AN ENDOCRINOLOGICAL APPROACH TO THE PROBLEM OF THE ORIGIN OF THE VERTEBRATES

by E. J. W. Barrington.

Department of Zoology, The University, Nottingham, England.

Summary. — It seems reasonable to look to the protochordates for at least some indications of the mode of origin of the endocrine system of the vertebrates, but the issues are not simple, because the protochordates are very specialized, and are well adapted to their own characteristic modes of life. We are not justified, therefore, in expecting these animals to show merely a continuous line of evolutionary progress (anagenesis), leading towards the establishment of the vertebrate endocrine system. We must expect them also to show a measure of specialized diversification (cladogenesis).

Since the pituitary gland is well differentiated in the lamprey and its larva, it seems likely that the protochordates may possess some forerunner of it; it is therefore significant that they and hemichordates develop gland-like invaginations near to the mouth. Each of the three types of organ (the preoral ciliary organ of the hemichordates, the neural gland of the urochordates, and the preoral pit of amphioxus) is specialized in relation to the particular mode of life of the group concerned, but one can nevertheless visualize that the adenohypophysis might have evolved from a similar organ in ancestral protochordates.

As regards the thyroid gland, the history of that organ, like that of the hypophysis, may well have been determined by the feeding mechanisms of the protochordates. Bound iodine is found in ascidians and amphioxus in localized regions of the endostylar epithelium, and perhaps also in other parts of the pharyngeal epithelium in ascidians. This, in conjunction with evidence of the presence of thyroxine and 3,5,3'-triiodothyronine in protochordates, suggests that the thyroid gland took its origin in the secretory activity of the pharynx of primitive chordates.

Bound iodine is also found in the tunic of ascidians, but the full significance of its presence in this specialized tissue is not yet clear. However, it seems probable that in *Dendrodoa* this binding is a phenomenon similar to that found in association with the structural proteins of the exoskeletons of invertebrates. It is difficult, however, at the present

moment to determine the precise relationship between the origin of the thyroid gland and the iodination processes in urochordates; the results of further investigations are needed before the problem can be usefully discussed.

1. — The stability of the vertebrate endocrine system.

Mr. President, it gives me a very special pleasure to be able to speak to your Society on the subject of comparative endocrinology, for it is only a few weeks ago that the second Reunion of European Comparative Endocrinologists was held in this city. Nobody who was present on that occasion, so brilliantly organized by Mme Herlant-Meewis, could have doubted the vigour of this rapidly advancing branch of the biological sciences, nor the importance of the researches that are being carried out in its name in all parts of the world.

Yet in deciding what to say to you today I have found myself in difficulties. The subject is so young, and its data still so meagre in comparison with the immense variety of animal life, that it is dangerous indeed to attempt broad generalizations. Nevertheless, I shall try to show you that comparative endocrinology can make a significant contribution to our understanding of the origin of the vertebrates, although I must ask you to forgive me if at this stage my conclusions have to be unduly cautious and tentative.

Surely the most remarkable feature of the endocrine system of vertebrates is that it has been able to provide massive support for the adaptive radiation of these animals without itself undergoing substantial changes in its organization (Barrington, 1964). Not only do the endocrine glands preserve in many of the vertebrate groups a histological structure that is recognizably the same wherever we find it. The hormonal molecules also remain in many instances quite unchanged, and in other instances are subject to no more than comparatively minor degrees of structural variation (Barrington, 1962 b). This is because the continued adaptability and flexibility of endocrine control in the vertebrates has depended very largely upon adaptive modifications of the target organs of the hormones, rather than upon adaptive evolution of the hormones themselves.

Birds, for example, unlike man, can drink sea-water without harmful results, because they possess nasal glands that can secrete a strongly hypertonic fluid. It is believed that the adrenal cortex is concerned in the regulation of these glands (PHILLIPS *et al.*, 1961), but no new hormone has been specially evolved to provide for this. On the contrary, the adrenocortical hormones remain remarkably constant throughout the vertebrates; what is new in birds in this particular instance is the target organ, the nasal glands.

