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Hormones and Vertebrate Evolution

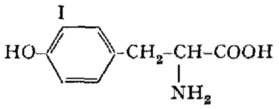
By E. J. W. BARRINGTON*

Endocrine systems depend for their operation upon highly specific relationships between hormonal molecules and the 'target cells' which they influence. The nature of these relationships is obscure, but their mere existence raises questions of great interest. How did these molecules first enter into the biochemical equipment of a particular group of animals? How, once they had entered, was it possible to maintain in their functional relationships a degree of flexibility sufficient to meet the varying needs arising out of the subsequent evolutionary history of the group? Did the hormonal molecules themselves evolve during the course of that history, or was it their target organs which changed? Such fundamental questions are simple enough to ask, but they are treacherously difficult to answer. Nevertheless, it seems worth while to attempt some consideration of them, for only when we have explored the evolutionary roots of endocrine systems can we hope to approach a full understanding of the specialized endocrine organization of the higher animals and of ourselves.

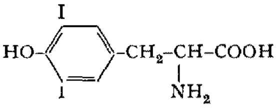
We may begin our examination of the problem by considering the hormones of the thyroid gland, for sufficient is now known about these to enable us at least to pose our questions clearly and without ambiguity. The sequence of events which takes place within the gland is now reasonably well understood[1]. It begins with the trapping of iodide from the blood stream, and is followed by the oxidation of this to free iodine, the combination of the iodine with tyrosine to form monoiodotyrosine and diiodotyrosine, and the polymerization of these molecules to form the two hormones, triiodothyronine and thyroxine (Figure 1). This organic binding of iodine takes place within the molecules of a glycoprotein, thyroglobulin, which constitutes the familiar colloid of the gland, and which is the site of storage of the active hormonal molecules. The release of these into the blood stream is brought about by proteolytic breakdown of the colloid; the iodinated tyrosines, however, are retained within the gland, where they are deiodinated by a specific enzyme.

This account of thyroidal biosynthesis, originally formulated in its essentials by HARINGTON, and subsequently confirmed and extended by the use of radioactive iodine, autoradiography, and paper chromatography, is now known to apply throughout the vertebrate series, from the primitive jawless cyclostomes (lampreys and hagfish) to man. Not only do all vertebrates possess a thyroid gland which, with its secretory follicles and colloid, is recognizable on the briefest inspection, but the identification in it of the iodinated tyrosines and thyronines shows that the constancy of the histological picture reflects an underlying biochemical uniformity. It follows that to enquire into the origin of the thyroid gland we have to search back into evolutionary stages preceding the appearance of the vertebrates.

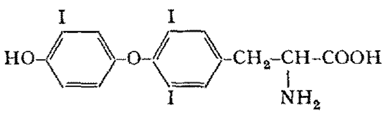
Here a valuable clue is provided in the cyclostomes by the ammocoete larva of the lamprey, an animal which lives in the mud of fresh water streams, and which feeds by filtering water drawn into its pharynx by muscular beating of a velum. This larva possesses a gland-like organ called the endostyle, a ventral out-



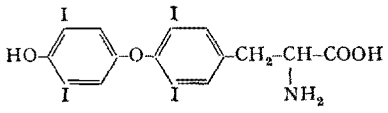
3-Monoiodotyrosine



3,5-Diiodotyrosine



3,5,3'-Triiodothyronine



Thyroxine

Fig. 1

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[1] J. ROCHE, Bull. Soc. Chim. biol. 42, 1155 (1960).

growth of the pharynx (Figure 2) which is believed by some observers to play some part in the feeding mechanism. The special interest of this organ is, however, that parts of it give rise during metamorphosis to the thyroid gland. This at once raises the question whether the endostyle is capable of thyroidal biosynthesis during larval life, and we now know the answer to this, for autoradiography[2–4] has clearly shown that much of its secretory epithelium is capable of the organic binding of iodine (Figure 3), while extracts of it have been shown by chromatographic analysis to contain the iodinated tyrosines and both of the hormones[5]. This is the more remarkable in that at first inspection the organ shows none of the histological characteristics of a thyroid gland. Closer study, however, has shown that, despite the absence of follicles with their contained colloid, such colloid can nevertheless be identified within the cells[3]. In fact, the major differences between this organ and a typical thyroid gland are that iodination of tyrosine takes place well within the cells of the former, rather than at their borders, and that some storage of the iodinated products also takes place within the cells, instead of in extracellular follicular cavities. These products are believed to be shed into the lumen of the endostyle and to pass through the pharynx into the intestine, where they are probably absorbed through the intestinal epithelium.

The particular importance of the ammocoete larva is that in certain features of its organization, and more particularly in its feeding mechanism, it closely resembles the marine animals known as the Protochordata. These animals, like the ammocoete, are microphagous feeders, filtering food material from the currents of water which they set into motion by ciliary activity. They, too, have an endostyle which is in the form of a groove running the whole length of the floor of the pharynx (Figures 4 and 7), and which shows such a close histological resemblance to the endostyle of the ammocoete that the homology of all three of these organs cannot reasonably be doubted. It is natural, therefore, to enquire whether this similarity extends in any way into the functioning of the protochordate endostyle. Does it, in other words, show any signs of being able to carry out thyroidal biosynthesis? We are now in a position to say quite confidently that it certainly does, and we may illustrate the evidence by reference to *Branchiostoma* (amphioxus), belonging to the Subphylum Cephalochordata, and to certain ascidians (seas-quirts), belonging to the Subphylum Urochordata.

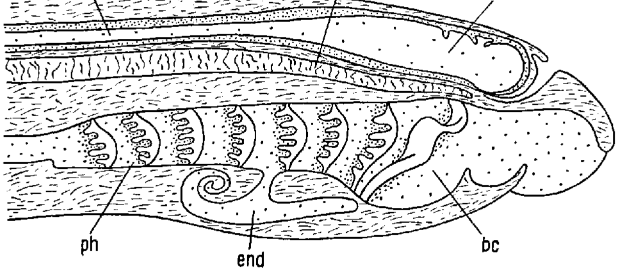

Fig. 2. Longitudinal section of anterior end of ammocoete larva (diagrammatic). b, brain; bc, buccal cavity; end, endostyle; nc, notochord; ph, pharynx; sc, spinal cord.

