitochondrial preparations to T₃ challenge at physiological ranges is not available, and thus most evidence at this time would support the concept that the hormone acts via its effects on gene activation in the nucleus (Lissitzky 1990).

(ii. Thyroid hormones and physiological processes)

The real history of science is a maze, in which most paths lead to dead ends and all are littered with the broken crockery of error and misconception.

T. Ferris (1988)
(Coming of Age in the Milky Way)

Aubrey Gorbman's (1969) review of the actions of thyroid hormones in fishes (Agnatha, Chondrichthyes and Osteichthyes) comprised 6 pages of text, and touched on five areas of physiology in which some action of thyroid hormones had been proposed (Table 1). His review, which represented the first major synthesis of the field since that of William Hoar in 1957, cited 49 original (i.e., non-review) publications dealing with the physiological effects of thyroid hormones in fish.

The late 1960s saw the advent of widely available sensitive radioimmunoassays for the measurement of thyroid hormone levels in small volumes of plasma or serum in fishes (and other vertebrates). This technological development prompted a renewed interest in comparative thyroidology, and in the fish thyroidology field, there was an outpouring of papers devoted to the re-exploration of the roles of the thyroid hormones in that group. It was this renewal of interest in the field that led to the publication of two reviews of thyroid physiology of fishes (Eales 1979, Leatherland 1982). While these reviews by Geoff Eales and myself were considerably longer than that of Aubrey Gorbman (1969) (each comprising some 12 pages of text devoted to the actions of thyroid hormones), and although each cited many more original papers than Gorbman's review, the list of possible areas of thyroid hormone involvement had not changed substantially in the decade between the early
and later reviews (Table 1). Major advances had been made in two main areas. Firstly, our understanding of the processes of thyroid hormone secretion and metabolism had been significantly enhanced by Eales and co-workers. Secondly, some movement had been made toward a better understanding of the biotic and abiotic factors affecting thyroid function in fishes, particularly the modifying influence of hormones other than TSH on the activity of the pituitary gland-thyroid tissue axis (see reviews by Eales 1979, Leatherland 1982, 1988).

During the last two decades there has been particular interest in the role of the thyroid hormones in three main areas of fish biology: (1) ontogeny and development (including salmonid smoltification), (2) reproduction, and (3) metabolism and growth. It is these topics that will form the basis of the following section of the essay.

Most of the advances that have been made in these three areas have been facilitated because of our better understanding of thyroid hormone economy in fishes, particularly as regards the factors that influence the negative feedback control of T₄ secretion, and of the factors involved in the synthesis of T₃ by peripheral tissues. These advances have shown how thyroid function is influenced by non-thyroid endocrine factors (e.g., the interaction between thyroid hormones and GH and cortisol) and thus related to the energy sparing activities and energy partitioning processes of fishes.

Table 1. Reported actions of the thyroid hormones in fish: Topics dealt with in the reviews published since that of Gorbman (1969).

<table>
<thead>
<tr>
<th>Topic</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seasonal changes in thyroid activity</td>
<td>2, 5</td>
</tr>
<tr>
<td>(including responses to changes in ambient temperature)</td>
<td></td>
</tr>
<tr>
<td>Migration</td>
<td>2, 5</td>
</tr>
<tr>
<td>Ontogeny and development</td>
<td>2, 5</td>
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<tr>
<td>Reproduction</td>
<td>2, 5, 6</td>
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<tr>
<td>Growth</td>
<td>1, 2, 4, 5</td>
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<tr>
<td>Intermediary metabolism</td>
<td>2, 3, 4, 5</td>
</tr>
<tr>
<td>Osmotic and ionic regulation</td>
<td>2, 4, 5</td>
</tr>
<tr>
<td>Nervous system development and function</td>
<td>2, 4, 5</td>
</tr>
<tr>
<td>Pigmentation</td>
<td>2, 4, 5</td>
</tr>
</tbody>
</table>

References:
1 = Donaldson et al. (1978); 2 = Eales (1979); 3 = Eales (1988);
4 = Gorbman (1969); 5 = Leatherland (1982); 6 = Leatherland (1987);
7 = Plisetskaya et al. (1983).
5. Thyroid hormones and ontogeny and development of lampreys and fishes

The central role of thyroid hormones (probably T3) in the initiation and regulation of the metamorphosis of the amphibian tadpole to the juvenile form (definitive phenotype) is one of the best known, unequivocal examples of thyroid hormone action in vertebrates. This remarkable effect of the thyroid hormones was first demonstrated by Gudernatsch early in this century in tadpoles that were fed pieces of horse thyroid gland (cited by Gorbman & Bern 1962). Numerous subsequent studies, using purified T4 and T3, have provided conclusive support for the hypothesis that these hormones are the main regulatory factors in all stages of the metamorphic process (see reviews by White & Nicholl 1982; Galton 1988).

Because of this striking action of thyroid hormones in amphibian metamorphosis, not surprisingly, there has been periodic interest in the possible role of the thyroid hormones in comparable “metamorphic” processes in other vertebrates. Among the non-tetrapod groups, several such events have attracted attention. In lampreys, the development of the filter-feeding ammocoete into a free-living or parasitic juvenile form, involving a complex array of morphological and physiological changes is usually considered a form of metamorphosis, as have several examples of ontogenic and/or developmental changes in teleostean fishes (see below).

The term “metamorphosis” simply means transformation. Traditionally, the term has been applied to describe those rapid morphological/physiological changes that follow a more stable period of slow development (Flegler-Balon 1989, Balon 1990). Since all organisms undergo a change in form (sometimes interrupted by relatively stable episodes) throughout their entire life cycle, one could argue that all organisms undergo continuous metamorphosis (but see Balon 1986). Thus, it is a non sequitur to restrict the consideration of thyroid hormone action to arbitrary ontogenic events in a limited number of species. In the following section, I examine the possible roles of thyroid hormones in the ontogeny of fishes generally, regardless of whether the event can be accurately described as metamorphic.

i. Transformation of lamprey ammocoetes

There is little evidence to support a stimulatory role of T3 in the transformation of lamprey ammocoetes into juveniles, in fact, if there is a role for the thyroid hormones in the metamorphosis of lampreys, they appear to have an inhibitory action. It is not possible to induce metamorphosis by treating lamprey ammocoetes with thyroid hormone preparations (a situation that is contrary to the situation in amphibians). Moreover, whereas in amphibians the plasma thyroid hormone concentrations (and T3 receptor gene expression (e.g., Shi & Brown 1993)) increase as metamorphosis progresses, such is not the case in lampreys. In both Petromyzon marinus and Geotria australis, serum thyroid hormone concentrations are highest in ammocoetes and fall precipitously at the onset of metamorphosis (Fig. 10; Wright & Youson 1977, Lintlop & Youson 1983a, Leatherland et al. 1990a, Youson et al. 1992).

In an attempt to determine whether thyroid hormones inhibit metamorphosis of lampreys, several groups have attempted to induce metamorphosis by treating ammocoetes with goitrogenic chemicals. In at least two studies (Suzuki 1986, Holmes & Youson 1994) immersion of ammocoetes of an appropriate size in goitrogens, such as potassium perchlorate promoted the onset of an apparently imperfect metamorphosis in Lampetra and Petromyzon species, respectively; in the case of the Petromyzon marinus study, this change was associated with a significant decrease in serum T3 concentration, but not in serum T4 concentrations (J. A. Holmes, J. H. Youson & J. F. Leatherland, unpublished data). However, this response is not always found. In similar studies with Geotria australis ammocoetes which were administered propyl thiouracil at levels that profoundly depressed serum thyroid hormone levels, there was no evidence whatsoever of initiation of metamorphosis (Leatherland et al. 1990a). Thus, the possible role of the thyroid hormones (if any) in the process if far from clear, but one is forced to conclude that it is not one that compares in any way to the role played by T3 in amphibian larvae.

Are there other possible explanations for the tantalizing, but tentative links that appear to be emerging between thyroid hormone withdrawal and the onset of metamorphosis? Two must be given some consideration. First, during metamorphosis the endostyle itself undergoes a radical transformation into the definitive thyroid tissue. The morphological changes in the endostyle begin shortly after the onset of metamorphosis, and the definitive thyroid tissue does not emerge until late in the process; could it be that the decline in serum thyroid hormone concentrations during this period simply reflects the altered ability of the endostyle/thyroid tissue to deliver thyroid hormones into the blood compartment? Second, although goitrogens are used experimentally to induce hypothyroidism, their site of action is not necessarily restricted to the
Figure 10.
The figure shows the changes in the concentrations (expressed as nmole L⁻¹; mean ± SEM) of thyroid hormones (thyroxine, T₄ and triiodothyronine, T₃) in the serum of the lamprey, Geotria australis as they undergo transformation from ammocoete to juvenile states. The animals were collected from streams in the southern regions of Western Australia. Both serum T₄ and T₃ levels were extremely high in the ammocoetes; in fact, the thyroid hormone concentrations found in lampreys represent some of the highest values found in any organism to date. A feature of the transition from ammocoete to transforming animal is the rapid fall in serum thyroid hormone concentrations, which then remain at relatively low levels throughout transformation. The increase in thyroid hormone levels in the stage 6 and 7 transformers compared with the stage 5 animals was significant, and may be biologically important. (Modified from Leatherland et al. 1990a).
thyroid epithelial cells (or endostyral cells, in the case of the ammocoetes). Moreover, we must remember that goitrogens are toxic materials. The possibility that the induction of metamorphosis by some goitrogens (such as the perchlorates), but not others (such as propyl thiouracil), is related to the toxicology of the goitrogens used, and not to the effects on thyroid hormone secretion per se, cannot be excluded. If, for example, the goitrogen causes metabolic (perhaps hepatic or endostyral) change, it is possible that it is these metabolic changes are the primary inducers of metamorphosis.

**ii. Transformation of teleost fishes**

**a. The thyroid hormones and early ontogeny**

As I am sometime reminded by the writings of my colleagues, Eugene Balon and Christine Flegler-Balon, the intervals of ontogeny of fish (and other animals) do not blend, one into the other, in a graded manner. On the contrary, during early ontogeny the development is characterized by a sequence of distinct, and usually abrupt, rapid changes that are interspersed by periods of relatively slow change (see Balon 1986, 1990, 1991, Flegler-Balon 1989). In most instances, these abrupt changes signify the start of a specific functional (and possibly functioning) tissue or organ system within the organism. As a result, the homeostatic controls, and interactions that previously existed between the tissues/organ systems is forever changed. This interrupted developmental pattern is essentially similar to that exhibited during the metamorphosis of amphibian larvae into juveniles, and begs the question as to whether the endocrine control (in particular, the action of the thyroid hormones) of the two events is similar.

Abundant evidence of an apparent direct affect of thyroid hormones on some aspects of the ontogeny of fishes has been derived from several sources, some specific examples of which have already been discussed above. In addition, the immersion of embryos in a solution of \( T_4 \) has been shown to increase the rate of yolk absorption in carp and tilapia representitive species (Lam 1980, Nacario 1983, Lam & Sharma 1985). However, paradoxically, Reddy & Lam (1991, 1992) showed that immersion of both denuded embryos, and embryos with the egg envelopes intact in a solution of either \( T_4 \) or \( T_3 \) delayed the secretion of hatching enzymes.

