1 Introduction

The book describes a method of transferring information within vertebrates. Such communication is necessary in order to coordinate physiological processes with each other and to the happenings in the external environment. Even unicellular organisms synchronize their various internal life processes. In such small creatures, however, local accumulations of metabolites may exert a direct control on biochemical reactions, whereas external stimuli have relatively widespread effects so that specialized pathways for communication may not be as necessary. Therefore, when the distances involved are short, physical processes such as conduction, convection, and diffusion may be adequate for the integration of the physiological processes. Nevertheless, even unicellular organisms possess specific coordinating systems such as that seen in the protozoan *Tetrahymena* (Blum, 1967), which possesses epinephrine. This hormone has similar metabolic actions in this protozoan to those that it has in vertebrates.

The problems of communication and coordination are greater in multicellular than in unicellular organisms. There are several reasons for this, especially their larger size. As the linear distances between the different parts of an animal increase, simple physical communications become relatively slower and less precise, and so not as effective. In multicellular organisms, the cells are usually specialized and perform different functions that, in combination, are essential for the animal's life. Thus, some tissues may be concerned with the formation of reproductive germ cells, several others with the preparation of suitable nutritive materials, and yet others with building morphological structures. The ultimate successful completion of these processes will be determined by the effectiveness of the communication between the tissues themselves and the external environment.

The transfer of information in animals

There are three principal ways by which cells in multicellular organisms can communicate with each other. First, when they are in close juxtaposition and are only separated by narrow fluid-filled spaces, direct electrical and chemical interactions can occur. Cells also maintain some structural connections with each other and secrete special excitants by which they may also communicate.