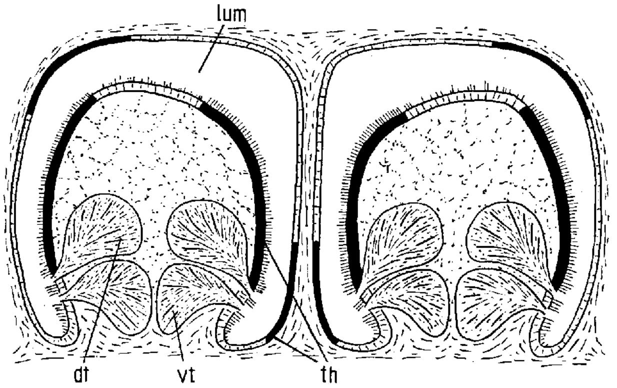

Fig. 3. Transverse section of endostyle of ammocoete larva; the chief regions of iodine binding are shown in black. dt, dorsal glandular tract; vt, ventral glandular tract; th, iodine-binding epithelium; lum, lumen of endostyle (× 100).

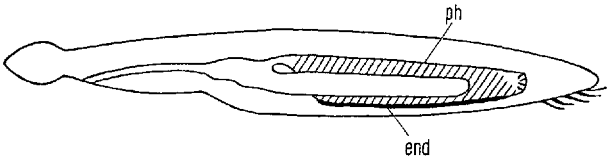

Fig. 4. Diagram of *Branchiostoma* in lateral view. end, endostyle; ph, pharynx (× 3).

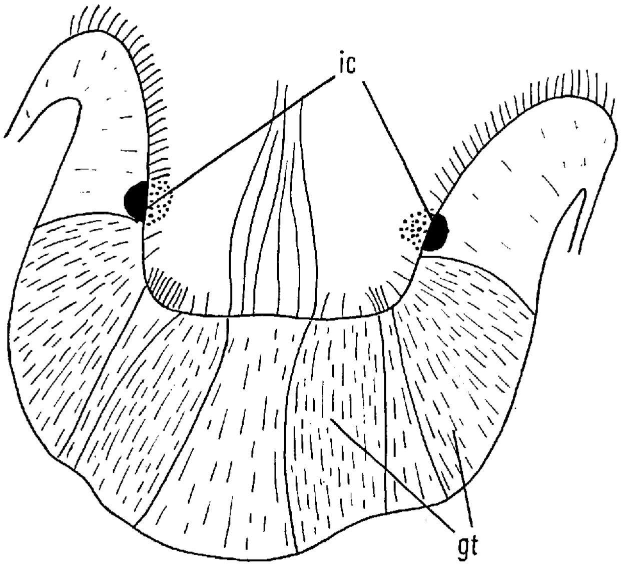

Fig. 5. Transverse section of endostyle of *Branchiostoma*. ic, iodination centres; gt, glandular tracts (× 500).

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[2] A. GORBMAN and C. W. CREASER, J. exp. Zool. *89*, 391 (1942).

[3] E. J. W. BARRINGTON and L. L. FRANCHI, Quart. J. micr. Sci. *97*, 393 (1956).

[4] M. CLEMENTS-MERLINI, J. Morph. *106*, 337 (1960).

[5] J. ROCHE, G. SALVATORE, and I. COVELLI, Comp. Biochem. Physiol. *2*, 90 (1961).

If autoradiograms are prepared from transverse sections of specimens of *Branchiostoma*[6] which have been immersed for up to two days in sea water containing radioactive iodine, two conspicuous spots of radioactivity can be seen in the endostylar epithelium (Figure 5), one on each side, immediately above the tracts of glandular cells which are considered to produce a secretion that aids in the feeding mechanism. These spots indicate the presence of bound iodine, but it might at first be thought that the binding is here only a random process, resulting from the association of iodine with the mucus-like secretion of the organ. This, however, is not so. At the points where the bound iodine is localized it is possible to identify well-marked groups of secretory cells which, curiously enough, seem to have escaped the notice of previous observers. The existence of these cell groups, together with the associated presence of bound iodine, and its virtual absence from the glandular tracts and from other secretory regions of the endostyle, suggest that this must be a specialized process, and one which is therefore of biochemical significance to the animal concerned.

Substantially the same state of affairs is found in the endostyle of the ascidians (e. g. *Ciona, Dendrodoa, Botryllus*) that have so far been investigated[7,8] (Figure 7). Here, too, autoradiograms disclose the presence of organically bound iodine in the epithelium on either side of the endostyle, lying, essentially as in *Branchiostoma*, immediately above the glandular tracts (Figure 6).

This provides the clearest possible evidence of the uniformity of organization of the endostyle in the protochordates, and of its essential homology with that of the ammocoete larva, but it does not, of course, provide adequate evidence for the occurrence of specialized thyroidal biosynthesis in the protochordate organ. Further evidence has, however, been obtained from chromatographic analysis, which has revealed the presence of the iodinated tyrosines and of the two hormones in extracts of the whole body of *Branchiostoma*[9]. It would seem reasonable to infer that these are arising within the areas of iodination in the endostyle, and some confirmatory evidence of this is to be found in the observation that implantation of dried endostyles of *Branchiostoma* into larvae of the amphibian *Ambystoma* produces some signs of metamorphic change[10]. The significance of this derives from the well-known fact that the thyroid hormones promote metamorphosis in these animals; we find here, then, some indication of thyroidal properties in the endostyle.

