The Stress Response in Fish

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I. Introduction 592
II. Hypothalamic-Sympathetic-Chromaffin Cell Axis 593
   A. Control of catecholamine secretion 593
   B. Functions of catecholamines 594
   C. Effects of stressors on catecholamines 596
III. Hypothalamic-Pituitary-Interrenal Axis 597
   A. Control of cortisol secretion 597
   B. Functions of cortisol 598
   C. Effects of stressors on cortisol 599
IV. Central Neurotransmitters, Neuroendocrine Peptides, and Hormones 601
   A. Central neurotransmitters 601
   B. Neuroendocrine peptides 601
   C. Pituitary hormones 602
   D. Other hormones 602
V. Stress and Hydromineral Balance 603
   A. Hydromineral control in fish 603
   B. Effects of stressors on branchial structure and function 603
VI. Stress, Growth, and Energy Balance 607
   A. Growth and stress 608
   B. Endocrine control of growth during stress 608
VII. Stress and Reproduction 609
   A. Hypothalamic-pituitary-gonadal axis 609
   B. Reproductive hormones and stressors 610
VIII. Stress and the Defense System 611
   A. Defense system of fish 611
   B. Defense system and stress 611
   C. Immunoneuroendocrine relationships 612
IX. Modifying Factors 615
   A. Mineral and ionic composition of water 615
   B. Additional stressors 615
   C. Social interactions and crowding 615
   D. Life stage 615
   E. Population and individual characteristics: acquired or inherited 615
X. Conclusions 616

Wendelaar Bonga, Sjoerd E. The Stress Response in Fish. Physiol. Rev. 77: 591–625, 1997.—The stress response in teleost fish shows many similarities to that of the terrestrial vertebrates. These concern the principal messengers of the brain-sympathetic-chromaffin cell axis (equivalent of the brain-sympathetic-adrenal medulla axis) and the brain-pituitary-interrenal axis (equivalent of the brain-pituitary-adrenal axis), as well as their functions, involving stimulation of oxygen uptake and transfer, mobilization of energy substrates, reallocation of energy away from growth and reproduction, and mainly suppressive effects on immune functions. There is also growing evidence for intensive interaction between the neuroendocrine system and the immune system in fish. Conspicuous differences, however, are present, and these are primarily related to the aquatic environment of fishes. For example, stressors increase the permeability of the surface epithelia, including the gills, to water and ions, and thus induce systemic hydromineral disturbances. High circulating catecholamine levels as well as structural damage to the gills and perhaps the skin are prime causal factors. This is associated with increased cellular turnover in these organs. In fish, cortisol combines glucocorticoid and mineralocorticoid actions, with the latter being essential for the restoration of hydromineral homeostasis, in concert with hormones such as prolactin (in freshwater) and growth hormone (in seawater). Toxic stressors are part of the stress literature in fish more so than in mammals. This is mainly related
to the fact that fish are exposed to aquatic pollutants via the extensive and delicate respiratory surface of the gills and, in seawater, also via drinking. The high bioavailability of many chemicals in water is an additional factor. Together with the variety of highly sensitive perceptive mechanisms in the integument, this may explain why so many pollutants evoke an integrated stress response in fish in addition to their toxic effects at the cell and tissue levels. Exposure to chemicals may also directly compromise the stress response by interfering with specific neuroendocrine control mechanisms. Because hydromineral disturbance is inherent to stress in fish, external factors such as water pH, mineral composition, and ionic calcium levels have a significant impact on stressor intensity. Although the species studied comprise a small and nonrepresentative sample of the almost 20,000 known teleost species, there are many indications that the stress response is variable and flexible in fish, in line with the great diversity of adaptations that enable these animals to live in a large variety of aquatic habitats.

I. INTRODUCTION

The definition of stress and stressors has a long history characterized by many controversies. Nevertheless, the fact that the concept of stress has not been abandoned and is widely accepted by cell biologists, physiologists, toxicologists, ethologists, and ecologists demonstrates its vitality and indicates the attraction of describing very different phenomena observed at the organizational levels of cells, organs, organisms, populations, and ecosystems on the basis of a unifying concept. In this review on teleost fishes, stress is defined as a condition in which the dynamic equilibrium of animal organisms called homeostasis is threatened or disturbed as a result of the actions of intrinsic or extrinsic stimuli, commonly defined as stressors (46). The actions of stressors are twofold: they produce effects that threaten or disturb the homeostatic equilibrium, and they elicit a coordinated set of behavioral and physiological responses thought to be compensatory and/or adaptive, enabling the animal to overcome the threat. If an animal is experiencing intense chronic stress, the stress response may lose its adaptive value and become dysfunctional, which may result in inhibition of growth, reproductive failure, and reduced resistance to pathogens. The physiological responses to a stressor are either specific for a single stressor or a group of related stressors, more general, or nonspecific, and they are commonly observed in reaction to many different types of stressors. These responses typically involve all levels of animal organization and are collectively called the integrated stress response.

The current paradigm of the integrated stress response is based on the pioneering studies of Cannon (41) on the role of the catechoamines (CAs) in the “fight/flight” response of animals to threat, combined with Selverstone’s concept of the general adaptation syndrome (224). The latter emphasized the nonspecific nature of the many reactive processes evoked by stressors and the important role of the glucocorticoids in this response. Although later studies showed many other hormones to be involved in the stress response, and that the neuroendocrine system in fact responds in patterns characteristic for each stressor (e.g., Ref. 133), the dominant role of CAs and glucocorticoids in this response is still generally recognized. These hormones are the primary messengers of the two major routes through which the brain coordinates the stress response: the hypothalamic-autonomic nervous system-adrenal medulla axis and the hypothalamic-pituitary-adrenal axis (46). Through the stress response, an animal tries to cope with a stressor by readjusting its biological activities. This implies the reallocation of energy, and this is reflected by the phenomenon that both neuroendocrine routes are also important neuroendocrine pathways for the control of mobilization and allocation of energy under normal as well as stress conditions (46, 81).

Although the conceptual framework of the stress paradigm has been based on mammalian studies, its relevance for fish has been well established (see reviews in Ref. 176). Early studies on fish have shown that the main neuroendocrine control mechanisms of the integrated stress response of fish are comparable to those of mammals and other terrestrial animals and thus conform to a general vertebrate pattern (54, 139). As will be shown, however, the stress response of fishes has many characteristics that are typical for this group. These are related to the intimate contact of these animals with the ambient water through the gills and, especially in seawater, the intestine. Most or all stressors affect branchial structure and as a result hydromineral balance, directly as well as indirectly (see sect. v), and this is one of the main reasons for the high vulnerability of fish to water pollutants. Another reason is the great variety and exquisite sensitivity of the sensory systems of the integument. Sensory perception of a stressor is a prerequisite for eliciting a stress response, in fish as well as in other vertebrates (218, 219). Fish respond to harmful chemicals and many other stressors at intensity levels frequently far below those that can be perceived by terrestrial animals (see sect. x).

The identification of stress in fish under field, aquaculture, or laboratory conditions is complicated. Selverstone (225) has already emphasized that there is a continuum in the responses of animals to challenging but mild, everyday events that cannot or can hardly be considered stressful (the mild form of stress that may even be stimulating, “eustress”), and the more intense responses to strong chronic and life-threatening challenges that may finally lead animals into a pathological state (“distress”). In this continuum it is hard to define the borderline between...
nonstress and stress situations, or between the adaptive responses to challenging but mild stimuli and the integrated stress response when stimuli become threatening and disruptive. This is the main cause of the fact that identification of stress is in principle an arbitrary decision, notwithstanding, or perhaps because of the multitude of behavioral, physiological, and structural parameters that have been used as indicators of stress. Other complicating factors are the temporal aspects of the stress response and the many factors ameliorating or aggravating the impact of stressors. There are substantial differences in etiology between acute and chronic stress conditions (249). Many modifying influences include variables such as temperature and water quality, season, age, gender, physiological condition, social factors, inherited or acquired individual characteristics, and strain or species differences. In this respect, the stress response of the vertebrates is highly flexible, as discussed for fish in section x.

The importance of modifying factors implies that the impact of a stressor is not only dependent on stressor intensity but also defined by the situation and, most importantly, by the way it is experienced by animals. As a matter of convenience, however, we follow common practice and call stimuli stressors when they have been shown to evoke an integrated stress response in fish. These imply sudden or extreme changes in the physical environment (temperature, turbidity, salinity), animal interactions (predation, parasites, intensive competition for space, food, or sexual partners), and human interference, including aquaculture practices (netting, handling, transport, and crowding) and water pollution (low water pH, heavy metals, and organic chemicals). Chemicals may have toxic effects at the cell and tissue level and, above a certain threshold, in addition elicit an integrated stress response. As discussed in sections v, vii, and x, it is frequently difficult to distinguish between the toxic actions of chemicals and their actions as stressors. Nevertheless, toxic chemicals are important as stressors for fish, more than for terrestrial animals (see sect. x), and therefore they have been included in this review.

For the integrated stress response in fishes, the distinction between primary, secondary, and tertiary responses has been introduced (e.g., Refs. 176, 182, 269). Primary responses are activation of brain centers, resulting in the massive release of CAs and corticosteroids, whereas secondary responses usually are defined as the manifold immediate actions and effects of these hormones at blood and tissue level, including increases in cardiac output, oxygen uptake, and mobilization of energy substrates and disturbance of hydromineral balance. Tertiary responses extend to the level of the organism and population: inhibition of growth, reproduction, and immune response and reduced capacity to tolerate subsequent or additional stressors. We follow this classification, although its rigidity, in particular concerning the distinction between secondary and tertiary responses, is increasingly difficult to combine with the more recent evidence on flexibility and complexity of the stress response in fish.

In this paper we review the stress response in teleost fish, with ~20,000 extant species representing >95% of all fish species and almost one-half of all vertebrate species. Particular attention is paid to the aspects that distinguish fish from the terrestrial vertebrates. This review is not intended to cover the available literature (preference is given to key publications and publications not covered by previous reviews), but concentrates on the physiological control mechanisms involved. For exhaustive and critical accounts of all the physiological changes used and for a description of the methods to assess these changes in fish, the reader is referred to reviews by Wedemeyer et al. (269), Donaldson (55), and several authors in Adams (1).

II. HYPOTHALAMIC-SYMPATHETIC-CHROMAFFIN CELL AXIS

In all vertebrate groups, CAs [epinephrine (Epi) and norepinephrine (NE)] are released into the general circulation under conditions that require enhanced blood oxygen transport and the mobilization of energy substrates. Therefore, the release of CAs is an integral part of the physiological response to stressors in all vertebrate groups (94). In this section, the control of CA secretion and the actions of CAs on respiration, the cardiovascular system, blood oxygen transport capacity, and the mobilization of energy substrates, the major functions of these hormones, is discussed. Actions of CAs related to hydromineral balance, reproduction, and immune function are discussed in sections v, vii, and viii, respectively.

A. Control of Catecholamine Secretion

The teleost homologue of the adrenal medulla, and the main source of circulating CAs in fish, is the chromaffin cells, which occur scattered or in small clusters in the head kidneys. These cells can be either closely associated with the steroidogenic cells around the walls of the posterior cardinal veins and their branches (Fig. 1) or in some species separated from the interrenal cells (45). Conversely, in mammals, a significant fraction of the circulating CAs, and in particular NE, originates as “overflow” from sympathetic nerves (217). Such overflow is restricted in fish (200). Although Epi and NE normally are produced in different cells, these same cells produce and release dopamine. At present, the functional significance of the dopamine release is unknown. Free as well as conjugated forms (sulfates and glucuronides) of these CAs have been demonstrated by Epple et al. (62) in superflusate of interrenal tissue of American eels (Anguilla rostrata). Whether the conjugated forms, which are also...
known from the blood of other vertebrate groups, have any physiological significance is unknown. In American eels, the CAs are collocated with neuropeptides such as methionine enkephalin, codeine, and morphine, which may act as autocrine regulators of CA release (61). Furthermore, immunoreactivity to atrial natriuretic peptide was demonstrated by Kloas et al. (113) in the A-type chromaffin cells of the common carp, Cyprinus carpio.

Resting levels of CAs can be obtained by sampling blood from cannulated fish and are generally <5 nM. Rapid and very marked increases in CA levels typically occur as an immediate response to almost any severe acute stressor, with blood Epi and NE rising in both species- and stressor-specific fashion. For example, levels >1,000 nM were reported within 1–3 min. In teleost blood, Epi usually dominates. The high CA peaks following acute stress drop rapidly, consistent with the brief duration of the effects of injected CAs, and reflecting the short biological half-life of circulating CAs (<10 min). During chronic stressor exposure, elevated CA levels may last hours or days [see reviews by McDonald and Milligan (145) and Randall and Perry (200)].

Although the effects of stressors on CA release from the chromaffin cells are mediated primarily via preganglionic cholinergic fibers of sympathetic nerves, sectioning of these nerves does not completely prevent CA secretion during stress. Such noncholinergic release is likely caused by actions of endocrine and nonendocrine blood-borne factors, including CAs themselves, cortisol, rises in plasma K⁺ and CO₂, or hypoxemia (200). Indeed, CA release in teleosts appears to be under complex control. Reid and Perry (206) showed for rainbow trout (Oncorhynchus kisutch) that the injection of the cholinergic receptor agonist carbachol selectively caused the release of Epi over NE, indicating that the innervation of the Epi and NE cells or their sensitivity to acetylcholine may be different. Intra-arterial injections of serotonin caused a dose-dependent increase in both plasma Epi and NE in rainbow trout, a phenomenon that was confirmed in a saline-perfused rainbow trout head kidney preparation. Substantial serotonin stores were demonstrated in the head kidneys (78). In cannulated American eels, Epple and Nibbio (60) observed a dose-related increase of plasma dopamine and NE within 3 min after infusion of Epi, and of Epi following NE infusion. During infusion of NE in common carp, Van Raaij et al. (254) observed a significant release of Epi, whereas the infusion of Epi did not influence plasma NE levels. They suggested that the stimulating effect of NE on Epi is mediated via neural stimulation, since NE, in contrast to Epi, is able to pass the blood-brain barrier in goldfish (Carassius auratus). Injection of cortisol stimulated the activity of dopamine β-hydroxylase in the chromaffin cells of rainbow trout. This enzyme activity catalyzes the hydroxylation of dopamine to NA. Cortisol had no effect on phenylethanolamine N-methyltransferase, the enzyme methylating NE to Epi.