![Figure 11.](image)

The figure shows the changes in the whole organism content of thyroxine (\( T_4 \)) and triiodothyronine (\( T_3 \)) (expressed as ng organism\(^{-1}\); mean ± SEM) in the eggs, embryos and larvae of chinook salmon. The asterisks and plus symbols represent the periods of first eye pigmentation and hatching, respectively. In this species, the tissue thyroid hormone levels fall steadily between the time of fertilization and hatching, but the most marked change is during absorption of the yolk sac. (Modified from Leatherland et al. 1989a).
In 1987, two laboratories, one in the U.S.A. and one in Japan independently published reports of remarkably high levels of T₄ in the eggs, embryos and larvae of coho (Kobuke et al. 1987) and chum salmon (Tagawa & Hirano 1987). Subsequent studies showed this to be true, not only for salmonid species, but also for a wide spectrum of freshwater and marine teleost fishes (Brown et al. 1987, 1988, 1989, Greenblatt et al. 1989, Leatherland et al. 1989a,b; Tagawa et al. 1990a,b, Reddy et al. 1992, Leatherland & Barrett 1993). Of particular interest was the observation that the egg content of thyroid hormones was related to maternal plasma thyroid hormone levels. If the broodstock females was administered T₄ or T₃, then egg T₄ or T₃ content was elevated (Brown et al. 1988, P. A. Flett, K. R. Munkittrick, G. Van Der Kraak & J. F. Leatherland, unpublished data). Conversely, when plasma thyroid hormone levels of the broodstock females were lowered by administering goitrogens, then egg thyroid hormone content was also lowered (Tagawa & Hirano 1991, Ayson & Lam 1993). In agreement with this, were observations made on coho salmon from Lake Erie which suffer epizootics of putative hypothyroidism (see below). The thyroid hormone content of eggs taken from goitered coho salmon from Lake Erie was significantly lower than in eggs of coho salmon from Lake Michigan and the Pacific northwest (Leatherland et al. 1990b).

In many species studied, there was clear evidence of a decrease in the thyroid hormone content between the eggs (early embryonic stages) and the time of hatching. Figure 11 illustrates the pattern of changes that are found in chinook salmon during this period. In Chinook (and also other salmon, such as coho salmon), the thyroid hormone levels begin to decline close to time of egg pigment appearance, and the levels fall progressively up until the time of complete yolk absorption and first exogenous feeding. Other species, such as the pink salmon have low egg thyroid hormone levels, and consequently, a pattern of change during embryogenesis is not evident.

The discovery of significant amounts of thyroid hormone (of maternal origin) in the yolk of many species of teleost fishes prompted speculation on the possible role of these, and possibly other maternal hormones, on developmental processes of the embryos and free embryos. Could it be that the adult females of these species provide a reservoir of hormone that can be "used" by the developing embryo as a means of regulating the process of ontogeny? Brown et al. (1989) provided evidence to suggest that an elevated egg thyroid hormone content increased the the rate of development of the eggs of striped bass. However, we have not been able to show such an effect in salmon (unpublished data). Moreover, two studies of lowered egg thyroid hormone content sheds doubt on the relevance of maternal thyroid hormones on either the developmental rate or the survival of the embryos:

(1) In medaka and rabbit fish embryos, the lowering of egg thyroid hormone content by treating the adult females with thiourea had no effect on embryo development (Tagawa & Hirano 1991, Ayson & Lam 1993).

(2) The embryos of goitered coho salmon from Lake Erie have lower thyroid hormone content than Pacific Ocean salmon stocks in the Great Lakes (Leatherland et al. 1989b), but the development rates of all Great Lakes coho salmon stocks are similar (Morrison et al. 1985).

Why is there this apparent discrepancy between thyroid hormone-treated eggs and embryos and eggs that have their thyroid hormone content manipulated by altering the maternal contribution to the eggs? The resolution of the apparent paradox depends, I believe, on the manner of processing of the thyroid hormones in these two situations. When eggs or embryos are immersed in solutions of thyroid hormone, the hormone presumably enters the blood stream via the gills and gives rise-to a marked hyperthyroid state. Conversely, when the thyroid hormone content of the eggs is manipulated via manipulation of the maternal sources of hormone, these hormones are probably associated with the yolk. The rate of release of the hormone is contingent upon the absorption of the yolk, and the consequent incidental delivery of thyroid hormone present therein into the vascular compartment. It is difficult to envisage how an unregulated hormone release (if indeed it takes place) from the yolk can be employed as a means by which the complex processes of teleostean embryonic development and growth can be controlled. A more likely explanation is that the appearance of the maternal thyroid hormone in the embryo (actually in the yolk) is a metabolic accident. Many nutrients are transported from the blood compartment of the adult female into the developing egg follicles during ovarian growth and maturation. Maternal hormones (thyroid and steroid hormones in particular) could well be an accidental component of this mass transfer. The amount of these compounds that are transferred would be clearly proportional to their availability for transport.

Rather than attempting to determine the role that these hormone reserves play in the developmental processes of teleost fishes, perhaps a more appropriate question to address is the mode of excretion of these excess, burdensome hormones. In the adult, much of the hormone is conjugated and excreted via the urine. This route of excretion is not available to the embryo until the gut has become functional, and thus the yolk hormones, rather than having a physiological significance, may represent a physiological embarrassment.

b. Special cases

Three "special" cases are briefly considered here: (1) transformation of leptocephali into glass eels, (2) transformation of pelagic bilaterally-symmetrical flounder larvae into asymmetrical benthic juveniles, (3) smoltification of salmonid fishes.

Because of the difficulty of obtaining leptocephali...
from ocean sources, studies of the transformation of leptoccephali into glass eels has been studied only infrequently; relatively little is known of the regulatory factors involved. With regard to the possible role of thyroid hormones, Gorbman & Bern (1962) make reference to the lack of a thyroid hormone effect on the metamorphosis of leptoccephali (they do not cite the origin of their information) but Kitajima et al. (1967) described an imperfect T₄-induced transformation of conger eel, Conger conger, leptoccephali. Moreover, Yamano et al. (1991) report significant changes in whole body thyroid hormone concentrations during transformation of the leptoccephalus of the same species. Both reports would suggest the possibility of some involvement of the thyroid hormones in the transformation of larval eels.

The transformation of flounder larvae into the juvenile form is an event that is commonly described as a metamorphic event. The transformation is clearly influenced by the administration of thyroid hormones to larvae, or by the elevation of endogenous T₄ levels by microinjection of bovine TSH, which induce a precocious onset of transformation, and enhance the rate of transformation (Inui & Miwa 1985, Miwa & Inui 1987, Inui et al. 1989, Yamano et al. 1991, de Jesus et al. 1993). Equally interesting was the recent report by Tagawa et al. (1990a) of the changes in thyroid hormone content of eggs, embryos and larvae of the flounder, Paralichthys olivaceus. Egg and embryo content of T₃ were high, falling progressively from >6.0 ng g⁻¹ tissue weight at the time of fertilization to <1.0 ng g⁻¹ within 1 day of hatching. Conversely, tissue T₄ content was much lower throughout the period (<1.0 ng g⁻¹). During the metamorphosis period (approximately 25 to 45 days after hatching) it is the tissue level of T₄, and not T₃ that increases during the pro-metamorphosis period and peaking (>12.0 ng g⁻¹) at the end of the climax period. Tissue T₃ levels were not detectable during the pro-metamorphosis period and became detectable only during climax. Can we assume from this evidence that it is T₄ that is the regulating factor in the process? If this is the case, it argues against the generally accepted role of T₄ as the pro-hormone, rather than a biologically active molecule, per se. Experimental evidence suggests that T₃ is far more potent than T₄ in promoting metamorphosis in this species (Miwa & Inui 1987), thus it is more likely that the tissues are sensitive to the low (undetectable) T₃ levels. Moreover, we should remember that the hormone levels were measured in whole organisms, and these may not necessarily reflect the hormone levels that are presented to the tissues. This dilemma is addressed in the consideration of the roles of thyroid hormones in the transformation of the embryo to larval stages of teleost fishes generally (see above).

Smolification of salmonid fishes describes the complex physiological and morphological changes that take place as the freshwater-adapted salmon parrs develop into smolts; the latter have a tendency to migrate downstream, and can tolerate and adapt to sea water. During smolification the parr marks on the flanks of the fish disappear, and there is a silvering of the ventral surface, there are marked changes in brain structure, and the smolts exhibit an increased level of activity (premigratory restlessness) (Barron 1986, Hoar 1988, Leatherland et al. 1993a).

Thyroid hormones have often been cited as regulatory factors in two aspects of smolification, namely a direct action on the physiological changes that are characteristic of the parr-smolt transformation (see above), and also the initiation of downstream migration of the smolts following a lunar phase induced "surge" of T₄ secretion (see Leatherland et al. 1993a). However, the evidence supporting such claims is contradictory, and direct actions of the thyroid hormones on most aspects of smolification have yet to be unequivocally established. Some of the contradictions are discussed below.

What is the evidence for a regulatory role of the thyroid hormones in parr-to-smolt transformation? Most of the evidence is indirect. The onset of smolification in Pacific salmon species has been attributed to an increase in plasma T₄ (but not T₃) concentration that is concomitant with the early stage of the process (e.g., Dickhoff et al. 1982). However, whether this drives the process or is simply an indication of the increased sensitivity of the thyroid tissue to TSH challenge is not clear. Cause-effect linkages have yet to be established.

There is no doubt of the potent action of exogenously administered T₄ (or the subsequent increase in endogenous T₃ produced by 5'-monodeiodination of T₄) on the silvering of parr. This response, which is the thyroid hormone-stimulated deposition of guanine in the skin) has been known for more than 30 years (see Gorbman & Bern 1962). However, silvering can be induced in salmonids at any stage of ontogeny, including the free embryos (e.g., Klein 1967), and does not necessarily correlate with other aspects of smolification, such as the ability to withstand a seawater challenge. Most recent evidence suggests that it is GH, rather than the thyroid hormones that are central to the ability of smolts to adapt to hyperosmotic milieu (e.g., Miwa and Inui 1985, Sakamoto et al. 1993).

Few, if any, of the developmental changes (other than guanine deposition) that we consider to represent smolification can be induced by thyroid hormone administration. Indeed, the onset of the morphological changes that epitomize smolification occurs prior to the recorded elevation in plasma T₄ concentrations. Thus, T₄ does not appear to induce parr-to-smolt transformation, even less, regulate the transformation process in a manner that resembles the manner in which the thyroid hormones affect the metamorphosis of amphibian larvae. Although many authors have attempted to draw parallels between smolification of salmonid fishes and metamorphosis of amphibian larvae (particularly the
endocrine control of the two events), the processes are very different from one another, they are certainly not homologous, and even analogies are difficult to justify.