Chromatographic studies of ascidians are leading to equally significant results, although some uncertainties and discrepancies remain to be resolved. Thyroxine has been identified in extracts of the endostyle of *Ciona*[11] and, although some doubt has been expressed as to whether or not it is accompanied there by the iodo-

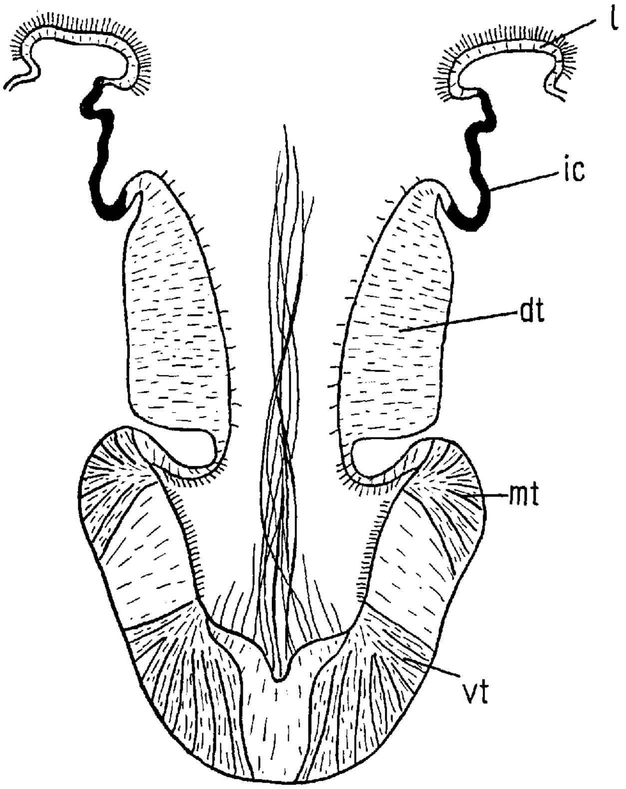

Fig. 6. Transverse section of endostyle of *Ciona*. dt, mt, vt, dorsal, median, and ventral glandular tracts; ic, iodination centre; l, lip of endostyle (× 300).

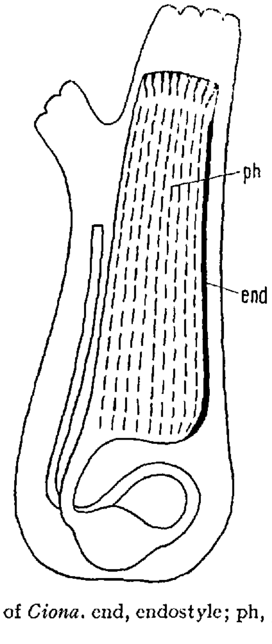

Fig. 7. Diagram of *Ciona*. end, endostyle; ph, pharynx (= $^2/_3$).

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[6] I. M. Thomas, J. mar. biol. Ass. U.K. *35*, 203 (1956). – E. J. W. Barrington, J. mar. biol. Ass. U.K. *37*, 117 (1958).

[7] E. J. W. Barrington, J. mar. biol. Ass. U.K. *36*, 1 (1957).

[8] E. J. W. Barrington, in *Comparative Endocrinology* (Ed. A. Gorbman, Wiley, New York 1959), p. 250.

[9] I. Covelli, G. Salvatore, L. Sena, and J. Roche, C. R. Soc. Biol. *154*, 1165 (1960).

[10] K. Sembrat, Zool. Polon. *6*, 3 (1953).

[11] J. Roche, G. Salvatore, G. Rametta, and S. Varrone, C. R. Soc. Biol. *153*, 1751 (1959).

tyrosines, we ourselves feel that these substances are present. Certainly the iodinated tyrosines and thyronines have been identified in extracts of the whole body both of this animal and of another ascidian, *Clavelina*[12].

We are thus led to the conclusion that the characteristic molecules of the thyroid gland were already established in the protochordate ancestors of the vertebrates. It seems likely, too, that they were of some biochemical importance to them, although this will remain uncertain until it has been possible to show that they have some function in present-day protochordates. Evidently, then, we cannot avoid extending our enquiries further if we are to determine how the chordate line of evolution first came to make use of them. Our ground here is much less certain, but there is good reason for suspecting that the key to the problem is to be found in the origin of life (and of the chordates) in the sea, in the relative abundance of iodine in that environment, and in the readiness with which that element can become bound to protein. It is a striking fact that diiodotyrosine was first discovered, not in the thyroid gland, but in some of the lowliest of metazoa, the gorgonid coelenterates. This is not to say that such animals are carrying on thyroidal biosynthesis. The explanation seems rather to be that iodine is readily bound to the tyrosine of the molecules of their scleroproteins, and it seems probable that between this process and the polymerization of the tyrosine residues to form thyronines there is a barrier which is not easily overcome[13]. This may be because the fibrous form of the scleroprotein molecule is less favourable for the interaction of the tyrosine residues than is the form of the thyroglobulin molecule, and it may be, too, that the process needs the support of some enzyme system which is lacking. The situation awaits further clarification, and it is important that it should be clarified, for autoradiographic evidence indicates that the iodination of scleroproteins may be a widespread phenomenon[14], occurring, so it is suggested, simply as a chance consequence of the association of iodine with protein, and not as a biochemically purposive act such as we seem to find in the protochordate endostyle. Some writers, because of the paucity of evidence that this random iodination can lead to the formation of active thyroidal molecules, are inclined to regard the two sets of events, random iodination and thyroidal biosynthesis, as being quite unrelated, but it is too early to be sure that this view is the correct one. It is a fact of great interest that trace amounts of thyroxine and triiodothyronine have been identified in the gorgonid *Eunicella*[15]. Thyronines are also said to be present in seaweeds[16], which are well known to take up large amounts of iodine, although the chemical events involved here are still in need of further clarification. While, therefore, doubt has recently been cast[17] on the correctness of the suggestion that large amounts of thyroxine are formed in certain invertebrates as the result of such chance iodination of scleroproteins, the possibility of trace amounts being formed has clearly by no means been excluded. It is important in this connexion to appreciate that the evolution of adaptations has depended, as far as we can judge, upon the natural selection of mutations which were rare in themselves, and the selective advantage of which may have been quite minute. Indeed, natural selection has been described as a mechanism for the generation of improbability! Given, then the undoubted occurrence of random iodination, the rare appearance of thyronines would provide a possible basis for the evolution of thyroidal biosynthesis. It is not inconceivable that the existence of the thyronines within the body of early protochordates or their ancestors, formed there perhaps at the body surface by random iodination, or possibly ingested with food from the world outside, might have proved to have some biochemical advantage sufficient to promote the establishment through natural selection of more efficient means of producing them. In this way thyroidal biosynthesis could have been established as a characteristic feature of vertebrate organization. Such an argument is speculative, but it is not wholly divorced from the evidence. Indeed, it already receives some support from the fact that iodination in *Ciona* occurs not only in the endostyle but also in the tunic, which forms a protective covering to the body. Here it results in the formation of a thyroxine-like substance, which, in our most recent investigations, is proving to have the chromatographic properties of the acetic acid analogue of the hormone[18].