Little is known about the effects of chronically elevated CA levels on the clearance of CAs. Gamperl and Boutilier (83) investigated the effect of repeated Epi injections in rainbow trout on in vivo CA clearance and metabolism, an approach used to mimic the effects of acute (1 day) and chronic (4 days) repeated stress on these parameters. They concluded that neither the rate constants for CA clearance nor the postinjection proportions of unmetabolized tritiated Epi and its metabolites were changed, indicating that CA clearance and metabolism are not affected by repeated exposure to acute stressors. Fløysand et al. (75) showed that in Atlantic salmon (Salmo salar) stressed by crowding, handling, or taking out of water, the cardiac content of undegraded Epi increased significantly within a few hours. This was interpreted as the result of the uptake of Epi for reuse by the sympathetic nerves innervating the heart.

B. Functions of Catecholamines

1. Respiratory and cardiovascular effects

The stress response in fish includes a marked increase in the oxygen uptake rate of the gills as a result...
of increased ventilation rate, stimulated branchial blood flow and branchial oxygen diffusing capacity, and increased oxygen transport capacity of the blood. Most of these effects are caused by circulating CAs acting through β-adrenergic mechanisms (see review by Randall and Perry, Ref. 200). The role of circulating catecholamines in the stimulation of the ventilation rate is unclear (279).

The teleost heart is under inhibitory cholinergic and stimulatory adrenergic (β-adrenoreceptor-mediated) control, with inhibitory and α-adrenoreceptor-mediated adrenergic mechanisms in a modulatory role in some species (200). Neural adrenergic innervation dominates, but the high circulating CA levels that follow stressor exposure can have additional stimulatory effects on cardiac output. In contrast to the vascular control of the gills, where β-adrenergic mechanisms dominate, the vascular resistance of the systemic blood vessels mainly is controlled by α-receptor-mediated adrenergic innervation, with high circulating CA levels performing an auxiliary function (200).

2. Effects on blood oxygen transport capacity

In rainbow trout, Epi increases erythrocyte pH, via a β-receptor-dependent process that is effected by stimulation of Na+/H+ exchange and inhibition of Cl-/HCO3⁻ exchange across the plasma membrane. This results in blood plasma acidification and cytoplasmic alkalination and increases the affinity of hemoglobin for oxygen (see reviews by Nikinmaa, Refs. 157, 158). After administration of an Epi/NE mixture to rainbow trout, the activation of Na+/H+ exchange, as concluded from the lowering in whole blood pH, is followed by transient reductions in partial pressures of CO₂ and O₂ reflecting a shift in the CO₂/HCO₃⁻ equilibrium in the cells, and the subsequent binding of O₂ to hemoglobin. As a result, oxygen transport properties are improved (246). This is a very prompt response, reaching a maximum within 5 min, which can compensate rapidly for the effects of reduced arterial oxygen levels on the degree of hemoglobin oxygen saturation. This rapid adrenergic control of hemoglobin oxygen affinity by stimulated Na+/H+ exchange has been demonstrated only in fish, and important species differences have been reported. The salmonids seem to be specialists in this capacity (see Ref. 158 for references). In American eels, proton extrusion is minimal under normoxic conditions and only 10% of that of rainbow trout under comparable concentrations of hypoxia. These observations are expected from the much lower numbers of surface β-receptors on the red blood cells of eel than on those of trout (172).

Interrenal CA release raises blood hematocrit by causing erythrocytes to swell and by increasing circulating red blood cell numbers. As a result, the blood hemoglobin concentration is increased. Much of this is effected by contraction of the spleen, an erythrocyte store, and, under chronic stress, by anamitotic division of the erythrocytes (233). Whereas the endocrine control of this cell proliferation is unknown, the contraction of the spleen is mediated by α-adrenoreceptor binding of Epi released from sympathetic nerves (200). When splenectomized rainbow trout were forced to swim at critical swimming velocity, arterial blood oxygen tension was significantly reduced while blood hematocrit was unchanged, whereas in sham-operated fish, the oxygen tension was maintained and the hematocrit significantly increased (82). The decreased resistance of erythrocytes to blood flow reported for Epi is mediated by β-adrenoreceptor stimulation (270).

3. Effects on blood glucose and free fatty acid levels

The stress-related hyperglycemia reported in many species of teleosts (24) is mediated mainly by the effects of CAs on glucose release from the liver, the main carbohydrate store in fish, with Epi being more potent than NE (e.g., Ref. 254). The hyperglycemia observed during hypoxia in rainbow trout can be prevented by infusion of adrenoreceptor antagonists (281). Glycogenolysis is the main process accounting for the CA-induced hepatic glucose release. Gluconeogenesis performs a minor role (3–20%), at least in rainbow trout and in the short term (107, 151). Indeed, this process may become more important when hepatic glycogen has been depleted. In vitro exposure of hepatocytes to Epi and NE has shown that CAs promote glycogenolysis through stimulation of glycogen phosphorylase, a β₁-receptor-mediated process (e.g., Refs. 49, 151, 281). Indications for an additional role of α-adrenergic receptors have been presented by Fabbri et al. (65) for American eel, bullhead (Ictalurus nebulosus), and catfish (Ictalurus melas) liver. It is interesting to note that in rats glycogenolysis is stimulated by α-receptors rather than β-adrenergic receptors (217).

There is also some evidence for the involvement of CAs in the mobilization of free fatty acids (FFAs), which are important energy substrates for fish (251; review by Pickering and Pottinger, Ref. 182). However, after reviewing the literature, Sheridan (227, 228) concluded that the results of studies on the effects of CAs on FFA levels in fish are too variable to permit any general conclusion. Recently, Van Raaij et al. (254) reported that NE infusion decreased the accumulation of FFA levels in hypoxic carp, whereas Epi infusions had a stimulatory action. Interestingly, aerial exposure and net confinement of hatchery-reared turbot (Scophthalmus maximus) led to a rise in plasma FFA levels instead of glucose and lactate (268). The absence of a rise in plasma glucose concentration has been reported earlier for some other marine fish but has been interpreted as posttrawling trauma or inability to adapt to captivity (see Ref. 268, for references). Although not typical for marine fish, it may point to a fundamental difference in energy metabolism between fish dur-
ing stress. This aspect of fish physiology needs more attention. So far, no relationship between FFA mobilization and CAs in marine fish has been investigated. The main functions of CAs in fish are listed in Fig. 2.

C. Effects of Stressors on Catecholamines

Acute stress caused by capture, handling, transport, forced exercise, hypoxia, osmotic and temperature shocks or social stressors, or exposure to water pollutants such as acid water containing aluminium has frequently been reported to result in a rapid rise in muscle and plasma lactate and decreased blood pH and oxygen content. Typically, these changes were associated with massive CA release from the chromaffin cells and were followed by a rapid rise in ventilation, branchial blood flow, gas exchange, and blood glucose levels (24, 36, 139, 182, 200, 278). In rainbow trout, plasma Epi and NE levels vary from <1 nM to >200 nM during acute stress (158). An interesting exception was reported recently by Davison et al. (50). They showed that the 5-min handling of the Antarctic teleost *Trematomus bernacchii* did not produce a rise in circulating CAs, whereas heart rate, ventral aortic blood pressure, and hematocrit were elevated. These fish likely depend on increased sympathetic nerve activity during acute stress.

Perry and Reid (173) showed that in cannulated rainbow trout acute hypoxia induced a preferential release of Epi over NE, with the lowering of arterial oxygen content/saturation (rather than PO₂) as an important stimulus causing CA release. Reduced arterial oxygen content can also be caused by gill damage, and this may contribute to CA release by stressors such as toxic chemicals and water acidification. Acidosis seems insufficient to induce CA release, as evidenced by the fact that when hyperoxic and normoxic rainbow trout were made acidotic by hypercapnia concentration, blood pH decreased more in the hyperoxic than in the normoxic fish, whereas the CA levels increased only in the normoxic animals, which had a decreased arterial oxygen content (171). It remains to be established, however, whether immediate CA release during acute stress is exclusively mediated via effects of the stressor on arterial oxygen levels, or can also be elicited through the perception of external sensory stimuli.

Stressors do not only stimulate the release of CAs; they can also indirectly modify the actions of these hormones. The high circulating CA levels that may occur during chronic stress may lead to desensitization of regulatory or target cells, mainly through downregulation of receptors on these cells. Downregulation of red blood cell β-adrenoreceptors was reported by Gilmour et al. (86) in rainbow trout following experimental chronic elevation of plasma catecholamine levels for 72 h and decreased responsiveness of chromaffin cells to cholinergic stimulation after prolonged physical stress (206). Acute handling stress in rainbow trout decreases the responsiveness of liver cells to stimulation by CAs as well as glucagon at 3 and 6 h poststress. This may reflect a mechanism for conservation of hepatic glycogen stores in stressed fish and might explain why these stores do not become depleted in fed, acutely stressed trout, notwithstanding high CA levels. This interpretation was supported by the finding in the poststress period of increased responsiveness of the liver to insulin, which promotes glycogen formation (261).

The effects of chronic stress may not be restricted to moderation of CA actions. For example, hypoxia stimulates the expression of cytoplasmic β-adrenoreceptors at the surface of the red blood cells of rainbow trout, thereby increasing their sensitivity to adrenal stimulation (132). Furthermore, Reid et al. (204) showed that the cytoplasmic pool of internalized β-adrenoreceptors was stimulated by chronically elevated cortisol levels, and con-
confirmed that these receptors moved to the cell surface, and thus became functional, only under hypoxia. Chronic cortisol elevation prevented hypoxia-induced reduction of β-adrenoreceptor affinity (205). Some toxic agents may not only induce a stress response but may also affect CA release or CA functioning, and in this way impair the stress response, by specific direct or indirect actions. Chronic cadmium exposure interferes with CA release in the American eel (85). As has been reviewed by Nikinmaa (157), many chemicals impair the functional capacity of the erythrocytes, and this may stimulate CA release via reduction of the arterial oxygen content. Water pollutants, including heavy metals and pulp mill effluents, may stimulate the breakdown of erythrocytes, which results in reduced hematocrit and a compensatory overproduction of immature erythrocytes. Different mechanisms are implicated. For instance, lead pollution leads to reduced erythrocytic aminolevulinate dehydratase activity, tributyltin inhibits the Na+/H+ exchange, and nitrite induces the formation of methemoglobinemia, and many toxic agents induce an abnormal cell shape (157).

### III. HYPOthalamic-PITuitary-INTERREnal AXIS

For many vertebrates, the involvement of the hypothalamus and pituitary gland in the control of corticosteroid secretion has been well established, with corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH) as the most important secretagogues and cortisol or corticosterone as the adrenocortical end products of the brain-pituitary-adrenal axis (46). This general vertebrate pattern also applies to teleost fishes (241). In fish, corticosteroid production is located in the interrenal cells. These cells do not form a compact gland comparable to the adrenal cortex, but are located in layers, strands, and cords around the walls of the posterior cardinal veins and its branches running through the head kidneys (45; Fig. 1). The homologous neuroendocrine system in fish is therefore referred to as the brain-pituitary-interrenal axis. The main end product in teleosts is cortisol (45). In this section, the corticotropic pituitary hormones and their hypothalamic control are reviewed, as well as the functions of cortisol and its implication in the stress response.

#### A. Control of Cortisol Secretion

The endocrine control of cortisol secretion in teleost fish is complex. Atrial natriuretic factor, angiotensin II (8, 9), growth hormone, thyroxin, arginine vasotocin, and CAs (221, 283) have corticotrophic actions, whereas cortisol was shown to effect self-suppression by negative feed-back of its secretion directly at the level of the interrenal gland (34). Interleukin-like factors of the immune system may have inhibitory activities (17). However, the effects of these factors are probably no more than modulating the corticotrophic actions of hormone(s) originating from the pituitary gland. Studies by Young (283) on hypophysectomized fish have indicated that the pituitary gland dominates the endocrine control of cortisol secretion, as has been concluded from the significant reduction in plasma cortisol levels of the hypophysectomized fish. α-Melanophore-stimulating hormone (α-MSH) and ACTH are the main candidates for this type of pituitary control, with perhaps β-endorphin as a potentiating factor.

1. **ACTH and its hypothalamic control**

The role of ACTH in controlling cortisol secretion has been well established in teleost fish, and there is also extensive histological evidence for the involvement of ACTH in response to stressors in these animals (see review by Donaldson, Ref. 54). However, the results of the few studies on plasma ACTH levels are inconsistent. A rise in plasma ACTH levels has been observed in coho salmon (Oncorhynchus kisutch), rainbow trout, and brown trout (Salmo trutta) subjected to handling, thermal shock, or confinement, in conjunction with a rise of cortisol (19, 185, 238). As shown by Balm et al. (16), in Mozambique tilapia (Oreochromis mossambicus), two types of peptides accounted for most of the ACTH immunoreactivity released by the pituitary pars distalis, with similar corticotrophic potency (16). Confinement in the presence of a dominant peer stimulated plasma ACTH and cortisol levels in subordinate fish. The rise in cortisol following handling stress in tilapia was not preceded or accompanied by a rise in ACTH (16). Thus the relationship between ACTH and cortisol release in tilapia may be less exclusive than in mammals. As in the higher vertebrates, the ACTH secretion in fish seems to be under stimulatory control of the hypothalamic neuropeptide CRH (79, 163, 164). For vasotocin, isotocin and neuropeptide Y stimulatory actions have been reported (79), and inhibitory effects for melanophore-concentrating hormone (Fig. 3; see sect. ivB).