Are the thyroid hormones involved in initiating downstream migration of smolts? Some stocks of Pacific and Atlantic salmon smolts exhibit a "surge" in plasma T₄ concentrations that is associated with the new moon nearest the spring equinox (see Leatherland et al. 1993a, for review). This very interesting finding, first reported from Howard Bern's laboratory at Berkeley (Grau et al. 1981), has led to the speculation that the lunar phase may act as a Zeitgeber that synchronizes a plasma T₄ "surge", which in turn stimulates the downstream migratory behaviour of smolts. Although this is an attractive hypothesis, there are data that do not support such an idea. Firstly, the lunar-related "surge" is not always evident, and there are large difference among stocks of the same species (see Leatherland et al. 1993a, Fujioka et al. 1990 for reference citations). Moreover, the presence or absence of the "surge" does not appear to affect the synchronized down-stream migration of the smolts, and at least in Atlantic salmon, Salmo salar, smolification is not a prerequisite for catadromous migration. Secondly, attempts to simulate the "surge" have had variable effects on the timing or synchrony of downstream migration, either none (Nishioka et al. 1985), or even reducing the downstream movement of Atlantic salmon smolts and juvenile steelhead trout, Oncorhyncus mykiss (Birks et al. 1985, Youngson et al. 1989). While it is true that some authors have reported behavioural responses (downstream orientation, or phototaxic behaviour) in salmonid fish in response to thyroid hormone administration (Barron 1986, Iwata et al. 1989), it must be emphasized that these responses fall far short of actual migratory activity. Thirdly, the variations in plasma/serum thyroid hormone concentrations that are reported during smolification or during the premigratory "surge" are limited to changes in T₄ concentration, while T₃ concentration is more or less unchanged. If T₃ is the biologically active thyroid hormone (as most evidence now suggests), it is difficult to envisage a regulatory role for the thyroid hormones in smolification and downstream migration of the smolts when the blood hormone levels do not change.

Does the change in plasma T₄ levels induce premigratory (and migratory) activity, or is the reverse true, i.e., does the onset of premigratory activity cause and increased secretion of TSH, and thus give rise to the elevated plasma T₄ concentration? There is convincing evidence from studies of Atlantic salmon, presented by A. F. Youngson, T. H. Simpson and colleagues, to suggest that the latter is the case (Youngson 1989, Youngson et al. 1986, Youngson & McLay 1989). There appears to be a direct correlation between plasma T₄ concentrations of Atlantic salmon smolts and the velocity of the stream flow from which they were taken. In experimental situations, plasma T₄ concentrations of smolts could be manipulated by altering water velocity, but the sensitivity of the T₄ response to such stimulation varied with season, the animals becoming more sensitive around the time of onset of smolification. A similar increase in the sensitivity of the thyroid to TSH stimulation was found in coho salmon between the presmolt period and the period of smolification (Specker & Schreck 1984).

What then is the relationship between the smolification, downstream migration, the lunar cycle and the thyroid gland of salmonid fishes? The most plausible (and parsimonious) explanation, based on present knowledge, is one that was iterated in part by Youngson (1989). As smolification progresses, the thyroid becomes more sensitive to pituitary stimuli (presumably TSH, but other factors may also be involved), probably by way of increased receptor activity at the thyroid level. The changes appear to occur at the level of the thyroid since the increased response of the thyroid to stimulation can be measured following administration of exogenous TSH (Specker & Schreck 1984). The steady increases in plasma T₄ concentrations that are reported during smolification are not reflected in altered plasma T₃ levels, suggesting that T₃ is not the main driving force behind the transformation. There is no obvious parallel between the endocrine regulation of smolification of salmonid fishes and metamorphosis of amphibian tadpoles.

Following smolification, and prior to the onset of downstream migration, the animals exhibit increased activity, the so-called "premigratory restlessness". The downstream migration itself is highly correlated with the phase of the moon; lunar Zeitgebers appear to be used as triggers possibly enabling migrants to enter the river estuary at times that enable them to take advantage of the highest tides, thus facilitating and optimizing their dispersal (Leatherland et al. 1993a).

Taken together, the evidence supports the argument that the lunar-induced premigratory restlessness is the stimulus that elicits the elevation ("surge") in plasma T₄ concentrations that we find in some stocks prior to their downstream movement; the T₄ surge is not the driving force of smolification or downstream migration as was originally supposed. This series of events is yet another example of the misinterpretation of events that are concomitant (and even related), but not cause-effect related.

Of some considerable interest is the recent report of changes in thyroid hormone economy (plasma thyroid hormone concentrations and hepatic 5'-monodeiodinase activity) in Atlantic salmon during smolification (Eales et al. 1993, Morin et al. 1993). In this study, although these alterations in thyroid hormone physiology were undoubtedly concurrent with the smolification event, there is little to indicate that they were other than concomitant. There was no evidence of a thyroid hormone induction of smolification, in fact, in these studies smolification was induced by photoperiod manipulation.
6. Thyroid hormones and reproduction in fishes

Most people are culturally more skilled as analysts than synthesists.

A. Toffler (1980) (The Third Wave)

Assertions that the thyroid hormones are somehow involved in the reproductive efforts of fishes have been a recurrent and widely accepted theme in comparative endocrinology for many years (see reviews by Gorbman 1969, Eales 1979, Leatherland 1982, 1987, Pickering 1992). However, the evidence in support of such assertions is, at best, limited. What then is the basis of the thesis? There appears to be two explanations for the resurrection of the proposal. Firstly, there is some evidence for an indirect thyroid hormone involvement in the gonadal and reproductive function of mammals, and secondly, there may be a correlation between the thyroid activity and the stage of gonadal maturation in some fish species.

As discussed earlier, that T₃ might be involved in some aspect of mammalian reproductive physiology is irrelevant as regards our interest in the reproductive biology of fishes. However, the second line of arguments warrants further comment and consideration.

The relationship between gonadal growth and maturation in fishes and thyroid tissue "activity" is based on histological studies of seasonal changes in the appearance of thyroid tissue of several (mostly temperate) species. In these species, the thyroid tissue appears "more active" during those periods of the year in which the fish undergo gonadal enlargement. But what does this mean? There is no reason to assume that the two events are correlated. One cannot avoid the obvious conclusion that the onset of gonadal growth in most temperate species of fishes is associated with both an increase in ambient water temperatures and an increasing daylength. Indeed, there is still a vigorous debate among fish reproduction specialists as to the relative importance of these two abiotic factors on seasonal gonadal recrudescence. With increasing water temperature, ectothermic animals are faced with increasing metabolic demands leading to an increased food intake, and usually an increased in general activity; clearly, all of these factors are interrelated events which cannot be readily dissected. As regards thyroid tissue physiology, there is ample reason to believe that the general activity of fish and the level of thyroid function are related, and there is unequivocal evidence of a link between the level of food intake and thyroid hormone economy of fishes. Consequently, the reported seasonally-related thyroid tissue changes almost certainly reflect adaptive responses to the metabolic events that result from the increased feeding and activity associated with a warming of the environment. They may even represent a direct response to the increased ambient temperature. They almost certainly do not link up with the gonadal changes that occur at the same season.

What then, is the evidence for a direct role of the thyroid hormones in reproductive function in fishes? In short, there is very little. Surgical thyroidectomy of the dogfish, Scyliorhinus canicula, prevents the development of vitellogenic ovarian follicles (Dodd 1983), chemically-induced hypothyroidism impairs gonadal function in several species (see Leatherland 1987, for review), and there is some evidence of T₃ binding to Leydig cells nuclei in vitro (Jana & Bhattacharya 1993). However, none of these responses can be readily attributed to a direct action of the thyroid hormones on gonadal function, because of the putative actions of the thyroid hormones on general metabolism; impaired metabolic function could readily account for any changes in vitellogenesis, without necessitating a direct action of the thyroid hormones in the process.

The one aspect in which T₃ has been shown to have a consistent effect on gonadal function is the potentiation of GTH-stimulated gonadal steroid hormone secretion by ovarian follicles in vitro (Hurlburt 1977, Cyr & Eales 1988, 1990, Cyr et al. 1988b, Soyano et al. 1993). However, even in these studies, the biological significance of the observations is questionable. For example, in rainbow trout, T₃ had a bimodal effect, with low levels (15 nmoles/l) stimulating and higher levels (>30 nmoles/l) suppressing GTH-stimulated 17β-estradiol secretion, in vitro. Moreover, GTH-stimulated steroid hormone secretion by oocytes in vitro takes place at very high levels even in the absence of T₃ in the culture medium. In the medaka, there was similarly some doubt as to the significance of the T₃ effect; there was no dose response, and the effect was evident only at 32 hours before ovulation even though the endogenous "surge" in plasma thyroid hormone levels occurred some 20 hours later, at 12 hours before ovulation. It is difficult to comprehend how such a relationship can have significance in the regulation of gonadal function in vivo.

Furthermore, if one attempts to relate seasonal changes in blood thyroid hormone levels with gonadal function, the patterns of change do not support, ipso facto, an essential role of T₃ in gonadal steroidogenesis. In fact, as I will try to show, there is considerable evidence to show a suppressive action of 17β-estradiol on T₃ production in several species of teleost fishes (see review by Flett et al. 1994).

Some papers report an increase in plasma thyroid hor-
Figure 12.
The series of figures show the seasonal pattern of changes in plasma triiodothyronine ($T_3$), thyroxine ($T_4$) and 17β-estradiol ($E_2$) concentrations (shown as mg ml$^{-1}$) in female brown bullhead in relation to gonadosomatic index (GSI = gonad weight/body weight x 100) and ambient water temperature. The peak $E_2$ values in mid-May precede the peak GSI values; and there is a rebound in $E_2$ values during reduction in ovary size following spawning. The changes in plasma $T_4$ and $T_3$ concentrations tended to increase from January through to late summer (reflecting the changes in ambient water temperature) except for an unexpected $T_4$ peak in April that is concomitant with the springtime increases in ambient water temperature. In this species, both $T_4$ and $T_3$ were most strongly positively correlated with ambient water temperature (see Fig. 13), although the major increases in plasma $T_3$ concentrations in the spring through early summer occurred following the pre-spawning highs in plasma $E_2$ concentrations.
The figure shows the relationship between the seasonal mean plasma triiodothyronine (T₃, stars) and thyroxine (T₄, crosses) concentrations in female brown bullhead in relation to ambient temperature. The data are the same as those shown in Figure 12. There is a very significant relationship between ambient temperature and plasma thyroid hormone levels in this species. The only outlying point is that for T₄ in fish sampled in early April associated with early warming of the lake.

mone levels associated with ovarian maturation in some annually spawning salmonid species (Pickering & Christie 1981, Cyr et al. 1988a), but no such relationship was found in annually spawning catfishes collected from the wild (Ictalurus nebulosus: Burke & Leatherland 1983) (Fig. 12) or raised in pond culture (Ictalurus punctatus: MacKenzie et al. 1989). In fact, rather than providing evidence of a thyroid hormone-gonad function link, the results of both catfish studies add support the hypothesis of a direct relationship between ambient temperature and plasma thyroid hormone levels (Fig. 13). Moreover, in several species for which data sets are available, there is an intriguing link between the phase of ovarian growth (associated with gonadal 17β-estradiol) and low plasma T₃ concentrations (Fig. 14-15); high plasma 17β-estradiol concentrations are invariably associated with low plasma T₃ levels. A particularly good example of this relationship was found in rainbow trout held under constant ambient temperatures over several reproductive cycles (Cyr et al. 1988b) (Fig. 14-15). As 17β-estradiol concentrations fall (usually following the end of gonadal growth) there is commonly a marked rebound of plasma T₃ concentrations; the plasma T₃ levels thereafter usually stabilize at a lower plateau than the post-estradiol peaks (Fig. 14). These observations reflect what is well established in salmonid fish that are experimentally administered 17β-estradiol (Cyr et al. 1988b, Flett & Leatherland 1989a, b, Cyr & Eales 1990); in these studies, plasma T₃ concentrations and hepatic T₃ production were suppressed in acutely 17β-estradiol-challenged rainbow trout (Fig. 15). This direct experimental evidence, combined with the indirect evidence
derived from studies of plasma T₃ changes that take place during the reproductive period, together support the hypothesis that 17β-estradiol acts to suppress the animal's ability to produce T₃, and hence plasma T₃ levels fall. It must be emphasized, however, that in the absence of elevated plasma 17β-estradiol levels the plasma T₃ concentrations of a given animal could be either high or low, depending on the physiological state of that animal. There is no evidence to support an inverse correlation between the 17β-estradiol and T₃, merely an impaired synthesis of the latter when the former is elevated.

