The Anatomical Organization of the Nervous System of Enteropneusta.

By

Theodore Holmes Bullock,
Department of Zoology, University of California,
and
The Section of Neuro-Anatomy, Yale University School of Medicine.

With Plates 2–8, and 1 Text-figure.

CONTENTS.

INTRODUCTION  55
MATERIALS AND METHODS  58
NEURO-ANATOMICAL DESCRIPTION  58
  Non-nervous Elements  59
  Fibrous Matter of the Nervous System  62
  Nerve-cell Bodies  66
  Regional Description  70
    Proboscis  70
    Peduncle  72
    Collar  74
    Trunk  82
  Nerve-cords  83
  Epithelium of the Gut  87
  The Internal Nervous System  87
DISCUSSION  89
  Anatomical Concepts  89
  Functional Correlations  94
  Phylogenetic Speculations  96
SUMMARY  104
LITERATURE CITED  106
EXPLANATION OF PLATES  108

INTRODUCTION.

The zoological interest attached to the Enteropneusta stems from the suggestion made by Bateson in 1885 that these worm-like creatures are actually chordate and that they occupy a strategic position as the most primitive of the invertebrate relatives of the vertebrates. In spite of an active interest in
their morphology, the animals remain far from well known. They are commonly designated balanoglossids after the earliest well-known genus, and comprise a highly homogeneous group of five to six dozen species divided into twelve genera and three families.

Our knowledge of the nervous system has grown up largely through a series of taxonomic and morphologic studies. The nervous system was first identified by Spengel in 1877. Kowalevsky in 1886 had designated a ‘Kopfganglion’ in the base of the proboscis, but his structure turned out to be the proboscis complex, including ‘notochord’, heart, cardiac vesicle, and glomerulus. The accounts published by Spengel in 1877 and in his monograph of the group (1893 and preliminary communication, 1884) stand to-day, almost without modification or addition, as the sum of our knowledge of this system. Spengel recognized a thin layer of ‘Punktsubstanz’ at the base of the ‘Epidermis’ everywhere over the body. (The integument is a pseudo-stratified, columnar, ciliated epithelium immediately underlain by the body muscles.) The basi-epithelial plexus he considered to consist of some kind of sensory cell and some kind of associative cell and nerve-fibres. Thickenings of this layer in certain regions he described as follows. A mid-dorsal and a mid-ventral longitudinal cord in the trunk region are joined by a circular connective at the junction of this region and the collar. The ventral cord ends at this level but the dorsal cord is continued anteriorly as the collar nerve-cord, which leaves the surface and plunges into the coelom of the collar (Pl. 2) to traverse it as a solid or a hollow strand (the ‘Kragenmark’). If it is solid, there are usually to be found small, unconnected cavities (‘Markhöhle’) scattered through it. Some species were described as having unpaired mid-dorsal extensions of the collar cord by which it communicates with the overlying epidermis. Giant nerve-cells were found in this cord. It returns to the surface at the anterior end of the collar and becomes continuous with the thin nerve layer of the outer epithelium of the collar and the deep layer on the dorsal side of the proboscis stalk. At the anterior end of this stalk or peduncle, where it is continued into the proboscis, the thickened nerve
layer extends down the sides and even ventrally, constituting the so-called anterior nerve ring. This latter in turn is continuous with the general plexus under the proboscis epithelium.

Bateson (1884, 1885, 1886a, 1886b) described the development of some parts of the nervous system and submitted classical phylogenetic speculations. Koehler (1886) and a long series of authors after him described new species and showed in what points variation was to be found within the group. Schneider (1902), Hilton (1919), Clayton (1932), and de Renyi (1934) each added a few histologic details, but most of the observations in the last forty years have been in the nature of gross morphology, both because these are the important taxonomic and phylogenetic aspects and because finer details were not demonstrable. The first study of the nervous system in life was made by Crozier (1915, 1917), who reported a few observations on chemical, mechanical, and photic irritability and phosphorescence. Hess (1931, 1936, 1937, 1938) exploited the photic reaction in a series of experiments on whole animals and fragments of various constitution and applied specific nerve stains for the first time to this group. Cary (1933) successfully cultured tissues of one species in vitro; he mentions nerve-cells as the first cell-type to show activity, elongating and sending out processes in the same manner as do those of warm-blooded animals. Bullock (1940) offered an analysis of the physiologic organization of the nervous system based on simple experiments and considerations of natural history. An anatomical study of the giant fibre system, made as an outgrowth of the present investigation, has been published (Bullock, 1944). The admirable monograph recently completed by van der Horst (1927–39) brings together all available information on these organisms and is of great aid in the study of any phase of balanoglossids.

The present investigation was undertaken because of the conspicuous lack of understanding of the balanoglossid nervous system from a neurologic standpoint. Virtually nothing is known of the system except the gross anatomic facts outlined above. The motivating interest in this investigation has been in the neurologic pattern or organization, the nerve-cell
types and their relations and the general level of complexity achieved.

It is a pleasure to acknowledge my debt to Professor S. F. Light, under whose guidance the work was pursued, for his generous and stimulating interest in every ramification of the problem. A number of individuals and institutions have kindly made loans or gifts of material used in this study. To these, as well as to those who have aided me in numerous ways in the collection of living enteropneusts, I am greatly indebted. I wish particularly to mention the generous assistance of Mr. J. E. Gullberg, who gave persistent and expert aid in all microscopical, mechanical, and photographic problems.

The work was done for the most part at the University of California, but further observations were made at Yale University where much material assistance was received from the staff of the Section of Neuro-Anatomy.

**Materials and Methods.**

An extensive series of species, representing nine out of twelve known genera, plus one manuscript genus, has been studied. A variety of general and special technics has been employed. These are described elsewhere (Bullock, 1944). The descriptions to follow are chiefly based on Saccoglossus pusillus (Ritter), prepared (1) cytologically, (2) with protargol impregnation, and (3) with intra vitam methylene blue.

**Neuro-anatomical Description.**

The nervous system in Enteropneusta occupies its primitive position within the superficial epithelium of the body. The epithelium of balanoglossids is extraordinarily highly developed. It is typically of a complex pseudo-stratified type. The cell outlines are vague, but the crowded nuclei indicate a densely packed tissue. Although the epithelium in various regions and species is highly diversified, the aspects shown in Text-fig. 1 and Pl. 6 are more or less typical. It will be seen that the 'Punktsubstanz' or nerve-fibre layer is localized at the base of the epithelium but that the nerve-cell bodies are not separated from non-nervous cells. A brief consideration of certain non-
nervous elements is necessary, therefore, before describing the nerve-fibre layer, nerve-cell bodies, and local differentiations.

**Non-nervous Elements.**

The most conspicuous formed elements in the epithelium are certain heavy fibres, oriented uniformly at right angles to the plane of the epithelium and attached at their innermost ends to the limiting membrane. The staining affinities of these fibres are, with almost all methods, diametrically opposed to those of nerve-fibres. They appear to be derived from the inner ends of the ordinary ciliated epithelial cells. This is well seen in teased or macerated preparations and in certain methylene blue stains (figs. 26, 27, Pl. 8). The degree of development of these epithelial cell feet varies from the extreme of simplicity, where the
epithelium is a low, simple, columnar layer and the broad basal ends of the ciliated cells rest directly on the limiting membrane, to a level of complexity represented by long, discrete, formed fibres traversing the nerve-fibre layer (fig. 10, Pl. 4) in regions where the epithelium is deep and crowded. The significance of these fibres is probably that of a supporting tissue. Similar radial supporting fibres are present in the deeper epithelia of phoronids and echinoderms and some other groups of invertebrates.