2. **α-MSH and β-endorphin and their hypothalamic control**

Both ACTH and α-MSH cells share the same hormone precursor molecule, proopiomelanocortin (POMC). In the α-MSH cells, this precursor is processed into a number of peptides, of which α-MSH and β-endorphin are now considered to be the most important (Fig. 3). There are three hormonally active forms of α-MSH: des-, mono- and diacetylated α-MSH (79). Several reports have suggested a role for α-MSH in stress adaptation. In brown trout, plasma levels of α-MSH, β-endorphin, and cortisol were...
raised when the fish were subjected to handling and confinement combined with a thermal shock. Without thermal shock, the stressors only induced a rise in cortisol. When rainbow trout were kept restrained out of water, plasma ACTH, α-MSH, and cortisol became elevated (238, 240). Brown trout adapted to a black background showed higher α-MSH levels than fish from a white background. In these fish, no effect could be demonstrated of subsequent acute confinement or thermal shock on plasma α-MSH levels. It was suggested that stimulation of α-MSH cell activity may modulate the pituitary interrenal axis, but on a limited number of observations in a few species only. AVT/IT, arginine vasotocin and isotocin; NPY, neuropeptide Y; CRH, corticotropin-releasing hormone; MCH, melanophore-concentrating hormone; TRH, thyrotropin-releasing hormone; DA, dopamine; ACTH, adrenocorticotropic hormone; MSH, melanophore-stimulating hormone; β-END, β-endorphin (see sects. iiiA and iv for references).

Stimulatory (+) and inhibitory (−) effects of (neuro)endocrine messengers of brain-pituitary-interrenal axis. Evidence is based on a limited number of observations in a few species only. AVT/IT, arginine vasotocin and isotocin; NPY, neuropeptide Y; CRH, corticotropin-releasing hormone; MCH, melanophore-concentrating hormone; TRH, thyrotropin-releasing hormone; DA, dopamine; ACTH, adrenocorticotropic hormone; MSH, melanophore-stimulating hormone; β-END, β-endorphin (see sects. iiiA and iv for references).

FIG. 3. Stimulatory (+) and inhibitory (−) effects of (neuro)endocrine messengers of brain-pituitary-interrenal axis. Evidence is based on a limited number of observations in a few species only. AVT/IT, arginine vasotocin and isotocin; NPY, neuropeptide Y; CRH, corticotropin-releasing hormone; MCH, melanophore-concentrating hormone; TRH, thyrotropin-releasing hormone; DA, dopamine; ACTH, adrenocorticotropic hormone; MSH, melanophore-stimulating hormone; β-END, β-endorphin (see sects. iiiA and iv for references).

Cortisol has broad activity spectrum in fish. Aldosterone, the mineralocorticoid of the terrestrial vertebrates, has only been demonstrated in minute amounts, without apparent physiological significance (45). Gills, intestine, and liver are important targets for cortisol in fish. These organs reflect the two major actions of cortisol in fish: regulation of the hydromineral balance and of energy metabolism. In this respect, cortisol combines actions in fish comparable to those of the mineralocorticoid aldosterone and the glucocorticoids in the terrestrial vertebrates. Other activities of cortisol include reduction in growth rate and suppression of reproductive and immune functions (Fig. 2), and are discussed in sections v–vIII.

1. Mineralocorticoid functions

Cortisol has often been considered a "seawater" hormone, because of its stimulatory effects on branchial Na⁺ and Cl⁻ extrusion, which is essential for hydromineral control in the marine environment. In euryhaline fish, such as salmonids and eels, the interrenal cells proliferate, and cortisol secretion and clearance are stimulated after transfer to seawater, and this may continue long after
acclimation has been achieved (45). However, in the last decade, the prominent role of cortisol in the hydromineral control of freshwater fish has been established, with a stimulatory effect on the uptake of ions such as Na\(^+\) and Cl\(^-\) rather than the exclusive action of the extrusion of these ions in seawater (122). Surgical removal of the interrenal glands of eels disturbs hydromineral balance in freshwater as well as seawater eels. Cortisol promotes the differentiation of the chloride cells, the main ion-transporting cells of the gills, and increases the specific activity of ion-transporting enzymes, in particular Na\(^+\)-K\(^+\)-ATPase in gills, intestine, and kidneys [see reviews by Chester Jones et al. (45) and McCormick (142)]. The important role of the hydromineral actions of cortisol during stress is discussed in section V.

2. Glucocorticoid functions

In fish, cortisol has effects on carbohydrate, protein, and lipid metabolism, in line with, although perhaps less prominent than, the main glucocorticoid actions in the terrestrial vertebrates (46). Cortisol administration is frequently followed by hyperglycemia in fish, but the mechanisms involved are unclear. The rapid rise in plasma glucose concentration following an acute stressor is mainly or exclusively caused by the glycogenolytic action of the CAs (see sect. III) rather than that of cortisol. The reported effects of cortisol on hepatic glycogen levels are inconsistent; increases as well as decreases have been described after cortisol administration [see reviews by Randall and Perry (200) and Van der Boon et al. (251)].

Stimulatory actions of cortisol on hepatic glucose production may be limited to gluconeogenesis, as has been reported for different species (for references, see Ref. 258), and this contributes to the weight loss that may occur during chronic stress. Nonprotein sources may be the preferred gluconeogenic substrates. In experiments on chronically cortisol-treated brook charr, Vijayan et al. (258) found indications for the use of amino acids or lactate as substrates for hepatic gluconeogenesis only in nonfed fish. Andersen et al. (6) studied carbohydrate and protein metabolism in rainbow trout with highly elevated cortisol levels (100–300 ng/ml), achieved by implanted cortisol-releasing osmotic minipumps. Activities of key hepatic enzymes for carbohydrate metabolism were unaffected, and the conversion of alanine and lactate into CO\(_2\), glucose, and protein was unchanged. However, recently Vijayan et al. (262) provided direct evidence for the implication of cortisol in gluconeogenesis in this species by showing that the corticosteroid antagonist RU-486 inhibited the increase in alanine gluconeogenesis of liver cells from cortisol-treated fish. Species differences are substantial, however, and the glucocorticoid actions of cortisol may vary, dependent on life-style and habitat, as indicated by the interesting studies by Vijayan and Moon (260) on the sea raven. In these fish, glucose production by isolated hepatocytes can be stimulated by both cortisol and CAs, in contrast to, for instance, salmonids, where only CAs have this activity (200). The rise in cortisol following stressor exposure in the sea raven (Hemitripterus americanus) is very slow, however, which implies that also in this species the CAs are mainly responsible for the rapid elevation of plasma glucose levels during acute stress.

Cortisol may stimulate plasma FFA levels by promoting lipolysis (see reviews by Sheridan, Refs. 227, 228). Sheridan (227) reported a reduction in hepatic and muscle lipid stores in coho salmon parr that had received cortisol implants. Cortisol treatment of hypophysectomized fish restored the reduced levels of liver lipase that followed the removal of the hypophysis. In a study on brook charr (Salvelinus fontinalis), Vijayan et al. (258) found evidence for a decrease in lipogenesis and an increase in lipolysis after 60 days of cortisol implants.

C. Effects of Stressors on Cortisol

An elevation of plasma cortisol is the most widely used indicator of stress in fish. Typically, plasma cortisol levels rise rapidly a few minutes after exposure to an acute stressor. Return to normal levels takes one or more hours. When the stressor is chronic, cortisol levels may remain elevated, although well below peak levels. Such cortisol responses have been reported for many fish species and after many different treatments, ranging from handling and disturbance, heavy metals, organic pollutants, rapid temperature changes and acid water, to confrontations with predators [see reviews by Barton and Iwama (24), Brown (36), and Donaldson (54)]. In rainbow trout, netting-induced cortisol secretion could be demonstrated up from 2 wk after hatching (Barry et al., Ref. 22). The elevation of plasma cortisol in general is a reliable and easily determined stress indicator because the basal or resting levels are very low (<5 ng/ml) in salmonids (Pickering and Pottinger, Ref. 178), whereas 10- to 100-fold increases have been reported after acute stressful experiences (see review by Barton and Iwama, Ref. 24). On the other hand, basal levels of 50 ng/ml or higher have also been reported. These high levels can be explained by differences in assay specificity, husbandry conditions, capture procedure, or natural factors (24). The capture procedure can greatly stimulate plasma cortisol levels, although to a lesser extent than plasma CA levels. Especially under field conditions, capture effects can hardly be avoided and are more difficult to estimate than in a laboratory because of the lack of appropriate controls. To date, the lowest values for wild fish (<8 ng/ml) have been reported for fish captured and sampled under water by scuba divers (167). High cortisol levels have been observed during the final stages of gonadal maturation and
during spawning (see sect. vii). Other sources of natural variation are the endogenous diurnal and seasonal cycles of plasma cortisol that have been demonstrated in many species. Many of the daily peaks reported are in the range typical for stressed fish (see Ref. 115 for references). Also, yearly cycles have been reported with highest values occurring frequently during wintertime. In migratory salmonids, increased levels of plasma cortisol in spring are associated with smoltification, the process of preadaptation for migration to seawater (e.g., Ref. 137). Cortisol levels may also vary with feeding (115). In farmed coho salmon, Maule et al. (137) found high cortisol peaks in the period before and immediately after these fish would have reached the ocean under free-living conditions. Only the second peak, probably the result of preventing the fish from entering the sea when they were physiologically pre-adapted to do so, was considered as a stress response.

Thus not all elevated levels of cortisol can be ascribed to stressors. Conversely, the absence of clearly elevated levels does not always guarantee the absence of stressors. First, in salmonids, slight but chronic elevations of cortisol levels (from 0–5 to 10 ng/ml, i.e., below concentrations frequently reported as unstressed levels) have been implicated in depression of immune function and disease resistance (136, 137, 181). Such low levels are often hard to distinguish from resting levels. Second, pollutants may have blunted the cortisol response. Poor water quality suppressed cortisol response of salmonids to handling and confinement (180). Exposure of the snakehead Channa punctatus to ammonium sulfate for 6–12 mo resulted in degenerative changes in theACTH cells and the interrenal cortisol-producing cells (199). Hontela et al. (98) concluded that a life-long exposure to chemical pollutants may lead to exhaustion of the pituitary-interrenal axis as a result of prolonged hyperactivity of the system. This was based on the finding of atrophied adrenocorticotropic cells and the absence of a serum cortisol elevation in response to capture in perch (Perea flavescens) and pike (Esox lucius) from field sites polluted by high levels of polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and mercury. Such fish showed an impaired ability to elevate plasma cortisol levels in response to an acute stressor (97). Specific toxic effects on these glands, rather than exhaustion, may be implicated, however, since organic xenobiotics have been shown to impair or alter steroid hormone metabolism in fish (88, 245). Third, the impaired cortisol response may be partially related to enhanced cortisol clearance or downregulation of ACTH or cortisol receptors in chronically stressed fish. Enhanced clearance, caused by, e.g., mixed function oxygenases and conjugating enzymes, may stimulate inactivation and excretion of corticosteroids (245). Vijayan and Leatherland (259) showed that interrenal tissue from brook trout reared at a high stocking density not only showed increased cortisol secretion, but also a lower response to ACTH stimulation than tissue from control fish. This was interpreted as indicating either maximum secretory activity in the fish kept at high density, or downregulation of the ACTH receptors of the interrenal tissue in these fish. Gill tissue of several salmonids was shown to have a reduced sensitivity to exogenous cortisol (as measured by Na+/K+-ATPase stimulation) in the migratory season in April/May, when the circulating levels are high, when compared with January/March (142). Long-term confinement or cortisol feeding decreased the number and affinity of coho salmon branchial cortisol receptors (135).

Fish, like other organisms, produce a variety of stress proteins when exposed to stressors, which are implicated in cellular restoration of affected functions and confer cellular protection and increased stressor tolerance. Of these highly conserved proteins, metallothioneins (metal-binding molecules produced in response to toxic metals) and heat shock proteins (chaperone-like molecules induced by rapid temperature changes and many other stressors) are best known (see review by Sanders, Ref. 216). Ubiquitins (mediators of nonlysosomal proteolysis in stressed cells) have also been demonstrated in fish (references in Ref. 211). Whereas corticosteroids facilitate the expression of these proteins in higher vertebrates, the reported effects of cortisol on stress proteins in fish in vivo are inconsistent (84, 182). Stimulatory effects of cortisol on metallothionein induction have been reported for primary cultures of rainbow trout hepatocytes (100). Although the term stress proteins suggests otherwise, these proteins can already be observed during exposure to adverse stimuli below the level that is required to initiate an integrated stress response. Fader et al. (66) reported significant seasonal difference in heat shock protein levels in four fish species under natural conditions and concluded that high expression of these proteins does not necessarily imply that the animals are stressed. The presence of stress proteins in the absence of an integrated stress response reflects that the expression of these substances can be controlled at the cellular or tissue level rather than at the level of the organism and induced by stimuli with an intensity below the threshold necessary to elicit an organismal stress response. Ryan et al. (211) detected free and conjugated ubiquitins in brain, gills, skeletal muscle, and erythrocytes of unstressed blue mao mao (Scorpsis violaceus). Thirty minutes after confinement, which elicited an integrated stress response as indicated by highly elevated plasma cortisol and lactose levels and disturbed plasma electrolyte concentrations, a significant rise of ubiquitin conjugates was observed in the erythrocytes only. Much research is going on in this field, and substantial progress with respect to the control and the physiological role of the stress proteins can be expected in the next few years.
IV. CENTRAL NEUROTRANSMITTERS, NEUROENDOCRINE PEPTIDES, AND HORMONES

A. Central Neurotransmitters

Many brain structures, including serotonergic, cholinergic, and adrenergic systems, become activated in the mammalian brain during stress (46, 277). Effects of stress on the brain of fish are only beginning to be explored. Mazeaud and Mazeaud (139) reported reduced CA content of rainbow trout hypothalamus after 5 min of struggling during emersion. Activation of serotonergic neurons and increased serotonin turnover rate have been reported in brains of Arctic charr (Salvelinus alpinus) stressed by netting or a subordinate position, and this was associated with reduced locomotory activity of the stressed animals (277). Activation of cholinergic brain systems was further observed after chronic cadmium and naphthalene exposure of Mozambique tilapia. This effect could be mimicked by intraperitoneal injection of Epi, a treatment known to stimulate cholinergic neurons in the mammalian brain (170).