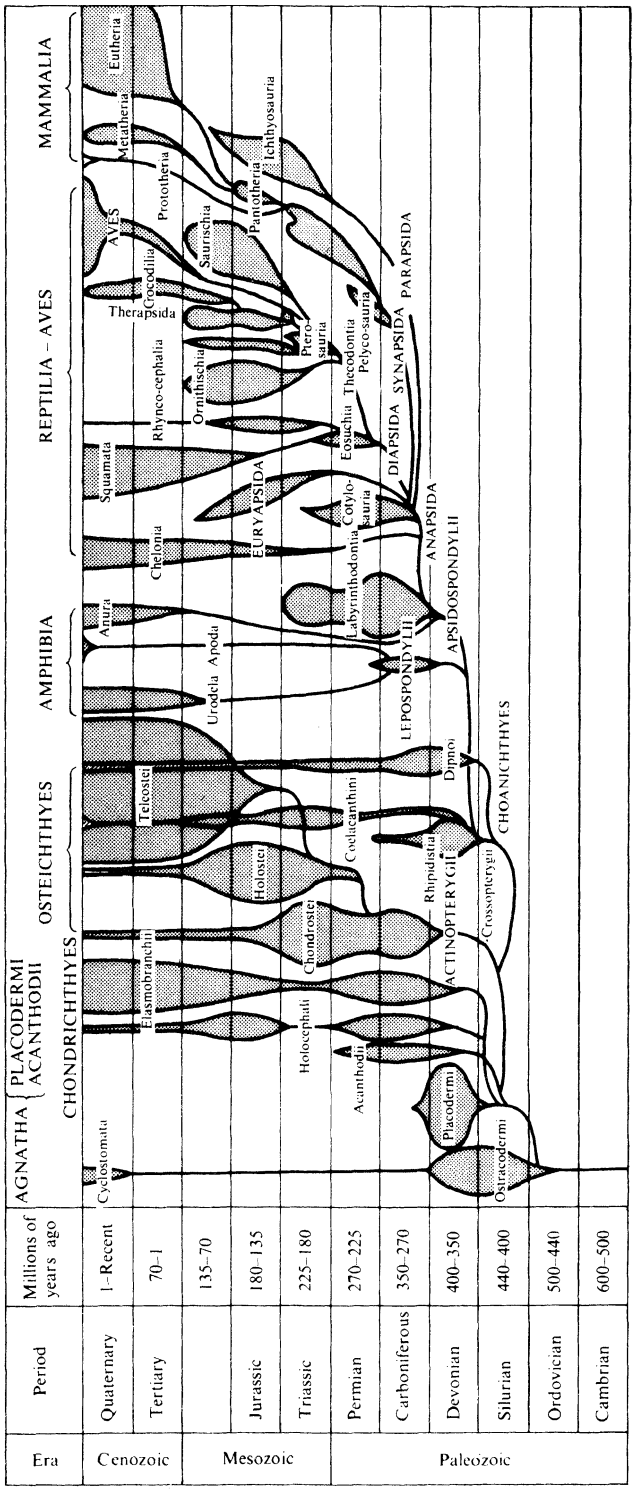

Fig. 1.1. A classification of vertebrates in relation to their phylogenetic origins and a time scale in terms of paleontological periods. (From Torrey, 1971.)

Such excitants are often called local hormones; if they affect a different type of neighboring cell their action is described as a paracrine one or, if it is a cell of the same type, it is described as an autocrine one. Second, contact between more remote cells can be maintained along tracts of nerve cells that are merely tissues specialized for such exchanges of information. Third, chemicals may be released, for example from the endocrine glands, into the blood, which carries them to special sites that are physicochemically programed to react and respond to them. The actions of hormones are slower than those of nerves but as they may persist in the plasma and at target sites their effects generally last longer. Nerves follow defined anatomical pathways but hormones are diffusely distributed. However, hormones attain precision in their actions by utilizing specific receptor molecules at their intended target sites.

The endocrine glands are tissues that, unlike exocrine glands, have no ducts but release their secretions, called "hormones," directly into the blood passing through them. It is with the diversity of such hormonally controlled processes that we will be principally concerned in this book. It should always be recalled, however, that the endocrine glands represent only a single facet of the animal's communication network and that nerves are also important. Nerves and hormones are often mutually interdependent and may even act together to control a single process. Nerve cells can respond to hormones in a manner that influences behavior, and endocrine glands often receive information and directions from the brain. Both hormones and nerves can act together to control the melanophores in certain fishes. Many hormones, including epinephrine, vasopressin, oxytocin, and the hypophysiotropic hormones, are even made by nerve cells.

What is comparative endocrinology?

Comparative endocrinology concerns the study of the endocrine glands in different species of animals, both vertebrates and invertebrates. Its aims are analogous to the older and more classical disciplines of comparative anatomy and comparative physiology. The prime academic objective is to reconstruct evolutionary pathways by the study of extant species. Figure 1.1 shows the phylogenetic relationships of the vertebrates and this emphasizes the extant groups that may be particularly interesting in such studies. The mere examination of the endocrine system of some bizarre and exotic vertebrate does not alone constitute "Comparative Endocrinology" (it may be "Animal Endocrinology") unless the data can be considered in relation to that in other, phyletically related species. Such information can be used to help to confirm, complete, and even extend our knowledge of the phylogenetic relationships between vertebrates and to follow the evolution of endocrine mechanisms. The lungfishes (Dipnoi) may afford us an example. These fishes have long been considered, on the basis of morphological information, to be close to the

original line of evolution connecting the bony fishes (Osteichthyes) and the Amphibia. As we shall see later, homologous vertebrate hormones often exhibit considerable differences in their chemical structure. Many such differences are apparent between the hormones in fishes and tetrapods. The structure of several hormones present in lungfishes, however, shows a greater similarity to those in tetrapods than those in other fishes. For instance, a neurohypophysial hormone called mesotocin is present in amphibians, reptiles, and birds, but in bony fishes the homologous hormone is isotocin (which differs from mesotocin by a single amino acid substitution), with the exception of the lungfishes, which have mesotocin. It has also been found that the growth hormone and prolactin present in lungfishes are more like those in tetrapods than in other fishes.