In certain regions of some species I have seen what appears to be direct continuity between these fibres and the intracytoplasmic fibres formed by the fusion of the ciliary rootlets.

In these structures, if anywhere, is represented the neuroglia of enteropneusts. If neuroglia may be defined as any differentiated supporting elements in the nervous tissue, then the term may be applied to these radial 'Stützfasern', different as they are from the usual invertebrate glia. Moreover, we have here the most primitive form of neuroglia to be found anywhere, for the cells which produce the supporting fibres are at the same time fulfilling the functions of ordinary ciliated epithelial cells. The same animal, indeed, presents all transitions to a condition so simple that structures differentiated for support are lacking and the term neuroglia is inapplicable. Even in the regions of highest elaboration the radial fibres do not achieve the complexity I have observed in the epithelia of phoronids and echinoderms.

The variations in elaboration of radial fibres between regions are correlated closely with variations in the development of the nerve-fibre layer. Exemplifying this correlation and indicating the highest level attained by the radial fibres in these organisms, is the condition found in the nerve-cords. Here the supporting fibres show a tendency to alinement. Rows of them, oriented in a direction corresponding to that of the nerve-fibres, cut the punctate substance into fascicles. However, the rows are loosely formed, uneven, and rarely extend as much as 75 μ, so that the fascicles are defined for only a short distance before splitting or anastomosing. This alinement is correlated not with bulk of nervous tissue but with unidirectional orientation
of nerve-fibres and thus indicates regions which are more in
the nature of conduction tracts and less in the nature of
neuropiles.

There occurs in the epithelium of certain species or genera
(in Balanoglossus and Ptychodera, but not in Saccoglossus and most others) a layer of fibres parallel and immedi-
ately peripheral to the nerve-fibre layer (fig. 17, Pl. 6),
which we may call the horizontal fibre layer. It was regarded
by Spengel (1903) and van der Horst (1924) as a cellular layer,
the latter author suggesting in 1927 (1927-39) that it may be
a layer of nerve-cells. The evidence from the present studies
indicates a non-nervous character and, moreover, a probable
common origin with the radial fibres. It is not regularly corre-
lated with any feature of the nervous system, and must be left
for the present purposes as a peculiar local specialization of the
supporting tissue of the epithelium of some forms.

Of more importance here is another supporting structure, the
limiting membrane. This is a continuous sheet underlying the
superficial epithelium throughout the body, forming the mesen-
terries and blood-vessels, extending into the linings of the gut
and gills, and locally thickened to form the skeletal masses of
the proboscis, collar, and gills. Previous study of this structure
has been centred around the question of its origin. Its impor-
tance in the present connexion lies in its conspicuous, sharp,
and ubiquitous separation of the nervous system from the
muscular, a separation which on physiologic grounds cannot be
absolute.

The limiting membrane is poorly seen in ordinary double
stains, but stands out brilliantly in suitable triple stains
(Mallory's, Masson's). It appears (Text-fig. 1) in the best fixed
and stained preparations as an uninterrupted, homogenous
membrane from one-half to several micra thick, consisting of
two distinct lamellae but otherwise structureless. It may be
folded or creased, and in oblique sections may appear discon-
tinuous (fig. 16, Pl. 6) owing to the conspicuous creases and
dge-views and very inconspicuous flat-view. Many prepara-
tions will present oblique sections in which no sharp boundary
between epithelium and muscle can be made out. But in general
it appears from careful observation of the best preparations that
the limiting membrane is always present, continuous, and un-
interrupted. Any structures communicating between epithelium
and underlying tissues must pierce the membrane. But by all
ordinary (critical) methods nothing can be seen to penetrate
it (Pis. 3, 4, 5). The best cytological preparations have been
repeatedly searched and no structure or fibre has been seen
definitely to cross this boundary. Of course, it is not to be
expected that single nerve-fibres would be seen in cytological
stains, but the significant result of these observations—with
stains which show the membrane well, whereas selective nerve
stains do not—is that whatever does cross it must be of the
order of single nerve-fibres. No ‘nerves’ or even small bundles
have been found to pierce it.

Fibrous Matter of the Nervous System.
The stratum of punctate tissue at the base of the epithelium,
shown in all the figures, represents the most conspicuous, in-
deed, the only obvious constituent of the nervous system of
balanoglossids. It is found in all but the thinnest regions of the
superficial epithelium and the more specialized levels of the
gut, and masses of nerve-fibres are found only in the epithelia.
No internal sub-epithelial masses or cords are definitely
known. The punctate tissue consists of fine, horizontally
oriented nerve-fibres and the heavier radially arranged support-
ing fibres already described. Its importance is twofold. In it
are developed the great nerve-cords, indeed, all nervous com-
unication between parts, adjacent and distant; and in it are
located, in all probability, the synapses or whatever form of
physiological continuity between neurons exists in this group.

As is seen in the photographs, the layer is rather sharply
defined on its outer as well as its inner surface. It is charac-
terized, with limited exceptions, by the lack of differentiations
within its thickness; there is no stratification; fibres passing in
one direction are not segregated from those passing otherwise.
It is significantly simpler in organization than the neuropile of
most other invertebrates.

The intimate structure of the nerve-fibres themselves (Pis. 7,
NERVOUS SYSTEM OF ENTEROPNEUSTA

has been best observed in preparations stained with methylene blue (intra vitam), protargol, and osmic ‘acid’ by the Mann-Kopsch Golgi body method. They are thin and rather uniform in diameter; at least they lack marked irregularities. The thorny projections and warty excrescences of some nerve-cell processes are absent. The degree of sinuosity is characteristically gentle, not acutely angular. In all these respects, all nerve-fibres of enteropneusts resemble in general the axon of vertebrate neurons as opposed to the dendrites, a resemblance shared by the nerve-fibres of coelenterates, platyhelminths, and other lower invertebrates. I have not made out neurofibrils except in the giant fibres (Bullock, 1944) and in the cell-bodies of methylene blue preparations (Pl. 7). De Renyi (1934), in a preliminary note, reports the observation of neurofibrils in living cells of Ptychodera by micro-dissection; and Hess (1937) figures them in the giant cells of Saccoglossus. All nerve-fibres, except the giant fibres, appear to be alike. No differentiations of axon and dendrite, of ‘Zellenfortsatz’, ‘Markfortsatz’, and ‘Nervenfortsatz’ (of many invertebrate unipolar neurons) have taken place. Nor have boutons terminaux or other specialized endings been seen.