Then again, the amphibians have a very characteristic property, the water-balance response, which enables them to absorb and store fluid. This response is regulated by one of the hypothalamic polypeptide hormones (HELLER, 1963; SAWYER, 1961), a group of hormones that we believe to have undergone changes in their molecular structure during vertebrate evolution. Yet this molecular variability, involving amino-acid substitution, has not been drawn on in the establishment of the water-balance response. On the contrary, the hormone concerned, arginine vasotocin, is present in cyclostomes, and is widely distributed throughout many vertebrate groups; what is new in the response is the specialization of the target organs, in this instance the skin, the kidney, and the bladder.

It appears, in fact, that the fundamental organization of much of the endocrine system of vertebrates is as distinctive a characteristic of the group as are the notochord, for example, or the dorsal nerve cord, and it is likely, therefore, that some components of the system may well have originated when the other basic features of vertebrate organization were being established. For comparative endocrinologists, then, the problem of the origin of the vertebrate endocrine system becomes part of the wider problem of the origin of vertebrates, while comparative endocrinology itself is revealed as what it truly is : part of the great and expanding field of modern zoology.

This imposes a heavy responsibility on all of us. The comparative endocrinologist must realize that however specialized may be his techniques, and however limited the immediate field of his investigations, his work must be planned, and his results interpreted, in conformity with the fundamental principles of zoological science. Equally, the zoologist who wishes to debate the problem of the origin of vertebrates must take full account of the findings of comparative endocrinologists, and must correlate their viewpoints with his own.

2. — Progress and diversification in evolution.

This correlation is by no means easy to achieve, partly, I suggest, because we all tend at times to take an over-simplified view of the course of evolution. In discussing the Vertebrata, we must clearly begin by recognizing that these animals constitute a Subphylum of a larger group, the Phylum Chordata. The remaining members of this Phylum constitute two other Subphyla, the Urochordata (Tunicata) and the Cephalochordata. These Subphyla, which are jointly referred to as the protochordates, are essentially invertebrate chordates closely related to another group, the Hemichordata, which can be regarded as a wholly independent Phylum of invertebrates. This Phylum can be placed, with the Chordata, the Echinodermata, and the Pogonophora, in a great assemblage called the Deuterostomia, an assemblage in which the vertebrate plan of organization emerges out of its invertebrate ancestry.

Our central problem is the analysis of the path along which this emergence has taken place, and it is here that we over-simplify the issue if we regard the protochordates as merely lower animals, attempting, so to say, to achieve vertebrate status. In reality, of course, they are very specialized and very diversified, closely adapted to their individual modes of life, and this means that we must make a clear distinction between two aspects of their evolution. One of these aspects is represented by the advances in organization that we regard as constituting evolutionary progress; this is the aspect for which Huxley (1957) has recently used the name *anagenesis*. The other aspect, no less important, is the branching of a group into a diversity of highly adapted forms; this he has called *cladogenesis*. It is, I suggest, of the utmost importance to bear this distinction clearly in mind when we attempt to interpret the history and the phylogenetic relationships of the chordates. We must surely expect to find in hemichordates and protochordates not only some measure of anagenetic progress towards vertebrate organization, but also a substantial element of cladogenetic diversification and specialization.

3. — The problem of the origin of the pituitary gland.

Consider, from this point of view, the problem of the origin of the pituitary gland, an organ of central importance in the vertebrate endocrine system. Its characteristic feature is the intimate association of an ectodermal ingrowth, the adenohypophysis, with a neural structure, the neurohypophysis, an association so fundamental that it is already established in the most primitive of living vertebrates, the lamprey and its ammocoete larva. This association is not fortuitous. It is ultimately to be ascribed to the importance of the hypothalamus in the regulation of visceral function (BARRINGTON, 1963), a regulation which it has long been known to carry out through the mediation of the autonomic nervous system, and which we now know it also carries out through the mediation of the endocrine system. The association of the neurohypophysis and the adenohypophysis allows hypothalamic secretions to be distributed to the adenohypophysis, in part through neurosecretory pathways and in part through the hypophysial portal vessels. Thus the hypothalamus can regulate the release of the adenohypophysial tropic hormones, and in this way it is able to exert a widespread control over the endocrine system as a whole.

