Hormones and Endocrine Glands of Fishes

Studies of fish endocrinology reveal major physiologic and evolutionary problems.

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To treat the endocrine systems—or any other aspect of comparative physiology—of fishes apart from vertebrates as a whole requires some caution. The fishes can hardly be considered a single group of "lower" forms. In fact, it is precisely their heterogeneity which makes it desirable to call special attention to the fishes. In their endocrine systems, as in all other aspects of their anatomy and physiology, the fishes reveal a broader range of variation and a longer history of adaptation than do the "landliving" (tetrapod) vertebrates. There is an elementary and unjustified tendency to view the fishes, in view of their collective ancestral position, as being much like their landliving descendants, only more primitive.

If we make a diagram at the level of Haeckelian comparative anatomy to illustrate the endocrine systems of the trout and of man (Fig. 1), we are indeed struck by the close similarities between these two atypical types. It is my purpose in this article to examine some of these similarities critically, in an attempt to undermine, at least in part, the uncritical generalization that one vertebrate is the same as any other, and to reinforce the idea that only on the basis of an extensive comparative biology can an authentic general biology emerge.

There are several ways in which the endocrine systems may differ in different groups of vertebrates. (i) Glands may be present in one group and absent from another. For example, fishes possess a caudal neurosecretory system and corpuscles of Stannius, for which there is no evidence among tetrapods. Tetrapods possess parathyroids and many mammals have a hormonogenic placenta, for which there is no evidence among fishes. (ii) The same gland may be present throughout the vertebrates, but specific hormones may be secreted in some groups and not in others. This may be the case with aldosterone from the adrenocortical (interrenal) tissue; almost all evidence indicates that this is a tetrapod hormone, essentially lacking in fishes (*1*), despite a small quantity demonstrable in elasmobranch and ratfish interrenals (*2*). (iii) A hormone itself may be represented throughout the vertebrates but may have acquired different biological and chemical properties in different groups. Thus, the "paralactin" of fishes may affect lower vertebrates in the same way that the "prolactin" of tetrapods does, yet lack the effects of the latter on higher vertebrates (*3–5*). The heterothyrotropin of fishes does not stimulate the thyroid of mammals, despite its presence in the pituitaries of mammals (*6*). (iv) The development of new "target organs" ("tissue sensitivities") is evident throughout the vertebrates. The same products of endocrine glands may be hormones in some species yet not definable as such in other species. Progesterone is unquestionably present in all vertebrate ovaries (*7*). However, there is no evidence that the oviduct of fishes responds to its presence, whereas the oviducts of birds and mammals are highly responsive.

In reviewing the chemical (molecular) evolution of vertebrate hormones one is struck by two trends: the apparent constancy of some hormonal agents (for example, the iodinated thyronines, including thyroxine; the catecholamines) throughout the vertebrate series and even extending into the protochordates (*8*), and the development of a series of peptides which differ among themselves by the substitution of one or two amino acids (for example, the neurohypophysial octapeptides: vasotocin, isotocin, mesotocin, oxytocin, vasopressins, and so on), more or less characteristic of different groups of vertebrates (*9*).

In our laboratory, attention has been focused on three principal endocrine areas in fishes. For some years now, we have been investigating the piscine homolog of the mammalian adrenal cortex: the interrenal tissue. In considering its contribution to organismal economy, we have been compelled to reexamine the corpuscles of Stannius—originally considered to be "posterior interrenals." A second area of interest is a mass of neurosecretory neurons in the caudal spinal cord terminating in a neurohemal area delimited as the urophysis in teleost fishes. Despite its questionable etymology, this term is useful in conveying the analogy of the caudal urophysis with the neurohypophysis at the cranial end of the vertebrate animal. A third area of interest concerns one of the several peptide and protein hormones secreted by the epithelial part of the pituitary—the hormone "prolactin" and its equivalent in fishes. The material presented in this article is an incomplete sampling of information currently available from our laboratory and from other laboratories, which may serve to illustrate some of the general points referred to above.

The Adrenocortical (Interrenal) Tissue and the Corpuscles of Stannius

The interrenal tissue (gland)—the homolog of the mammalian adrenal cortex—in fishes is morphologically extremely diverse (*10, 11*) but apparently biochemically rather invariable. In some groups (cyclostomes, lungfishes) the interrenal tissue has been localized only with great difficulty (*12*). In others, the chondrichthyeans, it is present in great abundance, as well-organized encapsulated glands of various shapes and sizes (*10*) located between the posterior ends of the kidneys (hence *interrenal*). In the fishes so far mentioned, the interrenal tissue has no evident relation to the second component

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of the adrenal gland of higher vertebrates: the catecholamine-secreting adrenomedullary or chromaffin tissue. Comparative biochemists have hoped to gain some understanding of the possible cortex-medulla relation by a study of chondrichthyean interrenal glands, which generally lack a chromaffin component.