B. Neuroendocrine Peptides

1. CRH

Neurons secreting CRH are widely distributed in the mammalian brain, with important concentrations in the paraventricular nuclei of the hypothalamus. Intracerebral administration of CRH elicits complex behavioral and physiological responses including arousal, anxiety, reduced appetite, and activation of the pituitary-interrenal axis as well as the sympathetic nervous system. Corticotropin-releasing hormone probably is the main mediator of the reduction in growth and reproduction during stress (46, 276).

Although the dominant position of CRH in the neuroendocrine integration of the stress response has still to be demonstrated for fish, at least its role in the hypothalamic-pituitary-interrenal axis has now been indicated for these animals (see sect. 3A and review by Lederis et al., Ref. 123). In most species examined, immunoreactive CRH is mainly present in the parvocellular and magnocellular nuclei of the nucleus preopticus (NPO). In goldfish and the white sucker Catostomus commersoni, colocalization of CRH with arginine vasotocin and isotocin has been reported. In these fish, the CRH-like peptide urotensin-I was localized in the nucleus lateralis tuberis (NLT) and, in goldfish, in two other lateral hypothalamic nuclei. Immunoreactive tracts of the NPO innervate the pituitary regions of the ACTH and α-MSH cells, and those of the NLT, the α-MSH cells (123). However, in the gilthead seabream Sparus aurata, most CRH/urotensin-like immunoreactivity is found in the NLT and not in the NPO. Immunoreactive fibers were only ending in the vicinity of α-MSH cells (128). Both CRH and urotensin-I have ACTH- and α-MSH-stimulating properties (see sect. 3A; Ref. 247). The predicted amino acid sequence of white sucker CRH has been determined and appeared very well conserved, with only 2 of the 41 amino acids different from rat CRH (161).

2. Urotensin-I

The CRH-like peptide is not only produced by hypothalamic nuclei, but also, and predominantly, by the urophysis, a neuroendocrine system unique for jawed fish. Because the release of urotensin-I was inhibited by transfer of the fish from freshwater to seawater and stimulated by the reverse procedure in Gillichthys mirabilis (119), the urotensins produced by the urophysis may only have significance during exposure to osmotic stressors and may not be involved in nonspecific stress responses.

3. POMC-derived peptides

Neuronal systems producing POMC-derived peptides, including α-MSH- and ACTH-like transmitters, have been demonstrated in fish (164, 214). Immunostaining with ACTH was reduced in the brain perikarya as well as in the pituitary corticotropes of cortisol-treated European eels (Anguilla anguilla), indicating possible involvement of the neural ACTH-like peptides in stress regulation (164). Salbert et al. (214) detected two POMC genes in hypothalamic neurons of rainbow trout. In sexually inactive fish, only one gene product was detectable, whereas both genes were expressed in sexually active fish.

4. TRH

The effects of TRH on ACTH and α-MSH release were mentioned in section 3A.

5. Arginine vasotocin

Arginine vasotocin (AVT) is the teleostean representative of the vasopressin family. Elevated brain levels of AVT, a likely antidiuretic factor in fish, have been reported for brook trout from acid lakes (99). In an earlier study on medaka (Oryzias latipes), hydromineral disturbance induced by freshwater to seawater transfer caused AVT perikarya of the AVT cells in the preoptic nuclei, whereas the reverse transfer resulted in AVT storage, indicating the stressor specificity of the AVT response (95). Evidence for a relation of AVT with the nonspecific integrated stress response has been reported for goldfish (79). This effect may be comparable to the role of hypothalamic vasopressin in mammals, where it potentiates the release of ACTH by CRH (96).
6. MCH

Melanophore-concentrating hormone is a rather recently discovered peptide from the vertebrate brain. It is also secreted from the teleost hypothalamus into the blood circulation and is known to antagonize the melanophore stimulating activity of α-MSH at the pituitary and at the target cell level. When administered to rainbow trout, it depressed the release of CRH and ACTH in addition to α-MSH (see reviews by Baker, Refs. 13, 14). Plasma MCH levels showed a marked increase after multiple injections as a stressor in these fish, which could be antagonized by the synthetic corticosteroid dexamethasone, suggesting a negative-feedback control of cortisol on MCH secretion (90). Groneveld and co-workers (92, 93) confirmed the inhibitory action of MCH on α-MSH secretion in Mozambique tilapia, at least at low concentrations. They further studied MCH gene expression in the two main MCH producing hypothalamic centers, the NLT and the nucleus recessus lateralis (NRL). In fish from strongly acidified water, with highly elevated cortisol and ACTH levels and disturbed ion regulation, a rise of preproMCH mRNA levels was found in the NLT but not the NRL. Conversely, repeated disturbance increased the preproMCH mRNA levels in the NRL, and not in the NLT (92). MCH may have a modulatory role on the hypothalamus-pituitary-interrenal axis in teleost fish.

7. Opioid factors

Enkephalin- and endorphin-like substances have been demonstrated in the brains of fish, but their role during stress is unknown. Some evidence for opioid receptor-mediated inhibitory and stimulatory effects on the hypothalamus-pituitary-interrenal axis has been reported for rainbow trout (152). Pituitary endorphins have already been discussed in section IIIA. Opioid actions on the immune system are mentioned in section VIII.D.

C. Pituitary Hormones

The POMC-derived pituitary hormones, which are part of the hypothalamus-pituitary-interrenal axis, were discussed in section III. Other pituitary hormones that have been directly implicated in the integrated stress response in fish are the three members of the growth hormone/prolactin family in fish: growth hormone, prolactin, and somatolactin.

1. Growth hormone

The function of growth hormone in teleost fish has been related to growth as well as hydromineral control in seawater fish, both functions that are greatly influenced by stressors, and are discussed in sections V.A and VI.

2. Prolactin

The main function of prolactin in fish is the inhibitory control of the permeability of the integument to water and ions in freshwater environment. Synthesis and release of the hormone are stimulated by reduction in water osmolarity or, in some species, water calcium concentration and other treatments that affect the integumental permeability, such as acid water and some pollutants (see review by Wendelaar Bonga and Pang, Ref. 274). Little is known about the effects of nonpollutant stressors on prolactin secretion. Avella et al. (12) reported a rise in plasma prolactin, associated with an increase in plasma cortisol, in freshwater and seawater coho salmon following acute handling or chronic confinement. A rise in prolactin, in addition to cortisol, was also reported for rainbow trout acutely subjected to hyperosmotic saline (28). However, Pottinger et al. (194) reported a reduction in plasma prolactin in confined rainbow trout. Kakizawa et al. (111) could not demonstrate a change in plasma prolactin in rainbow trout after acute lowering of the water level and chasing, conditions which stimulated plasma cortisol, somatolactin, and growth hormone levels.

3. Somatolactin

Somatolactin is a pituitary hormone of teleost fishes belonging to the growth hormone/prolactin family. Its function has not been clarified but has been related to gonadal maturation and spawning, hydromineral and acid-base regulation, and energy mobilization (for references, see Kakizawa et al., Ref. 111). Salmonids exposed to reduced water levels and chasing showed a rapid increase in plasma somatolactin levels (202), which was confirmed for rainbow trout by Kakizawa et al. (111). The rise in plasma levels was noticeable already after 2 min. Conversely, plasma somatolactin levels were not elevated in red drum (Sciaenops ocellatus) and Atlantic croaker (Micropogonias undulatus) in response to acute stressors, including netting, confinement, and transport. In these species, plasma levels increased in fish following transfer to dark background (285).

D. Other Hormones

Several other hormones have been reported to respond to stressors, which is not surprising given the profound physiological changes that may accompany the specific and nonspecific responses to stressors. For instance, prolonged exposure of Mozambique tilapia to high water osmolarity, high pH, or high temperature increased pineal weight and serum melatonin levels (207). Stressors have been reported to reduce or inhibit thyroxine release, but no clear picture has emerged to date (see Refs. 36, 177...
for references). Hormones involved in control of growth and reproduction will be dealt with in sections vi and vii.

V. STRESS AND HYDROMINERAL BALANCE

A. Hydromineral Control in Fish

Although in terrestrial vertebrates stressors have many disturbing effects on hydromineral parameters, in primary aquatic animals such as fish, the impact of stressors on water and ion homeostasis is much more profound, and in fact, disturbance of water and ion homeostasis is one of the most characteristic aspects of stress in fishes. This is mainly caused by the very intimate relationship between the body fluids in the gills and the ambient water. Briefly, the gills consist of two sets of four branchial arches, each with rows of filaments at right angles to the arch, and each filament with two rows of lamellae at right angles to the filament. Each arch contains a complex vascular system, consisting of afferent filamentary arteries and arterioles, opening in extensive flat blood sinuses in the lamellae, which are drained by efferent filamentary arterioles and arteries. The efferent arteries of each filament anastomose with a central venous sinus, found on the lamellae, the skin epithelium covering the branchial structure can also be affected by stressors, during acute as well as chronic exposure, with negative effects for all branchial functions. In this section, these effects are discussed, with emphasis on the role of the gills in hydromineral control.

1. Branchial structure

Water-borne toxic chemicals; low water pH, in particular in the presence of high aluminium concentrations; rapid and substantial changes in water temperature; or chronic handling cause marked structural changes in the gills, with pronounced effects on its major functions. In an extensive review, Mallatt (127) concluded that most of the changes in gill structure are nonspecific in nature, partially representing damage, and partially compensatory responses of the fish. Necrosis (cell death associated with swelling phenomena) of the branchial epithelial cells, epithelial lifting (i.e., fluid infiltration in the intercellular spaces and separation of the epithelium from its basal membrane), dilation of the blood sinuses of the branchial lamellae, and lamellar aneurism are examples of the first. Compensatory responses include hypertrophy and hyperplasia of epithelial respiratory and chloride cells, hypersecretion of mucus followed by depletion of mucus cells with differentiation of new mucus cells in the branchial
The high rates of necrosis and apoptosis in combination with mitosis in stressed fish reflect increased turnover or accelerated aging of the branchial epithelial cells during stress. Similar phenomena occur in the skin epithelium of stressed fish. In this tissue, stressors such as heavy metals, acid water with aluminium, or rapid temperature elevation also produce increased rates of necrosis, apoptosis, and mitosis of the pavement cells (the upper layer of the skin epithelium), hypersecretion of mucus, dilation of intercellular spaces, and infiltration of leukocytes in these spaces (e.g., Refs. 102, 103).

One could argue that apoptosis and necrosis of the gills are pathological phenomena caused directly by chemical (toxic agents) or physical (osmotic forces as occur after transfer of fish from freshwater to seawater) interaction with the epithelial cells of skin and gills. Such effects will certainly occur. For instance, in rainbow trout exposed to acid water containing a low aluminium concentration, no change in ACTH, α-MSH, or cortisol secretion could be demonstrated, although some structural damage to the gills, including elevated rates of apoptosis of the epithelial cells, as well as compensatory adjustment of branchial structure did occur during the 2-wk exposure period (18). This indicates that damage to the integument area, and infiltration of the dilated intercellular spaces by leukocytes. All of these structural changes in the gills are similar for freshwater and seawater fishes, with exception of epithelial lifting. This is more common in freshwater, indicating that lifting is an osmotic phenomenon (Mallatt, Ref. 127). More recently, ultrastructural examination of the gills of Mozambique tilapia exposed to rapid seawater transfer, cadmium, and acid water with aluminium has indicated that a common effect of stressors on fish gills is the high incidence of apoptotic (physiologically controlled) cell death in addition to necrosis [see Wendelaar Bonga and Lock (273) for references; Fig. 6]. Stressors have been reported to increase, to have no effect, or to reduce both branchial chloride cell numbers and Na"-K"-ATPase activity during chronic stressor exposure. We have discussed previously (273) that these responses of the fish are dependent on the balance between mitotic and cell death rates and the resulting number of functionally mature cells. This balance is related to stressor intensity rather than the type of stressor. As a result, highly increased cell numbers may not be correlated with high Na"-K"-ATPase activity or branchial ion-exchange activity, because of the predominance of differentiating and degenerating, and thus non-functional or suboptimally functioning cells (273).
FIG. 5. Diagrammatic representation of chloride cell function in freshwater (A) and seawater (B) fish. Active transport and cotransport are indicated by solid lines and diffusion by dashed lines. Tight intercellular junctions are indicated as multistranded; leaky junction (B; between AC and CC) is single stranded. This leaky junction permits Na⁺ efflux driven by serosa-positive transepithelial potential. PC, pavement cell; CC, chloride cell; AC, accessory chloride cell. Mechanisms of excretion of acid-base equivalents in seawater fish are unclear. Whether proton pump and Na⁺ channel (indicated in apical membrane of freshwater PC) is located in this cell, in CC, or in both is still under discussion. [-] Active transport. [Modified from Flik et al. (71) and Marshall (131).]

can be induced by negative stimuli at an intensity level that is too low to elicit a clear stress response. However, most changes mentioned above for gills exposed to toxic irritants (increased necrosis, apoptosis and mitosis, dilatation of intercellular spaces, leukocyte infiltration, and hypersecretion of mucus) have also been reported for fishes suffering from chronic isolation or a submissive position in social hierarchy (271; Fig. 6). Peters and Hong (175) have reported severe necrosis of branchial chloride cells of European eels which had lost fights for dominance. This indicates that such phenomena not only result from the direct toxic or otherwise damaging actions of water pollutants, but may also be induced indirectly, without chemical or physical interaction with the stressor. Our recent results indicate that high cortisol levels may be implicated. After administration of the hormone via the food to rainbow trout, which resulted in a transient rise of plasma cortisol to concentrations occurring in severely stressed fish, the mitotic and apoptotic rates of the pavement cells of the skin were dramatically increased (101), indicating increased turnover of these cells. For apoptosis, this observation has been confirmed for pavement cells and chloride in Mozambique tilapia gills in vivo (unpublished data) and for primary culture of tilapia gill filaments (38). Necrosis was not influenced by cortisol in these experiments, but it cannot be excluded that the hormone can promote this process indirectly, for instance, via its immunosuppressive actions, which may stimulate the incidence of necrosis by infection with pathogens (see sect. viii). A similar explanation has been put forward by McBride and Van Overbeke (141) for the severe tissue necrosis characteristic of Pacific salmon at the end of their upward migration to their spawning grounds, when plasma cortisol reaches extremely high levels. If the high apoptotic rates observed in submissive and cadmium-exposed fish are mediated, fully (submission) or at least partially (cadmium), by high cortisol levels, additive effects may be expected when fish are confronted simultaneously with both stressors. Such effects were indeed observed (Fig. 7).