In semelparous Pacific salmon species, there is no such relationship between gonadal function and thyroid hormone economy. The period of maximal in vivo gonadal steroidogenesis of both males and females is associated with a rapid decline in plasma thyroid hormone levels (Fig. 16) (e.g., Leatherland and Sonstegard 1980, Dye et al. 1986, McBride et al. 1986, Leatherland et al. 1989, Flett et al. 1994). In these species, even when plasma steroid hormone levels fall (in both males and females) following completion of gonadal growth, plasma thyroid hormone levels remain at extremely low levels because of the down-regulation of the hypothalamus-pituitary gland-thyroid tissue axis. The most plausible explanation of this suppressed T₃ production at this stage in the animals' life cycle is that the animals are in a catabolic state at that time and need to mobilize their energy reserves to support the caloric needs of migration and spawning (Flett et al. 1994).

The bulk of available evidence does not support a role for the thyroid hormones on gonadal function in vivo; the physiological significance of the observed in vitro actions of T₃ on steroid hormone secretion from the ovarian follicles is questionable; at best, the thyroid hormone effect is indirect and permissive, but it probably represents an experimental artefact, which although clearly present, has no physiological meaning in vivo.

Figure 14.

The figures show the seasonal pattern of changes in mean plasma triiodothyronine (T₃), thyroxine (T₄) and 17β-estradiol (E₂) concentrations (shown as ng ml⁻¹, ng 10⁻¹⁰ ml⁻¹ x 10 and ng 10⁴ μg⁻¹, respectively) in captive female rainbow trout during their reproductive cycle; the two figures represent measurements made over different years of fish maintained under different photoperiod regimes, and are taken from Cyr et al. 1988). An interesting factor in this study is that the fish were maintained under constant temperature conditions throughout the study period, thus none of the changes can be attributed to seasonal temperature shifts. It is difficult to see a repeatable pattern in the concentrations of the three hormones; however, the tendency is for the plasma thyroid hormone concentrations to rise shortly after the fall in plasma E₂ concentration, and for the thyroid hormone levels to decline as E₂ levels rise.
Figure 15.

The correlation between the mean concentrations of 17β-estradiol (E2) and triiodothyronine (T3) concentrations (shown as ng mL⁻¹) in the rainbow trout study described in the legend of Figure 14, is shown in Figure 15a.

Figure 15b shows a similar correlation between plasma E2 and T3 concentrations in rainbow trout administered different levels of E2 in the form of a slow release implant. These data are taken from Flett & Leatherland (1989), the points represent individual fish, and the values are shown as ng mL⁻¹.

Both data sets suggest that under high plasma E2 concentration situations, the plasma T3 levels are suppressed; conversely, when E2 levels are low, then there is a wide range of T3 concentrations.
The figures show the seasonal changes in serum thyroxine (T4, hatched bars) and triiodothyronine (T3, open bars) concentrations (shown as ng ml⁻¹, mean ± SEM) in coho salmon collected from Lake Ontario and vicinity during the final seven months (during year 3) of their life cycle. All of these animals exhibited thyroid enlargement (goitre) when examined histologically, and some had overt lesions (visible by gross examination). Overt lesions were evident in some fish sampled during the early summer, although the prevalence of the overt lesions increased progressively during the seven month sampling period.

The salmon collected between May (as lake water temperatures rise) and August were actively feeding and rapidly growing; during this period the gonads were small. Salmon taken during September and October were congregated at the mouth of their spawning river prior to upstream migration. During this period feeding activity had diminished, the gonads were nearly full-grown and secondary sexual characteristics were evident. The salmon taken in November were sexually mature and either preovulatory or had recently ovulated and were captured during their upstream migration. The salmon taken in January were of post-ovulatory salmon that had not found spawning sites and had therefore not spawned.

The capability of the salmon to secrete large amounts of thyroid hormones despite the thyroid enlargement is evident in the fish sampled during the spring and summer periods; these values represent some of the highest recorded thyroid hormone levels in teleostean fish. The decline in thyroid hormone levels between September and January occurred during the period when the animals cease to feed and become resident in the spawning river. In this example, the decline is NOT related to changes in salinity, nor to prolonged migration (the salmon are not blocked from moving upstream by a dam located within 2 miles of the river mouth). It IS likely related to the altered metabolic states of the fish and they transform from actively feeding (anabolic) animals to fasting (catabolic) forms. (Adapted from Leatherland & Sonstegard 1990).
7. Thyroid hormones, growth and energy partitioning in fishes

i. Growth

The process of animal growth is superficially simple, but inherently complex, and it is far beyond the scope of this essay to deal adequately with the relative influences of the genetic and abiotic factors that we generally consider to be the primary growth regulators. Suffice to say, growth of most fish species is indeterminate, and although growth rate and growth potential are undoubtedly heritable characteristics, environmental factors, such as ambient temperature and food availability exert a potent epigenetic influence, both in the determination of growth per se, but also in the expression of morphological characteristics. Some aspects of this interaction are illustrated simply in Figure 17.

Growth is often expressed in simple terms as weight gain per unit time. Weight gain represents the net difference between energy input and energy expenditure; however, we normally differentiate between weight gain caused by the assimilation of metabolites, such as lipids, and weight gain based on protein (mostly muscle) accretion. When food of appropriate quality and quantity is available, nutrients that are not required for immediate energetic purposes are available for purposes of body growth. When food is rationed, several events take place, the animals adopt calory sparing approaches (e.g., reducing activity levels), the efficiency of absorption of nutrients from the intestinal tract is increased, and growth ceases. If food is severely rationed, then the animal mobilizes metabolite reserves (initially liver carbohydrate and lipid, and then skeletal muscle lipid, perirenal fat reserves, and eventually tissue protein stores). When food becomes available, the liver reserves are rapidly replenished, the immediate caloric needs are met by the incoming nutrients, and eventually the protein (and lipid) accretion re-occurs. Thus, this process of “growth” of fishes is, in effect, a flexible energy partitioning event with energy transfer taking place between compartments (see Fig. 18). Equally, this energy transfer is affected by many exogenous and endogenous factors (see Fig. 18) resulting in greatly enhanced or reduced growth rates. Thus, growth of most fishes is remarkably plastic, and the simple approaches to growth that are still used by numerous investigators (i.e., temporal changes in body weight and/or length), although convenient, have limited value, unless they are accompanied by other types of data (see Weatherley 1990 for an interesting and innovative overview of the process).

![Flow Chart](image)

This schematic flow chart illustrates, in a simple fashion, the interactive nature of major factors that are involved in the “growth” process of fishes. The central column illustrates the basic components of energy uptake via food, production of macromolecules and the incorporation of these molecules in tissues in the form of metabolite reserves, or for somatic growth. Even somatic growth processes represent a form of metabolite reserve, since they can be mobilized as required (hence the two way arrow connecting the “whole fish” and “organs and tissues” operational boxes in the figure). The nutrient needs for growth change with ontogeny, represented by the reference to juvenile and adult stages within the “whole fish” box.

Growth rates are heritable and the processes represented in the central column are significantly influenced by the genetic program; moreover, most of the processes are coordinated by hormones. However, this internal regulation is greatly influenced by environmental factors.
mones. The thyroid hormones cause weight gain, or weight loss depending on dosages applied and the mode of application (e.g., Higgs et al. 1982). However, this does not mean that these hormones have a direct influence on growth, per se. Clearly, if T3 effects a change in energy distribution (as would appear to be the case), then a growth rate change might occur if the animal is administered hormone via the diet or via injection; however, this growth response will be an indirect one that follows on from the energy repartitioning event. Despite the considerable speculation and respeculation in the assorted reviews of this topic, there is no unequivocal evidence of a direct role of the thyroid hormones in in vivo growth regulation in fishes. Consequently, for the purposes of this essay, I will not consider the issue further. All evidence presented to date supports the concept that if T3 is an active player in growth regulation in fishes, it acts synergistically with other factors (including growth regulating hormones, such as insulin, GH and IGF, as well as environmental stimuli), or, as is equally likely, it influences growth by virtue of its energy partitioning actions.

ii. Intermediary metabolism

The role of the endocrine system in the regulation of catabolic and anabolic metabolism of animals has been the subject of intense study since the turn of this century. The nature of the control is complex, and the manner of the control poorly understood. In general terms (and at the risk of being overly simplistic), insulin, somatostatin, GH, IGF and gonadal steroid hormones tend to stimulate anabolic processes, whereas the adrenocortical steroid hormones, glucagon and the catecholamines act predominantly to promote the mobilization of energy reserves. Although the thyroid hormones are commonly assumed to play a central role in several aspects of the intermediary metabolism, the details of their actions are obscure. Moreover, as with its actions on growth, T3 has a bimodal effect, enhancing anabolic (e.g., enhancing amino acid incorporation) or catabolic (e.g., enhancing the utilization of metabolite reserves) depending on the dosages of thyroid hormone applied.

The experimental design that has been employed for investigations of thyroid hormone actions in intermediary metabolism of fishes tend to fall into one of two categories. In the first category (the direct approach), thyroid hormones are administered to the experimental ani-
mal and changes in intermediary metabolism are examined. In the second category (the indirect approach), animals are fed experimental diets (containing variable levels of carbohydrate, protein, lipid, etc.) and the subsequent responses of the pituitary gland-thyroid tissue axis are monitored. Both approaches have limitations that often confound the interpretation of the results that emanate from the studies. With the direct approach, the major problem is that intact fish are normally used; thus, the responses to the administered T₃ are responses to superphysiological or even pharmacological dosages of the hormones. Most investigators now take great pains ensure that in such studies the blood thyroid hormone levels are elevated within a range that has been found naturally in the species of interest (the so-called "physiological range"). Nonetheless, it cannot be assumed that the measured total plasma thyroid hormone levels necessarily reflect the physiological impact of an exogenously administered T₃ burden. We simply do not know whether the administered hormone levels in fact represent a bone fide physiological challenge.