We pass now to another of our questions, which arises from the fact that the thyroid hormones have remained unchanged throughout the whole course of vertebrate evolution, for we have next to consider how they have helped to meet the diversity of functional needs which have arisen during the course of that evolution. The interpretation of thyroid function has tended to be dominated by the well-known action of thyroxine in promoting an increase in the basal metabolic rate of mammals, the so-called 'calorigenic effect', but most investigators (although not all) have concluded that no such effect is detectable in fish. There are indications, however, that the thyroid hormones have other effects in mammals; in particular, it is believed that they directly promote growth (the so-

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[12] G. SALVATORE, G. VECCHIO, and V. MACCHIA, C. R. Soc. Biol. *164*, 1380 (1960).

[13] J. ROCHE, Pubbl. Staz. zool. Napoli *31*, Suppl. 176 (1959).

[14] A. GORBMAN, Physiol. Rev. *35*, 336 (1955).

[15] J. ROCHE and P. JOUAN, C. R. Soc. Biol. *150*, 1701 (1956).

[16] C. B. COULSON, J. Sci. Food Agric. *11*, 674 (1955).

[17] W. TONG and I. L. CHAIKOFF, Biochim. biophys. Acta *48*, 347 (1961).

[18] E. J. W. BARRINGTON and N. BARRON, J. mar. biol. Ass. U.K. *39*, 513 (1960). – E. J. W. BARRINGTON and A. THORPE, unpublished.

called 'maturation effect')[19], and in this respect there is good evidence for a similar action in lower forms[20]. Thus, treatment of rainbow trout with thyroid powder, or immersing them in thyroxine solution, results in marked stimulation of growth both in length and in weight (Figure 8), and there are clearly marked effects upon individual tissues also, such as thickening of the epidermis and dermis.

It is not yet known how this growth effect is produced, but it recalls what is in some ways the best-known of all of the actions of the thyroid hormones, their influence upon amphibian metamorphosis, which will not take place in the absence of the thyroid gland, and which can be accelerated by feeding tadpoles with thyroid material. Attempts to interpret the role of the thyroid hormones in metamorphosis have varied, and it has sometimes been argued that their action involves no more than the production of metabolic changes analogous to that of the calorigenic effect in mammals. However, even if such action is a factor in the situation, it is also clear that the influence of the hormones on metamorphosis involves a precise and localized effect upon the tissues, or even upon single cells, which necessarily implies the existence of a highly diversified specialization of the target organs. At a relatively crude level of analysis this is sufficiently obvious in the contrasting behaviour of the tails of the anurans and urodeles, the former undergoing regression while the latter remain intact, but much more striking is such an example as that provided by Mauthner's cells[21], a pair of giant cells lying one on either side of the hind brain of the frog tadpole. These cells specifically regress at metamorphosis, while neighbouring cells undergo further development; in fact, implantation of thyroid tissue adjacent to these cells evokes rapid enlargement and mitosis of all of the cells of the hind brain except these particular ones, which become reduced.

Similar evidence of specialized responses can be readily drawn from other groups of vertebrates. There are indications, for example, of an involvement of the thyroid in the metamorphosis and migration of teleost fish[22], although it is impossible here to do more than touch very briefly upon this aspect. The metamorphosis of the salmon parr into a smolt is a profound physiological transformation which has the effect of changing an essentially fresh water fish into a marine one, the latter being more active, with an increased oxygen consumption, and being capable of osmo-regulating in the sea. There is good evidence of increased thyroid activity during this metamorphosis, and, although this does not necessarily mean that the gland is a causal factor, there are indications that it may well be so. For example, the parr-smolt transformation is accompanied by a silvering of the body, resulting from an increased deposition of guanine in the skin, and it is known that treatment of salmonids with thyroid hormones will evoke just such a silvering.

There is evidence also of an involvement of the thyroid in osmo-regulation. Sticklebacks (*Gasterosteus aculeatus*) may migrate from sea to fresh water early in the year in preparation for spawning, and return later to the sea when their reproductive processes are accomplished. These movements are accompanied by appropriate changes in salinity preference, and it has been shown that these preferences can be modified by immersion of the fish in thyroxine solution[23]. Thus marine forms, with a preference for salt water, may develop a preference for fresh water after as little as three days immersion in thyroxine solution. An interesting illustration of the high level of specificity in such responses is seen in the fact that young salmonids are affected by thyroxine treatment in exactly the opposite way to this; they show an increased tolerance of sea water after such treatment, which is, of course, in good accordance with the evidence of increased thyroid