Since the pituitary gland is so well differentiated in the cyclostomes it seems likely that the protochordates may have possessed some forerunner of it, just as they show us the beginnings of the notochord and spinal cord. Thus it is surely more than a coincidence that hemichordates and protochordates possess glandlike ectodermal invaginations, situated close to the mouth in positions that immediately bring to mind the way in which the adenohypophysis develops in vertebrate embryos. In the enteropneustan hemichordates we find the preoral ciliary organ (BRAMBELL and COLE, 1939), in the urochordates we find the neural gland, and in the larva of amphioxus we find the preoral pit, which contributes at metamorphosis to HATSCHEK's pit and the wheel organ.

Various workers have considered these organs to have secretory or sensory functions related to the currents of water and food particles that are set up by the ciliary feeding mechanisms of their possessors, but arguments have also been advanced for homologizing them with the pituitary gland. These arguments have been based in part on morphological considerations, the most convincing example of this being GOODRICH's (1917) demonstration of a remarkable similarity between the preoral pit of amphioxus and the developing adenohypophysis of certain vertebrates. The preoral pit opens into the most anterior coelomic pouch, and the adenohypophysis may have a similar, although transient, coelomic connection. This striking resemblance is of no obvious adaptive value, and, since it seems unlikely to be a mere coincidence, it strongly suggests that the two organs may have had a common origin. It is, however, more doubtful whether there is a homologue of the neurohypophysis in amphioxus. Secretory cells in the cerebral vesicle have been compared with the neurosecretory cells of the vertebrate hypothalamus, but it now appears that they are concerned with the secretion of a possible homologue of REISSNER's fibre (OLSSON, 1962), and they certainly seem to have no functional relationships with HATSCHEK's pit.

From this point of view it is the neural gland of urochordates that presents at first sight a more attractive field for phylogenetic speculation, for this organ is closely connected with the neural ganglion to form a complex that superficially recalls the compound structure of the pituitary gland. Closer and more critical scrutiny, however, gives little support to the possibility that the neural gland and ganglion may respectively be homologous with the adenohypophysis and the neurohypophysis. The relationship lacks the functional basis that determines the association of the two components of the pituitary gland, for although supposed neurosecretory cells have been described in the urochordate ganglion, their significance remains doubtful (MAZZI, 1952; DAWSON and HISAW, 1956). Moreover, the embryological situation is, to say the least, obscure, for it has been shown that the gland and ganglion may both develop at metamorphosis from the nervous system (ELWYN, 1937).

Morphological and embryological considerations cannot, of course, be conclusive, and it is true that from time to time biochemical and physiological evidence has been brought forward to support the supposed homology of the neural gland complex and the pituitary gland. It has been said, for example, that the complex secretes substances resembling, or identical with, oxytocin and vasopressin, two of the hypothalamic polypeptide hormones of the vertebrates which I mentioned earlier. These assertions, however, find no support in the most recent investigations. DODD (1959) and SAWYER (1959) have independently investigated the supposed oxytocin-like and vasopressin-like properties of extracts of the complex; they find that they differ in detail from the known properties of the vertebrate hormones, and that they are probably of no physiological significance at all. It has further been suggested that the complex may secrete gonadotropins, but this again receives no support from another recent study (HISAW *et al.*, 1962), in which the gland, or the ganglion, or both, were very carefully removed from ascidians without disturbing the attachment of the animal to the substratum. The operations were found to produce no effect at all upon the development and maturation of the gonads.

In fact, the function of the neural gland remains obscure. PÉRÈS (1943) believes it to be phagocytic, as also does GODEAUX (1953). CARLISLE (1950), on the other hand, suspects that in the salps it may be an important part of the feeding mechanism, testing the concentration of particles in the incoming water, and discharging signals that control the rate at which water is taken into the pharynx. The two views are not, of course, irreconcilable, for, as GODEAUX points out, the gland might phagocytize particles taken up as they pass the ciliated tubercle on which the organ opens.