I have two major concerns over interpretation of the results of "indirect" experiments, and they are equally hard to resolve. Firstly, any observed perturbations in the pituitary gland-thyroid tissue axis in fishes that are fed a particular diet could be explained on the basis of indirect compensatory responses to alterations in other components of the endocrine "system". Secondly, even though comparable experimental diets are usually described as being isocaloric, it is simply not possible to determine whether the diets are equally digestible and taken up with equal facility by the different experimental groups. The diets can be considered as isocaloric only if the absorbed metabolites are of comparable nutritional value. If this criterion is not satisfied (and in almost every study to date, it is not), then the comparisons between treatment groups are, as with apples to oranges, of no physiological meaning. As will be shown later, the pituitary gland-thyroid tissue axis of teleost fish is exquisitely sensitive to a reduced satiety level, and thus observed diet-induced changes probably represent differences in effective food intake levels, and not, per se, evidence of thyroid hormone involvement in specific aspects of intermediary metabolism. The use of such "indirect" studies (which I have also been known to make) is yet another example of a design that obfuscates, rather than enlightens our understanding of thyroid hormone function in fishes. For this reason, I will consider only those studies that have employed the "direct" approach.

In ectothermic vertebrates, the reports from different laboratories concerning the effects of thyroid hormone administration have been conflicting, and they are often difficult to compare because of profound differences in the range of hormone levels applied, the methods of hormone administration (via diet, via diffusion from the ambient water, or via injection) and the range of species used. How can we therefore proceed to determine at least some of the underlying principles of thyroid hormone actions on metabolism of fish? One approach has been to revert to the "comparative" mode, with all of its restrictions (see Part 1, above). Can we take anything from the mammalian (terrestrial endotherm) model that we could apply to fishes, and that will provide us with an insight into the roles of thyroid hormones in aquatic ectotherms, particularly given the central role of these hormones in the control of endothermic processes (Stevens 1973)? Probably not, but it is clear that in mammals, T₃ has a profound catabolic effect on lipid, carbohydrate and protein metabolism, and is particularly significant in the regulation of metabolic rate and thermogenesis (Ingbar & Woeber 1981, Lissitsky 1990). At least in the case of the action of T₃ on protein metabolism, the response is bimodal, with the hormone stimulating amino acid uptake and incorporation by various tissues when administered at dosages that can be considered as "physiological" (euthyroid), and effecting the loss of proteins when administered at dosages that are in the hyperthyroid range. In fishes, the evidence for thyroid hormone affects on catabolic and respiratory (thermogenic) processes in fishes is far from consistent, and more often than not is unconvincing.

a. Lipid metabolism

There are reports of a catabolic effect of thyroid hormones on lipid metabolism in fishes, but the evidence is conflicting. Some reports describe thyroid hormone-stimulated lipolysis (based on changes in tissue lipid reserves and/or plasma fatty acid (PFA) concentrations), but other studies report either increases in lipid stores or no effect of thyroid hormone administration on lipid reserves or PFA levels (Eales 1979, Leatherland 1982, Plisetskaya et al. 1983, Matty & Lone 1983, Sheridan 1986). Some of the differences in the reported responses is undoubtedly related to the variability of the dosages and nature of the particular thyroid hormone used, methods of hormone administration, prior metabolic condition of the experimental animals, levels of feeding, etc. Equally, the reliance on relatively insensitive parameters, such as measuring changes in total tissue or carcass lipid content, does not facilitate a precise measure of short-term changes in lipid metabolism. A more sensitive measure of hormone effects on intermediary metabolism is gained from studies of altered activities of key enzymes, rather than dependence on changes in total metabolite reserves. Recent studies in four fish species, in which the altered activities of one or more key metabolic enzymes were used as indicators of altered lipid metabolic states all suggest that the thyroid hormones exert a lipolytic effect, both in short-term (acute) and chronic studies [triacyl-glycerol lipase in
coho salmon (Sheridan 1986), malic enzyme in bowfin, *Amia calva* and lake charr, *Salvelinus namaycush* (Ballantyne et al. 1991), and malic enzyme and 3-hydroxyacyl CoA dehydrogenase (HOAD) in brook charr (Scott-Thomas et al. 1992).

### b. Carbohydrate metabolism

If the effects of thyroid hormones on lipid metabolism in fishes are controversial, the evidence for putative effects of these hormones on carbohydrate intermediary metabolism is even more questionable. The field is reviewed by Eales (1979), Leatherland (1982), Matty & Lone (1983) and Plisetskaya et al. (1983), but it is fair to say that no clear pattern emerges from the published studies to date. Only a few studies have been made of the direct action of the thyroid hormones on the activity of enzymes involved with intermediary metabolism of carbohydrates of fish. In our preliminary studies of T₃ affects on key hepatic and red muscle enzymes of brook charr in vivo, the thyroid hormone effects on carbohydrate metabolism can best be explained on the basis of T₃ exerting a gluconeogenic effect, using lipid substrates (Scott-Thomas et al. 1992). However, the picture is not altogether clear, since glycogen content of livers of T₃-treated fish was lower than that of controls, and in in vitro primary liver tissue cultures from T₃-fed brook charr, glucose release to the medium was significantly higher than that of controls. It should also be noted that the response was variable, and could be influenced by other factors, such as the level of endogenous cortisol secretion, another hormone that has been shown to play a significant gluconeogenic role in fish.

### c. Protein metabolism

The several reports of increased somatic growth of fishes following thyroid hormone administration (usually via the diet) lend support to the concept of these hormones having at least a permissive abalone effect on protein metabolism, and have led several authors to propose their use as growth promoters (see reviews by Donaldson et al. 1978, Higgs et al. 1982).

The literature pertaining to the thyroid hormone actions (some of which are physiological, but most are largely pharmacological) on amino acid incorporation by various tissues in fish has been amply reviewed previously (Donaldson et al. 1978, Eales 1979, Higgs et al. 1982, Leatherland 1982, Plisetskaya et al. 1983) and need not be reiterated here. Details of mode of action of thyroid hormones on these processes in fishes are still lacking; as with most aspects of thyroid hormone influenced intermediary metabolism it is most likely that the effects of T₃ are permissive, and facilitate the direct actions of other anabolic hormones.

### iii. Tissue respiration (thermogenesis)

The dose-related increase in tissue thermogenesis in mammals and birds in response to thyroid hormone challenge, in vitro and in vivo, has been well known for nearly half a century. That these effects were apparent in most (but not all) tissues suggest that there is a common subcellular site of action in all responsive tissues; this conclusion led investigators to seek reductionistic (subcellular) explanations that are common to all intermediary metabolic pathways (including those in ectotherms). The logical candidate is the mechanism of ATP production or ATPase, and the mitochondria, as primary sites of ATP production, were the obvious candidates. However, most attempts to demonstrate direct actions of T₃ on mitochondrial respiration required the use of very high (most certainly pharmacological) levels of the hormone, and can hardly be considered as representative of the action of the hormone in vivo. Such actions can probably be best explained by hormone-induced changes in the mitochondrial membrane phospholipids, and a subsequent alteration in the permeability of the inner mitochondrial membrane to protons (i.e., an increase in state 4 respiration) (Hoch 1988).

Following thyroidectomy of rodents, the decrease in metabolic rate, characteristic of the hypothyroid state, occurs steadily over a period of several days. Similarly, when T₃ is administered to thyroidectomized rodents, there is a latency period of approximately 12 hours between hormone administration and the observed recovery of metabolic rate. These well established findings suggest that RNA and protein synthesis are the intermediary steps in the instigation of the metabolic rate response to the thyroid hormone. Thus, the increased metabolic (and respiratory) rate exhibited by T₃-treated mammalian tissues is probably best explained by an increased production of metabolic enzymes. The major singular consumer of cellular ATP (estimates range from 25 to 45%) is the transmembrane electrolytic pump ("sodium pump"), which actively transports Na⁺ out of the cell, in exchange for the movement of K⁺ into the cell; the electrolytic pump appears to be a Na⁺-K⁺ dependent ATPase system. Greater than 90% of the thyroid hormone-increased respiration of some tissues can be attributed to the increased utilization of ATP for active transport of electrolytes across the cell membrane (Edelman & Ismail-Beigi 1974). Thus, this profound catabolic action of the thyroid hormones in mammals and birds appears to be linked directly to the T₃-stimulated increased ATP consumption at the level of the cell membranes.

In fishes, the evidence for an effect of thyroid hormones on tissue respiration is ambiguous at best. Some studies (e.g., Peter & Oommen 1989) report the effect of thyroid hormones on oxidative metabolism of fishes.
iv. Thyroid hormone economy during reduced dietary intake

One of the most consistent findings in the field of fish thyroidology has been the down-regulation of the hypothalamus-pituitary gland-thyroid tissue axis in fishes subjected to a reduced ration, or an enforced fast. These responses include a reduction in plasma thyroid hormone levels (Fig. 19) and a reduced sensitivity of the thyroid tissue to TSH challenge. In addition, food-deprived fishes exhibit a lower hepatic 5' monodeiodinase activity, and a reduced GH-stimulated increase in hepatic 5' monodeiodinase activity than well fed fish (Eales 1988, Farbridge et al. 1992, Leatherland & Farbridge 1992, Eales et al. 1993, Leatherland 1993a, Leatherland et al. 1993b).

The most immediate response to short-term food deprivation is a reduction in plasma $T_3$ levels, probably coming about by a reduction in hepatic 5' monodeiodinase activity. Given our limited understanding of the role of $T_3$ in various aspects of intermediary metabolism (see above), clear explanations for this change are not forthcoming. However, a logical explanation is that the reduction in $T_3$ production permits the animal to avoid anabolic events, and either promote catabolic processes (to supply immediate energy needs), or, in the case of calory-limited animals, conserve calories (and employ them only as absolutely needed to supply the animal's immediate energy needs). Having said this, it has to be emphasized that $T_3$ is only one of an entire orchestra of hormones that are involved in metabolic homeostasis. Moreover, of equal significance is the fact that the food-deprivation "state" in aquatic ectotherms is characterized by a series of distinctive physiological conditions as it progresses from short to medium to long term, and these events are markedly influenced by ambient temperature. Short-term food deprivation can be accommodated by partitioning energy away from growth, medium term food-deprivation is accompanied by mobilization of readily accessible reserves (liver carbohydrate and lipid, and muscle lipid) followed by less accessible reserves (adipose tissue), and long term food deprivation is associated with gluconeogenesis and tissue protein mobilization. It is still not clear to this investigator as to when the physiological state of "starvation" is applicable.