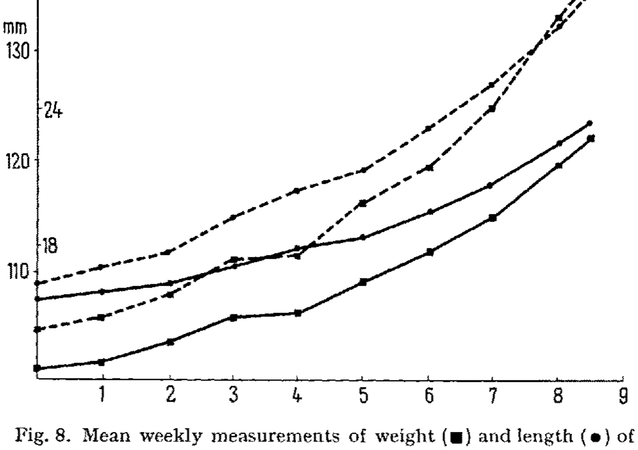
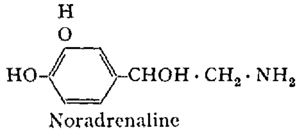
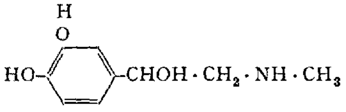


Fig. 8. Mean weekly measurements of weight (■) and length (●) of 9 thyroxine-treated (---) and 10 control (——) rainbow trout[20].



Noradrenaline



Adrenaline

Fig. 9

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[19] R. Pitt-Rivers and J. R. Tata, *The Thyroid Hormones* (Pergamon, London 1959).

[20] E. J. W. Barrington, N. Barron, and D. J. Piggins, Gen. comp. Endocrin. *1*, 170 (1961).

[21] P. Weiss and F. Rossetti, Proc. Nat. Acad. Sci. *37*, 540 (1951).

[22] M. Fontaine, Exper. *16*, 433 (1960). - A. D. Woodhead, J. mar. biol. Ass. U.K. *38*, 407, 417 (1959). - E. J. W. Barrington, Amer. Zoologist *1*, 97 (1961).

[23] B. Baggerman, in *Comparative Endocrinology* (Ed. A. Gorbman, Wiley, New York 1959), p. 24.

activity associated with parr-smolt transformation, a transformation which ends with the passage of the fish into the sea.

The nature of this suggested relationship of the thyroid hormones to ionic movements is far from clear, and the same can be said of all of their observed effects in fish. These may well involve complex interrelationships with other elements of the endocrine system, and it is possible, too, that there may be some action on the nervous system, perhaps a sensitizing of the receptor systems with a resultant modification in the behaviour of the animals. Or again, the thyroid hormones may be promoting in some way the metabolism of the cells concerned, a possibility suggested by the observation that the oxygen consumption of the starry flounder (*Platichthys stellatus*) is higher in salt water than in fresh, and that the thyroid gland is more active in the former environment[24]. What is clear, however, is that constancy of molecular structure of these hormones throughout the vertebrate series is no barrier to their involvement in an almost endless variety of adaptational responses. What light, then, does this shed on the relation of the hormonal molecules to their target cells? It is difficult to see any other explanation than that the thyroid hormones must be acting upon fundamental metabolic pathways in the organization of the cells, and that the expression of this action at the level of the whole organism depends upon the nature and functional status of the particular cells with which the hormones are interacting. Unfortunately, we know little of the mode of action of the thyroid hormones at the cellular level, but it is of interest that current theories tend to fall into this general pattern of thought. One suggestion, for example, is that they influence cell metabolism by modifying the permeability of mitochondria, and WILLMER[25] has pointed out that their molecular pattern suits them for incorporation into the phospholipid layers of cell membranes, a process which might well influence permeability relationships.

We have drawn upon the thyroid gland in illustration of the constancy of hormones during long periods of evolutionary time, and of the consequences which flow from this, but the principles are applicable also to other vertebrate hormones. The catechol amines, adrenaline and noradrenaline (Figure 9), remain equally unchanged, and it is significant that these are tyrosine derivatives of simple structure, and ones which in this case are certainly not confined to the Chordata[26]. No less striking an example is provided by the steroid hormones. In the vertebrates these are characteristically the product of the gonads and of the adrenocortical tissue, both derivatives of the coelomic epithelium, but they are based upon a plan of structure, the perhydro*cyclo*pentenophenanthrene (steroid) nucleus (Figure 10), which is widely distributed in nature, in plants as well as in animals. Here, too, we find that a remarkable constancy of molecular structure has been maintained during vertebrate evolution, the adrenocortical steroids cortisol (Figure 10) and corticosterone, for example, having now been identified in all the major groups[27]. As with the thyroid hormones, then, flexibility of relationship between these molecules and their target cells must have depended upon the capacity of the latter for adaptive modification, and certain aspects of salt-electrolyte metabolism provide an instructive example of this.

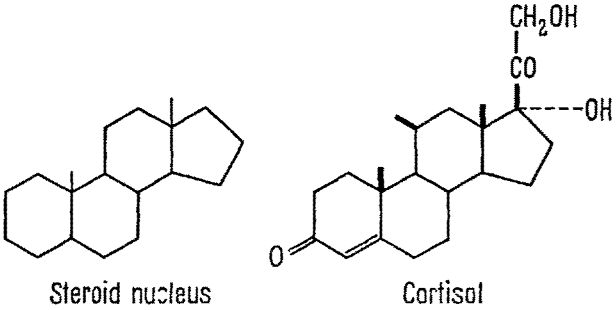

Fig. 10

It is well recognized that teleost fish in a marine environment need to replace the water which they are continuously losing to their hypertonic environment. This they do by swallowing sea water, and by excreting sodium and other ions through their gills. Man, unfortunately for shipwrecked mariners, cannot survive such a process, the swallowing of sea water leading in his case to still greater stress. This is because, even with the highly developed mammalian capacity for secreting hypertonic urine, the amount of water needed to remove the surplus salts of the sea water is in excess of the amount of water swallowed. The situation of sea birds in this regard is, however, quite different[28]. Whether they normally drink sea water is not clear, but they can certainly survive its ingestion under experimental conditions, and this capacity they owe to their possession of specialized nasal glands. These secrete a fluid which contains sodium at a higher concentration than that of sea water, and the activity of these glands in birds which have been given a salt load is made evident by the appearance of drops of fluid at the end of the beak, fluid which has been secreted by the glands and discharged through the nasal passages.