Although there is a clear relationship between stressor exposure and branchial damage, the relation between structural damage and the initiation of a stress response is not always clear. Rather than by damage to the integument, the immediate rises in plasma cortisol and catecholamines evoked by acute exposure to acid water containing high aluminium levels may be mediated by sensory perception of the metal rather than by damage to the integument, as is indicated by behavioral responses (see references in Refs. 10, 11).

2. Branchial function

Earlier reports on the effects of stressors on osmotic and ionic regulation have been reviewed by Eddy (56). In general, handling, rapid changes in salinity, heavy metals and pollutants, ammonia, water acidification, or sudden temperature changes cause passive ion losses and water uptake in freshwater fish, increase passive ion influxes and water loss in seawater fish, and inhibit active ion exchanges in both. These effects are associated with disturbed plasma ion homeostasis and acid-base balance. More recent reviews, in general confirming these results, can be found in Evans (64) and Wendelaar Bonga and Lock (273). Toxic water pollutants and acidified water containing aluminium also impair oxygen uptake. This has been ascribed to both the disruption of the branchial epithelium, causing hemodynamic malfunction, and clogging of the branchial cavity by accumulation of mucus (64,
Fig. 6. Generalized diagram of filamental epithelium (fe) and lamellae (la) of gills of unstressed (A) and stressed (B) freshwater fish. bs, Lamellar blood sinuses lined by cytoplasmic extensions of pillar cells (pc); r, respiratory epithelium covering lamellae; e, erythrocyte; f, filamental cells. Lamellae of control fish are smooth and slender. In stressed fish, lamellae have an irregular surface, and layer of connective tissue between pillar cells and respiratory epithelium shows edematous swelling (s); intercellular spaces in filament epithelium are enlarged (is). Number of chloride cells (c) is higher in stressed fish; number of apoptotic (ac) and immature (ic) chloride cells is enhanced, indicating a higher turnover rate of these cells. During stress, macrophages (ma) and lymphocytes (ly) extravasate and are found in connective tissue of lamellae or in intercellular spaces of filamental epithelium. Circulating neutrophils (ne) increase in number. Necrotic swelling (ns) may occur in all cell types. Mitosis (mi), which is limited to inner layers of filamental epithelium, is more frequently observed. Chloride cells, usually confined to filamental epithelium, migrate in respiratory epithelium of stressed fish. There they are very common in salmonid fish. These diagrams are based on the author's studies on common carp, tilapia, and trout (e.g., Refs. 271, 273; unpublished data) and on literature (e.g., Ref. 127) and show changes that different types of stressors (e.g., toxic substances, acidified water, low hierarchical position, rapid temperature changes) have in common. Stressor-specific effects are not shown.

Structural damage to the gills by stressors such as handling, isolation, or a subordinate position develops slowly. What then causes the increased permeability of the gills to water and ions that is associated with acute exposure to stressors that do not interact directly with the branchial epithelia? Most likely the increased oxygen demand during stress compromises the hydromineral balance of fish, with the high levels of circulating catecholamines during stress as the main causal factor. As mentioned above, the vascular resistance of the gills is reduced by circulating Epi. The increased blood flow, together with distension of perfused lamellae and the recruitment of branchial lamellae that are hardly or not perfused in the resting state, increases the effective respi-
A central aspect of stress adaptation is the reallocation of metabolic energy away from investment activities (i.e., growth and reproduction) and toward activities that require intensification to restore homeostasis, such as respiration, locomotion, hydromineral regulation, and tissue repair. This reduces the performance capacity of the fish during chronic stress and the recovery phase following stress (218, 219). Reduced or negative growth is commonly observed during stress, and growth rates, expressed as changes in body weight or derived parameters such as condition factors or food conversion efficiency, are generally considered as reliable indicators of stress and have been widely applied to fish (see review by Goede and Barton, Ref. 87).
A. Growth and Stress

Inhibited growth has been reported for physical, social, and chemical stressors, including rapid temperature changes, capture, handling, crowding, and many kinds of pollutants, among which include low water pH and aluminum, heavy metals, and organic chemicals. For the toxic stressors, a distinction between the direct toxic effects and effects mediated by neuroendocrine messengers as part of the integrated stress response cannot always be made, but both types of effects have been demonstrated (24, 87). Growth rates reflect food availability, appetite and consumption, intestinal uptake, and metabolic rate, and these factors can all be influenced by stressors.

1. Appetite and food intake

Reduced appetite is often mentioned, although less well documented, in studies on stressed fish. A marked reduction in feeding activity was recorded for brown trout during the first 3 days after 2-min handling stress (184). Food intake was considered one of the main causes of the growth compression in brown trout under crowded conditions (187) and a main cause of reduced growth in subordinate eels (175). Exposure to pollutants can reduce food intake substantially (208). In aquaculture, stocking density is a major factor influencing food intake, in a species-specific way. For instance, high densities reduce feeding activity and growth rates in coho salmon, brook char, and rainbow trout but have a positive effect on these parameters in Arctic char. In the latter species, agonistic behavior is reduced at high densities (see Ref. 4 for references).

2. Food assimilation

During stress, the food assimilation capacity may become impaired. Peters (174) reported that the low food intake of subordinate eels was associated with extensive intestinal lesions.

3. Metabolic rate

Acute and chronic stress are typically associated with increased metabolic rate. Although direct measurements of metabolic rate are scarce, the frequent occurrence of accelerated metabolism during stressor exposure can be deduced from the many reports of hyperglycemia during stress, because plasma glucose levels are positively correlated with metabolic rate (e.g., Ref. 23). Brief disturbances of steelhead trout (Oncorhynchus mykiss) increased metabolism to ~25% above standard metabolism (25). Rice (208) calculated for largemouth bass (Micropterus salmoides) that a 10% increase in metabolic rate reduced growth rates by 22%, and a 20% increase by 42%.

Increased metabolic rates are further reflected by stimulated respiratory movements and oxygen consumption. A 20% increase in respiratory rate was associated with a reduction of 8–16% in growth rate of coho salmon (255). Respiration rates of juvenile steelheads were more than double control levels 1 h after several disturbances of 2 min (26). Volpato et al. (264) have shown that acute grouping leads to a higher respiratory rate in subordinate Nile tilapia (Oreochromis niloticus) than in the dominant animals. Subordinates also showed higher consumption of liver carbohydrate reserves (51) and died earlier than dominants during progressive hypoxia (264).

Not all stressors increase metabolic rate under all conditions. Instead of showing a stress response, some fish species respond to hypoxia with downregulation of the metabolic activity well below the standard metabolic rate, an adaptive reaction called metabolic depression. The capacity for metabolic depression is species-dependent, with salmonids being poor performers and eels and carp being specialists. This phenomenon may be mediated by the release of opioid substances (see review by Van den Thillart and Van Ginneken, Ref. 250).

B. Endocrine Control of Growth During Stress

Growth is under multiple endocrine control, with growth hormone as the pivotal factor. Insulin, thyroid hormone, and gonadal steroids, in particular during the initial phases of sexual maturation, also have growth-promoting capacities (177). Catecholamines and cortisol are the major hormones with growth-inhibiting effects (177; see sects. II and III).

Molecular structure, control of release, and physiological function of growth hormone in fish have been studied extensively in the last 10 years. Its growth-promoting effects, role in hydromineral control in seawater fish, stimulation of gonadal development, and the dependence of its actions on insulin-like growth factors have been established [see reviews by Le Gac et al. (124) and Sakamoto et al. (213)]. The relationship between plasma growth hormone levels and growth rate is unclear. Whereas growth hormone injections stimulate growth, several authors have reported an inverse relationship between circulating growth hormone and somatic growth, for instance, in strains of rainbow trout with different growth rates (177) or during starvation (201). Conversely, a strongly positive relationship between plasma growth hormone and growth was found in Atlantic salmon during parr-smolt transformation (30), whereas in transgenic coho salmon expressing a sockeye salmon growth hormone gene construct, growth hormone levels were 40-fold and body weights 11-fold higher than in controls (52). Given this complexity, it is not surprising that the reports on circulating growth hormone levels in stressed fish are inconsistent and difficult to interpret. Plasma growth hormone levels have been
reported to increase after injection stress in goldfish (47) and to remain unaffected in chum salmon (Oncorhynchus keta) after handling stress (265). In rainbow trout, a decrease was observed after acute handling and confinement, when plasma ACTH and cortisol levels were elevated. However, chronically low water oxygen levels, which also led to elevated ACTH and cortisol levels, stimulated an increase in growth hormone levels in these animals (186). A rapid transient rise, already noticeable after 2 min, and concurrent with a rise in plasma cortisol, was observed in rainbow trout following lowering of the water and chasing (111). These observations all concern freshwater fish, and thus this growth hormone response cannot be related to the actions of the hormone on hydromineral control, since these actions are most likely limited to seawater fish (142).

Catecholamines and cortisol inhibit somatic growth by stimulating energy consumption, gluconeogenesis, and lipolysis (see sects. II and III), and perhaps, via effects on growth-promoting hormones. However, evidence for the latter mechanism is scarce and conflicting. Cortisol increased the secretion of growth hormone from Mozambique tilapia pituitary in vitro (160). On the other hand, growth hormone secretion was inhibited immediately after exposure of these fish to acid water, which caused a rapid rise in plasma cortisol (272). Effects of CAs and cortisol on insulin, thyroid hormone, and gonadal steroids are also inconsistent (254; see sects. III and IV). In salmonids, experimentally induced high circulating cortisol levels inhibit growth (26), or induce negative growth, by promoting cellular atrophy in a wide range of body tissues (141). In rainbow trout fibroblasts in vitro, high cortisol concentrations (within the range found in blood of stressed fish) inhibited proliferation by delaying the entry of cells into S phase, a phenomenon well known from glucocorticoids in mammals (125). Thus, high cortisol levels may affect growth processes via different mechanisms.

VII. STRESS AND REPRODUCTION

Successful reproduction is dependent on complicated neuroendocrine control mechanisms as well as on many environmental variables. Temperature and daily photoperiod are most important. Food availability, water quality, social environment, or suitable substrates for spawning may also be decisive. Thus the many prerequisites for successful reproduction make reproduction very dependent on environmental conditions (e.g., Ref. 29). Reduction of reproductive performance is a common phenomenon associated with stress in the vertebrates (150), including fish (24, 55). It reflects that the reallocation of energy during stress not only affects the anabolic processes of fish but also their investment of energy in progeny. Analogous to the environmental changes affecting hydromineral control and growth, a distinction can be made between factors that are inhibiting reproduction directly, without a manifest stress response, and factors that elicit a stress response and impair reproduction indirectly. The existence of the first situation results from the fact that successful reproduction frequently requires more stringent conditions than other body functions. For instance, relatively few teleost species are able to reproduce spontaneously under most farming conditions or other forms of relative confinement such as laboratory holding facilities, without showing other characteristics of a stress response. For some species, normal gonadal growth and development may proceed, but gonadotropin-releasing hormone analogues or pituitary extracts are necessary to induce ovulation and spermiation, and gamete stripping and artificial fertilization are usually required for successful reproduction. For many other species in captivity, such as eels, even gonadal development does not occur without the administration of different hormones (35). The literature on the effects of stressors on reproduction has been reviewed previously by Billard et al. (29), Barton and Iwama (24), and Donaldson (55) and, for toxic substances, Suter et al. (242). The latter authors (242) concluded that reduction of fecundity was the most sensitive parameter for toxicity, more than survival of early life stages. Many chemical agents have toxic effects rather than stressor effects on reproduction, and these are frequently mediated by toxic interactions with the endocrine control mechanisms of reproduction (e.g., Refs. 74, 88, 147). However, here we concentrate on what we consider the main new development of the last 15 years in this area, the analysis of the endocrine mechanisms involved in the nonspecific impairment of reproduction by stressors, at the level of the hypothalamic-pituitary-gonadal axis.