Among the teleost fishes, the interrenal tissue is associated with the anterior end of the head kidney, and the spectrum of morphologic variations includes the full gamut of possibilities, including patterns suggestive of various tetrapods (*11*). The interrenal may be organized as a collar around the postcardinal veins, as in eels; or organized around branches of these veins, as in the mullet or *Tilapia*; or spread out in the head kidney tissue, as in some salmonids and wrasses. The chromaffin tissue may be undetectable in the head kidney; or even more widely dispersed than the interrenal in some cases; or detectable as islands of chromaffin cells intermingled among the interrenal tissue, as in birds, anuran amphibians, and some reptiles; or enclosed within the interrenal collar, thus resembling a true medulla around a vein; or enclosing the interrenal collar, thus resembling a true cortex (*13*). Additional variations exist in the nature of the head kidney tissue, which may be entirely hemopoietic (lymphoid or myeloid, or both) and which may also contain nephric tubules, including glomeruli (*14*). Investigators have concluded that there are no evolutionary trends of great importance evident in these highly variable patterns.

Insofar as interrenal secretory products are concerned, information is available from extraction of the pertinent tissues themselves (which in teleosts may contain much more than interrenal tissue); or of the media after incubation with or without appropriate (or even inappropriate) substrates, cofactors, and tropic agents; or of blood plasma or whole blood. The technique of analyzing the venous effluent has not yet been applied to study of interrenal areas in fishes.

In general, as noted above, the piscine interrenal produces no major surprises for the comparative endocrinologist (*15–17*). Secretion of cortisol (Fig. 2) is characteristic of teleosts, some cortisone and corticosterone also being produced (*18*). Cortisol appears to be essential for interrenalectomized eels in seawater (*19*). Minor components in some species include 11-dehydrocorticosterone, 11-deoxycorticosterone, and 11-deoxycortisol. There is some evidence that "new" steroids may be formed in appreciable amounts by fish interrenals—11-ketotestosterone and 1α-hydroxycorticosterone are among these (*20*); adrenosterone has also been reported (*20*). What their biological significance may be is open to question, although 11-ketotestosterone is an active androgen in salmon (*20*). Indeed the physiologic (as opposed to pharmacologic) effects of all the corticosteroids reported in fishes remain largely undetermined.

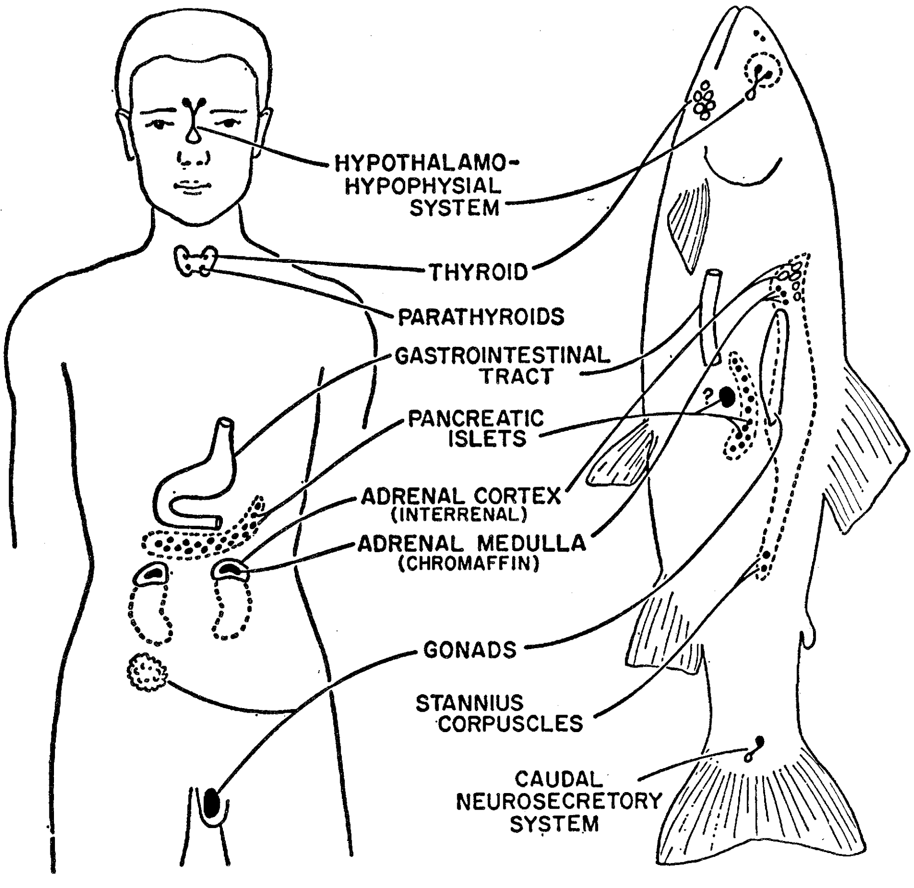

Fig. 1. Comparison of endocrine system of man and trout. Note that the parathyroids are lacking in the fish, and that the Stannius corpuscles and caudal neurosecretory system are lacking in man (and in all other landliving vertebrates). The functions of these latter two structures are still incompletely defined.