Whatever the precise function of the neural gland complex, however, I should not wish to reject completely the possibility of linking these hemi- and protochordate organs with the pituitary gland. On the contrary, I believe that it can be plausibly argued that we owe our possession of that gland to the mode of life of the earliest chordates. Conditions in hemichordates and protochordates suggest that ciliary feeding encourages the development, close to the mouth, of glandular organs such as those that I have mentioned, serving to test the incoming stream of water (much as do the osphradia of lamellibranch molluscs), and to add secretions to it. We must expect, however, that such organs will have become specialized in relation to the modes of life of the groups possessing them, and that they will show much cladogenetic diversification.

Because of this we may never know exactly how this type of organ could have given rise to a pituitary gland, supposing that we are correct in believing that it did so. But perhaps we might speculate so far as to suggest that an organ sensitive to incoming water and food particles might have become sensitive to secretions of other individuals of the same species. Thus it might have come to play some part in intraspecific coordination, as, for example, in the synchronization of spawning behaviour. CARLISLE (1951) has suggested just such a function for the neural gland of ascidians, and his interesting theory merits fresh experimental analysis. Further speculation suggests that an organ that had become sensitive in this way to external secretions might then have become sensitive to internal ones as well, and thus the internal regulating functions of the pituitary gland might have become initiated at an early stage.

4. — Iodine binding in the protochordates.

You may well feel at this point that the comparative endocrinologist's contribution to any discussion of the origin of vertebrates consists largely of criticism and speculation. Both of these are, indeed, desirable activities, and essential for the framing of fruitful hypotheses, but there is a good deal more to comparative endocrinology than this, as I should like now to demonstrate by discussing the problem of the origin of the thyroid gland.

The histological structure of this gland is simple in comparison with that of the pituitary gland, but the simplicity is misleading, for the production of the thyroid hormones involves the operation of complicated and beautifully adapted biochemical pathways. These comprise the trapping of iodide, the oxidation of this to iodine, and the binding of the iodine to tyrosine with the formation of the biologically inactive iodo-tyrosines and of the two hormones, thyroxine and 3,5.3'-triiodothyronine. Finally, the thyrogobulin in which these reactions largely take place must be hydrolyzed, and the products of hydrolysis controlled in such a way that only the two hormones pass into the blood.

It is well known that the lamprey larva has an endostyle, and that this organ is transformed into the thyroid gland at metamorphosis. There is now good evidence that this endostyle is itself able to bind iodine and form thyroid hormones during larval life (Barrington, 1963, 1964; Gorbman and Bern, 1962). Thyroidal biosynthesis, then, is as much a primitive characteristic of vertebrates as is the pituitary gland, so that here again we are bound to enquire if its foundations are being laid in the protochordates. Indeed, it is difficult to believe that the whole of its biochemical and histophysiological machinery could have arisen in one evolutionary step. We should rather expect to find it emerging as the result of the transformation and elaboration of an initially simpler biochemical situation. For this reason it is of no small interest to find that the binding of iodine to tyrosine occurs in the Cephalochordata, in the Urochordata, and in the Hemichordata.

The situation is most clearly defined in amphioxus (Thomas, 1956; Barrington, 1958), where, in animals that have been immersed in radio-iodinated sea-water, bound iodine is to be seen in autoradiograms at a sharply localized region of the endostylar epithelium. This suggests, of course, that the endostyle of amphioxus, like that of the lamprey larva, may be engaged in thyroidal biosynthesis, and there is, indeed, good reason to believe that this is so, for the chromatographic studies of COVELLI *et al* (1960), confirmed independently by TONG *et al.* (1962), and by ourselves (unpublished), has established the presence in amphioxus of monoiodotyrosine, diiodotyrosine, and the thyroid hormones.