It is well established that the selective transport of ions in the kidneys of vertebrates and in the gills of fish is regulated by the steroid hormones of the adrenocortical tissue, and evidence is now accumulating that

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[24] C. P. HICKMAN, Can. J. Zool. *37*, 997 (1959).

[25] E. N. WILLMER, Biol. Rev. *36*, 368 (1961).

[26] E. ÖSTLUND, Acta physiol. Scand. *31*, Suppl. 112 (1954).

[27] I. CHESTER JONES and J. G. PHILLIPS, Symp. zool. Soc. Lond. *1*, 17 (1960).

[28] K. SCHMIDT-NIELSEN, C. BARKER JØRGENSEN, and H. OSAKI, Amer. J. Physiol. *193*, 101 (1958). – J. G. PHILLIPS, W. N. HOLMES, and D. G. BUTLER, Endocrinology *69*, 958 (1961).

they are also concerned in the regulation of the nasal glands of birds. For example, total adrenalectomy of domestic ducks results in complete elimination of the response of the nasal gland to a salt load. Removal of one adrenal reduces the level of the response, while administration to such animals of cortisol will restore that response almost to normal (Figure 11). Here, then, is a clear illustration of the situation postulated above, in which the action of hormonal molecules upon some aspect of cell metabolism (in this instance, ion transport) is manifested in different adaptive responses of gills, kidney, and nasal glands, the nature of these responses being determined by the properties of those cells with which the molecules are in direct functional relationship.

We must now turn to consider another group of hormones to which the above principles seem at first sight not to apply. One example of these is found in the growth hormone[29] secreted by the pars distalis of the pituitary gland, and known to be a factor in the growth of fish as well as of mammals. This is a protein hormone, with a very large molecule, but it has none the less been possible to prepare it in a homogeneous form which may be regarded as the hormone itself. Fortunately, it has been possible to obtain it from teleost pituitaries as well as from mammalian ones, and as a result it has been demonstrated that it shows marked variation in its properties, that of the teleost having a molecular weight of 22000–26000, while that of the ox is 44000–47000. Moreover, even within the mammals the hormone shows some significant variations in physical, chemical, and physiological properties, and these are associated with highly specific antigen reactions.

No doubt such variations reflect differences in the structure of the hormonal molecules, and examples of the possibilities of such structural variation have been very clearly shown in studies of the molecule of corticotropin (adrenocorticotropic hormone, ACTH), another product of the pars distalis. In this instance the molecule is much smaller, and has been shown to consist of 39 amino acid residues. The structural sequence of these has been established (Figure 12), and the result has been to show that the corticotropins of the pig, ox, sheep, and man differ from each other either in the sequence of residues or in actual composition. The analysis can, however, be taken further than this, for there has been found to be a close structural relationship between these corticotropins and the hormone which has been generally known to zoologists as intermedin, or the B-substance[30].

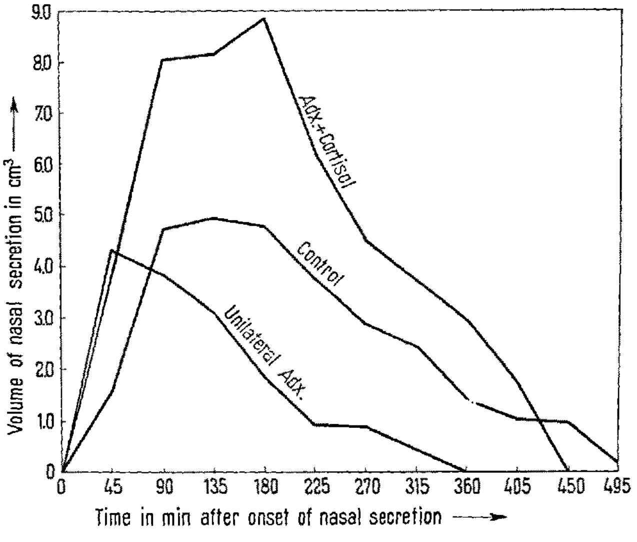

Fig. 11. The nasal secretion of domestic ducks subsequent to saline loading. (a) unilaterally adrenalectomized; (b) intact controls; (c) totally adrenalectomized with cortisol replacement therapy. (From Phillips et al.[28])

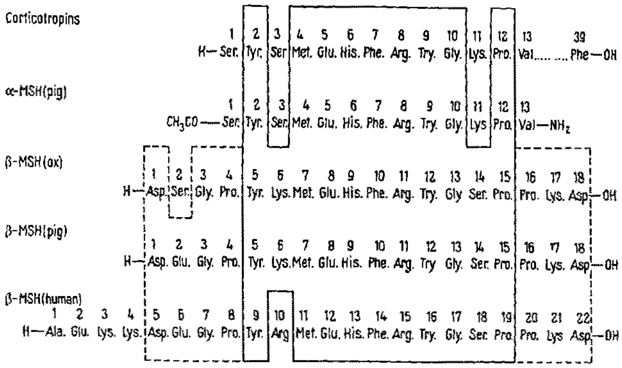

Fig. 12. Amino-acid sequences of corticotropin and melanocyte-stimulating hormones from mammalian pituitary glands (from Harris[30])

Intermedin, now increasingly referred to as the melanocyte-stimulating hormone (MSH), is secreted in the pars intermedia of the pituitary gland, and is responsible for the darkening of the skin which is evoked under certain environmental conditions in amphibians, elasmobranch fish, and certain teleosts and reptiles. The structure of this hormone is now becoming well-known, and it appears that the pituitaries of the pig, sheep, and ox each contain three melanocyte-stimulating hormones, known as α-MSH, β-glutamyl-MSH, and β-seryl-MSH (Figure 12). The last two of these differ from each other in respect of a single amino acid residue, but both differ markedly from α-MSH, which is highly basic, whereas they are slightly acidic. Human β-MSH differs again, having four additional amino acid residues in comparison with the other β-MSH molecules. Despite these variations in structure, however, all of these hormones evoke dispersion of the melanin granules in the melanocytes (pigment cells) of the frog, and it has been suggested that this is because

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[29] I. I. Geschwind, in *Comparative Endocrinology* (Ed. A. Gorbman, Wiley, New York 1959), p. 421. – A. E. Wilhelmi, in *The Hypophysial Growth Hormone* (McGraw Hill, New York 1955), p. 59.