Branching occurs, and in this respect also the enteropneust fibres suggest the axon of heteropolar neurons. The branching is in general dichotomous and relatively infrequent. The branches are, like collaterals, equivalent in size to the main fibre, if indeed either one can be distinguished as the main fibre. Hilton (1919) apparently had a different conception, for he considered the fibre matter of enteropneusts to consist mostly of short lateral twigs of the main processes. He further regards this condition as more highly developed than that which he found in echinoderms, whose fibre matter consists mostly of ‘strands from cells’, but it is not clear how he was able to make out any of these details, not having used special stains. Our knowledge of the echinoderm nerve elements is as meagre as for the present group, and no one has described the fibrous matter from selective nerve stains. But my own examinations of cytologically prepared material of Leptosynapta, Piasaster, and other genera have revealed no less complexity.
and, in fact, greater heterogeneity of fibre structure and arrangement than in balanoglossids.

No indications whatever have been found of sheaths around the nerve-fibres.

There now arises the question of the nature of the anatomical relations between neurons. Is the nervous system of balanoglossids synaptic or asynaptic, or a mixture of these conditions? Unfortunately I am unable to give a satisfactory answer. Good methylene blue stains are the most reliable preparations for this purpose, but my results with this stain on Saccoglossus pusillus and Saccoglossus kowalevskii have never been complete enough to justify an exclusive generalization. Such stains as were obtained never showed a nerve-fibre which could be traced to a neuron in both directions (i.e. an anatomically continuous nerve-net), but did show many long and delicate fibres passing, apparently independently, among each other, through the plexus. These pictures, per se, would not seem to be more than suggestive. But their resemblance to such figures as those of Bozler (1927), on which Hanström (1928) places the greatest weight as demonstrating synaptic relations in coelenterates, and their complete divergence from figures offered by proponents of the theory of continuity in the nerve-net (listed and reviewed in Hanström and in Bozler), lend some support to an interpretation of these observations on enteropneusts in favour of the synaptic theory.

Sections of protargol material, cut parallel to the plane of the nerve-fibre layer, show that plexus beautifully, although thin sections impose limitations on the tracing of fibres and on the interpretation of apparently free nerve endings. Here, again, after careful search, I am unable to rule out the possibility that anastomoses of fibres occur, but on the other hand, neither was it possible anywhere to demonstrate a case of anastomosis, as opposed to mere contact or crossing of fibres. The fibres could often be followed for long distances, and the rich plexus studied fibre by fibre, the general impression always remaining that any anatomical continuity that did exist must be very rare. The only opinion to be found in the literature is that expressed by Hess (1937), who believes that no true nerve-net but ... a
plexus of ... neurons ...' is involved in Saccoglossus kowalevskii. Neither interpretation of the structure has been demonstrated. At present, however, the evidence seems to lean towards the assumption of a discontinuous, synaptic condition.

It is of some interest to determine where in the nervous system synapses or other forms of physiologic connexion between neurons occur. In successively higher groups of animals a striking process of concentration of synaptic fields is to be noted. In the lowest groups possessing nervous systems the connexions between neurons are scattered throughout the system; but early, beginning with the lowest worms, these areas of interneuronal junctions are restricted to certain parts of the system. The indications for balanoglossids are that all or nearly all neuron-to-neuron communications occur in the nerve-fibre layer, and that such junctions are unlocalized within this layer but are scattered everywhere over the body. This conclusion is strengthened by the evidence, described in the next section, of wide distribution of nerve-cell bodies, and even more by the physiologic evidence of local reflex arcs and neuromuscular autonomy of small pieces (Bullock, 1940).

The problem of communication across the limiting membrane between epithelial nervous system and sub-epithelial muscles has already been introduced by the statement that nothing can be seen to cross this boundary in critical preparations by ordinary methods. Remarkably enough, even in successful protargol stains, such as clearly show individual nerve-fibres, nothing can be traced from the epithelium into the muscles. Again, great care in the use of sections and adequate optical resolution is necessary, for many confusing and uncertain appearances are encountered. But while it is true that the general integrity of the limiting membrane is emphasized in these preparations, there have been encountered many situations where fibres of the nerve-fibre layer turn inwards and apparently penetrate the limiting membrane, though they have not been followed definitely into the muscles in any case. These pictures are the only visible evidence of crossing I have found and they are unsatisfactory. Although the problem of the shallow focal plane is here acute, photographs of such situations
are reproduced in figs. 20 and 21, Pl. 6, as the only objective anatomical evidence offered, here or heretofore, for the existence of a motor-nerve supply. It is interesting to recall that in no group possessing a superficial nervous system and no internal ganglia—for example, coelenterates, echinoderms, phoronids—have the motor connexions been established. The negative statement that nowhere in the body of the balanoglossid is there to be found a localized inflow of nerve-fibres from the epithelium to the muscles is more easily established than the positive suggestion given above. These two arguments, however, converge towards the presumption that the motor-nerve supply of enteropneusts is a generalized, diffuse, fibre-by-fibre crossing of the limiting membrane, a condition that would account for the degree of local reflex autonomy found experimentally (Bullock, 1940).

Nerve-cell Bodies.

By ordinary staining methods nerve-cell bodies are not with certainty distinguishable from other cells of the epithelium among which they are scattered. Only the giant cells and the larger ganglion cells of the collar cord are recognizable in such preparations. But in any section adequately preserved to show the infraciliature, glands, and nuclei, strongly presumptive evidence of nerve-cells is easily obtained.

First, it must be said that extended examination has indicated the probability that three cell-types, nerve-cells, gland-cells, and ciliated cells account for the entire epithelium. No other cell-type has been recognized in the animals studied and none is reported in the literature. But one possibility has been suggested from the histology of certain other invertebrate groups, namely, that undifferentiated reserve cells may be present throughout the epithelium. A special search for these was in vain. Further evidence that there are few, if any, such cells is given on another page. Second, it seems safe to assume, from study of the simpler epithelia and maceration preparations, that all the cell-types are normally uninucleate.

Now, we may obtain the total number of cells in any portion

---

1 A recent paper by J. E. Smith (1945) promises a description of these elements in asteroids in a forthcoming issue of the Philosophical Transactions.
of the epithelium by counting nuclei. The total number of ordinary epithelial cells may then be obtained by counting the conspicuous converging bundles of ciliary rootlet fibrils. Likewise, we may count the gland-cells. True, there is the possibility of error in this count, due to the presence of collapsed glands; however, several considerations render it likely that the error is not large. Thus, individuals, treated in various ways designed to encourage secretion of mucus just before fixation are not greatly different in respect to numbers of gland-cells visible from specimens fixed by rapid freezing in liquid air. The existence of many empty but uncollapsed glands, and the ease with which the nuclei that belong to the commonest type of gland (small, peripheral goblets) may be recognized, likewise argue that this error is not large. If the number of ordinary epithelial cells plus the number of gland-cells be subtracted from the total number of nuclei, the remainder should represent the number of nerve-cells in that portion of epithelium. Such counts have proved more than instructive and little short of astounding.

Thus, an extent of lateral trunk epithelium, bounded by deep grooves, in an 8 μ section measured 81 μ in length, contained 167 nuclei, 50 rootlet bundles, and 20 glands. No significant estimate of the error in gland-cell count is possible, but even if it is 100 per cent. or more the small figure involved does not change the general result materially. There remain a number of nuclei—65 to 120—exceeding that accounted for by any other cell-type, which would seem to belong to nerve-cells. A portion of proboscis epithelium in the same section, 74 μ in length, not encroached upon by grooves, contained 284 (±5) nuclei, 50 (±20) rootlet bundles, and 12 gland-cells. About two-thirds of all the cells in this area of the proboscis epithelium apparently are nerve-cells.