Figure 3 indicates that there is little phylogenetic significance to the distribution of major corticoids among the vertebrates, other than among the sauropsids (reptiles and birds), which all appear to be secretors of corticosterone. However, one major problem continues to plague comparative adrenal biochemists, and this concerns the occurrence of the potent salt-retaining (in mammals) steroid aldosterone. The few reported occurrences of this steroid in fishes are open to question (*21*), and the minute amounts reported for chondrichthyean fishes give little suggestion of a significant physiologic role for this steroid if it does occur (*2*). It seems most reasonable at present to suggest that aldosterone may be an "invention" of landliving vertebrates, playing a role in sodium retention for animals living in freshwater or in terrestrial environments demanding such conservation (see *1*, *22*). The urophysis may play a similar role, where needed, in fishes, and indeed other factors may also be sodium-retaining in environments favoring sodium loss. The literature on the osmo(iono)regulatory influences of the endocrine system in teleost fishes is confused and diffuse; Fig. 4 summarizes diagrammatically (and questioningly) the many claims that have been made.

The most astonishing aspect of studies of the interrenal glands in sharks, rays, and ratfishes (chondrichthyeans) is the observation that, for all the mass of lipogenic tissue present, they seem to produce so little detectable steroid

(*2, 23*). In these fishes, in any case, osmoregulatory problems are solved by the maintenance of blood osmotic pressure in part by considerable amounts of urea and trimethylamine oxide and by the excretion of excess sodium with the aid of the rectal gland (*24*)—an apparent caudal analog of the cephalic salt glands of birds. Several aspects of possible interrenal steroid activity in these fishes demand investigation: (i) Do interrenal steroids affect metabolism, leading to the formation of nitrogenous compounds? (ii) Do interrenal steroids regulate rectal gland function in a manner resembling their effect on the salt glands of birds (*16*), interacting with a nervous control mechanism (*25*)? (iii) Is the interrenal gland, which is apparently so poor in corticosteroids, a source of other biologically active steroids—gonad-like, for example—and, in any case, what is the role of all the lipid in such tissue [almost 40 percent of the wet weight of the interrenals in *Squalus acanthias* (*26*) is extractable lipid]? (iv) Is the regulation of carbohydrate metabolism the primary role of corticosteroids in chondrichthyeans (*27*)?

In the earliest studies of teleostean adrenal tissues, attention was also focused on bodies located posteriorly in the kidneys and derived from the embryonic pronephric ducts. These masses of epithelioid cells—the corpuscles of Stannius, once called the "posterior interrenals"—are another example of a possible endocrine gland confined to fishes and apparently not to all of them. They have been found also in ganoid fishes, but not in cyclostomes or chondrichthyeans or lungfishes. The need for more comparative studies—morphologic as well as physiologic—is strongly indicated (*28*).

In mammals the adrenal cortex is generally clearly demarcated into zones of apparent functional significance. The zona glomerulosa is largely a source of aldosterone, and attempts have been made to homologize the Stannius corpuscles of fishes with this zone, without success (see *17*). Removal of the Stannius corpuscles may result in changes in sodium, potassium, and calcium concentrations, and indeed calcium ion regulation may be an important function of the corpuscles (*29*), a function somewhat like that of the tetrapod parathyroids—glands which are lacking in fishes. Studies of the effects on calcification processes of removal of the Stannius corpuscles or of treatment with Stannius corpuscle extracts are needed before we can press any parallelism with parathyroid function.

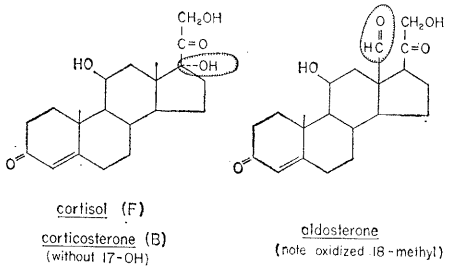

Fig. 2. Chemical structures of the three principal vertebrate corticosteroids. The α-hydroxyl function on the 17 carbon increases the gluconeogenetic activity of the molecule. (Cortisol and corticosterone are "glucocorticoids," and aldosterone is the most potent naturally occurring "mineralocorticoid"—as judged by mammalian assay systems.)

Assessment of the steroidogenic potential of these organs has been most troublesome. Despite positive and negative claims for corticoid and estrogen synthesis (*30*), based on extraction and incubation of Stannius corpuscles, it has generally appeared that, at most, steroid storage may occur. Materials mimicking steroids have complicated chromatographic separations, but these materials, including something that first suggests aldosterone, are eliminated by subsequent analytic procedures. Steroid-3β-hydroxy-dehydrogenase activity was not detected in eel corpuscles (*31*). Most recently, Idler and Freeman (*32*) found definite conversion of pregnenolone to progesterone and have isolated deoxycorticosterone from Stannius corpuscles of pollack, Ogawa (*33*) claims to have found deoxycorticosterone in goldfish corpuscles, and Nandi and Pieprzyk (*34*) have found some evidence of this steroid in trout corpuscles.

In assessing steroidogenesis by as yet incompletely defined glands, alternative concepts as to how steroid formation may occur as a result of glandular activity are needed. The "ordinary" vertebrate steroidogenic organs—gonads, adrenal cortex, possibly some placentas—are able to synthesize steroid hormones from acetate or cholesterol. Other tissues, however, may have the ability to carry on only one or two critical and ultimate steps in the conversion of an inactive steroid precursor into a potent hormone. The "transforming gland" could accomplish this *in situ*, or by secreting a needed enzyme which then acts at the periphery to accomplish the conversion. To examine biochemically or ultrastructurally such organs as isolates for evidence of steroidogenic apparatus may be misleading. These comments may be found relevant when attempts are made to assess the steroidogenic potential of the corpuscles of Stannius. The ultrastructural evidence does not suggest a full steroidogenic role but suggests, rather, protein-secretory activity (*35*). However, that these organs make a partial or terminal contribution to steroid hormone biogenesis remains a real possibility in the terms just described.