Apart from contributing to the overall phyletic study of vertebrates, the comparative endocrinologist aspires to reconstruct the lines of evolution within the endocrine system itself. This can be done by examining and comparing in different species, the morphology of the endocrine tissues, the structures and biological activities of their secreted hormones, receptors and their different physiological roles.

Occurrence of vertebrate hormones in other organisms

It is now generally accepted that vertebrate hormones and their receptors may have evolved from identifiable counterparts among the invertebrates. The increasing awareness of this concept has been promoted by the use of immunocytochemical techniques to identify minute amounts of related molecules at specific sites in various invertebrates, especially among the molluscs and insects. Such an identification of related hormones has been aided by microbioassays and recombinant DNA techniques. The latter have been used to produce probes to identify coding nucleotide sequences that can be deciphered to describe the polypeptide molecules. Often, the genes themselves have been identified and chemically dissected so that their structural organization can be compared with those of their putative vertebrate relatives. Even the most sceptical may be impressed by their possible relationships. Molecules that are identical or are close chemical relatives to vertebrate hormones have been identified among the invertebrates (see for instance, LeRoith *et al.*, 1986; de Loof and Schoops, 1990; Pertseva, 1991; Renaud *et al.*, 1991).

Vertebrate hormones have even been identified in plants (Le Roith *et al.*, 1986). Thyrotropin-releasing hormone has, for instance, been found in alfalfa (Jackson, 1981a). Such occurrences obviously do not indicate a phylogenetic relationship though they could reflect the reutilization of a pool of available genetic material. When they have been identified in plants, the role of such vertebrate hormonal substances is usually obscure. However, if

consumed by animals they may influence their physiology. Examples include a toxic deposition of calcium in the tissues of cattle caused by vitamin D in their forage (Wasserman *et al.*, 1976) and an inhibition of seasonal reproduction in desert quail by plant estrogens (Leopold *et al.*, 1976).

The presence of molecules related to vertebrate hormones among the invertebrates is not surprising when one recalls that the principal neurotransmitters acetylcholine, catecholamines, 5-hydroxytryptamine, and γ-aminobutyric acid (GABA) are all shared on many occasions by both of these major groups of animals. Insulin-like molecules have been identified in the gut tissues of some molluscs and a honeybee (Plisetskaya *et al.*, 1978; Moreau, Raoelison, and Sutter, 1981), calcitonin and somatostatin in nerve tissue of a sea squirt (a tunicate, Protochordata) (Falkmer and Ostberg, 1977; Girgis *et al.*, 1980), and cholecystokinin (CCK) in the brain of a blowfly (Duve and Thorpe, 1981). The occurrence of iodinated tyrosine molecules with activity like that of thyroid hormones in protochordates is also well known. A molecule that cross-reacts with antibodies to mammalian vasopressin (antidiuretic hormone, ADH) has been found in the brain and other tissues of a locust (Proux and Rougon-Rapuzzi, 1980). The physiological roles of such substances in these invertebrates are uncertain, but it has been suggested that they may act as neurotransmitters or hormones and, in the instance of insulin and epinephrine, they may even have comparable metabolic effects to the ones they have in vertebrates.

It is tempting to suggest that the presence of vertebrate types of hormone in invertebrates may reflect some evolutionary relationship. In the instance of the protochordates and the molluscs this may be so. Their presence in insects, however, makes such common origins appear rather tenuous. It seems likely that nature has been somewhat frugal or has a limited inventiveness in its provision of molecules and their encoding genes, that are suitable for roles in chemical coordination mechanisms. They may then have been reutilized by nature on several separate occasions. However, the possibility that such similar molecules may arise owing to convergent evolution should not be forgotten. Common molecular requirements may have limited structural solutions.