These counts vary in a highly significant manner from one part of the body to another. The specific results will be mentioned below in the regional survey. But some generalizations may be made here. (1) The number of ciliated epithelial cells per unit of surface area is approximately the same everywhere on the body. (2) The number of gland-cells varies greatly but
is never more than a small proportion of the total number of cells. (The situation in the collar epithelium may form an exception; here the glands cannot be counted owing to their richness and indefinite outlines, but I believe their nuclei number fewer than those of the ciliated cells.) Thus, a nearly constant figure represents the number of non-nervous cells per unit of surface area anywhere in the epithelium. The difference between this figure and the total nuclear count varies greatly—from zero to three or four times the 'constant'. (3) This variation corresponds closely to the thickness of the nerve-fibre layer, to the threshold of irritability in life, and to the abundance of one of the two chief types of nuclei. This is the chromatin-rich cigar-shaped form as opposed to the round or oval lighter-staining nuclei characteristic of the ciliated cells. Thus, in the sensitive epithelia of the proboscis, the difference between ciliated plus gland-cell count, and total nuclear count is great, the nerve-fibre layer is thick (the epithelium over the nerve-cords forms a special case), and dark cigar-shaped nuclei are numerous. In the abdominal areas between glandular islands few or no gland-cells are present, the nuclear count is so close to the ciliary rootlet-bundle count as to be within the probable error of that figure, no nerve-fibre layer is visible, and no cigar-shaped nuclei occur. These correlations would hardly be expected of undifferentiated reserve epithelial cells and constitute, therefore, presumptive evidence that no considerable number of such cells exist. The hypothesis may then be suggested that these great variations in cell population of the enteropneust epithelium are due primarily to variations in the abundance of a single cell-type, and that this is probably a sensory nerve-cell.

Turning now to special nerve stains, the neurons most consistently demonstrable in both methylene blue and protargol preparations are just such elements—primary sense-cells (figs. 18, 19, Pl. 7; figs. 22, 23, 25, Pl. 8). These are elongate, upright cells, that is, perpendicular to the plane of the epithelium. One process, a typical nerve-fibre, extends from the inner end of the cell into the basi-epithelial plexus. The outer end of the cell-body is much constricted and tapers until, near its termination just below the surface, it often exhibits a slight
swelling. A bristle-like projection is sometimes apparent, often not. The nucleus is well shown in protargol stained sections and is usually of the elongate, cigar-shaped type which is most abundant in the zone just peripheral to the deep-lying round and oval nuclei. Although slight individual variations are common, I was unable to subdivide these cells into morphological types. There seems to be but one kind of sensory cell in the balanoglossid. The primary sense-cells are much the most numerous neurons encountered (absolute figures on their abundance are not possible with these methods). They account for the majority of fibres making up the basi-epithelial plexus.

The other nerve-cell types found in the epithelium may collectively be termed ganglion cells, although no true ganglion exists in the Enteropneusta. They are all lodged in that zone of the epithelium immediately peripheral to the nerve-fibre layer (or the horizontal fibre layer when present), that is, they are not scattered in the plexus but are sharply segregated from it. Occasional exceptions are found, particularly in the peduncle of large species, where a significant number of nuclei may be seen within the thick nerve-fibre layer. But this is rare elsewhere. At present no distinction can be made between intermediate and motor neurons; both are probably present in the epithelium, side by side. Ganglion cells of bipolar, multipolar, and unipolar varieties occur, but the last named are confined to the collar nerve-cord and adjacent levels of other nerve-cords. Bipolars are the most common (figs. 28, 29, 30, Pl. 8) and for the most part are rather large (15 to 20 μ long) with round or short oval nuclei and processes arising abruptly from two sides of the cell. A few smaller spindle-like cells have been seen (under 10 μ). No differences were noted between the two poles of the cell. In the cell-bodies of some individuals in methylene blue preparations, clearly defined sinuous threads were visible which suggest neurofibrillae. The typical orientation of bipolar neurons is parallel to the nerve-fibre layer.

In the abdomen, where the best methylene blue stains were obtained, a very few multipolar neurons were found (fig. 31, Pl. 8). These were simple isopolar cells with large bodies, processes leaving each of the corners, and delicate fibrillae
filling the cell except for a circular space in the centre, which
doubtless accommodates the nucleus.

Unipolar neurons have not been identified with any certainty
in the general epithelium but occur clearly in the nerve-cord
of the collar. No methylene blue pictures of them have been
obtained, and only fragmentary pictures of the cell-body with
protargol. But they are often relatively large and can be found
in any cytological preparation. These cells vary in outline but
usually have a rounded end on the side nearest the surface of
the epithelium and a single process emerging from the opposite
side. They have a fairly large proportion of cytoplasm and a
round or short oval nucleus of average size, i.e. 3–5 \( \mu \) in diameter,
or larger, even reaching 7 \( \mu \) to intergrade with the giant cells.

The remarkable giant cells and fibres have been described
elsewhere (Bullock, 1944).

Regional Description.

Proboscis (figs. 4, 5, Pl. 3; fig. 11, Pl. 4; fig. 17, Pl. 6).—
Two general features characterize the nervous system of the
proboscis. It is comparatively well developed and is compara-
tively uniform over the entire organ. The nerve-fibre layer is
here thicker than anywhere else in the body, excepting the
nerve-cords. Its uniform thickness, anterior to the peduncle
and base of the proboscis, is striking. No local specializations,
dorso-ventral differential, or thickened spot at the active
anterior tip can be demonstrated. In some species an exception
of questionable significance is formed by the occurrence of a
mid-dorsal thickening. Assheton (1908) noted such a thickening
in \textit{Saccoglossus serpentinus}, also Hinrichs and Jacobi
(1938) in \textit{Saccoglossus pygmaeus}, and van der Horst
(1930) in \textit{Saccoglossus otagoensis} and \textit{Glosso-
balanus ruficollis}, the last-named species having also a
mid-ventral thickening. A mid-dorsal differentiation is com-
monly present in \textit{Saccoglossus pusillus}, but not in my
specimens of \textit{Saccoglossus kowalevskii}. In the other
species I have examined only occasional suggestions of a cord
are encountered. They either extend through a few sections only
or are not definite enough to merit recognition. It may be said
therefore, that there are essentially no nerve-cords in the proboscis in most enteropneusts.

Whereas the fibre layer of the proboscis epithelium of any individual—as represented by sections—has a rather definite thickness, that measurement varies considerably between specimens of the same species. This is noteworthy, in connexion with the common practice of including in taxonomic descriptions a figure for the depth of the fibre layer and another for the depth of the entire epithelium, or a ratio of the two. I find that in the same species these two measurements may vary apparently independently. Such ratios have little or no significance therefore. Doubtless, the state of the animal at the time of fixation and other technical factors are largely responsible for the apparent variation. It is true that the measurements of the nerve-fibre layer vary less than do those of the epithelium as a whole; still, the variation is almost as much within as between species.