The reduction in plasma $T_3$ concentration seen in the experimental food-deprivation studies to date (most of which have been carried out with salmonid fishes), is also found in salmonid fishes that are raised at a high stocking density. Under such conditions, some salmonid species exhibit impaired growth rates despite an unlimited supply of food, and therefore under conditions that are not food-deprivation or food rationing (Leatherland 1993a, Leatherland et al. 1993b). In these types of studies, the physiological/behavioural basis of the growth inhibition is not clear. Do the animals feed at...
Figure 19.
The figure shows the temporal changes in plasma T₃ and T₄ concentrations (shown as nmol l⁻¹, mean ± SEM) in rainbow trout during chronic fasting and refeeding. The open bars represent fasted animals, and the hatched bars comparable fed groups sampled at the same time. Groups of trout were fasted for up to 6 weeks (wk), and subsequently fed to satiety three times daily; samples were taken daily for the first 3 days (d), and up to 4 weeks after refeeding. Controls were fed to satiety three times daily throughout the study period. Plasma thyroid hormones in the fasted groups were depressed within 1 week of food deprivation, and had returned to levels that were not significantly different from the controls within 1 week of refeeding. (Modified from Farbridge & Leatherland 1992).
a maximal level, but convert less energy into growth? Do the animals "choose" to feed at a lower level (i.e., have a reduced appetite)? Do the animals feed at a lower level because the acquisition of food is made difficult by the high density conditions? These questions have not been adequately resolved, although recent work shows that rainbow trout stocked at high densities exhibit some of the same physiological signs as ration-limited animals (Leatherland 1993a). This observation begs the question as to whether the trout are unable to feed adequately and thus are in a ration-restricted (fasting) state, and therefore show a concomitant reduction in plasma T3 level, or whether the trout lower plasma T3 concentrations in response to the high stocking density, with a resulting decrease in appetite and associated reduction in food intake. Whatever the answer, it would appear that under conditions in which incoming (dietary) energy sources are restricted, there is an associated reduction in T3 secretion, suggesting that the hormone is an important regulator of energy partitioning, possibly acting as a metabolic switch.

There are particularly interesting situations in which animals in the wild adopt will cease feeding. During the reproductive periods of their life history, many fish species will cease feeding, or severely reduce their food intake. The Pacific salmon have usually ended their feeding activity before they begin their upstream spawning migration. During these "natural" (i.e., "self-imposed") fasting states, the pituitary gland-thyroid tissue-hepatocyte axis of these species is profoundly down-regulated, and this period is associated with a marked mobilization of metabolic reserves that they animals have accrued during the feeding phase of their life cycle. Our studies of introduced stocks of Pacific salmon in the Great Lakes of North America have showed that as the animals migrate from the lake into the rivers there is a progressive and sustained reduction in the plasma concentrations of T4 and T3, a reduced sensitivity of the thyroid to TSH challenge, and low hepatic 5'-monodeiodinase activity (Leatherland & Sonstegard 1980, Leatherland & Flett 1991, Flett et al. 1994) (Fig. 16). Similar progressive reductions in plasma thyroid hormone levels have also been reported during the anadromous migration of Pacific salmon stocks from Oregon and British Columbia (see Leatherland et al. 1989 for references).

These animals are in a profound catabolic state; their biological role is to deliver the gametes to appropriate spawning areas and undergo the competitive process of spawning. Their growth has ended, and their total energy sources to power the migration and spawning activities are contained within their body tissues. The down-regulation of the pituitary gland-thyroid tissue axis during this period of the animal's life history is perhaps one of the strongest indirect arguments in support of the concept that T3 is principally used to regulate anabolic processes.

Whether this is the case for all developmental stages is not yet clear. A noteworthy (and key) life history phase is the early developmental period of fishes, which in some ways resembles that of the migratory adult, since both phases rely on accrued (endogenous) energy reserves. However, the energetics of teleostean embryos differ from those of the migratory adult in some important ways. While it is true that the embryo mobilizes its endogenous energy reserves (from the yolk) to supply its caloric needs (and is therefore in a catabolic state), they also use these metabolites as building blocks for development and growth (and therefore for anabolic purposes). These two processes, anabolism and catabolism, are mutually exclusive, and suggests an interesting partitioning of the two processes within the same organism. (It will be interesting to see if the free embryos exhibit the same ultradian rhythms of catabolism and anabolism to temporarily partition these mutually exclusive processes; such rhythms are, of course, a requirement for normal metabolic functioning of juvenile and adult stages of fishes.) If T3 is a metabolic switch in juveniles and adults, could it also be involved in play a similar regulatory function in free embryos?

Embryos that are immersed in solutions of thyroid hormones rapidly deplete their yolk reserves, suggesting that the hormones exert a catabolic effect. However, it is far from clear whether this reflects the in vivo situation since the dosages used are generally high, and immersion will undoubtedly elevate blood hormone levels because the hormones readily pass through the gills. Even though the embryo contains substantial amount of thyroid hormones, the vast majority is contained within the the yolk compartment. We do not know what levels of thyroid hormones are found in the circulatory system of the animal, and we still do not know whether the maternally deposited thyroid hormones, as they are released from the yolk during yolk absorption, provide the necessary hormones that regulate the thyroid hormone-sensitive anabolic processes.
8. Thyroid gland function in fishes: environmental considerations

An environmental source of iodide is required for the manufacture of thyroid hormones. Vertebrates that inhabit environments that are deficient in iodide, or contain factors that inhibit iodide incorporation into thyroglobulin have a dysfunctional pituitary gland-thyroid gland axis. This final section of the essay considers some of the major environmental factors affecting thyroid hormone economy in vertebrates, with a particular emphasis on problems encountered by fishes in the wild.

i. Iodide deficiency and “simple goitres”

Terrestrial vertebrates satisfy most of their iodide needs via dietary sources, whereas aquatic vertebrates derive their iodide from the ambient medium by active transport across the gills or skin (amphibians). Without a sufficient supply of iodide, the ability of the thyroid gland to synthesize T$_4$ is impaired, and thus blood T$_4$ levels eventually fall as the thyroglobulin reserves of T$_4$ are depleted. As a consequence, there is a subsequent chronic increase in the secretion of TSH, the thyroid gland enlarges (by increases in follicular cell size, and the number of follicles) under the influence of the hypersecretion of TSH, and a benign “simple goitre” develops.

The thyroid tissue responses to hyperstimulation with TSH include a reduction in thyroglobulin content, an increase in protein synthesis with an associated increase in the relative amount of endoplasmic reticulum (and an associated increase in the size of the follicle cells), and an increase in mitotic activity (and a subsequent increase in the number of follicle cells).

By using iodide-free practical (prepared) diets, it is possible to induce experimental goitres in mammals and birds. There is usually a time-lag in the development of such goitres because of the reserves of hormone accrued during periods of iodide availability. As the thyroglobulin thyroid hormone reserves are progressively mobilized without being replaced, and the as the iodide resources eventually disappear (this is slow to occur because there is an efficient recycling of iodinated compounds by enterohepatic routes - conjugated iodinated thyronine compounds are excreted in the bile, the compounds are further degraded in the intestinal tract, and the iodide is reabsorbed).

The prophylactic use of iodide in salt prepared for human consumption has eliminated the occurrence of these types of goitres in many parts of the world. However, naturally occurring endemic goitres are still a major consideration in some human communities, particularly those associated with mountainous areas in central land masses of Africa, Asia, India and South America where environmental sources of iodide are negligible, or possibly bound in a form that cannot be liberated for metabolic use. In those communities, not only is the prevalence of overt goitres high (particularly in women) in the adults, but also in the newborns who exhibit the impaired developmental problems associated with thyroid hormone deficiency during early ontogeny (cretinism).

Iodide deficiency may not be limited to these particular central continental regions of the world. Recent studies in some parts of Europe (which employ prophylactic iodide) have provided evidence to suggest that the present levels of iodide intake are not sufficient to prevent hypothyroidism in some parts of the human population. The problem is of particular concern for pregnant women and the newborn since the embryo’s iodide needs are supplied by the mother via the placenta. If the mother’s iodide intake is insufficient to meet both her own and the fetus’ needs, there is the potential for hypothyroidism in the mother, and more important, for hypothyroidism of the fetus at critical developmental stages; this has been suggested by clinical studies in Belgium (Glinoer et al. 1993) and Switzerland (Baltisberger et al. 1993).

For aquatic ectotherms, such as fishes, it is unlikely that iodide deprivation, in nature is a serious problem, and thus “simple goitres” are unlikely to occur naturally. Fish species adapted to marine or brackish water are exposed to high ambient iodide concentrations, and thus are more likely posed with a problem of iodide excretion, rather than assimilation. Even in freshwater fish species several characteristics work against the development of iodide deficiency. First, iodide assimilation via the gills occurs against a steep diffusion gradient, so that even small concentrations of iodide in the ambient medium provide a sufficient resource. Second, predatory fish species, particularly those that prey on other vertebrates, obtain significant amounts of iodide from the prey species. Third, enterohepatic recycling of iodinated compounds by fish is highly efficient.

Even in the laboratory, the induction of experimental hypothyroidism in aquatic ectotherms by iodide deprivation is problematical. In my laboratory, attempts to induce simple goitres in salmonid fish by feeding them iodide-free practical (artificial) diets and maintaining them for prolonged periods (up to 5 months) in deionized water were singularly unsuccessful (J. Hilton & J. F. Leatherland, unpublished data).
Figure 20.

These two photographs show the gross appearance of the thyrocotyle guilure in sexually mature coho salmon from Lake Ontario. The operculum has been removed from both fish, also, in the lower figure the first gill arch has been removed. The upper figure illustrates the early stage of overt guilure development. A small purple coloured swelling is evident at the base of the gill arch. The lower figure illustrates the later stage of overt guilure development with a large growth pushing out from the base of the first and second gill arches. There has also been some erosion of the gill filaments on the second gill arch.
The photomicrographs of sections of the thyroid tissue of goitred coho salmon collected from the Great Lakes illustrate the different histological appearance of the tissue (probably representing different degrees of hyperstimulation of the thyroid tissue by TSH). Figures 21a and 21b show trabeculate forms, probably brought about by tissue compression; the follicles take on a tubular appearance, and in these specimens the colloid is largely depleted. Figures 21c and 21d show microfollicles that may have their origin from the trabeculate form; note the similarity in the appearance of these follicle to that of "normal" tissue shown in Figure 1. Figure 21e shows an afollicular form of thyroid tissue; the follicles are impossible to distinguish in this tissue, but under the electron microscope, the follicular form can still be identified.

Figures 21f and 21g were immunostained as described in Figure 1f. Figure 21f shows a section of the thyroid tissue of a coho salmon from Lake Ontario in which the immunoreactive $T_4$ sites are restricted to the follicular lumina, but there is residual $T_4$ content. Figure 21g shows a similar preparation from a coho salmon from Lake Erie; this section is depleted of immunoreactive $T_4$. 

47
some of the highest recorded serum T₃ levels were found in
goitred Lake Ontario
coho salmon collected during the early
summer months (Leatherland &
Sonstegard 1980; Fig. 16). If iodide had
been in short supply, it is unlikely that the
animals would have been able to synthe-
size these levels of hormones. Second,
these salmon are top
predators in a food
web; in the last years
of their life history
they are piscivorous
and will have access
to iodide in their
prey, as well as from
the ambient water. In
fact, tissue iodide lev-
els of coho salmon
from some of the
Great Lakes were
found to be in the
same range as those
of salmon taken from
the Pacific Ocean
(Fig. 21). Third, there
are marked differ-
ences in the size of
the thyroid lesions in
salmon occupying dif-
ferent Great Lakes,
differences which
cannot be accounted
for by variations in
the lake water iodide
levels (Fig. 23).