To appreciate this situation fully it is necessary to understand how feeding and digestion take place in amphioxus. The function of the endostyle is to produce a secretion that is swept like a net up the side walls of the pharynx, where it traps food particles from the water that passes through it. Iodinated products of the endostyle therefore become included in the food cord, and can be detected in autoradiograms. I have shown that food particles from the food cord accumulate in vacuoles within the cells of the mid-gut caecum (BARRINGTON, 1962 a), where they undergo intracellular digestion. Iodine is also found at the same level in these cells, so that it seems likely that the thyroidal hormones secreted by the endostyle are absorbed through the digestive epithelium and distributed in the blood stream. This may well account for TONG *et al.* (1962) finding the thyroid hormones in other parts of the body as well as in extracts of the pharynx. However, we cannot yet exclude the possibility that they may be manufactured in other tissues as well as in the endostyle.

The Urochordata also have an endostyle in their pharynx. In the ascidians it contributes to the feeding mechanism in essentially the same way as in amphioxus, and I have shown (BARRINGTON, 1957) that in these animals it binds iodine, and does so in a region corresponding closely with the iodinating region in the endostyle of amphioxus. The situation is less clear, however, than in that animal, for in ascidians some iodine is also found quite abundantly over the pharyngeal epithelium, and particularly on the ciliated regions that help to drive the endostylar secretion up the wall of the pharynx. I have found it difficult to decide whether this is a result of the adsorption of iodinated secretion on to the cilia, as I originally suggested, or whether iodination is actually taking place in those areas. I am inclined to believe that the latter is at least part of the explanation, and that the capacity for binding iodine is not restricted to the endostyle in the ascidians, but is distributed also over other parts of their pharyngeal epithelium. If this is so, there is here a significant difference from the condition in amphioxus, where pharyngeal iodine binding is certainly restricted to a localized area of the endostyle.

A further complication in the Urochordata is the presence of their remarkable tunic. This external protective coat is in part a secretion and in part a vascularized tissue, laid down by the epidermis and by wandering amoebocytes. It is composed of acid mucopolysaccharide, cellulose, and protein, the protein being particularly well developed as a thin but distinctive layer over the surface. The presence of this protein component makes it easier to understand the otherwise remarkable fact that when urochordates are immersed in sea-water that contains radioactive iodide, a great deal of bound radioiodine accumulates in the tunic. Some of this is certainly organically bound, and it is noticeable that in *Ciona* it is largely concentrated in the surface layer of protein (Barrington, 1960).

The occurrence of iodine binding in the tunic has led to the suggestion (Roche *et al.*, 1962) that this tissue may be carrying on thyroidal biosynthesis. The admitted relationship of urochordates and vertebrates makes this an attractive proposition, yet it is not easy to understand how such a specialized biochemical process could have developed in a tissue that is itself specialized to produce a primarily skeletal material. In this connexion it seems helpful to remember that the relationships of urochordates are not only with the vertebrates. As we have seen, they are invertebrate members of the Phylum Chordata, so that in some respects they may have at least as much in common with other invertebrates as they have with the vertebrates. Indeed, if we forget this we are in danger of neglecting my warning against over-simplification.

The importance of this consideration lies in the fact that the binding of iodine to tyrosine is widespread in the invertebrates (Roche, 1959). Typically it occurs at the body surface, particularly where the structural proteins of skeletal materials are being secreted. Whether it gives rise to significant quantities of thyroid hormones is a matter of dispute, but it has been shown that at least in some species it does not do so, and that only the iodinated tyrosines are formed (Tong and Chaikoff, 1961; Roche *et al.*, 1963). It seems likely that in these cases the iodine binding is a chance by-product of the secretion of proteins that contain tyrosine. Tong and Chaikoff (1961) have suggested that one reason for this association of iodine with structural proteins is that these substances are often accompanied by quinones, which bind the protein molecules together through the process known as quinone tanning. They find that quinones promote the oxidation of iodide to free iodine, so that the coexistence of proteins and quinones may be expected to facilitate the binding of free iodine to the tyrosine residues of the protein molecules.