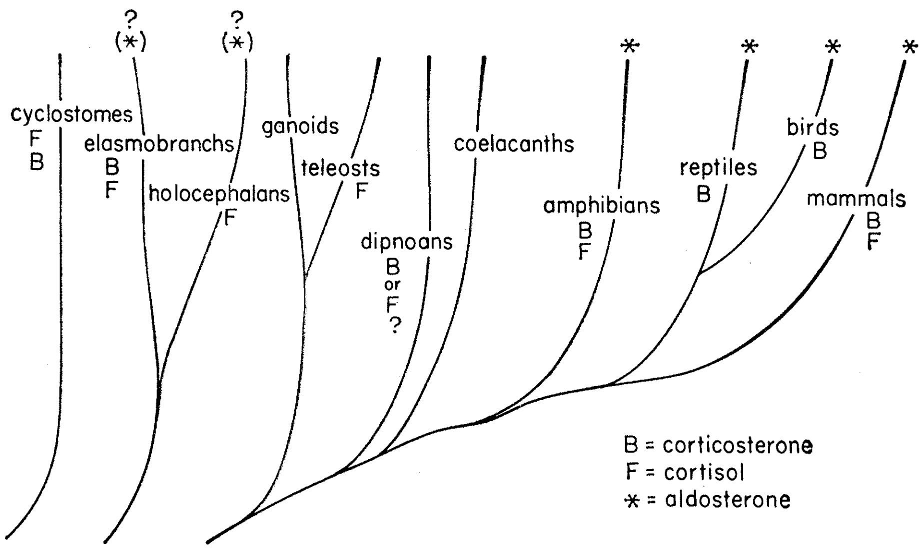

Fig. 3. Distribution of the major corticosteroids among the vertebrates. Some mammals secrete corticosterone, others secrete cortisol, and still others secrete both. This also seems to be the case for most groups of nonteleostean fishes. Cortisol is generally the principal corticoid of teleost fishes, and both reptiles and birds are characteristically secretors of corticosterone.

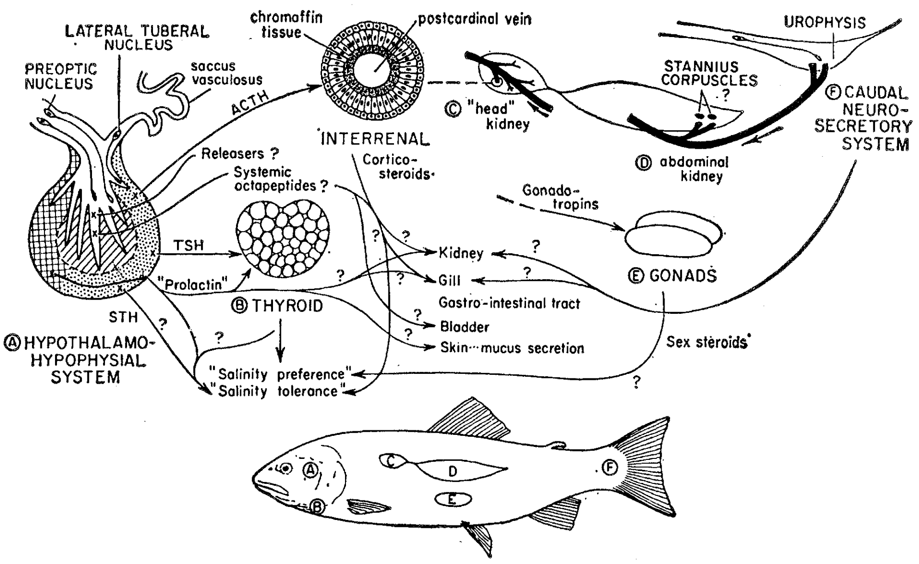

Fig. 4. Possible endocrine factors in osmoregulation in teleosts. In this diagram most of the available information on possible endocrine regulation of water and ion metabolism in teleost fishes is combined, in order to portray the extent of the intractions that can be visualized. In fact, the role of only one hormone, prolactin, has been firmly established in a physiological sense, and its locus of action is still being debated. [Adapted from Nandi and Bern (*17*)]

More significant at the moment than the possible limited steroidogenic contribution of the corpuscles is the discovery by Chester Jones and his colleagues (*36*) of an important pressor activity, partially reminiscent of the renin-angiotensin system of mammals, associated with these bodies. As with the urophysial system described below, drainage from the corpuscles of Stannius is also presumably through the kidney (the renal portal system), and the target for this hormone may be within the kidney itself.

The degree of dependence of the interrenal (and even the Stannius corpuscles) upon the pituitary and the importance of an adrenocorticotropic function is difficult to estimate in fishes. There are recurrent morphologic indications (*37*) of some regulation by the pituitary; the effect, however, may be minimal and only indirectly related to steroidogenesis, as has been suggested for other nonmammalian vertebrates (*38*).