Molecular evolution and the endocrine system

The phyletic distribution of hormones and their precursors, their synthesizing enzyme systems, and receptors among the vertebrates reflects the processes of evolution. While such components of the endocrine system have retained characteristics in common, they frequently display changes in their chemical structures. The structural homologies of the hormones, their precursor prohormones, preprohormones, and their receptors often permit the construction of molecular phylogenetic trees (cladograms) and trace their evolution

among vertebrates, from cyclostome fish to mammals, and sometimes even include invertebrates. Estimates of the time when such changes may have occurred can sometimes even be proffered.

The evolution of protein families (see for instance Wilson, Carlson and White, 1977; Doolittle, 1981, 1989; Niall, 1982) began in prokaryotic organisms which, probably, possessed a 'library' of less than 1000 proteins. These archetypes are thought to be the progenitors of the 'superfamilies' to which many contemporary proteins belong. Their evolution and proliferation have involved various types of genetic event but were probably initiated by gene duplication followed by mutations on their DNA sequences to code for novel proteins. Such modifications include point mutations and rearrangements such as tandem duplications, gene conversion, and exon shuffling. Viruses may be involved in such transposition of genetic material. There appears to be a tendency to increase the amount of DNA in the genome as a result of such events. Such a process may provide the organism with more opportunities to produce proteins, including hormones, that may aid their adaptation to the environment.

Homologies in the amino acid sequences of the proteins of the endocrine system reflect the nucleotide sequences of their genes. When describing such relationships in the endocrine system it is more usual to compare the sequences of their amino acids, but those of their nucleotides are also used. Similarities are usually expressed as a percentage (%) identity or homology. Thus, two identical polypeptides will have a homology of 100%. Lesser values suggest a more distant relationship but may still be consistent with a common ancestor. Two proteins of similar length and amino acid composition would be expected, on average, to display a 5% identity by chance alone. (For comparisons using nucleotide sequences, this value would be 25% as only four bases are involved, compared with 20 amino acids.) Greater values than these may reflect an ancestral relationship, though it may sometimes be quite remote. The reliability of low homology values depends on various factors and can be influenced by the particular amino acid composition of each molecule (the proportions may vary), the chain length, and the alignment used for such comparisons. 'Tails' of unmatched amino acids may be present and there may be interruptions, or 'gaps', in the sequences. Such factors can, however, be compensated for. Computer programs are available for making such comparisons and can provide statistical limits of the reliability of a particular value (Doolittle, 1990). Criteria for assessing the relationship of molecules vary but an identity of between 15 and 25% in amino acid sequence is usually considered as being 'suggestive' and over 25% is highly likely. Such pronouncements can be aided by statistical information. The possibility that convergent evolution may be contributing to similarities in molecular structure should be considered (Doolittle, 1989). Hormones and receptors may on the basis of such infor-

mation be classified into families and superfamilies. The latter involves clear evidence of a homology, though with percent identities often less than 50%. Several families may make up a superfamily and an arbitrary percent identity of greater than 50% has been suggested as one such criterion for a family, but there are others.

The usefulness of phylogenetic information derived from differences in the amino acid sequences of peptide and polypeptide hormones varies depending on the size of the molecule and the relative number of conserved amino acid sites that it contains (see Hedges, Moberg, and Maxson, 1990; Dores, Rubin and Quinn, 1996). The latter may be integral, and indeed essential, to the molecule's biological activity and, therefore, they may be invariant over a broad phyletic range of species. As a result, such sites can be relatively 'uninformative' with respect to the detailed tracing of phylogenetic trees. (Though they are very useful for identifying functionally important regions of peptides and polypeptides). Small molecules such as the neurohypophysial hormones, gonadotropin-releasing hormones, and even insulins have a high proportion of such conserved amino acids. The phylogenetic information such molecules provide may then even be inconsistent with the fossil record and comparative morphology. In such instances, the structures of their preprohormones and prohormones may provide more useful cladistic information. Larger hormones such as growth hormone and prolactin usually have a greater number of variable or 'informative' sites for such analyses.