It occurred to us that this characteristically invertebrate process might well be operative in the tunic of urochordates. Some preliminary observations on *Ciona* were inconclusive, but we have now tested this possibility further by studying the tunic of the ascidian *Dendrodoa*. This animal has a particularly tough and fibrous tunic, and it is noticeable that bound iodine, while still particularly concentrated at its surface, is also abundantly present throughout its thickness. It seems reasonable to associate this with the extensive development of the fibrous content of the tunic in this species. How these fibres are laid down we do not yet know, but we have considerable evidenec that quinone tanning is an important factor in the differentiation of the tunic.

Tanning is held to involve the production of polyphenols, and the oxidation of these to quinones by polyphenol oxidase. Now the presence of polyphenols can be investigated by using certain well-known histochemical procedures; thus they may be expected to give a positive argentaffin response, to develop a brown colour in the presence of potassium dichromate (the chromaffin response), and a golden-brown colour in the presence of potassium iodate (LISON, 1953). Applying these methods to the tunic of *Dendrodoa,* we find that in the region of the mantle epithelium there is a very substantial accumulation of amoebocytes which contain granules that give a positive response with all three of those tests. These cells pass through the mantle epithelium into the tunic; there they can be seen to break up, and it seems highly probable that they are contributing polyphenols to the developing tissue.

As regards the polyphenol oxidase, we have applied to the tunic of *Dendrodoa* the test devised by SMYTH (1954) for the detection of that enzyme. This has given clear-cut positive results, pieces of tunic becoming reddish-brown when they are immersed in catechol solution, an indication of the oxidation of the catechol and the tanning of protein in the tunic. The enzymic character of the reaction is shown by the fact that it can be blocked by the presence of cyanide. As a further test we have used the manometric method of BHAGVAT and RICHTER (1938), in which extracts of homogenates of the tunic are incubated with catechol. In this procedure, checked by appropriate controls, we have found that the presence of catechol produces a very large increase in oxygen consumption of the extracts, a further indication that polyphenol oxidase is present.

I believe the urochordates to be very complicated animals, and I am sure that we are still far from a full understanding of the biochemical basis and physiological significance of the results that I have briefly reviewed. Moreover, ascidians show a great deal of specific variation in the composition of their tunic, so that it would be unwise to generalize from conditions in only one species. Nevertheless, the facts so far established certainly suggest that iodine binding in the tunic of *Dendrodoa* is not typically thyroidal, and that it may have something in common with the iodination processes found in other invertebrates. This is the conclusion that we draw also from our chromatographic studies, which have so far failed to demonstrate the presence of either of the thyroid hormones in extracts of the tunic of the animal, although monoiodotyrosine, diiodotyrosine and various unidentified products are certainly present. Whether this will prove to be true also of other urochordates remains to be seen, for there have been reports of the presence of thyroid hormones in *Clavelina* and *Ciona* (SALVATORE *et al.*, 1960; ROCHE *et al.*, 1962), notably in the tunic of the latter.

In any case our own results are not yet final or exhaustive. For example, we do not yet know what are the optimal conditions for iodine binding in these animals. Moreover, we cannot exclude the possibility that thyroxine and triiodothyronine may be present in amounts that are below the limit of sensitivity of our methods, and it is important to bear this possibility in mind. One can conceive that the production of trace quantities of these molecules in the chordate line might well have been a first step in the evolution of thyroidal biosynthesis, and that improved paths for their production could have been evolved later through the agency of natural selection. This remains a matter for further study. It is, however, of great interest that traces of thyroxine and triiodothyronine have been shown to accompany the much larger amounts of the iodotyrosines that are formed by the iodination of the scleroproteins of gorgonids (ROCHE *et al.*, 1959). Clearly there is much material here for further investigation, so that it would seem more profitable now to pass from this rather specialized survey of experimental results to the more general field of zoological interpretation with which I began.

5. — The problem of the origin of thyroidal biosynthesis.