Figure 22.
These photomicro-
graphs show sections of
goitred thyroid tissue
illustrating colloid
goitres from a saltmarsh
topminnow, Fundulus
jenkinsi (Fig. 22a) and
guppy, Poecilia latipinna
(Fig. 22b), respectively,
and a papillomatous
lesion in a salmonid
species (Fig. 22c).
ii. Goitres that do not have an iodide deficiency aetiology

Until recently, most goitres that were found in association with a reduced ability to secrete T\textsubscript{4} were considered to be “simple goitres”, and assumed to have an endemic iodide-deficiency aetiology. However, there is increasing evidence to link certain goitrous outbreaks with the presence of chemicals (so-called goitrogenes, or antithyroid compounds) in the environment.

Antithyroid compounds that act either by inhibiting iodide metabolism by the thyroid (e.g., some monovalent anions, notably thiocyanates and perchlorates), or impairing iodination (e.g., thionamide compounds, such as propylthiouracil, thiourea and methyl mercaptoimidazole), have been known for several decades. Similarly, naturally occurring goitrogenes, most of which are thiocyanate compounds, are prevalent in some plant species [rutabagas, cassava and canola (rape seed) are perhaps the best known examples].

As discussed above, iodide deficiency (“simple”) goitres are unlikely to occur in most fish species in the wild, and are difficult, if not impossible to induce experimentally. Nevertheless, thyroid neoplasms represent the most commonly reported tumours in both bony and cartilaginous fishes taken from the wild, or held in captivity (Leatherland et al. 1994). Even more surprising is the fact that this holds true, even for species that inhabit iodide-rich environments, and in the case of captive animals, fishes that are fed iodide-enriched diets. Most of these neoplasms have the cytological characteristics that we associate with “simple goitres”, but an iodide deficient aetiology is highly unlikely. A far more plausible explanation postulates the presence of environmental goitrogenes; the following section of the paper outlines the arguments in support of this hypothesis.

Thyroid lesions are relatively rare in fishes taken from the wild, regardless of their natural ambient salinity (ies). Does this mean that they do not occur, and does this mean that we can unequivocally argue that iodide deficiency goitres do not occur in fishes in the wild? The answer is no! We can argue that we have no direct evidence to indicate the presence of such lesions, but we cannot subsequently deduce that the lesions do not occur. Given the vicissitudes of comparative pathological studies, it is probable that the majority of thyroid (and other) lesions in wild fish populations may go undiscovered. First, there have been so few pathological studies of any type on wild populations of fishes; most studies, for very practical reasons, focus on captive species, and most of the 25,000 or so known species of fishes have not been examined pathologically at all! Moreover, most lesions of the thyroid can only be detected by histological examination of lower jaw tissue, and most pathological studies do not consider the endocrine system as a priority. Thus, even if present, thyroid lesions are likely to be undetected. Furthermore, one could readily argue that those fish that develop a dysfunctional condition (e.g., impaired thyroid function) are more susceptible to predation, and would therefore be less available for pathological screening, even if such a screening were undertaken.

It must be emphasized that pathological data from fish populations cannot be used in the same manner as pathological data of human populations. The latter form an invaluable source of information for epidemiological studies [i.e., studies of the distribution of diseases that afflict human beings (from the Greek word for “people”, demos). In most Western cultures, autopsies are a common post-mortem procedure and generate a massive data base on diseases of the single species, Homo sapiens. This is clearly not the case for the plethora of fish species. Pathological data bases for fishes, even the extensive ones curated by the Smithsonian Institution in Washington, cannot be used in the same way to assess the extent of epizootics (disease prevalence in non-human animals species). Consequently, we do not know whether freshwater fish species in the wild suffer from epizootics of iodide-deficiency related “simple goitres”, although the evidence to date would not suggest that this is the case.

Most reports of thyroid enlargement in fish are of captive animals held in recirculating aquarium systems; goitres in fish taken from the wild are relatively rare. This has led us to ask whether the thyroid enlargement is associated with housing fish in standard aquarium conditions. Accumulating evidence from studies of some human communities suggests that metabolic by-products of bacteria and algae have potent goitrogenic activity (Gaitán 1973, Vought et al. 1974, Gaitán et al. 1980). It is possible that the epizootics of goitres in captive fish stocks have a similar aetiology, particularly since a microflora is an essential component of aquarium filter systems, and since microorganisms are a natural part of the “ecosystem” of an aquarium.

Although epizootics of thyroid enlargement in fishes collected from the wild are rare (Leatherland et al. 1994), there is one stark exception, that of the stocks (populations) of Pacific salmon (coho, chinook and pink salmon) introduced into the Great Lakes of North America (see Fig. 20a-b, 21a-g). Every single adult Great Lakes salmon that we have examined over the last 15 years, regardless of species or lake of origin, has had a thyroid lesion, characterized by hyperplasia and hyper trophy (see Leatherland 1992, 1993b, for reviews); salmon taken from the Pacific northwest regions of North America do not exhibit these lesions. The aetiology of the lesions is still unknown, but there is convincing evidence to suggest that the lesions have an environmental aetiology other than that of a de facto iodide deficiency. First, during the summer, these fish were able to secrete very high levels of thyroid hormones. In fact,
What then, is the cause of the thyroid lesions? Since Great Lakes salmon are renowned for their body burdens of man-made toxic substances, particularly heavy metals and aromatic halogenated hydrocarbons (AHHSs), these materials were obvious candidates for consideration. The AHHSs were initially suspected to be involved because they are known to alter thyroid hormone economy in mammals (Bastomsky 1977, Potter et al. 1983, McKinney et al. 1985a, b, Leatherland 1992, 1993b, Morse et al. 1992, 1993), and we focused on these compounds. However, the evidence from interlake comparisons of the size and prevalence of the lesions in coho salmon did not support the argument that they were the main culprit; lesion size and prevalence were not correlated with the body burden of these man-made toxicants. Moreover, in an extensive series of laboratory studies in which we fed juvenile rainbow trout and coho salmon either with diets containing Great Lakes coho salmon (“fish-to-fish” studies), or diets contaminated with PCBs and Mirex, we could find no convincing evidence of an anti-thyroid effect of either the “naturally occurring” or artificially introduced dietary AHHSs (Leatherland 1992, 1993b). We therefore lack the necessary evidence to link these compounds to the omnipresent goitres of Great Lakes salmon. Something else appears to be responsible.

Even though the “fish-to-fish” studies were refractory, it is still possible that the Great Lakes salmon bioaccumulate a factor(s) that has biological activity in animals that feed on the salmon (e.g., human beings), and thus in addition to the “fish-to-fish” studies we carried out “fish-to-rodent” studies in which we fed Great Lakes coho salmon to mice and rats. Surprisingly, the rodents developed goitres and had low blood thyroid hormone levels (Cleland et al. 1988, Leatherland 1992, 1993b) (Fig. 24c), suggesting the presence in the salmon of a goitrogenic factor(s). The rats and mice also developed marked hepatomegaly (enlarged livers) (Fig. 24a), and greatly elevated hepatic mixed function activity (Fig. 24b), clear evidence of the significant toxic burden of the Great Lakes coho salmon.

Evidence of another kind also points to the presence of goitrogenic substances in Great Lakes fishes; field studies of piscivorous birds and mammals (mink) taken...
from the Great Lakes basin showed that, in addition to severe developmental problems, the birds had enlarged thyroid glands (Colborn et al. 1990). (In the case of the piscivorous birds, salmon are unlikely to be a major vector for uptake of the goitrogenic factor(s) because they are not the prey of choice, except for situations in which gulls feed off the carcasses of post-spawned salmon in the rivers. However, the evidence from the bird studies does suggest the presence of a goitrogenic factor(s) in the Great Lakes environs.)

If mammals and birds that feed on (or are fed diets containing) Great Lakes fish show thyroid responses, why don't the salmonid fish that are artificially fed diets containing Great Lakes salmon? The most parsimonious explanation is that the birds and mammals are responding to the presence of AHHs in the salmon, but the fish do not. The different responses probably relates to the manner in which the AHHs are thought to affect thyroid function in mammals (and birds). In mammals, the molecular shape of some forms of the AHHs allows them to bind to the blood proteins that are involved with the transport of the thyroid hormones in the blood and occupy the binding site that is normally occupied by the thyroid hormones (McKinney et al. 1985a, b). The net effect is a reduction in the thyroid hormone carrying capacity of the blood of the animal, and a significant cascade effect that alters the entire thyroid hormone economy of an animal. Changes in the binding capacity of the thyroid hormones will alter the free to bound ratio of both $T_4$ and $T_3$, modulate the negative feedback control of $T_4$ release, and possibly influence peripheral $T_3$ production. The thyroid hormone binding (and the turnover rates of thyroid hormones) in endotherms is markedly higher than in ectotherms, and therefore the interactions between toxicant and blood proteins would not elicit nearly as profound a response in salmonid fish as it might in mammals. Another explanation for the differences between the "fish-to-fish" and "fish-to-rodent" studies relates to the manner in which lipophilic substances such as the AHHs are dealt with by the recipi-

**Figure 24.**

The figures show the effects of feeding Great Lakes coho salmon (30% of diet mixed with rodent chow) to two strains of mice for 2 months. The two strains of mice were used because one of the strains (C57B1/6) is known to be genetically susceptible to the effects of aromatic halogenated hydrocarbon compounds; the other strain (DBA/2) is known to be relatively resistant. PO, E, M and O represent the sources of salmon used to prepare the diets [Pacific Ocean (=control), Lake Erie, Lake Michigan and Lake Ontario]. The asterisks indicate significant differences from the Pacific Ocean salmon-fed mice. (Modified from Cleland et al. 1988.)

Figure 24a shows the relative changes in liver size of mice fed diets containing Great Lakes coho salmon; the hepatosomatic index (HSI - liver weight/body weight x 100) of mice fed the Lake Ontario salmon diets were significant enlarged in both susceptible and resistant strains of mice.
Figure 24b shows the hepatic ethoxyresorufin-o-deethylase (ERR) activity of the same mice. ERR is one of the enzymes involved in detoxification and is a principal factor in metabolism of xenobiotics such as the aromatic halogenated hydrocarbon compounds. Significant ERR inductions were evident in both the resistant and susceptible strains of mice fed the diets containing Lake Ontario coho salmon, although ERR activity was markedly higher in the susceptible strain. It is worth noting that since the liver size of the Lake Ontario salmon fed susceptible strain of mice was significantly elevated, the total body ERR activity of this group is remarkably higher than that of any other group.

ent. Many teleost fishes "deal" with toxic burdens by storing them away in lipid-rich tissue depositories, such as the liver (see Leatherland 1992, 1993b, for reviews); thus, although body burdens of these lipophylic chemicals may be high, the blood levels of the toxicants are relatively low, and therefore the carrier protein binding-inhibition effects of compounds such as the AHHs are considerably lessened.