The Caudal Neurosecretory System and the Urophysis

The caudal neurosecretory system is an example of a presumed endocrine apparatus apparently limited to the fishes. It is evident as specialized glandular neurons in the caudal spinal cord and reaches its maximum development in the higher bony (teleost) fishes (*39*). In these animals the secretory neurons send processes to an organized, often lobate, neurohemal structure—the urophysis—in which the axon terminals are associated with a rich vascular bed. Similar secretory neurons also occur in elasmobranch fishes, and indeed were described in these forms by Speidel in 1919 (*40*); this was the first description of neurosecretory neurons in any animal. An extensive neurohemal area is present in elasmobranchs, but it is not organized into a distinct organ (*41*). The diffuse neurohemal area may be looked upon as primitive or as a secondary evolutionary development consistent with isospondyly (a gradual posterior tapering of the vertebral column and the contained spinal cord); among teleosts, some primitive forms and the eels also have diffuse neurohemal areas. It remains uncertain whether caudal neurosecretory cells are also present in cyclostomes, ratfishes, ganoids, and lungfishes, although there is some evidence for their occurrence in the last two groups. Considerable attention has been devoted to the comparative anatomy and histology of the urophysis in teleosts; there is considerable variation in "exterior" morphology (Fig. 5).

The urophysis itself was recognized in the early 19th century by pioneer comparative anatomists such as Weber and Serres; however, ganglionic function was assigned to it, and it was not until Enami in 1955 (*42*) integrated cytologic and anatomic observations that the existence of a neuroendocrine system—analogous to the hypothalamo-neurohypophysial system located anteriorly—was first propounded. From the time of first recognition of the system as such, data of ambiguous nature have been forthcoming to suggest an osmo(iono)regulatory role for the urophysis (*43*). The principal basis for the earlier arguments seems to be that, inasmuch as the urophysis looks like the neural lobe of the hypophysis situated anteriorly, it ought to act like it. Another major anatomic feature, first emphasized by García Romeu (*44*), is the drainage of the vascular bed of the urophysis into the caudal vein and thereby into the renal portal system. Proponents of a gill action for the urophysial hormone (*45*) have to consider this anatomic fact. The more recent data supporting the view that the urophysis contributes to ionic homeostasis are also open to some question. An effect of urophysectomy can be demonstrated when the experimental animals (the tropical cichlid fish *Tilapia mossambica*) are subjected to highly deleterious conditions (placed in a 0.9-percent sodium chloride solution); such effects are not demonstrable when Ca^{++} concentrations are kept high (*46*). The important observations (*45*) of increased Na^{+} influx against the osmotic gradient across the gills in goldfish injected with urophysial extracts need confirmation and leave a possible renal role for the postulated neurohormone at best uncertain. The discovery, by electrophysiologic methods, that there are at least two kinds of fiber in the caudal system in *Tilapia*—those which increase firing when blood concentration of Na^{+} is raised and those which respond similarly to decreased Na^{+} concentration—is of interest (*47*). Interpretation of the data is predicated on the view that the caudal system is involved in ionic regulation and on the belief, recently substantiated to some degree, that impulse conduction is related to discharge of neurosecretory product (*48*). Some morphologic data suggest that major activation of the system could occur in responses to frequent changes in salinity rather than in response to a stable hypo- or hypertonic environment per se (*49*).

The urophysis-neurohypophysis analogy (in fact, almost serial homology!) has led to a search for biologically active octapeptides in the urophysis, similar to the vasotocin-isotocin-mesotocin-

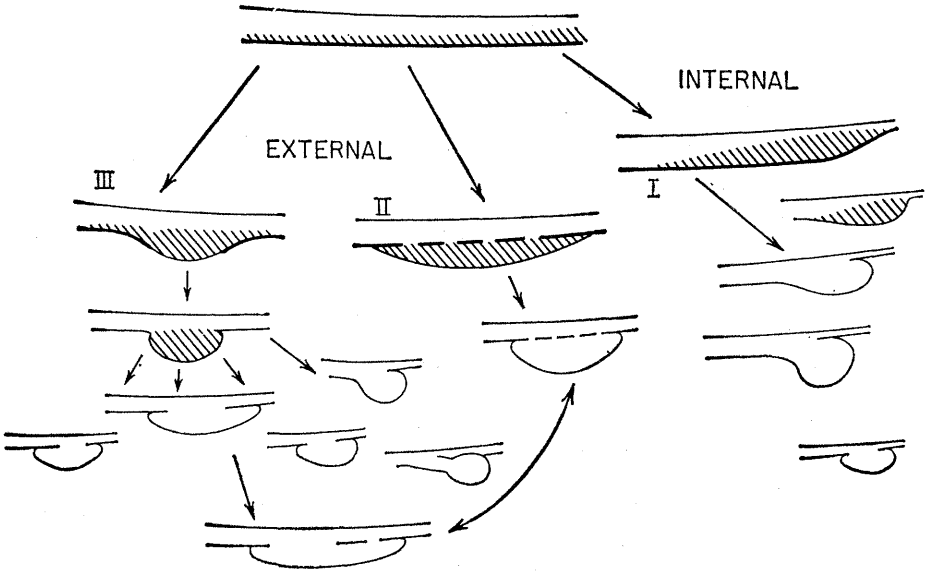

Fig. 5. Varieties of urophysial structure in teleosts and possible derivation of different types by ventral and caudal expansion of neurohemal tissue (hatched area) into or through the meningeal membranes (heavy black line) to produce lobate urophyses. The "ancestral type" is an exaggeration of the elasmobranch situation and is encountered in some isospondylous teleosts (for example, the clupeoid *Elops*). Type I is exemplified by *Conger*; type II, by *Salmo*; type III, by *Albula*. [From B. Hart McLean, unpublished thesis, University of California, Berkeley]