I must at once admit that endocrinological data relating to the origin of vertebrates are difficult to interpret with any assurance. This is partly because we have too little information and partly because we are so uncertain of the precise evolutionary relationships of the several groups from which that information has to be obtained. As a basis for argument, however, I am going to assume, following Garstang, Berrill, and others, that amphioxus and the vertebrates arose by neoteny from tunicate larvae, and I shall further assume, following Whitear, that the urochordates concerned were generalized and primitive protoascidians, probably not far removed from pterobranchs. This, of course, is only one of several possible interpretations of vertebrate origins, but, as I shall now show, it has the merit of fitting well with the facts that I have reviewed.

Chordates began as ciliary feeders, and I have already suggested that this may have been a major factor in influencing the origin of the pituitary gland. The hemichordates and protochordates show us, however, two important stages in the evolution of ciliary feeding mechanisms. In the first of these stages, the collection of food is mainly external, by means of ciliated tentacles (as in the Pterobranchia) or by a ciliated proboscis (as in the Enteropneusta). Our studies of the enteropneust *Saccoglossus* have shown that some iodine becomes bound to the external secretions of its epidermis, but, contrary to one recent suggestion (Thomas, 1962), this is certainly not a thyroidal process. It leads only to the formation of monoiodotyrosine (Barrington and Thorpe, 1963), and it is doubtful even whether it is an intracellular process at all; our evidence suggests that it may be simply a consequence of the taking up of iodine into the secretion after it has been discharged from the epidermal cells, but in any case it is clear that at this stage we are a very long way from the establishment of thyroidal biosynthesis.

In the second stage of the evolution of these ciliary feeding mechanisms, the collection of food has become internal, by means of the ciliated pharynx, and we find that this stage marks a definite advance towards the evolution of a thyroid gland. The pharyngeal epithelium now shows some capacity for binding iodine, and this leads, as we have seen, to the elaboration of more complex iodinated products than does the external binding in *Saccoglossus*.

In amphioxus this pharyngeal binding capacity is localized in a limited region of the endostyle, and probably yields thyroxine and triiodothyronine (see above). This, together with the evidence of resemblance between the coelomic relationships of the preoral pit and of the adenohypophysis, suggests that amphioxus and the vertebrates are very closely related, sharing amongst other things, a common and highly specialized property of their pharyngeal epithelium.

I must add, however, that we cannot feel certain that thyroidal biosynthesis evolved only once in the chordate line. It may well have done so, and it is attractively simple, and plausible, to assume that it was first fully established in the common ancestors of amphioxus and the vertebrates. Yet we cannot overlook the deepseated unity which the biochemist and the molecular biologist are now demonstrating beneath the diversity of living organisms, a unity that is expressed in common patterns of biochemical pathways and molecular structure. Because of this we must be prepared to concede greater possibilities of parallel and convergent evolution than would have seemed acceptable to earlier workers. Sex steroid hormones, identical with those of vertebrates, are present in echinoderms (Botticelli *et al.*, 1961), but we cannot take this as evidence for a close affinity between the two groups. The fact is that identical compounds occur in *Pecten* and in lobsters (Lisk, 1961), and also in plants, the result we must suppose, of the widespread capacity of living organisms for metabolizing sterols along particular pathways. Clearly, then, the presence of identical iodinated products in two groups of animals cannot in itself be decisive evidence of the common origin of those two groups.

This consideration applies also to the urochordates, but in any case this group needs much further investigation before we can safely generalize about its iodine binding and about the relationship of this to the origin of vertebrates. Present evidence leads me to believe that the iodine binding in the pharynx of these animals has something in common with that found in the pharynx of amphioxus, for our chromatographic studies of extracts of the endostyle of *Ciona* have shown monoiodotyrosine and diiodotyrosine to be present. We have also found traces of what may prove to be thyroxine in the endostyle, although further study is needed before we can regard the presence of this particular substance as being certain. This, however, is not the only available evidence. ROCHE *et al.* (1962) have identified thyroxine in endostylar extracts prepared from *Ciona,* but they were in doubt as to its significance, since, in contrast to our own observations, they were unable to identify either of the iodinated tyrosines. Clearly we need to learn more of the conditions that lead to the appearance of these various compounds. But even so, there is at least a possibility that the functioning of the endostyle of urochordates, litke that of amphioxus, is related in some way to the origin of thyroidal biosynthesis. If this relationship could be confirmed, we might reasonably regard it as a biochemical characteristic supporting the view that the urochordates are closely linked with the ancestry of amphioxus and the vertebrates.