A. Hypothalamic-Pituitary-Gonadal Axis

Gonadal development and gametogenesis are under control of the brain via the hypothalamic-pituitary-gonadal axis (155). Two isoforms of gonadotropin-releasing hormones have been demonstrated in most teleost species investigated so far, whereas three forms have been reported for the gilthead seabream Sparus auratus (195). They are produced in the hypothalamus and stimulate the secretion of gonadotropic hormones (GTH I and II) in the pituitary gland. Gonadotropic hormone I controls gonad development and the early stages of gametogenesis, and GTH II controls the final maturation stages. Most actions of GTHs are mediated through steroids, produced by the follicle cells of the oocytes. One of these is 17β-estradiol, which promotes oocyte growth and yolk accumulation and the production of vitellogenins, precursors to yolk proteins. Vitellogenins are calcium-phospholipoproteins that are produced in the liver, transported via the blood to
the liver, and taken up by growing oocytes. Gonadotropic hormone II is important for the meiotic maturation of teleost oocytes, and its action is mediated through 17α,20β-dihydroxyprogesterone or closely related steroids, also produced by the follicle cells (21, 155). In males, steroidogenic activity is located in the somatic cells of the testes, which produce 11-ketotestosterone as the main androgen controlling spermatogenesis; spermatozoa are capable of the conversion of 17α-hydroxyprogesterone, produced by the somatic cells, to 17α,20β-dihydroxyprogesterone, the steroid responsible for spermatiation in salmonids and many other fishes (55).

B. Reproductive Hormones and Stressors

Whereas the inhibitory effects of stressors on reproductive performance have been established for many types of stressors and for many different species, the data on gonadotropic hormones and gonadal steroids, mainly concerning salmonids, are inconsistent. Increases, no effect, or decreases have been reported in response to many physical or chemical stressors. For instance, acute handling stress for 1 h elevated plasma cortisol and ACTH levels and reduced plasma testosterone and 11-ketotestosterone concentrations. However, plasma gonadotropin levels were elevated (183). This confusing picture, further illustrated by previous reviews (29, 55), has not been clarified by more recent results. Plasma testosterone and 17β-estradiol levels were depressed 1 h after capture of female New Zealand snapper Pagrus auratus (42) and 24 h after capture of rainbow trout (166). Chronic confinement stress during the months before spawning reduced plasma testosterone levels in male but not female rainbow trout, and female but not male brown trout. The 17β-estradiol levels were not affected, although plasma vitellogenin levels were reduced. The survival rate of the fertilized eggs from these fish was significantly reduced for both species (40). Thus the degree of stressor-induced changes in plasma gonadotropic or gonadal steroid levels does not necessarily reflect the extent of impairment of reproduction, although there is a tendency for a reduction in gonadal steroid levels during stress. Careful analysis of hormone synthesis and kinetics is required to interpret the plasma levels of these hormones in stressed fish.

Cortisol has frequently been indicated as the major factor mediating the suppression of reproduction during stress, in analogy with mammalian glucocorticoids, for which the inhibitory effects at different levels of the hypothalamo-pituitary-gonadal axis have been established (46). For fish, the evidence is limited and not fully consistent. Carragher et al. (44) found that cortisol implants reduced circulating estradiol, vitellogenin levels, and gonadal size in sexually mature female brown trout and rainbow trout. On the other hand, the high levels of cortisol reported for sexually mature male brown trout during the spawning season (e.g., Ref. 179) did not point to an inhibitory effect of cortisol on gonadal maturation, nor the potentiation by cortisol administration of the induction of vitellogenin gene transcription by estradiol in tilapia, Oreochromis aureus (53). Pottinger and Pickering (192) found that cortisol administration to immature female trout did not change the low circulating estradiol levels in these fish. The estradiol binding capacity in the liver decreased, and they concluded that cortisol did not inhibit the secretion of estradiol but reduced its biological activity through stimulating production of binding proteins. Cortisol reports on androgens are more consistent: inhibitory effects have been reported after cortisol pellet implants in brown trout and rainbow trout (44) and Mozambique tilapia (76). In the latter fish, the effect was already noticeable after 1 day.

Indications that effects of stressors may be mediated via direct actions of cortisol were provided by in vitro studies. Once more, however, the evidence is inconsistent. Jalabert and Fostier (105) reported that high cortisol levels enhance the GTH stimulation of 17α,20β-dihydroxyprogesterone secretion from mature oocyte follicles of rainbow trout, this being in line with earlier in vitro studies suggesting stimulation by cortisol of oocyte maturation (e.g., Ref. 284). In contrast, Carragher and Sumpter (43) found a reduction of 17β-estradiol and testosterone secretion by cortisol in rainbow trout ovarian follicles. It may be of interest that such an effect was not found in a culture secreting 17α,20β-dihydroxyprogesterone. The conflicting results might then be partially explained by assuming that the inhibitory effect of cortisol is mainly restricted to the early development stages of the oocyte follicles and not to the maturation stage. This would also explain why high circulating levels of cortisol can occur in salmonids around spawning time (179). Recently, Pankhurst et al. (168) reported on the effects of cortisol on in vitro steroidogenesis of ovarian follicles of three nonsalmonid species: goldfish, carp, and New Zealand snapper. They were unable to demonstrate any inhibitory effect on 17β-estradiol productions. Barry et al. (21) could not find any effect of cortisol, alone or in combination with human chorionadotropin, on oocyte maturation or ovulation in gonadal tissue in vitro of walleye (Stizostedion vitreum). They concluded that the stress-associated inhibition of reproduction is not mediated by cortisol or, alternatively, that the hormone might exert its effects on the hypothalamic-pituitary-gonadal axis at a level higher than that of gonadal steroidogenesis, for instance, by directly inhibiting the release of maturational gonadotropin or of luteinizing hormone-releasing hormone. Sexual maturation may influence the intensity of the stress response. The elevation of plasma cortisol and ACTH following confinement in rainbow trout was less pronounced in sexually mature males than in immature males (190).
These observations support earlier reports that the corticosteroid response of different salmonid species to stressors is attenuated during the reproductive period. The effects may be due to inhibitory effects of androgens on ACTH release, as has also been demonstrated for mammals (see Ref. 190 for references).

VIII. STRESS AND THE DEFENSE SYSTEM

Stressors have profound and diverse effects on the defense mechanisms of fish. The inhibitory effects on disease resistance (immune suppression) have been demonstrated in many studies and are classically designated as a tertiary response (see sect. 1), of which the adaptive significance is questionable [see reviews by Anderson (7), Barton and Iwama (24), and Ellis (57)]. Less well known are the stimulatory effects on immune functions in fish, which are less prominent and therefore not easy to detect, but perhaps can be considered as secondary and adaptive responses. After a brief description of the defense system in fish, we concentrate on a summary of the effects of stressors on the different components of the immune system and on the mechanisms involved. This section concludes with a brief account of the interaction of the immune system and the neuroendocrine system, a field extensively explored in mammals but hardly in fish.

A. Defense System of Fish

Fish have melanomacrophage centers but not the lymph nodes and tonsils as found in the higher vertebrates. They are found throughout the body, although concentrated in spleen, liver, and kidneys and are supposed to be sites of antibody presentation. Bone marrow, bursa of Fabricius, or Peyer’s patches are absent. The thymus, that degenerates in maturity in many but not all teleost species, acts as a center of T-lymphocyte differentiation, and the spleen acts as a center of memory function, plasma cell development, and erythropoiesis. In addition, similar activities take place in the lymphomyeloid tissue of the head kidneys (112, 252).

The first defense line of fish against microorganisms is represented by the epithelia covering gills, skin, and gut. These epithelia secrete a layer of mucus that contains nonspecific and specific defense factors such as complement-like proteins (see below), lysozyme activity and antibodies (226), and peroxidase activity (103), produced by the cells of the immune system or (peroxidase) the epithelial cells (103). When microorganisms penetrate the body, tissue macrophages, circulating monocytes, and granulocytes will enter the infected area and attack the pathogens through phagocytosis, the release of oxidative and lytic enzymes, and reactive oxygen species (respiratory burst), as nonspecific defense activities. Phagocytosis by macrophages of antigenic material can start the specific humoral and cellular immune responses (222). After transport to melanomacrophage centers, the information is passed to lymphocytes, which leads to the differentiation of B lymphocytes into plasma cells (producing antibodies, in particular the immunoglobulin M type) and, probably, B-memory cells. Cell functions comparable to those of mammalian T-helper cells and T-suppressor cells have been demonstrated, as well as natural killer-like cells. Glycoproteins of the major histocompatibility complex are also expressed in fish (see review by Stet et al., Ref. 235). They may play a role in development of T-cell tolerance and in antigen presentation. A complement system is also present in fish. This system comprises several of the complement factors known from mammals. It is activated, as in mammals, by antigen-antibody interactions, or, by the so-called alternative route, via binding to microbial cell wall polysaccharides, which results in opsonization and/or lysis of foreign cells. Finally, immunoregulatory factors such as C-reactive protein and cytokines have been demonstrated in fish (7, 112, 223, 252).

B. Defense System and Stress

A conclusion of earlier reviews of the relationship between stressors and the defense system in fish is that many stressors (including acute or chronic handling, crowding, transport, confinement, or a subordinate position) in general cause a rapid increase in circulating neutrophils and reduction in circulating lymphocyte levels. At least part of the reduction of circulating lymphocytes and macrophages may be caused by the extravasation of these cells and their penetration into the epithelia of gills (Fig. 5), skin, and intestine of stressed fish. Both B- and T-cell functions become affected, and this is frequently associated with decreased resistance to opportunistic pathogens (bacteria, fungi, protozoa, or viruses) or to experimental infection with pathogens, resulting in diseases and mortality (e.g., Refs. 7, 24, 48, 57, 127). These observations have now been extended, partially with more elaborate methods. Using flow cytometry and specific antibodies for B and T lymphocytes and neutrophils, Ainsworth et al. (2) showed that handling and transport of channel catfish (Ictalurus punctatus) induced a decrease in circulating B lymphocytes but enhanced the blood levels of T lymphocytes. However, repeated handling of Atlantic salmon did not influence antibody production or resistance to Aeromonas salmonicida infection (63). In dab (Limanda limanda) turned upside down and disturbed for 1 h, circulating leukocytes, in particular phagocytes and damaged cells, increased and lymphocytes decreased. The phagocytic and respiratory burst activity of renal leukocytes of the stressed fish was stimulated (198). In channel catfish stressed by net confinement for 0–12 h, the
percentage of death by apoptosis in peripheral leukocytes was reduced (5). Aggression affected the immunocompetence of tilapia leukocytes (see references in Pulsford et al., Ref. 198).

Maule et al. (138) have shown that the response to an acute stressor such as transport may be more complicated than assumed on the basis of earlier results. Whereas coho salmon showed an increased mortality when infected with Vibrio anguillarum immediately after transport or 8 days later, infection in 24 h after transport resulted in improved survival. These experiments indicated a rapid immune depression, followed by a transient immunostimulation, and ending in a more chronic suppression of the immune functions. The effects of temperature may be also more complicated than previously assumed. There are many reports that abrupt and drastic temperature changes can evoke a stress response that includes a suppression of both specific and nonspecific immunity (see reviews by Ellis (57), Fries (77), and Kennedy-Stoskopf (112)). However, it has become clear that temperature changes can have profound effects on the immune system that are not related to a stress response and may represent adaptive changes related to seasonal temperature fluctuations. For instance, a decrease of water temperature of 12°C in 24 h, which may occur in winter and is then associated with increased mortality, suppressed B- and T-cell functions of channel catfish for 3–5 wk. These fish did not show notable indications of stress as judged by serum glucose concentration and blood leukocyte counts (32). Similar experiments on this species showed that the phagocytic ability of the neutrophils was less affected than lymphocyte activity, indicating that at low temperature the nonspecific defense may become more important than at optimal temperature, in contrast to the specific defense. There is evidence that this conclusion can be extended to other species (see references in Ainsworth et al., Ref. 3). Specific temperature effects on, for example, membrane lipid fluidity rather than stress-related effects may be the causal factor.

Seawater transfer of brown trout in autumn, which was associated with marked hydromineral imbalance, stimulated the phagocytic capacity of pronephric leukocytes as well as plasma lysozyme levels. The stimulation was higher after direct than after gradual transfer. Another parameter of the nonspecific defense, the activity of pronephric and splenic nonspecific cytotoxic cells, was unchanged (Marc et al., Ref. 130). Rainbow trout exposed to hyperosmotic saline showed a suppressed antibody responses to Yersinia ruckeri infection (28). Crowding produced a reduction in nonspecific immune parameters in carp (Cyprinus carpio), including serum protein concentration, lysozyme, bacterial complement, and respiratory burst activity. Leukocyte phagocytic activity and susceptibility of the fish to Aeromonas hydrophila were unchanged (282).

Most reports on the relationship between stressors and immunocompetence in fish are related to water pollution. Infections with a multitude of pathogens have been reported in relation to eutrophication, organic pollutants, heavy metals, and other chemicals, such as chlorine (see reviews by Barton and Iwama (24), Sindermann (229), and Snieszko (232)). More recent reports on the effects of organic pollutants and toxic metals on immune functions in general confirm and extend earlier studies (see review by Wester et al., Ref. 275). More recently, many studies have appeared on the effects of organic pollutants and toxic metals on specific and nonspecific immune functions. The results of these studies are variable and partially inconsistent. Stimulatory as well as suppressive effects have been reported, although at higher concentrations suppression of nonspecific and specific immunity dominates (see reviews by Kennedy-Stoskopf (112) and Wester et al. (275); see also Tahir and Secombes (244)). With respect to the mechanisms involved, frequently no clear distinction can be made between toxic and stress-related effects, although the toxic effects on the immune system clearly dominate. Unfortunately, most authors do not report on stress indicators that are unrelated to the immune response, and therefore, correlations between nonimmune and immune stress parameters have seldom been reported for toxic chemicals. Sindermann (229) concluded that low levels of toxic water pollutants affect fish primarily by predisposing them to diseases through immunosuppression by direct toxic actions. Although indeed toxic effects on the immune system may dominate, the actions of toxic chemicals on cortisol and CA levels (see sects. II and III) indicate that, in addition, a stress response is frequently elicited, in particular at higher concentrations of the chemicals.