glumitocin-oxytocin-vasopressin family in the neurohypophysis (*9*). Pharmacologic surveys toward this end have been notably unproductive (*45, 50*), and indeed the staining and cytochemical characteristics of the urophysial secretory protein complex are unlike those of the principal hypothalamic system (failure to stain with paraldehyde fuchsin, chrome-alum hematoxylin, or alcian blue; absence of appreciable sulfhydryl-disulfide). On the other hand, the ultrastructural cytology of the caudal system is in fact indistinguishable from that of the anterior system, suggesting peptide hormonal factors and a neurophysin-like carrier protein (*51*).

Two reassuring findings have recently been made, however, that indicate the possibility of a biologic contribution of this elusive neuroendocrine apparatus. At the Stazione Zoologica in Naples we have collected large numbers of caudal systems from *Mugil* species. Urophysial breis from these fish will do two things: (i) cause a consistent retention of water by toads (*Bufo bufo* and *B. viridis*) receiving injection of as little as one urophysis in their dorsal lymph sacs (*52, 53*), and (ii) cause a notable increase in blood pressure in eels receiving even a small fraction of a urophysis, accompanied by renal diuresis and natriuresis (*52*). It thus becomes possible to begin extraction and purification procedures, and to compare, in a realistic fashion, urophysial activity of fishes from different environments and under different physiologic conditions. The water-retaining effect differs from that associated with neurohypophysial hormones in its prolonged action, evident as much as 24 hours after a single injection. However, until some knowledge of the effective compound becomes available, one cannot be sure that the long duration of influence is not due simply to slow release of the active agent from the protein carrier.

It would seem bizarre that a system so constantly present in at least teleost fishes should have no function (*54*). Its very location in the tail region—the region of the fish most subject to natural trauma and predation—would tend to minimize the likelihood of an essential functional contribution. However, the defect of many previous experiments involving urophysectomy may lie in an underestimation of two major aspects of the morphology of this system. In the first place, as appears true of neurosecretory systems generally, this system regenerates rapidly. Even *total* removal of the caudal peduncle from *Tilapia* is followed eventually (in less than 21 days) by functional reestablishment of the caudal neurosecretory system; apparently new neurosecretory neurons differentiate from ependymal elements (*55*). Second, there is increasing evidence for the discharge of caudal neurosecretion into the central canal and the cerebrospinal fluid (*56*), and it is possible that cells temporarily deprived of their normal vascular outlet by urophysectomy can release amounts of neurohormone adequate for the organism by this alternative pathway. The secretory bipolarity of a neurosectory neuron should be no more surprising than the somewhat similar activity of other endocrine cells, such as the thyroid follicle cell.

The Adenohypophysis and Fish "Prolactin"

The mammalian pituitary is credited with secreting a variety of hormones: seven or eight from its epithelial portion and two octapeptide factors from its neural portion. Prolactin, one of the hormones from the epithelial part (adenohypophysis), is of particular interest from the standpoint of the comparative biologist. No other single hormone influences in a specific fashion a greater variety of target tissues and physiologic processes than prolactin does. Table 1 lists only those effects that have been described in fishes; consideration of other vertebrates would provide a list at least five times as long. Considerable attention has been given to finding a common basis for the multitude of actions ascribed to this hormone. Possibly it is entirely an integumentary hormone, acting upon obvious ectodermal derivatives such as mammary glands, brood patches, mucous glands, and even gills. The cropsac of pigeons, which provides the standard biologic assay for prolactin, is certainly derived from esophageal ectodermal epithelium, and it is possible that the "seminal vesicles" of fishes are derived ultimately from a proctodeal ectodermal contribution. However, there is no "germ-layer" explanation possible for the ability of prolactin to stimulate also the mesodermal corpora lutea of the mouse and rat ovary, or the mesodermal kidney, or the mesodermal tissues responding to its growth-stimulating influence in amphibian tadpoles. Prolactin is "simply" a hormone that affects a host of target tissues, including the nervous system as it is involved in behavioral responses.

Almost since the delineation of prolactin as a specific pituitary hormone, its biologic detection and assay have been based upon the reaction of the pigeon cropsac. This method, adapted to local rather than systemic application of the material to be tested, has recently been improved for more precise quantitative analyses (*57*). Mammalian prolactins will stimulate the cropsac, just as they will apparently induce all responses reported to date in both mammalian and nonmammalian vertebrates, including those listed for teleost fishes in Table 1.

It has been assumed for years, on the basis of the misreading of a paper by Leblond and Noble (*58*), that the pituitaries of all vertebrates secrete prolactin—that this hormone is a constant component of the vertebrate endocrine system (*7, 59*). Reexamination of this contention, with cropsac response the criterion for presence of the hormone, failed to reveal typical responses in material obtained from the pituitaries of fishes (*60*), with one exception. The African lungfish, *Protopterus aethiopicus*, does show prolactin (that is, cropsac-stimulating activity) in its pituitary, as do representatives of all classes of landliving vertebrates (*60*). Examination of pituitary glands for evidence of mammotropic activity (the ability to stimulate secretion by alveoli of the mammary gland) again failed to demonstrate evidence of a fully effective agent in the pituitaries of fishes, in this case the lungfish as well as teleost fishes (*5*). Here again a separation must be made between landliving vertebrates and fishes, with the lungfish this time on the fish "team" (*61*).