If AHHs are not the culprit in causing the goitres in Great Lakes salmon, then what is? While it is true that the relative size (or prevalence) of the thyroid lesions in Great Lakes coho salmon is not correlated with the toxic burdens of the fish from the different lakes, it is correlated with the relative degree of eutrophication of the lakes from which the fish was taken. The largest thyroid lesions are found in coho salmon from Lake Erie (the most eutrophic of the Great Lakes) and the smallest in coho salmon from Lake Superior (the least eutrophic) (Fig. 23). We have preliminary evidence that shows water collected from Lake Erie (and recirculated water taken from aquaria that housed goitred poecilid fish) to contain a factor(s) that has anti-thyroid properties, as assessed by in vitro bioassays based on pig thyroid slices (E. Gaitán, R. Sonstegard & J. F. Leatherland)
Figure 24c shows the relative changes in serum thyroid hormone concentrations (T₄ and T₃) in mice fed the Great Lakes salmon diets compared with those fed the Pacific Ocean salmon control diet. The C57B1/6 mice fed the Lake Ontario salmon diets had significantly reduced serum T₄ and T₃ levels, whereas in the DBA/2 groups, all Great Lakes salmon-fed groups had significantly lower serum T₃ concentrations compared with the Pacific Ocean salmon fed controls.

unpublished data). As yet, we have no idea of its chemical nature, but we suspect that it is a bi-product of the microflora of the lake system, and it would certainly explain the omnipresence of such factors in the Great Lakes basin.

If waterborne environmental goitrogens are responsible for the reported thyroid lesions in fish species sampled from the wild, can they account for the different forms of thyroid "tumours" that have been described in the pathological literature? The reported thyroid lesions in fish have been grouped into well-defined categories, depending on the size of the epithelial cells, the amount of colloid present in the lumina, and the size of the follicles themselves. The relationship between the various "forms" of the lesions is not fully elucidated, but as illustrated in Figures 25a-c, one can present a reasonable and logical argument for a relationship between the various forms of known lesions, based on the postulate that there is a seasonal exposure to the goitrogen(s). This would appear to be a reasonable assumption if the goitrogen(s) is a bacterial product. The alternating periods of high and low exposure to the goitrogen could account for the range of goitre "types" that are found.
iii Human health implications of the thyroid lesions in Great Lakes salmon

Disease states in wildlife species have been viewed by several groups as a valuable means by which we can identify the contamination of ecosystems with naturally occurring or man-made toxicants (see Colborn & Clement 1992). The use of such biomarkers for monitoring the "health" of an ecosystem has been promulgated by various groups as a most efficacious method of identifying areas of concern, since one does not require a prior knowledge of the nature of the chemicals that are the aetiological agents.

The significance of the thyroid lesions in Great Lakes salmon in terms of human health considerations for the human communities that cohabit the Great Lakes basin is still the subject of considerable debate (Colborn & Clement 1992). For example, can we necessarily assume that our interlake studies of the size of the lesions reflects the relative distribution of environmental goitrogens in the Great Lakes basin? In fact, the results of interlake studies of the type that we have undertaken for the past 15-20 years have to be interpreted with extreme caution, and cause-effect relationships should not be assumed as given. In this type of study, there are no bona fide control populations or stocks, and it could be argued that all differences are accounted for on the basis of the major variations in the ecological characteristics of the different Great Lakes. However, when the observations from the several sources are considered together, there is abundant (and increasing) evidence of an environmental problem. The Great Lakes salmon have exhibited a 100% prevalence of thyroid disorder for at least 15 years, piscivorous birds and mammals that feed on Great

![Diagram](image)

**Figure 25.** The figure offers a schematic representation of the proposed role of environmental goitrogens in the formation of different types of thyroid lesions that have been identified in fishes. The initial insult gives rise to the simple hyperplasimal form (Figure 25a). Continued exposure to the environmental insult induces further growth of the thyroid tissue, and compression of the tissue giving rise to trabeculate, microfollicular and "afoolicular" forms (Figure 25b). A seasonal removal of the environmental insult could, in theory, ameliorate the hypersecretion of TSH, leading to the development of the colloid goitre form in the already enlarged thyroid tissue. Reimposition of the environmental insult, with a concomitant reintroduction of TSH hypersecretion might account for the papillary forms of goitres seen in some teleost fish (Figure 25c) (Modified from Leatherland et al. 1993).
Lakes fish develop thyroid problems, and in well controlled “fish-to-rodent” studies there is unequivocal evidence of anti-thyroid factors in the Great Lakes salmon.

The toxic nature of the Great Lakes salmon is recognized by federal, state and provincial government fisheries and health agencies in both Canada and the U.S.A. as evidenced by the fish consumption advisories that are issued to the sportfishing communities. Those advisories are still in effect despite the reported decreases in total toxic burdens of salmon during the last decade. More sinister, is the apparent rebound of levels of specific toxicants (Mirex and DDT) in Great Lakes fishes in recent years (Colborne et al. 1990); levels are no longer decreasing, but are tending to increase. Moreover, recent developments in our understanding of the relative toxicology of different co-planer congeners of AHHs have caused us to reconsider the parameters that we use as the basis of fish consumption advisories.

Wildlife species, particularly aquatic species such as

the introduced Pacific salmon, represent a valuable diagnostic tool for environmental assessment of Great Lakes (and other) water quality. As sensitive indicators of environmental insult, they offer a means of monitoring changes in the degree of environmental insult, and as top predators, they bioaccumulate and biomagnify at least some of the xenobiotics to which they are exposed. The biomagnification effect is extremely significant, for example, in terms of the PCB content, one pound of Lake Ontario salmon is the equivalent to 1.6 million gallons of Lake Ontario water (hence the significance of the fish consumption advisories!).

The particular value of aquatic biomarkers is that they can be used even in situations in which the chemical nature of the putative toxicant(s) is not known, and they offer an inexpensive means of screening for environmental “hotspots”. Moreover, because of the biomagnification process, they deliver to the chemist a concentrated sample of environmental contaminants for identification. As the complexity of the mix of chemicals that we add yearly to our environment increases, biomarkers will continue to play a vital role in initially monitoring the effects of man-made toxicants, particularly in aquatic habitats.

![Diagram of thyroid conditions](https://via.placeholder.com/150)

- [Simple hyperplasia]
  - enlarged follicles
  - columnar epithelial cells
  - partially depleted colloid

  ▼

- increased hyperplasia
- hypertrophy

  ▼

- compression of tissue

  ▼

- [Trabeculate goitre]
  - tubular follicles
  - hyperplasia and partial depletion of colloid

  ▼

- "budding"

  ▼

- [Microfollicular goitre]
  - small follicles
  - partial depletion of colloid

  ▼

- [Afollicular goitre]
  - colloid depletion
  - loss of follicular morphology

25b
[Afollicular goitre]
: colloid depletion
: loss of follicular morphology

- TSH hypersecretion
- ameliorates or ends

- fusion of follicles?

[Colloid goitre]
: cuboidal epithelial cells
: large, colloid-replete follicles

Re-imposition of environmental insult

[Papillary goitre]
: large follicles
: columnar epithelial cells
: depleted colloid

Removal of environmental insult
9. Finalé

This is not the end. It is not even the beginning of the end. But it is, perhaps, the end of the beginning.

W. Churchill (1942) (of the Battle of Egypt)

And the evening and the morning were the first day.

Genesis 1:1

The progress of the human race in understanding the universe has established a small corner of order in an increasingly disordered universe.

S. Hawking (1988) A Brief History of Time

In this essay, I have attempted to illustrate the scope of a very small field in the cosmos of comparative physiology. The essay is not meant to be a review, and I apologize to my colleagues who might feel slighted (it was not intended) by any perceived omission of their contributions to the field. Thyroidology in general, and fish thyroidology in particular, has attracted the interest of specialists in many diverse fields, including anatomy, biochemistry, developmental biology, ecology, genetics, histology, molecular biology, nutritional science, pathology, pure and applied physiology and toxicology, and it is simply not possible to refer to all significant contributions. Wherever possible, I have referred to reviews for literature citations, and, because I am most familiar with work originating from my own laboratory, I have used that work to illustrate the points that I wished to make.

During the post-World War II period, the "General Endocrinology" discipline, which was itself still in its infancy, spawned the "subdiscipline" of comparative endocrinology. Comparative endocrinology is now well established as a field of study in its own right, and the unique comparative view of the endocrinological universe has contributed immensely to our greater understanding of the evolution of hormone and receptor structure and function has therefore influenced our concepts of general endocrinology. The developments in our understanding of the relationships between the form and the function of the endocrine tissues, and the burgeoning use of bioassays for measurement of the actions of hormones (typical of the 1950s and 1960s) laid the foundations for the subsequent era of hormone assay development and application that typifies contemporary endocrinology. The first hormone assays were often insensitive and non-specific, but technological developments in immunology and protein chemistry have enabled the production of precise and sensitive assays that are routinely accessible. More recent technological developments in pharmacology, molecular genetics and other areas of molecular physiology are providing the tools with which to investigate protein hormone structure, receptor structure and function and signal transduction processes, enabling questions to be addressed that only a decade ago could not even be formulated.

Advances in the endocrinology field in the last 20 years have been fast and furious. As with most other areas of science, those advances have depended, in large measure, on technological break-throughs. We have progressed to where where we now stand in part by chance, and in part despite ourselves. Stephen J. Gould (1989), in his book "Wonderful Life", argues that chance plays a major role in fashioning the nature of organismal evolution (the so-called "contingency" theory). He argues that unpredictable, and often catastrophic events, clear the decks of dominant species thus enabling other forms of life to occupy and exploit the vacated ecological niches. If we turn back the clock to any point in time and allowed the process to occur again, the outcome would not be the same, and the surviving species would not be the same. We can make a similar argument for the development of any discipline or field of science, be it physics, chemistry or biology. Progress (however that can be defined) in any area of science is governed in large measure by technological advances that permit new questions to be asked, economic interests that determine which aspects of the field are the most "important" (and hence funded), and sheer luck as regards the fields in which the principal talents will become engaged. Not least, we are governed by the unpredictable (catastrophic?) "bandwagon" phenomenon whereby several laboratories, in short time, assuming a proprietary interest in the same field. David Hull (1988) in his book, "Science as a Process" emphasizes (amongst many other things) the manner in which progress in any discipline is influenced at least as much by the characters of the players in the game, as by the nature of the game. He also addresses at length the multifactorial nature of "science" and of the agendas of those of us who participate in the scientific process. Some researchers come to the scientific well seeking subject areas that have Nobel Prize potential, others to seek refuge in a non-competitive niche, and others to find a discipline that will provide them with abundant research resources; yet others allow their intellects to
wander at will [Dans les champs de l'observation le hasard ne favorise que les esprits préparés. (Louis Pasteur 1854)]. There is no large plan, and the contemporary form of any given field or discipline is the result of a complex, and largely unstructured process; paradoxically, the progress of science is one that many scientists would intuitively consider to be “non-scientific”. We are what our historical past has permitted us to become, within the omnipotent constraints and opportunities of chance. The perception of “truth” depends on the culture and prior experience of the observer and the observer’s position in space and time. It is not an absolute. At the beginning of each semester, I make a habit of telling my senior physiology students that at least half of the subject matter of the course will be wrong....but I don’t know which half. The same is true for this essay.

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