C. Immunoneuroendocrine Relationships

In relatively few studies has the functional relationship between immune parameters and cortisol, CAs, and other hormones been explored. These are discussed here.

1. Effects of hormones on the immune system

A) CORTISOL. Cortisol generally has been implicated in mediating the inhibitory effects of stressors on the immune response. High plasma cortisol levels are associated with increased susceptibility to pathogens, reduced circulating lymphocyte levels and antibody production, and, as established by in vitro assays, lower mitogen-induced proliferation of these cells and inhibition of phagocytic activity. Cortisol administration increases blood neutrophils but inhibits the migration of these cells to injured sites or inflammatory lesions, and slows down wound healing (see reviews by Barton and Iwama (24), Ellis (57), and Suzuki and Iida (243)). This knowledge has now been
extended with data on both the nonspecific and specific immune mechanisms.

Comparison of two lines of Atlantic salmon selected for high or low cortisol response to confinement showed that a high cortisol response was correlated with reduced nonspecific immunity and higher susceptibility to pathogens (68, 69). Reduced antibody production by head kidney lymphocytes was also demonstrated for Atlantic salmon showing elevated cortisol levels as a result of high rearing density (140). Handling-induced cortisol elevation was associated with reduced antibody-producing cell numbers in coho salmon, Oncorhynchus kisutch, and chinook salmon, Oncorhynchus tshawytscha (215). Saline injections, noise, transport, and confinement elevated cortisol levels and reduced phagocytic activity of splenic and head kidney macrophages of rainbow trout (156). The suppressed antibody response to pathogenic bacteria in rainbow trout exposed to hyperosmotic saline was associated with increased plasma cortisol and prolactin concentrations. After exposure for several weeks, the antibody titers were still reduced, although the hormone levels were back to normal (28). Maule and co-workers (136, 137) showed that the high cortisol levels of smolting coho salmon were associated with increased pathogen susceptibility and lowered ability to induce antibody-producing lymphocytes. This observation was not confirmed by Olsen et al. (165) for the specific immune response of smoltifying Atlantic salmon. They found high cortisol levels but unchanged proliferation activity of head kidney leukocytes in the presence of phytohemagglutinin and bacterial lipopolysaccharides (LPS). A similar observation was reported by Nagae et al. (153), who were unable to demonstrate reduced plasma antibody levels in smoltifying masu salmon (Oncorhynchus masou), although plasma cortisol levels were also highly elevated in these animals. Actions of thyroid hormone and growth hormone, both able to stimulate immune functions and showing elevated plasma levels during smoltification, were suggested to antagonize any immune suppressive action of cortisol (153). Crowded rearing caused elevated cortisol and glucose levels had suppressed values for several nonspecific immune parameters, and the susceptibility to pathogenic bacteria was unchanged (282). Nevertheless, although the data are not fully consistent, in most reports high cortisol levels and reduced immune responses were positively correlated.

This conclusion is in line with the effects of administration of cortisol or synthetic corticosteroids to fish. Pickering and Pottinger (181) found that even minor chronic elevations in plasma cortisol levels (≥10 ng/ml) reduced the disease resistance of brown trout to opportunistic antigens. Circulating lymphocyte numbers were inversely and neutrophil numbers directly correlated with the magnitude of the initial cortisol levels following confinement of rainbow trout. This was based on the comparison of progeny of fish selected for high or low cortisol response to confinement (191). Ellsaesser and Clem (58) reported for channel catfish receiving a single cortisol injection, resulting in transiently increased cortisol levels similar to those of acutely stressed fish (~100 ng/ml), that circulating neutrophils increased and lymphocytes decreased. The lymphocytes from these fish showed suppressed responses to B- and T-cell mitogens. In a similar experiment on this species, the circulating neutrophils showed decreased phagocytic ability after multiple injections of cortisol (2). Cortisol administered via the food to juvenile coho salmon produced the same changes in leukocyte distribution as acute stress (increases in thymus and anterior kidney and decreases in blood and spleen within 1 day after treatment), although the magnitude and duration of the elevated cortisol levels and leukocyte numbers were not correlated (134). Nagae et al. (154) showed that orally administered cortisol produced a slight reduction in plasma antibody concentrations in masu salmon. Repeated injections of dexamethasone, a cortisol analog, reduced the phagocytic activity of spleen and head kidney macrophages from rainbow trout (156). Oral administration of cortisol induced apoptosis of lymphocytes present in the skin epithelium of common carp (101). A single cortisol injection in Atlantic salmon did not influence the circulating numbers of lymphocytes and neutrophils, but caused a significant reduction of the mitogenic response of peripheral B lymphocytes to LPS in vitro (63).

These studies relate enhanced cortisol levels to immunosuppressive actions, and this is in line with the results of most in vitro studies. Stave and Roberson (234) found an 80% reduction in the respiratory burst response to bacteria in head kidney leukocytes of striped bass incubated for 1 h in the presence of cortisol, whereas Tripp et al. (248) reported that cultures of splenic and pronephric lymphocytes from coho salmon showed dose-dependent suppression by cortisol of the antibody and mitogenic responses to hapten conjugated LPS. This suppression could be overcome by the addition of conditioned medium supposed to contain an interleukin-like substance produced by macrophages (109). Wang and Belosevic (266) demonstrated inhibition by cortisol of mitogen-stimulated proliferation of peripheral blood lymphocytes in goldfish, and Espelid et al. (63) in Atlantic salmon. Pulford and co-workers (196, 197) showed inhibition by cortisol of unstimulated lymphocytes from the dab Limanda limanda. The hormone depressed phagocytic and phorbol ester-stimulated respiratory burst activity of kidney and spleen phagocytes, whereas basal respiratory burst was stimulated. Cortisol inhibited chemotaxis, phagocytosis, and nitric oxide production of a goldfish macrophage cell line (267). Conversely, the hormone had hardly any depressive effect on the mitogenic response of channel catfish lymphocytes or neutrophils (2, 58), nor on phagocytic
activity of rainbow trout peripheral macrophages in vitro (156). The inhibition of apoptosis in peripheral leukocytes as observed in confined channel catfish could not be affected by cortisol in vitro (5). Nevertheless, there is good evidence now that the immunosuppressive effects of stressors can be explained, at least partially, by high cortisol concentrations.

B) CATECHOLAMINES. Surprisingly little attention has been paid to the effects of CAs on the immune system of fish, especially because of the extensive literature on this subject in mammals, which have revealed many α- and β-adrenoreceptor-mediated effects on the activity and cytokine production of leukocytes [see reviews by Fuchs and Sanders (80) and Rothwell and Hopkins (210)]. Injection of killifish (Fundulus heteroclitus) with Epi simulated the repeated sequence of reduced and increased leukocyte levels induced by cold shock in these fish (188). The α-receptor agonist phenylephrine had marked and rapid stimulatory effects on the respiratory burst of rainbow trout phagocytes in vitro, whereas the β-receptor agonist isoproterenol had suppressive effects (27). The antibody-producing response of rainbow trout lymphocytes to LPS was stimulated by the α-adrenergic agonist clonidine and suppressed by the β-adrenergic agonist isoproterenol (73). Lesioning of the adrenergic nerves innervating the spleen stimulated antibody secretion in rainbow trout (72). Phenylephrine depressed the phagocytic activity of rainbow trout macrophages to the same extent as the β-receptor agonist isoprenaline (156). Thus CAs seem to have stimulatory and inhibitory actions on immune functions in fish.

C) OTHER HORMONES. In mammals, gonadal steroids suppress and growth hormone may promote the defense activities of lymphocytes and granulocytes (see references in Refs 110 and 266). In chinook salmon, testosterone was shown to suppress antibody production by lymphocytes (231), an effect mediated by specific high-affinity androgen receptors in the cytosol of lymphocytes (230). Estradiol inhibited the proliferative response of goldfish peripheral lymphocytes to mitogens in vitro and increased the susceptibility of goldfish to infection with trypanosomes (266). The hormone also inhibited chemotaxis and phagocytosis, but not nitric oxide production or respiratory burst activity, of a goldfish macrophage cell line (267). Growth hormone injection stimulated respiratory burst activity in rainbow trout leukocytes (110, 212). Given the inconsistency of the reported effects of stressors on circulating gonadal steroid and growth hormone levels, the significance of the interaction of these hormones with immune function during stress is unclear.

2. Effects of cytokines on the brain-pituitary-interrenal axis

For mammals, there is an extensive literature on neural and humoral messenger interaction between the brain, the neuroendocrine system (in particular the brain-pituitary-adrenal axis), and the immune system. The production of key mediators of the immune system such as interleukins (ILs) is not restricted to the cells of this system and may also occur in the brain and other tissues. Interleukins can modulate the activity of the brain-pituitary-adrenal axis. Conversely, the production of neuropeptides such as CRH, ACTH, and endorphins also occurs in the immune system, where these messengers may have local effects. When combined with the well-established effects of cortisol and CAs on the immune system and the immunoregulatory effects of other hormones such as prolactin, growth hormone, thyroid hormones, and gonadal steroids, a picture emerges of intensive interactive modulation and tuning of neural, neuroendocrine, and immune functions, which are of primary importance during stress [see reviews by Madden and Felten (126) and Wilder (276)].

There are several indications of the presence of cytokines in fish. The presence of interferon-like and IL-1 and IL-2-like molecules and inhibiting factors has been demonstrated (see review by Secombes et al., Ref. 223). Human IL-1α or IL-1β did not stimulate carp T-lymphocyte proliferation, in contrast to the IL-1-like factor isolated from carp phagocytes, although the anti-human IL-1 antibodies could block the action of the carp phagocyte factor, indicating similarity as well as structural differences between the bioactive parts of the human and carp cytokine (257). Burnett and Schwarz (37) provided evidence that macrophage-derived cytokines potentiate the response of red drum peripheral leukocytes to mitogens. Rainbow trout leukocytes were shown to secrete a variety of potent macrophage activating (tumor necrosis factor-α) and deactivating (transforming growth factor-β) factors (106).

In mammals, IL-1 is known to stimulate CRH, ACTH, and cortisol secretion via direct actions on the cells secreting these hormones (126). Comparable, although inhibitory, effects have been reported by fish IL-like factors and mammalian IL-1. Schreck and Bradford (220) cultured coho salmon leukocytes and used the media to incubate pituitary glands and interrenal tissue. They showed inhibition of the production of corticotrophic hormones by these media of the pituitary gland as well as the cortisol production of head kidney tissue in vitro. Balm and co-workers (17, 20) showed that four injections of murine IL-1α on alternate days inhibited ACTH and α-MSH release from the pituitary gland of Mozambique tilapia. In vitro LPS also inhibited the release of both corticotrophic hormones. Analogous to the action of LPS in mammals, this suggests that the endotoxins induce the release of an inhibitory IL-1-like factor in the pituitary gland, possibly from stellate cells. The immunosuppression produced by social confrontation in subordinate Mozambique tilapia could be partially reversed (cytotoxic and T-cell responses) by the opioid antagonist naltrexone. The concentration of a
blood-borne immunosuppressive factor was reduced. This indicates the release of an immunosuppressive opioid by the brain during social stress in fish. Administration of β-endorphin to peripheral leukocytes in vitro produced naltrexone-inhibitable reduction of mitogen-stimulated proliferation and cytotoxicity (67). There is also evidence for the production of α-MSH immunoreactive substances by the head kidneys of Mozambique tilapia (15, 20). This production was stimulated by LPS in vitro and by in vivo pretreatment of the head kidneys. Adrenocorticotropic hormone stimulated the respiratory burst of rainbow trout phagocytic leukocytes in vitro (27). Thus there are several indications that the similarity between the defense systems of mammals and fish includes production of cytokines and neuropeptides and the intensive humoral and hormonal interaction between the immune system and the neuroendocrine system.

IX. MODIFYING FACTORS

Factors modifying the impact of stressors, and thus contributing to the variability of the stress response, are numerous. In this section some of the major factors are discussed.

A. Mineral and Ionic Composition of Water

As discussed in section v, disturbance of hydromineral control is an important aspect of stress in fish. The extent of the disturbance following stress depends on the ionic and osmotic gradients across the integument and its permeability to water and ions. Lowering of the gradients can reduce the impact of stressors, as demonstrated by the aquaculture practice of adding salts to freshwater to reduce the impact of netting and transport, and to improve disease resistance and survival (139). External Ca\(^{2+}\) reduces the permeability of cell membranes including those of the gills, and therefore, high water Ca\(^{2+}\) levels moderate the impact of many stressors on freshwater fish (see sect. v). Water pH is an important factor modifying the impact of many pollutants, by influencing membrane permeability to chemicals, or metal speciation. Notable examples are the high toxicity of aluminium in acid water (especially at pH 4.8–5.2) and copper below pH 6.5, which is caused by the formation of toxic hydroxides or high free metal ion levels, respectively (e.g., Refs. 209, 236).