The concept of a 'molecular clock' has provided a method of utilizing observed differences in amino acid or nucleotide sequences among homologous proteins or polypeptides to estimate the period of time that may have elapsed since they diverged. Such a clock has been utilized to describe evolution in the endocrine system. It is calibrated from information available in the fossil record in units such as the rate of exchange of amino acids (or nucleotides) per year. By counting amino acid substitutions one can then estimate the time since the divergence of the two molecules occurred from a common ancestor. These estimates have sometimes been quite contentious (owing to possible reversions or back mutations) but they are, nevertheless, often very productive (see Lewin, 1988).

The methods used in comparative endocrinology

Many of the endocrine glands were described morphologically long before their functions were recognized. Establishment of a physiological role is usually initiated by observations of the effects of its surgical removal. Administered extracts of such tissues ("replacement") should be able to correct any observed deficiency. The particular active component in the extract then must be identified. The final criterion for a hormonal status is its identification in the blood and its observed release in response to appropriate stimuli. The

mere presence of a response to administration of a tissue extract or a hormone from another species need not reflect an endocrine role.

The scientific techniques utilized in the study of comparative endocrinology have usually been derived from the contemporary technology. At first it was anatomical, histological, and surgical extirpation. Pharmacology provided a variety of techniques, including ways to prepare glandular extracts and measurement of their activity using biological preparations ("bioassays"). It also furnished the concepts of "receptors" and the relationships between chemical structure and biological activity. Chemical methods were used to separate out and purify the hormones and often to determine their structures. Immunology provided antibodies to hormones and so fostered their immunocytochemical detection. It also offered methods for comparing the serological relationships of large protein hormones. The availability of radioisotopes has influenced virtually all aspects of comparative endocrinology by making possible the "labeling" of hormones; this facilitated their measurement by radioimmunoassay and their identification at tissue sites. The most recent technological contribution has been molecular genetic methods (recombinant DNA and molecular cloning techniques), which have provided knowledge of the nucleotide sequences that encode hormones, their precursors, and their receptors. Nucleotide sequences for hormone-like peptides and proteins that were not known to occur naturally have also been uncovered in this way. Such codes for peptides may be for as yet undetected hormones, "unused" ones, or even long extinct ones. This gene technology has even resulted in the identification of the genes that express the hormones and their receptors and has provided a view of the characteristic organization of their DNA and the patterns of the exons and introns present.

The uses of comparative endocrinology

The classic, or academic, aims of comparative endocrinology have been described. The provision of such intellectual satisfaction is not, however, sufficient justification for all! There are, indeed, a number of other contributions that such studies can make to biology, and some examples of these are given in this section.

The process of reproduction in vertebrates is dependent on the endocrine secretions, and an understanding of this relationship can provide information that may be usefully applied when, for esthetic or economic reasons, we may wish to increase, or decrease, the fecundity of a species. This type of study, therefore, constitutes a contribution to the field of "biological control" (Bern, 1972).

Knowledge of the endocrine system in humans has largely been made possible by experiments on other animals. This has principally involved mammals like rats, rabbits, and monkeys but also some more exotic and

bizarre creatures. Quantitative measurements of gonadotropins and melanocyte-stimulating hormone (MSH) were originally made using the responses of the clawed toad (*Xenopus laevis*), and prolactin levels can be measured by its effects on the pigeon's crop-sac or on the behavior of a newt. Oxytocin is assayed by utilizing its ability to decrease the blood pressure of chickens, and the rate of water movement across the toad's urinary bladder can be used to distinguish between two, chemically different, mammalian ADHs.

The responsiveness of a toad's urinary bladder to ADH and aldosterone is used to study the 'mechanism of action' of these hormones on membrane permeability. Such preparations provide useful "models" of hormonal effects on the mammalian kidney.