Table 1. Reported effects of prolactin on teleostean fishes.

A) On structures associated with the integument:
  1) Ion movements across gills* (*67*)
  2) Pigmentation (melanogenesis) (*79*)
  3) Skin mucus secretion (including "discus milk") (*69–71*)

B) On structures associated with reproduction or parental care:
  1) Seminal vesicle growth and secretion (*74*)
  2) Skin mucus secretion (*69–71*)
  3) Pigmentation (*79*)
  4) Antagonism to toxic effects of estrogen (*69*)
  5) Fin-fanning and nest-building behavior (*71*)

C) On nonintegumentary and nonreproductive structures:
  1) Renal excretion* (*67*)
  2) Thyroid stimulation (*80*)
  3) Fat deposition (*81*)
  4) Resistance to thermal stress (*82*)

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* Survival of hypophysectomized euryhaline fishes in freshwater (*64, 67, 68*).

More recent quantitative studies of the cropsac-stimulating activity of fish pituitaries, involving internal control both of weight responses and of biochemical parameters, indicate a minimum activity in some species, more than appears to occur as a nonspecific response to tissues other than pituitary. Similarly, a degree of mammotropic activity associated with the fish pituitary was also evident in our studies, and in both tests Chadwick (*61*) also has found minimum responsiveness. We conclude that a "partial prolactin" may be present in fishes, but one qualitatively different from the prolactin of tetrapods.

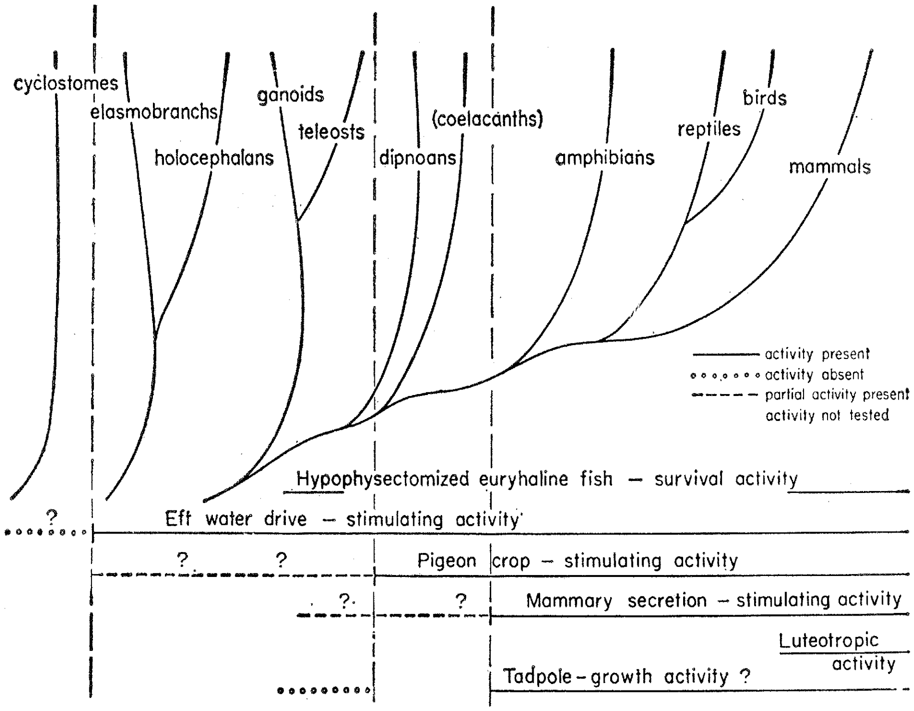

Fig. 6. Distribution of some of the activities associated with prolactin among vertebrates. The data on tadpole growth (see *83*) are taken from Enemar and von Mecklenburg (*84*), who interpret them as indicating the presence of a growth hormone (STH) effective in tetrapods, rather than prolactin, as is suggested here. [Adapted from Nicoll, Bern, and Brown (*5*)]

There are two other effects of prolactin which have been fairly well studied. This hormone—and extracts of pituitaries from almost all vertebrates tested—will induce premature "water-drive" in the landliving (eft) stage of the salamander, *Diemyctilus viridescens* (*4, 62*). This return to water—"second metamorphosis" (*63*)—precedes reproductive activity. Important behavioral, physiologic, and morphologic changes are manifested as a result of treatment with prolactin. The "water-drive" activity associated with prolactin appears to be a general characteristic of vertebrate pituitaries. Prolactin is also able to sustain certain euryhaline teleosts hypophysectomized and then replaced in their freshwater environment, which otherwise would die within a few days (*64*). Species of *Fundulus, Poecilia, Xiphophorus, Gambusia, Tilapia,* and other genera show this survival response, and mammalian prolactin, or the animal's own pituitary, or the extracts of pituitaries of certain teleost fishes, will keep the animal alive. No other hormone yet tested will do this. It appears likely that prolactin—or its piscine equivalent [the "paralactin" of Ball (*3*)]—is a most important hormone for osmoregulation in teleosts. Indeed, it may be the only hormone for which there is an incontrovertible physiologic—as opposed to pharmacologic—role in osmoregulation in fishes. Hoar and Lam (*65*) implicate prolactin in the ability of seawater sticklebacks to adjust to freshwater; this hormone may prove to be of critical significance in anadromous migration (*66*).