Let me repeat, however, my warning against over-simplification. Modern ascidians are highly specialized and diversified animals, and they cannot possibly be regarded as themselves ancestral to amphioxus and the vertebrates. Moreover, the urochordate tunic is a specialization peculiar to the group, the product of the cladogenetic tendencies to which I earlier referred, and it is not yet clear how its iodine-binding properties are related to that of the pharyngeal epithelium. It would be a great mistake, therefore, to assume at this stage that the iodine binding that we find in the surviving protochordates is an expression of simple anagenetic progress towards the establishment of the thyroid gland. It is unfortunate, no doubt, that the cladogenetic tendencies of evolution have left us at the present day with only highly specialized hemichordates and protochordates from which to deduce the paths along which vertebrate organization emerged. Because of this, however, we must await much further information about the biochemistry and physiology of these interesting animals before we can confidently generalize about them.

Mr. President, I warned when I began that my conclusions would be cautious. I hope, however, that I have said enough to show that such deductions as we can make regarding the history of the vertebrate endocrine system are fully reconcilable with current views of the origin of the vertebrates, and that they can give added weight and interest to those views. The voice with which I have spoken to you, and the ears with which you have listened, are derivatives of a perforated pharynx which was established in the remote past as a component of the ciliary feeding mechanism of our protochordate ancestors. We can, I believe, feel confident that some parts at least of our endocrine system have a no less ancient lineage, and are, indeed, products of those same feeding habits.

Résumé.

Il semble raisonnable de chercher chez les Procordés au moins quelques indications sur l'origine du système endocrinien des vertébrés. Mais le problème n'est point simple, parce que les Procordés sont très spécialisés, et qu'ils sont bien adaptés à leurs modes de vie caractéristiques. Il est peu prudent, dès lors, de s'attendre à ce que ces animaux évoluent suivant une ligne continue que nous puissions considérer comme un progrès évolutif (l'anagénèse), conduisant vers la création du système endocrinien. Nous devons nous attendre à rencontrer aussi, dans une certaine mesure, la spécialisation et la diversification (cladogénèse).

Vu la différenciation de l'hypophyse chez la Lamproie et sa larve, il semble probable que les Procordés possèdent quelque précurseur de cette glande. En effet, les Hémicordés et les Procordés présentent, près de la bouche, une invagination d'aspect glandulaire. Chacun des trois organes (l'organe préoral ciliaire des Hémicordés, la glande neurale des Urocordés, et la fossette préorale de l'Amphioxus) est spécialisé par rapport à un mode de vie particulier, mais on peut suggérer que l'adénohypophyse ait pu évoluer à partir d'un tel organe chez les Procordés anciens.

En ce qui concerne la thyroïde, il se peut que l'histoire évolutionnaire de cet organe, comme celle de l'hypophyse, ait été déterminée par les mécanismes alimentaires des Procordés. Chez les Ascidies, et chez l'Amphioxus, on trouve de l'iode lié dans une région localisée de l'épithélium endostylaire, et aussi, peut-être, dans d'autres parties de l'épithélium pharyngien chez les Ascidies. Chez les Procordés, on a identifié la thyroxine et la triiodothyronine; il es possible, alors, que la thyroïde a tiré son origine de l'activité sécrétrice du pharynx des Cordés primitives.

En outre, il y a de l'iode lié dans la tunique des Ascidies, mais on ne peut pas préciser actuellement la signification de sa présence dans ce tissu si spécialisé. Or, il est probable que chez Dendrodoa *cette liaison est un phénomène semblable à ce qu'on trouve à propos des protéines structurales des exosquelettes des Invertébrés. Il est difficile de déterminer dès maintenant les relations précises entre l'origine de la thyroïde et les processus de l'iodination chez les Urocordés; on doit attendre les résultats d'investigations ultérieures avant de discuter pertinemment ce problème.*

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