The relationship of the structure of a molecule to its biological activity is a field of considerable interest to biologists. The diversity, or polymorphism, in the structure of vertebrate hormones, together with their disparate effects on different tissues and in various species, offers a natural "laboratory" for such studies. Nature has had a long time and wide opportunities to experiment with the effects of changes in molecular structures on the activities of such excitants. At present, this is most clearly seen among the neurohypophysial hormones, of which there are 12 known chemical variants among the vertebrates. These hormones are peptides containing nine amino acid residues and often only differ from one another by a substitution at a single chemical locus. They are very reactive molecules and can exert actions at many different sites ranging from the uterus and mammary gland to blood vessels, the kidney, and the amphibian skin and urinary bladder. Analogous effector tissues in different phyletic groups exhibit different abilities to respond to each such hormone, be it a natural one or a variant made in the chemist's laboratory. There are available, and in use, more than 20 different effector preparations that can be used to study the effects of changes in chemical structure among these hormones on their biological effectiveness. Natural variants of hormones, in which the biological activity has been altered in some way, may be of potential use to humans or may provide information about structural modifications that may be medically advantageous. Calcitonin (a hormone concerned with the regulation of calcium in the body) from the ultimobranchial bodies of salmon differs from the hormone present in humans by 16 of its 32 amino acid residues and yet it is much more potent in humans than the human hormone itself. Salmon calcitonin is used therapeutically to treat Paget's disease of the bones. ADH from pigs is effective in humans. It differs from the human hormone by a single amino acid substitution. Early preparations of the synthetic pig ADH were found to be more chemically stable than the homologous human hormone and provided the blue print for a manufactured drug used to treat human diabetes insipidus.

Table 1.1. *The secretions of the endocrine glands*

Gland	Hormones	Principal target tissues/effects
Pituitary		
Adenohypophysis		
Pars distalis	Follicle-stimulating hormone (FSH)	Ovary and testis
	Luteinizing hormone	Ovary and testis
	Thyrotropic (TSH)	Thyroid
	Corticotropic (ACTH)	Adrenocortical tissue
	Growth hormone (GH) (somatotropic)	Liver (forms IGF-I), muscle, bone, adipose tissue, gills (teleosts). Proliferation, metabolism
	Prolactin	Mammary glands, fish gills, tadpole metamorphosis, corpus luteum, skin, etc.
Pars intermedia	Somatolactin (teleosts)	?
	Endorphins	Nerve and endocrine cells
	Melanocyte-stimulating hormone (MSH)	Melanocytes, pigmentation and color change
Neurohypophysis		
Pars nervosa	Vasopressin (ADH), vasotocin	Kidney, amphibian skin, urinary bladder
	Melanin-concentrating hormone (MCH)	Teleost melanocytes (other?)
	Oxytocin	Mammary gland, uterus
Hypothalamus	Pituitropins; GnRH, CRH, TRH,[a] somatostatin, etc.	Release of hormones by the adenohypophysis
Thyroid gland	Thyroxine (T_4), triiodothyronine (T_3)	Tissue metabolism and differentiation: thermogenic (homeotherms); morphogenetic (larval amphibians, teleosts)
Parathyroid glands	Parathyroid hormone (PTH)	Bone and kidney
Ultimobranchial bodies (C-cells in mammalian thyroid)	Calcitonin (CT)	Bone and kidney (gills?)
Adrenal glands		
Cortex (interrenals in sharks and rays)	Cortisol, corticosterone, cortisone, 1α-hydroxycorticosterone	Tissue metabolism (liver, muscle), proteins to amino acids, gluconeogenesis; gills, intestine (various)
	Aldosterone	Na^+ and K^+ in kidney, sweat and salivary glands, gut, amphibian skin and bladder
Medulla (chromaffin tissue)	Norepinephrine (noradrenaline), epinephrine (adrenaline)	Tissue metabolism (liver, muscle, adipose tissue), glycogenolysis, mobilization fatty acids, calorigenic, constriction and relaxation of smooth muscle
Islets of Langerhans		
A-cells	Glucagon	Liver (glycogenolysis); adipose tissue (fatty acid release); gluconeogenesis
B-cells	Insulin	Liver, muscle and adipose tissue (amino acids to protein, glucose to fat and glycogen)