The manner in which prolactin influences ionic balance in fishes is being actively investigated in several laboratories. In general, the absence of the pituitary in euryhaline fish results in lowered blood osmotic pressure when the fish is in a hypotonic medium, and in loss of sodium. Administration of mammalian prolactin partially prevents this loss (*67, 68*). This is true also for eels (*Anguilla anguilla*), a euryhaline species which does not die in freshwater when hypophysectomized but which does show changes in blood ionic composition similar to the changes in the species that do die. Maetz and his co-workers (*68*) have found that hypophysectomy enhances sodium efflux at the level of the gills; this efflux is reduced by injection of prolactin.

Both the eft water-drive effect and the fish-survival effect may involve osmoregulatory phenomena. The terrestrial eft must readjust physiologically to aquatic conditions. In the teleost, at least, prolactin has effects on renal excretory mechanisms as well as on the gill, so that, again, the concept of prolactin as solely an "integumentary" hormone is not tenable. Nevertheless, there are profound skin changes in the eft returning to water, and recently there has been interest in the mucus-stimulating influence of prolactin in teleosts. The interesting observation (*69*) that the cichlid *Symphysodon discus* will secrete "discus milk" (used in the nutrition of the young) after administration of prolactin has been extended to various cichlids by other workers (*70, 71*). Blüm and Fiedler claim to have observed an important increase in the number of epidermal mucus cells following injection of prolactin. In our laboratory, Bowman (*72*) has not been able to verify this phenomenon in the cichlid *Tilapia mossambica.* Hypophysectomy reduces the number of mucus cells in some teleosts (*73*) (but, again, not in *Tilapia*); evidence for the ability of prolactin to restore the normal picture, however, appears equivocal.

Studies of the comparative physiology of prolactin have been influenced by the notion that this hormone was *ipso facto* involved in reproductive and maternal activity. It appears evident that this may not be the primary contribution of the "primitive" prolactin in fishes, where its integrating influence seems to pertain more to survival of the individual than to reproduction. However, even among teleost fishes, some structures related to reproduction have developed sensitivity to prolactin. In addition to the discus milk secretion, the secretion of the seminal vesicles of certain fish (*74*) is conditioned by prolactin acting synergistically with androgen.

Tetrapod prolactins appear to possess the properties of fish prolactins (the effects on water drive in efts and on survival in fish), but fish prolactins appear not to have all the properties of tetrapod prolactins—at least the responses of the cropsac and of the mammary gland to fish pituitary preparations would seem to be qualitatively different (Fig. 6). There does, however, appear to be, in the pituitary of teleosts, a specific cell type concerned with secretion of the prolactin-like entity (*75*). Sage (*76*) was able to accentuate directly the secretory activity of these cells in organ-cultured pituitaries by lowering the osmotic pressure of the medium, and euryhaline fish in seawater show cytologic signs of inactivity as compared with the same species in dilute seawater or in freshwater (*75, 77*).

It is interesting to speculate on the possible evolution of the prolactin molecule, in terms of the development, in time, of active sites conveying the ability to stimulate cropsac and mammary glands upon a molecule initially involved in maintaining ionic balance. Whether the fish type of prolactin coexists with the tetrapod type in the pituitaries of higher vertebrates is difficult to decide at present. This is the situation with the fish type of pituitary thyrotropic hormone (heterothyrotrophin) and the tetrapod type (*6*). Two statements can be made, however: (i) fish prolactin appears to differ from its tetrapod counterpart in biologic activity, and (ii) the activities of the tetrapod hormone (*5*) would seem to have evolved before many of the target organs so far delineated had appeared.

Summary

The structures discussed above illustrate some of the evolutionary knowledge to be obtained from studies of the comparative biology of the endocrine system among the fishes (see also *17, 78*). The interrenal (adrenocortical) gland is an example of an endocrine structure which shows great morphologic variation in the vertebrate series, and among the fishes themselves. The structural variability implies little selective value for any particular pattern, and, in fact, biochemically the interrenal tissue would seem to be involved in much the same kind of steroidogenesis in the vertebrates generally. However, the hormone aldosterone may be a tetrapod novelty. The caudal neurosecretory system is a good example of a ubiquitous endocrine apparatus, among at least teleostean and elasmobranch fishes, for which a function has yet to be elucidated and which continues to challenge the comparative physiologist. This system, along with the Stannius corpuscles, is lacking in the tetrapods. The existence of these structures makes it clear that the endocrine biology of fishes cannot be tacitly summarized as being essentially similar to that of the tetrapods, only less well developed. The prolactin situation illustrates the existence of a gland —the pituitary—present among fishes as among tetrapods, which secretes a product only partly related to the tetrapod hormone and having a very different functional significance. In this case it is clear that "the hormone *and* the uses to which it is put" have undergone evolutionary change during vertebrate phylogeny.

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