If the hypothesis advanced in the previous section is correct, that the excess of nuclei over ciliated cells and gland-cells represents the number of nerve-cells in the epithelium of the balanoglossid, then for certain preparations of *Saccoglossus pusillus* the nerve-cells number approximately 37 per 100 square micra of surface. This figure means little as an absolute quantity, depending as it does on the state of the glands at the time of fixation, the contraction of muscles, and so on, but it is significant relative to the figures given below for other regions which were obtained in the same preparations. (These counts were made on stretches of epithelium 70–90 μ in length in 8 μ sections, thus involving several hundred square micra of surface and several hundred nuclei, the latter counted from camera lucida drawings.)

The directions taken by the fibres in the nerve-fibre layer are, in all parts of the proboscis except the dorsal cord of certain species, not restricted. Antero-posterior, circular, and oblique courses are equally demonstrable. I am not able to say whether there is a significant preponderance of fibres passing in one direction, but I find no segregation, for example, of outer longitudinal and inner circular elements. We have here, then,
the conditions for a true nervous plexus, and the anatomical basis for the diffuse conduction found in life. But this does not imply necessarily a network of anastomosing fibres, a feature frequently identified with, but not at all necessary to, a plexus.

If differentiated sense organs, as opposed to scattered sensory cells, were to be found in balanoglossids, they would logically be expected in the proboscis. It may be appropriate, therefore, to state at this point that I have found no structures which could be identified as sense organs, either in the proboscis or anywhere else in the body, in any of the species I have had opportunity to examine.

Peduncle.—The peduncle or stalk of the proboscis (fig. 1, Pl. 2; figs. 4, 5, Pl. 3; fig. 10, Pl. 4) depends for its existence on the state of extension of the specimen; it is never easily delimited. For the present purposes the term is applied to the region beginning, anteriorly, with the first significant modification of the general condition of the nervous system of the proboscis and ending at the commencement of the collar cord. The most anterior modifications are a gradual increase in thickness of the fibre layer, but not of the rest of the epithelium; an increase in the number of primary sense-cells at the expense of gland-cells; and a regimentation of the fibres in respect to orientation. Over the base of the proboscis the thickening of the nerve-fibre layer is essentially equivalent on all sides. Here the fibres tend to be oriented circularly, forming the ‘anterior nerve ring’ of some earlier authors. But as the constricted portion of the stalk is reached, an inequality is developed. Thus, the dorsal and lateral sides of the peduncle show the nerve-fibre layer at its greatest depth in the entire enteropneust body, reaching $50 \mu$ in Saccoglossus pusillus and $150 \mu$ in Balanoglossus occidentalis, whereas the ventral epithelium has a very thin fibre layer (varying in thickness according to species and at least partly dependent on the development of the ventral keel of the skeleton). In this constricted portion of the peduncle the nerve-fibres are aligned antero-posteriorly, which together with the fact that the great majority of fibres are patently not the processes of local cell-bodies but of distant ones distinguishes this region as a rather
advanced form of conducting tract. I am unable to demonstrate whether it is merely a conduction path or whether it acts also in the capacity of a 'centre' or ganglion; but reasoning from the strikingly parallel orientation of the fibres, and the lack of neuropile-like plexus, I prefer, tentatively, to regard the peduncle as essentially conducting in function, funnelling fibres from the proboscis into the collar cord.

Other epithelia of the peduncle are continuous with this highly nervous region. It is of interest, therefore, to search for nerve tissue in these structures. Interrupting the peduncular fibre tract in the region of its greatest development is the proboscis pore, which places the coelom of that organ in communication with the outside. The short canal between the coelom and the pore is lined with a low, ciliated epithelium. I have never found a trace of nervous tissue here, though a few micra of continuous epithelium bring us to the greatest concentration of nervous tissue in the body. In certain species of balanoglossids, notably those of the genus Ptychodera, there occurs in the anterior wall of the buccal cavity (the posterior ventral wall of the proboscis) a 'raccoe' or 'blumen-kohlähnliches' organ (fig. 5, Pl. 3). This consists, essentially, of a more or less folded thin outpouching of the ventral coelomic diverticulum of the proboscis. It thus comprises two layers—the epithelial lining of the coelom and the outer body epithelium. The function of this structure is unknown. Of its nervous system I may say that the thick nerve-fibre layer of adjacent epithelia is reduced in the racemose organ to invisibility. Finally, the 'notochord' may be mentioned at this point, although it extends posteriorly into the collar and anteriorly into the proboscis. The highly modified epithelium of this hollow diverticulum is typically continuous with the lining of the buccal cavity. It is ciliated, in large part at least. In three of the species I have examined, a distinct nerve-fibre layer is present in the 'notochord', though in the majority there is none. In Balanoglossus occidentalis the 'nuchal' portion of this organ has a conspicuous plexus of nerve-fibres about 2–3 μ thick, bounded externally by a well-developed plexus of horizontal fibres. Both layers disappear anteriorly
as the ‘notochord’ passes into the proboscis and changes its histologic character. In Ptychodera bahamensis and an undetermined species of Glandiceps, the most posterior levels of the organ, close to its opening into the buccal cavity, display a visible nerve layer, but it diminishes sooner than in the first-named species. Willey (1899) found a similar condition in Balanoglossus biminiensis. No function has been suggested for this nerve supply to the cavity of the ‘notochord’.

Collar.—The superficial nervous system of the collar (figs. 4, 5, Pl. 3; figs. 13, 14, Pl. 5) is very simple. No specialized regions or concentrations have been found; the nerve-cords do not occur here; the orientation of fibres is unrestricted, that is, fibres run in all directions within the plane of their plexus; and the fibre layer does not vary greatly in thickness over the whole surface but is everywhere thin. In Saccoglossus pusillus and other species with a highly modified glandular collar epithelium, the number of nerve-cell nuclei is difficult to ascertain, because the glands cannot here be counted. However, they may be estimated. The total number of nuclei minus the number of ciliated epithelial cells (rooted bundles) is about 21 per 100 square micra of surface. This represents gland-cells and nerve-cells. There are at least a few gland-cells, but probably not more than eight (= the number of ciliated cells in this area). Probably not more than eighteen and not less than thirteen nerve-cells exist in this unit of epithelium, therefore. It is clear that the density of nerve-cells in the collar of Saccoglossus pusillus is significantly less than that in the proboscis, and about equal to that of the trunk.

Other species show considerable variation in the histology of the collar epithelium. In Ptychodera, for example, no great development of glands occurs in the collar, the epithelium being like that of the proboscis and anterior regions of the trunk. Here the significant diminution of nervous tissue characteristic of Saccoglossus does not take place, the thickness of the nerve layer and number of nerve-cells remaining very close to the level found in the proboscis. In Balanoglossus, however, the great differential between proboscis and collar nerve layers does exist, although the epithelia of the
two regions are very similar. Both are highly glandular and exceptionally tall in this form, recalling the collar of Sacco-glossus.

As in the coelomic pore of the proboscis, no visible nervous tissue can be made out in the epithelium of the canals of the collar pores.