B. Additional Stressors

Because the integrated stress response comprises many nonspecific elements, most notably the drain of metabolic energy and susceptibility to diseases, many effects of stressors are additive. Such effects may be mediated through elevated cortisol and CA levels (Fig. 6; Ref. 148). Stressors can also exert complicated interactions with respect to their specific effects, as is known from the toxicological literature, and these may interfere with the nonspecific effects. Additive, more than additive, less than additive, independent, and antagonistic effects have been described for exposure to a combination of stressors (169). Several mechanisms may be involved. The inhibitory or otherwise disturbing effects of chemicals on corticosteroid or CA synthesis and release, the downregulation of receptors for these hormones during chronic stress, and the alterations of hepatic steroid catabolism, which will influence the response to any additional stressor, have already been mentioned (see sects. ii and iii). An adaptive mechanism is the formation of stress proteins in response to both toxic and nontoxic stressors, which may confer resistance to other stressors. Induction of metallothioneins by a toxic metal may provide protection against other metals, as is indicated, for instance, by the less than additive effects of copper and zinc on growth of fathead minnows, Pimephales promelas (169).

C. Social Interactions and Crowding

A low rank in social hierarchy may represent an intense stressor (see sects. ii and iv). In groups with a hierarchical structure, a stressor will therefore have a differential impact on the group members in relation to their hierarchical position (Fig. 6, A and B) and the size of the group (193). Such phenomena, as well as crowding (see sect. vi), increase the variability in the response of experimental groups to stressors.

D. Life Stage

Early life stages (embryos, larvae, early juveniles) are more sensitive than adults to stressors such as water pollutants and temperature changes. Handling and transport have less impact, and this may be related to the absence of a fully functional hypothalamus-pituitary-interrenal axis, which in rainbow trout could not be demonstrated until 5 wk posthatching. During the reproductive period, the cortisol response to stressors may be reduced (190). Effects of smolting on salmonid stressor responsiveness have been mentioned in section v.

E. Population and Individual Characteristics: Acquired or Inherited

Acquired individual characteristics that may modify the stress response include nutritional status and energy reserves (see sect. vi) and earlier experience with other stressors that may have increased, through adaptations
(i.e., induction of stress proteins or acquired immunity to diseases) or reduced (see under additional stressors) the resistance to subsequent adverse stimuli. This is also reflected by differences in rearing conditions. For instance, wild coho and chinook salmon juveniles showed higher cortisol levels and reduced antibody-producing cell numbers than hatchery-reared fish with the same genetic background and collected as fry from the wild environment (215).

There is also evidence for innate differences in the stress response in fish, as has been demonstrated by the successful selection of salmonid strains with a high or low cortisol response to a standard stressor (68, 69, 191). Iwama et al. (104) found differences in the responses of subpopulations of coho salmon to standardized stressors (salt water, low water pH, disease, and handling) and concluded that each subpopulation displayed a unique and genetically based response profile. Genetically isolated strains of lake trout showed substantial differences with respect to changes in plasma concentrations of cortisol, glucose, sodium, and chloride following net confinement (146).

In mammals, there is a growing literature on genetically determined individual differences in coping strategies with respect to stressors. Individuals with an active or aggressive (fight/flight) approach and a pronounced catecholaminergic response, or with a passive strategy (conservation/withdrawal), associated with high corticosteroid levels, are two extremes of a genetically stable continuum of coping styles that has now been demonstrated in several species (114). Recently, Van Raaij et al. (253) studied the effect of severe hypoxia on rainbow trout fitted with an aortic cannula for repetitive blood sampling. In the recovery period, 40% of the fish died. During the preceding hypoxia period, these fish displayed strenuous avoidance behavior, whereas the surviving fish stayed quiet. Moreover, in the nonsurviving fish, plasma CA levels were much higher, and cortisol levels were less elevated than in the surviving fish. The latter fish practised metabolic depression, a prompt and drastic reduction in metabolic rate that prevents a serious drop in plasma pH during hypoxemia (250). These results probably are the first well-documented evidence for the presence of active and passive coping strategies within a group of fish.

X. CONCLUSIONS

When it comes to drawing conclusions from the available literature, one should keep in mind that to date only a very small and far from representative fraction of the ~20,000 species of teleosts has been investigated. Among these, the salmonids, one of the less advanced families, are highly overrepresented, together with some other commercially interesting and widely cultured fish species including eels, tilapia, and carp. For most studies, fish from domesticated stocks have been used, and such fish may have a blunted stress response when compared with wild-type strains of the same species (280). Nevertheless, dramatic species differences in the responses to stressors have been revealed. The absence of a chromaffin cell response in an Antarctic fish, the 50-min delay in the cortisol response of the sea raven, and the rise in plasma FFAs instead of glucose and lactate in turbot after acute exposure to stressors (see sects. ii and iii) are but a few examples, probably representing genetic adaptations to the exceptionally varied habitats of teleost fishes, and pointing to the wealth of adaptive variations still to be discovered.

Although the evidence may not be based on a fully representative sample of the teleosts, this review confirms earlier conclusions [see reviews by Pickering (176) and Barton and Iwama (24)] that there are many similarities between the integrated stress responses of aquatic and terrestrial vertebrates. This indicates that these physiological mechanisms, of fundamental importance for adaptation to physical, chemical, and biological factors, have an evolutionary history of at least 400 million years. This is exemplified, for instance, by the structural identity (CA’s) or similarity (cortisol in teleost fish, cortisol, or corticosterone in the terrestrial vertebrates) of the primary neuroendocrine messengers involved in the control of the stress response. This is in marked contrast to the variability displayed in the molecular structure found for almost all other neuroendocrine messengers. Also, the functions of the primary messengers are similar, with the rise in plasma CAs as the first neural messengers, indicating a nonspecific alarm or activation response leading to rapid mobilization of energy substrates and increased uptake and transfer of oxygen. The rise in circulating cortisol follows more slowly and more sustained, facilitating (glucocorticoid) or moderating (restoration of disturbed hydromineral balance) the effects of the CAs. The similarities also pertain to the inhibitory effects of stressors on growth, reproduction, and immune function.

There is a large gap between the state-of-the-art stress research in fish and mammals. In fact, this gap is widening, in particular with respect to the psychological, behavioral, and immunological aspects of stress.

The current concepts of stress as applied to mammals and humans have an important emotional dimension, and emotional stressors are the most frequently reported stress stimuli in human stress literature (46, 249). As a result, the psychological approach to the stress response has developed into a fully developed subdiscipline alongside the physiological and clinical approaches. It seems well accepted that factors such as perception and awareness are important determinants of the integrated stress response in fish, as can be deduced from experiments on sensory perception and stress reduction by training (see discussion in Schreck, Ref. 218). Nevertheless, such topics
have been investigated only incidentally. An example is represented by a study of Järvi and Uglem (108), who demonstrated that seawater Atlantic salmon smolts trained to avoid predators were physiologically less stressed when confronted with predators than predator-naive smolts, as was concluded from plasma chloride, glucose, and lactate levels in the trained fish. The scarcity of such observations suggests that notions such as the subjective experience of stressor and stress (awareness, appraisal) and controllability and predictability of the situation (from the point of view of the exposed animal) are difficult to make operational for fish given the current state of the art. In fish, stress is still almost exclusively defined and described in physiological terms. Moreover, brain research on fish under stress conditions is still in its infancy (see sect. iv).

Although substantial progress has been made in fish immunology in the last decade, the shortfall with mammalian research is immense and increases rapidly. The evidence for an immunosuppressive role of cortisol, and for suppressive as well as stimulatory actions for CAs, is in line with mammalian studies (126, 249). However, the mechanisms involved have hardly been explored. In the short term, the immununosuppressive actions may be adaptive by preventing overstimulation of the immune system, but in the long term, they may become maladaptive by promoting diseases. Whereas there are now some papers indicating the presence of cytokines in fish, their numbers contrast markedly with the >2,500 articles on cytokines of mammals that were available by early 1995, with one-half of these published in the two previous years (210). Nevertheless, the available evidence for fish indicates a similar intensive interaction by humoral and hormonal messengers between the immune system and the neuroendocrine system, as has now been demonstrated so convincingly for mammals (see sect. viii).

Even though the similarities between fish and mammals are more conspicuous than the differences, the latter have been clearly identified and, not surprisingly, these primarily relate to the aquatic environment of fish.

1) Stressors induce hydromineral disturbances in fish. Several mechanisms are probably involved. One important mechanism is represented by the increased permeability to water and ions of the branchial epithelium as a result of the high circulating CA levels that can be evoked by all kinds of stressors. The stimulation of passive water and ion fluxes by the CAs may be considered a negative side effect of the excessively high levels of these hormones during acute or chronic stress, comparable to the suppression of disease resistance associated with high cortisol levels. A second mechanism might be the induction of apoptosis by chronically high cortisol levels. If the first indications for such a mechanism (see sect. v) can be confirmed, this would explain the apoptosis of branchial cells observed in fish exposed to stressors such as isolation or a subordinate position in hierarchy. The direct toxic action of chemical stressors on the branchial cells is a third mechanism. This may lead to epithelial lesions as well as specific inhibition of ion transport mechanisms or increased membrane permeability. When toxic chemicals such as heavy metals produce a stress response in addition to their toxic effects, all three mechanisms may be involved.

2) The role of cortisol in fish differs from that of cortisol or corticosterone in terrestrial vertebrates, by pronounced effects on hydromineral control, in addition to glucocorticoid effects. In both freshwater and seawater fish, long-term maintenance as well as adaptive changes in ion transport capacity are under control of cortisol. In this respect, it has an important function in the integrated response to stressors by moderating and compensating the disturbing effects of CAs on hydromineral balance. Thus cortisol combines mineralocorticoid and glucocorticoid functions in fish, and this may reflect the intimate relationship between energy metabolism and hydromineral control that exists in fish (see sect. v). It further explains the absence of a distinct mineralocorticoid in fish comparable to deoxycorticosterone or aldosterone of the terrestrial vertebrates, and this reflects the close relationship between stress and hydromineral disturbance in fish. The glucocorticoid actions of cortisol in fish are more directed to gluconeogenesis and the long-term maintenance of energy reserves than to short-term energy mobilization through promotion of glycolysis. However, the latter function has also been called into question for glucocorticoids in mammals (e.g., Ref. 59).

3) Of the other hormones involved in the stress response, prolactin and growth hormone show important specializations in fish with respect to hydromineral control. Both prolactin (controlling membrane permeability in freshwater) and growth hormone (of importance for active ion transport in seawater) have been implicated in the compensation of hydromineral disturbance during stress. Species differences have been reported, however. The function of somatolactin, the third member of the prolactin/growth hormone family and, as a pituitary hormone, unique to fish, has not been clarified so far. However, the rapid and marked stimulation of somatolactin release reported after stressor exposure points to a specific function in stress adaptation that may be typical for aquatic animals.

4) The brain-pituitary-interrenal axis in fish has many functional and structural aspects in common with the brain-pituitary-adrenal axis in mammals including the role of ACTH as an important regulator of corticosteroid secretion. Differences may be present in the involvement of other neuroendocrine messengers, with MCH and α-MSH perhaps in a more important role in fish than in mammals. In fish, enhanced cortisol levels are not always associated with elevated ACTH levels. However, α-MSH has also
been implicated in the control of glucocorticoid release in mammals (e.g., Ref. 263).

5) The function of the CAs during stress is comparable to that of higher vertebrates, with the rapid mobilization of glucose and stimulation of oxygen uptake and transfer to the tissues as major functions. The limited overflow of CAs from synaptic endings into the general circulation seems typical for fish, as well as the rapid adrenergic stimulation of the oxygen affinity of hemoglobin through stimulated Na\(^+\)/H\(^+\) exchange in the erythrocyte membrane, as demonstrated in salmonids and some other fish (see sect. ii). The presence of this mechanism in fish may be related to the circumstances where the oxygen and carbon dioxide content of the environment can fluctuate markedly, in seawater as well as freshwater (158, 229). This adrenergic action will promote survival in hypoxic waters as well as contribute to successful coping with other stressors. The disturbing effects of high circulating CA levels on membrane permeability, which are also known for mammals, but which have much more serious consequences for aquatic animals possessing gills, were mentioned above (see point 1).

6) Toxic substances are part of the stress literature on fish much more than in mammals, and this is related to the fact that many chemicals occur as water-borne pollutants, affecting fish continuously across an extensive and delicate surface. A stress response is evoked in conditions that cause discomfort, fright, or pain, and thus sensory perception of the adverse stimulus or its effects is essential (218, 219). The large variety of perceptive mechanisms typical for the integument of fish (including light, mechanos-, temperature, pain, touch, pressure, electro-, and chemoreceptors) explains the immediate rises in plasma CA and cortisol levels after acute exposure to many types of water pollutants. This exquisite sensitivity to water-borne chemicals has been exploited by using fish as bioindicators for water pollution, utilizing behavioral parameters as the end point (31). The reports on sensory perception of heavy metals and aluminium at low water pH may explain the immediate rises in cortisol and CA levels following acute exposure to these water pollutants. In addition to, and perhaps more than, the damage incurred by the gills, this sensory component may account for the strong stress response of fish to many pollutants. This response is much more pronounced than that of terrestrial vertebrates, where awareness of this kind of stressor plays a limited role, with exception perhaps of airborne pollutants. The nonspecific stress effects of toxic pollutants are frequently difficult to identify because of their association with their toxic effects. This makes toxic chemicals poor stress models. On the other hand, their toxic actions to fish cannot be fully understood when their stress effects are ignored.

There are few exceptions to the rule that the activation of the brain by stressors results in stimulated release into the circulation of cortisol and CAs. The release and the subsequent actions of these hormones can be considered the common neuroendocrine framework of what is still called, even in recent fish studies, the general stress response. It has become clear, however, that “general” cannot be equated with “identical,” because of the many specific effects of stressors evoking stressor-specific physiological reactions, and of the many factors modifying this response (see sect. ix). The stress response is above all a highly adaptable, flexible response in fish as well as in terrestrial vertebrates.

I am indebted to E. M. Jansen-Hoorweg, D. Nolan, and W. Atsma for assistance during the preparation of this manuscript.

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STRESS IN FISH

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