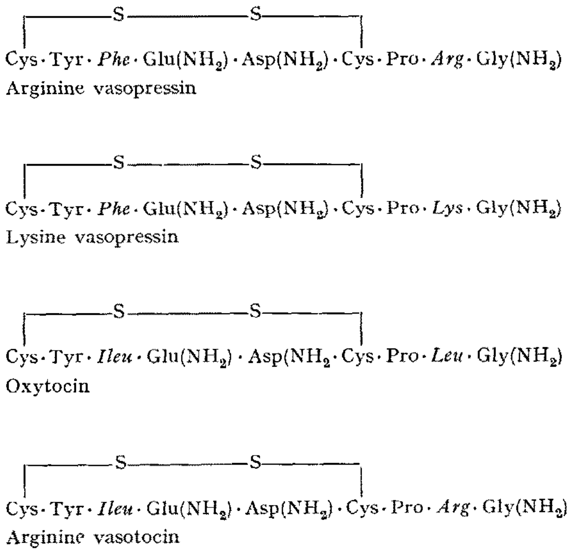
[30] I. Harris, Brit. med. Bull. *16*, 189 (1960). -A.C.J. Burgers, Endocrinology *68*, 698 (1961).

they all possess the common heptapeptide sequence Met. Glu. His. Phe. Arg. Try. Gly. (Figure 12). Some confirmation of this is to be found in the fact that this sequence is also present in the molecule of corticotropin (Figure 12), for it has been established that this molecule, too, has a small, but definite, capacity for stimulating dispersing melanin. Moreover, it has been possible to prepare a synthetic pentapeptide which contains five of the amino acid residues of this sequence, and this, too, has a similar property, although its activity is weak.

Now it will be noticed that these variations are appearing in hormones which differ fundamentally from those that we were considering earlier in that they are proteins or polypeptides, and it seems very likely that, at least in the larger molecules, such variations are related to some extent to the high degree of protein specificity in living organisms[31]. It is probable that each species is characterized by the possession of a unique assemblage of proteins, and, if this is so, we must expect that protein hormones will vary in relation to such specificity. In other words, some degree of structural evolution in large protein molecules may be necessary merely to enable them to retain a particular function in a continuously evolving internal environment, and this could account both for their variation between one species and another, and also for the persistence of a constant core upon which their characteristic properties depend. We might expect that variations in this core would so drastically modify the properties of a hormone that they would be rejected by natural selection. Variations in the remainder of the molecule, however, could take place without such extreme results, and it is for this reason that such variations would be able to persist. Such is the basis of the interpretation of the active core of the polypeptide molecule, but this should not be taken to imply that the remainder of the molecule is unimportant, or that variations in it are necessarily without function. This part may well be needed to contribute to the specificity of relationship between the molecule and its target cells, or, perhaps, to preserve the active core from destruction by enzymes during its passage through the body. Indeed, we cannot really assume that the form in which the molecule is isolated from extracts of its gland is necessarily identical with its final active form in the living body; the possibility of further transformation after release from the gland is certainly not excluded.

However this may be, the situation which we have outlined clearly provides extensive scope for molecular evolution, and it is attractive to speculate that there may be a close evolutionary relationship between the corticotropins on the one hand and the melanocyte-stimulating hormones on the other. The pars distalis and pars intermedia, from which they are respectively secreted, are derived from a common embryological rudiment, Rathke's pouch, and it is at least possible that their joint possession of a common heptapeptide core is a consequence of this. In other words, just as Rathke's pouch has become specialized into two glandular regions, so an initial capacity for secreting a particular form of polypeptide might have become correspondingly specialized towards the production of two distinct types of hormonal molecule, with a common central sequence, but differing in their properties by virtue of other structural specializations. If this were indeed the actual course of events, it could be said that changing adaptive needs have been met in part by adaptive evolution at the molecular level, and not solely through the changing properties of the target organs. Great caution, however, is needed in applying this concept, and the reason for this can be well seen if we consider the case of the polypeptide hormones of the neurohypophysis[32].

This is the region of the pituitary which develops as a downgrowth from the brain, and which comes to form, with the pars intermedia, the so-called 'posterior lobe'. We now know that the hormones which can be extracted from it are produced, mainly if not entirely, in neurosecretory centres in the hypothalamus, and are conveyed along neurosecretory nerve fibres into the neural lobe for storage and eventual release. These hormones are two in number in mammals, oxytocin and vasopressin; they are relatively simple polypeptides, and their structure has been determined, and confirmed by synthesis (Figure 13). They, like the other hormones which we have just considered, are



$$\overbrace{\text{Cys}\cdot\text{Tyr}\cdot\textit{Phe}\cdot\text{Glu(NH}_2)\cdot\text{Asp(NH}_2)\cdot\text{Cys}}^{\text{S}\text{———}\text{S}}\cdot\text{Pro}\cdot\textit{Arg}\cdot\text{Gly(NH}_2)$$

Arginine vasopressin

$$\overbrace{\text{Cys}\cdot\text{Tyr}\cdot\textit{Phe}\cdot\text{Glu(NH}_2)\cdot\text{Asp(NH}_2)\cdot\text{Cys}}^{\text{S}\text{———}\text{S}}\cdot\text{Pro}\cdot\textit{Lys}\cdot\text{Gly(NH}_2)$$