The nerve-cord of the collar (fig. 1, Pl. 2; fig. 6, Pl. 3; figs. 12, 13, 14, Pl. 5) presents for examination the only internal mass of nervous tissue in the enteropneust body. This cord is commonly thought of as the central nervous system of the balanoglossid, and is the only candidate for the distinction of being called a ganglion. It exhibits a combination of characters unique among the nervous concentrations of animals and correspondingly difficult to interpret. It is simply a submerged strip of epithelium traversing the dorsal mesentery, and continuous at both ends of the collar with the superficial system in the mid-dorsal line. It neither gives off nor receives nerves (using the term to mean discrete bundles of nerve-fibres). No cellular sheath encapsulates it, but only a limiting membrane precisely like, indeed, continuous with, that bounding all the epithelia of the body. It is dorsal to the digestive tract and in some few species is hollow, which perhaps is its primitive condition. It is neither terminal nor related to important sense organs, but is stretched along the principal nervous pathway of the body. Although it has developed specialized giant cells and many smaller unipolar neurons, its fibre matter resembles a conduction tract more than a neuropile, and its cross-sectional area is far smaller than that of the peduncle and but little greater, sometimes smaller, than that of the dorsal and ventral cords of the trunk combined.

Some further anatomical features may be mentioned briefly. (1) The shape of the cord is highly variable within the same species. It is generally flattened dorso-ventrally, but is apparently subject to pressure from the surrounding musculature and changes shape freely. It, therefore, has no characteristic gross form, as do most internal nerve masses elsewhere. (2) In some species there occur median, unpaired medullary strands connecting the cord with the overlying superficial epithelium.
These so-called dorsal roots or crests will be described in greater detail below. (3) As would be expected from the epithelial character of the cord and its dorsal position, the fibrous portion is ventral and the cellular portion dorsal. But the cord is always more or less rolled up at the edges. Thus it might be expected that fibrous matter would be found on all sides of the cellular portion. This condition is rarely achieved, the fibre layer usually being undeveloped dorsally. (4) The adjacent coelom of the collar is almost empty, dorsal and lateral to the cord, but ventrally the cord is in contact with the perihæmal pockets of the trunk coelom which project anteriorly into the collar and are almost filled with muscle-fibres. Ventro-lateral to these is the often considerable musculature of the wall of the buccal cavity. Since there are no other important effector organs in the vicinity and no special sense organs, these muscles are the only organs likely to be innervated by the collar cord. They are responsible for movements of the collar, but their importance in the life of the balanoglossid is not known. (5) At the anterior and posterior ends of the cord, where it emerges to the surface, there may sometimes be distinguished deeper or shallower invaginations of the outer epithelium. These have usually been called neuropores, especially in species wherein they are continuous with an axial canal, but Spengel (1893) refers to them as ‘vordere und hintere Epidermistasche’ and shows them to be often independent of and dorsal to the nerve-cord itself. Willey (1899) distinguishes a ‘neuropore (sstr)’ and an ‘epidermal pouch’, which two invaginations may coexist in the same individual or may coincide as an ‘anterior neuropore’, in the wider sense. These and later authors have shown that great variation exists between species with respect to this character. My observations emphasize the variability within a species, dependent on the state of contraction and the position of the flaring margins of the collar when the animal was fixed.

A continuous axial canal traverses the collar cord in the genus Ptychodera, many members of the genus Glossobalanus, and in one species of the genus Balanoglossus, all belonging to the family of the Ptychoderae, and in a single species outside this family, Glandiceps malayanus of
the Spengeliidae. As seen in Ptychodera bahamensis (fig. 5, Pl. 3), the lumen (axial canal) is large and unobstructed, widely open to the outside at both ends. Its lining is a ciliated glandular epithelium little or not at all modified from that typical of the outer surface. All members of the Harrimaniidae, all but one species of the Spengeliidae, and most of the Ptychoderaidae, however, exhibit collar nerve-cords which are essentially solid. In all these species there are scattered through the cellular portion of the cord isolated cavities, often small (3 μ in diameter) or only potential. (One species, Schizocardium peruvianum, has been supposed on the basis of Spengel’s description (1893) to lack even these cavities. I have been fortunate enough to be able to section and study the only balanoglossid known to me to have been taken from the Pacific coast of continental South America since that time, a specimen which is unquestionably of the same species as Spengel’s. Numerous small and virtual cavities, recognizable by the radiating lining cells, are present in the collar cord of this specimen.)

Ritter (1930) stated that the nerve-cord of Saccoglossus pusillus was without cavities. In the numerous examples, indubitably of this species, which I have sectioned, I have never failed to find numbers of small lacunae, and they can be found in Ritter’s original slides, also. Most of the lacunae are imperfect, that is, the cavity is virtual and the prominent row of basal granules does not form a closed figure, being usually open dorsally. Cilia are present, together with intra-cytoplasmic fibrils, but in relatively small number and in poorly developed state. The rootlet fibrils do not converge into cones as in epithelial cells, and the free portions of the cilia, which project into the lacuna, are tangled, giving the appearance of a non-functional apparatus. A definite, characteristically staining cuticle may be present. Gland-cells which appear to be functional may be found in the walls of the lacunae, emptying, therefore, into blind spaces.

The number of lacunae in the collar cord of Saccoglossus pusillus varies between limits of about 40 and 100. In Balanoglossus occidentalis there are several hundred.
The lacunae are distributed in most species in two longitudinal rows, one on each side of the mid-line, about evenly divided as to number, but not paired or evenly spaced.

Although the nerve-cord of the collar is essentially a tube of epithelium, the nervous portion is usually confined almost entirely to the floor; the roof is often poorly developed even as a simple epithelium. The floor of the collar cord is always thicker than the roof, and in species with scattered cavities the latter may be evident only laterally.

For convenience sake many authors speak of the ‘cellular portion’ of the cord, meaning that tissue which includes the cell-bodies of the nerve-cells, the cytoplasmic portions of the ordinary ciliated epithelial cells and gland-cells, as opposed to the ‘fibrous portion’, that is, the nerve-fibre layer (see Pl. 5). Nerve-fibres certainly originate in the cellular region, but there is no evidence that nerve-fibres terminate in it to any considerable degree. It must be assumed, therefore, that this region is not one of synapse and functional interrelationship of neurons, but simply a concentration of the cell-bodies of neurons whose important nervous functions are discharged almost entirely in the fibrous layer. The cellular region may be subdivided into more or less clearly marked zones corresponding to the strata of the outer epithelium. A reduced ciliary apparatus and infra-ciliature bounds the lacunae. A zone of cytoplasm, chiefly of the epithelial cells, includes a concentration of pigment granules (often very conspicuous, see Willey, 1899; Spengel, 1904a), osmiophilic granules, eosinophilic granules, and granular mitochondria. (How many of these groups of elements are the same structures it is impossible at present to say.) Most of the nuclei of the cord occupy the zone next to the fibre layer, although minor topographic differences occur between species. The nuclei are all of the round or short oval type; none of the dark-staining cigar-shaped forms, identified earlier with primary sense-cells, occur in the collar cord. In species which possess horizontal supporting fibres in the general epithelium a layer of these elements is interposed between the nuclei and the nerve-fibres. Sometimes it is only moderately developed here, as in Ptychodera, but, again, it may be
extraordinarily thick and modified in staining affinities from the same elements elsewhere, as in Balanoglossus occidentalis.

The resolution of cell boundaries is as difficult in the collar cord as elsewhere and it is not possible to count the ciliated cells. But it is apparent, on the one hand, that considerable numbers of non-nervous cells occupy the cord, and, on the other hand, that the majority of cells are nervous. No evidence of sensory cells has been encountered and, in fact, nuclei of the type characteristic of primary sense-cells are lacking. It has been impossible to separate motor and intermediate neurons. The ‘ganglion’ cells of the cord include unipolar and multipolar forms and perhaps bipolars, but the first named, I believe, predominate. Included in the unipolar category are the giant cells, described elsewhere (Bullock, 1944).

Finally, it should be said that the nerve-cells of the collar cord appear to be distributed uniformly through the region they occupy. No differential distribution of certain types is apparent, except for the posterior concentration of giant cells. No special ‘nuclei’, strata, or ‘corpora’ of nerve-cell bodies occur.

The nerve-fibre layer is composed of fine processes of nerve-cells and heavy radial supporting fibres. It occupies a little less than half the cross-sectional area of the cord. The nerve-fibres have the same character as in the superficial system. They are gently sinuous, rather smooth, dichotomizing, and without thorny side branches or elaborate endings. They present a conspicuously parallel orientation and run longitudinally. No real segregation, differentiation, or stratification is detectable, with the exception of the giant fibres. Van der Horst (1929) describes an outer concentration of transverse fibres, and an inner of longitudinal fibres in the posterior half of the cord in Glandiceps talaboti, but I have seen nothing of this kind in other species. Thus, the fibrous mass in the collar cord differs in no essential from the same layer in other cords; it shows no morphologic evidence of specialization nor do its fibres form a tangled neuropile.

All members of the Ptychoderidae possess one or more
strands of cellular tissue which traverse the dorsal mesentery of the collar, and connect the collar nerve-cord with the mid-dorsal outer epithelium (fig. 1, Pl. 2; fig. 13, Pl. 5). These median unpaired structures have long been called ‘dorsal roots’. Spengel (1893) wished to express approval of and to use Bateson’s term, but to avoid the impression of concurring in Bateson’s homologies; so he simply used the name ‘Wurzel’. Some of the Harrimaniidae have essentially similar strands, which have long been distinguished as ‘dorsal crests’. In no species of the Spengeliidae has a root or crest been found to reach the epithelium, though several forms have short vestigial outgrowths of the collar nerve-cord.

The dorsal roots and the dorsal crests (or ‘keels’) differ chiefly in antero-posterior extent. The former structures are always short in this axis, as both types are in the transverse axis, so that a dorsal root is a round or nearly round strand of tissue, longest dorso-ventrally. The dorsal crest is long in the antero-posterior direction, usually one-quarter to three-quarters of the length of the collar. Furthermore, the crest is characteristically short dorso-ventrally, since the cord is close to the epithelium in these regions and species. There may be several roots, but usually only a single crest. The latter may, however, open to the epithelium several times, being for most of its length separated by the limiting membrane from direct continuity therewith. Except for these topographic features, dorsal roots and crests are essentially similar structures and will be treated together. The crests are structurally simpler, but there is no good evidence as to which is more primitive. In either case their nature is that of a dorsal extension of the tissue which forms the cellular mass of the collar cord medially. This extension pushes apart the laminae of the dorsal mesentery to reach the dorsal limiting membrane. Breaking through this, the cellular tissue of the root or crest becomes continuous with the outer epithelium. Instances occur, however, where a dorsal root is independent of the mesentery, for example, in front of its anterior margin. The opening in the limiting membrane of the dorsal epithelium by which direct continuity is established may be exceedingly small, often less than 10 µ in any direction.
in the crest of Saccoglossus pusillus. The root always contains nuclei, though what kind of cells these represent I am unable to say. Spengel (1893) felt sure they were not nerve-cells. Frequently, as in Saccoglossus, this is virtually all that makes up the structure, though a few nerve-fibres do pass through it as well (Spengel, loc. cit., to the contrary notwithstanding). But in many species the root has a well-defined external fibre layer and an internal cellular cord. The former condition is found in some roots and all crests; the latter represents the root at its highest development. Thus the fibre layer of the collar is clearly put into direct continuity with that of the outer epithelium through these roots.

Many dorsal roots exhibit either diverticula of collar-cord cavities or small, blind lacunae of their own. Successive roots in the same individual may possess such cavities or be entirely solid. Species with a continuous canal in the collar cord usually have only isolated lacunae in the roots, but may rarely have a continuous canal in the root opening into that of the cord. The fate of the cavities dorsally is naturally of interest. In the great majority of species the cavities do not enter the epithelium or open to the outside. An interesting exception has been reported by Willey (1899) and confirmed by Maser (1913). In Balanoglossus carnosus, and to a lesser degree in Balanoglossus numeensis, the dorsal-most cavity is continued into the epithelium as a horizontal, blind-ending, ‘intra-epidermal canal’ for distances up to 180 μ. Only a single instance has been reported of a dorsal root canal opening to the exterior. Van der Horst (1924) found one large specimen of Ptychodera bahamensis in whose dorsal root there was a continuous canal which communicated ventrally with the axial canal of the cord and dorsally with the outside.

Thus, histologically, the dorsal roots and crests are very simple structures. But they vary greatly between species in number, arrangement, topography, length, size, and constitution. There may be up to eighteen roots (Glossobalanus ruficollis), but most species have less than six; individuals having no roots but belonging to species which usually possess them have been encountered (Spengel, 1904a, Ptychodera
flava funafutica). Roots may divide, anastomose, end short of the epithelium, or rejoin the nerve-cord. They have never been found paired. The variations between species will not be catalogued here; they are chiefly of taxonomic significance and may easily be found in the systematic literature or in Van der Horst's monograph (1927-39). Their morphologic significance and their role in the life of the individual worm are discussed below.

Trunk.—Regions of very different histologic character are included in this, the longest section of the body. The histologic character, furthermore, varies considerably from species to species. But with respect to the nervous system, over most of the surface of the trunk both these regional and specific differences are essentially quantitative. As over the entire surfaces of proboscis and collar, so for most of the trunk, the nervous system is essentially generalized and without local differentiations. Only in the mid-dorsal and the mid-ventral lines is this condition modified; in these positions there are concentrations of fibrous matter which constitute genuine cords. They will be separately described below.

The lateral plexus exhibits, in general, a gradient of development from anterior to posterior. Thus, the branchio-genital region of Saccoglossus pusillus has a nerve-fibre layer 4-7 μ deep, and a nerve-cell concentration averaging about 15 per 100 sq. μ of surface. These quantities decrease posteriad until, in the abdomen, between glandular ridges or islands, no visible nerve-fibre layer is present and no excess of nuclei over ciliary rootlet bundles can be detected. The glandular islands, besides exhibiting a sharp change in the staining affinities, a lengthening of cilia, and a general transformation in the character of the epithelium as compared with the surrounding non-glandular epithelium, show a well-defined nerve-fibre layer, 2-3 μ thick. These spots represent, in fact, the only places in the lateral epithelium where nervous tissue is demonstrable, in ordinary sections. A continuity between fibre layers of adjacent islands cannot be traced, though it is a priori probable that such exists. How tenuous it must be is evidenced by the appearance of the epithelium in interglandular areas. Simple, cuboidal,
faintly ciliated cells, 5–10 μ in height, rest directly on the ever-prominent limiting membrane, showing no constricted ‘cell feet’ or any trace of punctate substance.