Lysine vasopressin

$$\overbrace{\text{Cys}\cdot\text{Tyr}\cdot\textit{Ileu}\cdot\text{Glu(NH}_2)\cdot\text{Asp(NH}_2\cdot\text{Cys}}^{\text{S}\text{———}\text{S}}\cdot\text{Pro}\cdot\textit{Leu}\cdot\text{Gly(NH}_2)$$

Oxytocin

$$\overbrace{\text{Cys}\cdot\text{Tyr}\cdot\textit{Ileu}\cdot\text{Glu(NH}_2)\cdot\text{Asp(NH}_2)\cdot\text{Cys}}^{\text{S}\text{———}\text{S}}\cdot\text{Pro}\cdot\textit{Arg}\cdot\text{Gly(NH}_2)$$

Arginine vasotocin

Fig. 13

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[31] C. B. ANFINSEN, *The Molecular Basis of Evolution* (Wiley, New York 1959).

[32] W. H. SAWYER, Rec. Prog. Horm. Res. *17*, 437 (1961); Pharmacol. Rev. *13*, 225 (1961). – H. HELLER and B. T. PICKERING, J. Physiol. *155*, 98 (1961).

subject to modification by amino acid substitution, as is shown by the fact that the pig, and probably the hippopotamus, secrete their vasopressin in the form of lysine vasopressin, while that of the other mammals so far studied is arginine vasopressin; the difference lies in a single substitution, but this is sufficient to create a detectable difference in properties.

The most remarkable fact about these particular hormones, however, according to evidence which is becoming increasingly convincing, is that vasopressin is a characteristically mammalian hormone, and that its place is taken in lower vertebrates, and also in birds, by arginine vasotocin, a substance which was first prepared by synthesis, and only afterwards shown to possess properties corresponding to those found in the neural lobe extracts of the groups concerned. It is impossible to review the evidence for this conclusion here, and it must suffice to say that certain amphibians have a highly specialized and very characteristic capacity for the uptake and retention of water, an adaptation associated with their highly permeable skin and their consequent danger of dessication. This 'water-balance effect' is regulated by their neural lobe hormones, and it has been shown that arginine vasotocin possesses the necessary level of activity, whereas neither oxytocin or vasopressin do.

The evidence which is now beginning to take shape (and much more is needed before we can safely take the picture as being complete) is that arginine vasotocin is present in all vertebrate groups from lampreys up to birds (but excluding elasmobranch fish, which have polypeptide hormones of still unknown constitution), that oxytocin appears at the fish level of evolution and persists thereafter throughout the vertebrates (but again excluding elasmobranchs), and that vasopressin is substituted for arginine vasotocin in mammals. Scrutiny of the structural formulae of all of these molecules (Figure 13)[32] shows that the transition from one to another need involve no more than a single amino acid substitution on each occasion, and there is good reason for supposing that such a change might well be effected by the occurrence and selection of a single gene mutation. Here, then, at first sight are tempting grounds for supposing that molecular variation of these hormones might have served to meet the changing demands of the vertebrates at different stages of their evolution.

If, however, we examine the situation more closely, we find that it is very difficult to correlate the changes in the hormonal molecules with the major changes in vertebrate evolution. The water-balance effect in amphibians, for example, arises out of the stresses of terrestrial life at a stage when the vertebrates were still imperfectly adapted to dry land, but it is not at this stage that arginine vasotocin appears. On the contrary, it was present long before. So also, to take only one other example, with lactation in mammals. Oxytocin plays an important part in regulating milk ejection, but it was present long before this particular demand arose, and does not seem to have undergone any structural alteration in relation to it. One might, perhaps, suppose that this same hormone, which promotes uterine contractions in mammals (an effect of doubtful physiological significance), might also promote the contractions of the oviduct which in birds aid the expulsion of the eggs, but, curiously enough, even this simple expectation is not fulfilled, for it is arginine vasotocin which seems the more important in this regard.

We must re-emphasize that the present evidence is very incomplete, and that any generalizations regarding the evolutionary or functional significance of molecular variation in polypeptide and protein hormones must be very tentative. Within those limits, however, it certainly appears that the capacity of such hormones to undergo structural variation has not removed the need for adaptive change in their target organs. In this respect, then, there may be no fundamental difference between the functional relationships of these complex molecules and the relationships of the simpler ones with which we opened our discussion. As regards the amino acid substitutions in the former, we have suggested that these may be the direct expression of gene mutation, but in this connexion it must be remembered that the most obvious effect of a gene may not be its only one, nor even necessarily its most important one. The natural selection of particular variants of hormonal molecules, therefore, may not always be determined exclusively by their own properties. Moreover, we cannot assume that the only effects of a particular hormone which are of significance to an organism are the ones which are most readily detected by our laboratory methods. In fact, those methods, being developed in order to measure certain specific properties, can easily introduce false simplifications into our conclusions. Finally, there may well be polypeptides in the hypothalamus and neurohypophysis which still await detection (in teleosts[33], for example, and in elasmobranchs), and other functions of the known molecules may also be awaiting investigation. Particularly important in this respect is the growing belief that hypothalamic polypeptides, not necessarily identical with those already characterized, may be concerned in the humoral regulation of the activity of the pars distalis[34], the suggestion being that, in lower vertebrates as well as in the higher ones, they are transmitted from neurosecretory centres in the hypothalamus through the hypophyseal portal system, thereby bringing the pars distalis under neural control[35]. Confirmation of these beliefs would

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[33] H. HELLER, B. T. PICKERING, J. MAETZ, and F. MOREL, Nature, Lond. *191*, 670 (1961).

[34] C. B. JØRGENSEN and L. O. LARSEN, Ergeb. Biol. *22*, 1 (1960). – E. J. W. BARRINGTON, Symp. Zool. Soc. Lond. no. 2, 69 (1960).

[35] G. W. HARRIS, *Neural Control of the Pituitary Gland* (Arnold, London 1955).