Local variations in degree of development of the nervous tissue occur over the surface of the trunk. Thus, over the gonads the epithelium may become thin and the nerve layer disappear; the superficial annulations of the integument may be reflected in thickenings of the nerve-fibre layer; or deep grooves, such as those between hepatic caeca, may lack that layer. Species like the Ptychodera with gill bars exposed have a distinct nerve layer on the outer side of the gills, whereas forms with well-developed branchial chambers and small, secondary gill-pores (SaccoGLOSSUS) have no visible nerve layer on the gills. In no species, apparently, do the anterior and posterior faces of the gills have visible nervous tissue. Again, the species of Ptychodera, as opposed to SaccoGLOSSUS, have no extensive non-glandular areas on the abdomen. The epithelium and its nerve layer are well developed right back to the anal end. Only in the grooves between hepatic caeca is a simple, cuboidal, non-nervous epithelium to be found.

The orientation of nerve-fibres in the lateral plexus of the trunk is nowhere restricted to any one direction. No predominance, certainly, of antero-posterior elements exists, though dorso-ventral fibres may sometimes appear in the majority. Some fibres pass in every direction, however, and no segregation could be demonstrated.

Nerve-cords.—Elongated concentrations of fibrous nerve tissue occur in the trunk along the mid-dorsal line, directly over the attachment of the dorsal mesentery, along the mid-ventral line, likewise marked by a mesentery, and in the transverse plane around the sides at the junction of trunk and collar. These three strands of thickened fibre layer will be spoken of as the dorsal (nerve) cord, the ventral (nerve) cord, and the circular connective, respectively.

The dorsal and ventral cords may be considered together for the sake of contrast. The dorsal cord (figs. 1, 2, 3, Pl. 2; figs. 4, 7, Pl. 3; fig. 9, Pl. 4) is represented by a thickening in the fibre layer of the epithelium in the mid-dorsal line. The
increase in thickness of the fibre layer occurs at the expense of the superficial zones of the epithelium. In contrast, the mid-ventral epithelium as a whole is greatly thickened. The nerve-fibre layer makes up most of this increment in depth, but the nuclear zone also is usually thicker. Comparison of direct measurements of the two cords reveals a consistent difference in total cross-sectional area in favour of the ventral cord. The amount of this difference is not fully realized by a comparison of depths, for another highly characteristic distinction between dorsal and ventral nerve-cords is in the lateral extent of the fibrous thickening. The dorsal cord is always recognizable by its narrow, sharply delimited cross-section abruptly passing, laterally, into the general plexus, whereas the ventral cord is not only much wider, but its transition into the lateral plexus is often gradual. The ventral cord may, in fact, be actually shallower in certain regions than the dorsal, but its greater lateral extent always brings its total cross-sectional area to a figure higher than that of the dorsal cord. In one individual of Balanoglossus occidentalis a maximum depth of 110 \( \mu \) for the dorsal cord, and only 80 \( \mu \) for the ventral was recorded for a certain region; but the respective widths were such as to bring the total sectional area of the former to 29,000 sq. \( \mu \), of the latter to 87,000 sq. \( \mu \). Usually, however, the ventral cord is at least as deep as the dorsal, and the disproportion in area greater. Absolute figures mean little, because of the great variation between regions and species. For the sake of comparison with previous figures, however, we may mention typical measurements of Saccoglossus pusillus. The nerve-fibre layer of the dorsal cord in the branchial region may reach a depth of 50 \( \mu \), the sectional area amounting to 8,000 sq. \( \mu \), as against 75 \( \mu \) and 18,000 sq. \( \mu \) in the ventral cord in the same section. These figures diminish progressively posteriad until in the abdomen the depths may be no greater than 5 \( \mu \). Hess (1937), like others before him, reported that ‘... the dorsal (cord) is larger and better developed than the ventral’. But my own measurements of his species (Saccoglossus kowalevskii) agree entirely with those above in respect to a consistent preponderance in size of the ventral cord; whether the
The nervous system of enteropneusta 85

dorsal cord is ‘better’ developed or not, I will not argue; it certainly is differently developed from the ventral.

The fate of the cords in the terminal abdomen is interesting. In Ptychoder a bahamensis, where a well-developed lateral plexus persists throughout the abdomen, the dorsal and ventral cords, a few micra anterior to the anus, are about 25 μ deep. Laterally, directly over the end of the abdomen the fibre layer is thickened to at least 20 μ deep. We may think of the cords here as extending right to the lips of the anus, there to bifurcate and meet around its sides. I was unable to detect, however, such an orientation of fibres as would suggest a circumanal ring. An interesting contrast is seen in Balanoglossus occidentalis in which the abdomen is differentiated into a thin-walled post-hepatic region and a conspicuously swollen terminal region. Further differences are seen in this species in the presence of a ‘pygochord’ (Willey, 1898), and in the character of the lining of the terminal intestine, which here is a deep, glandular epithelium possessing a very thin nerve-fibre layer, whereas that of Ptychoder a is low, non-glandular, and without a visible nerve layer. It is perhaps surprising that in the more specialized terminal abdomen of Balanoglossus the dorsal cord should diminish until, several sections before the first breaking through of the anus in a transverse series, it is completely indistinguishable from the lateral plexus. The ventral cord persists somewhat longer, but there is no circumanal thickening by which the cords are continuous.

Differences between the dorsal and ventral nerve-cords extend to finer details as well. Over both cords the epithelial glands are significantly reduced in number, but this is more true of the dorsal than of the ventral. The radial fibres of indifferent epithelial cells are concentrated to each side of the greatest depth of fibrous matter in the dorsal cord, thus exaggerating its delimitation from the lateral plexus. They frequently divide the peripheral-most nerve-fibres into conspicuous fascicles occupying alcoves between the radial elements but broadly continuous with the rest of the cord. The radial fibres of the ventral cord are equally well developed, but more numerous and more
uniformly distributed. Reference has been made to the differences in number of nuclei of the two cords. Whereas in the ventral cord nuclei are considerably more numerous than in the adjacent lateral epithelium, in the dorsal cord of *Saccoglossus pusillus* the nuclei are less than half as numerous as in the immediately lateral epithelium. A typical figure for the branchial region is 10 nerve nuclei per 100 sq. \( \mu \) of surface over the mid-dorsal line, 18 in a similar area parasagitally. In other species there may be, on the contrary, a concentration of nuclei in the dorsal cord, but not to exceed that of the ventral cord. The reduction in gland-cells and the proportional or absolute increase in the number of nuclei indicate that it is nerve-cells which are being concentrated in the cords. Corresponding with its greater number of nerve-cells and greater size, the ventral cord may differ from the dorsal with respect to fibre orientation. In both, the predominant direction is longitudinal, but the ventral cord may exhibit a greater number of diversely oriented fibres with but a central zone of longitudinal ones.

Although the dorsal and ventral nerve-cords are connected with each other throughout their extent by the lateral plexus, there occur in all species of enteropneusts thickenings of the nerve-fibre layer at the anterior end of the trunk where the ventral cord abruptly ends and the dorsal cord is continued into the collar cord. These thickenings extend through the lateral plexus from the ventral to the dorsal nerve-cord on each side. In some species they are poorly delimited. In no species are they very thick, both cords together probably never equal the cross-sectional area of the ventral cord at this level. In *Saccoglossus pusillus*, they rarely exceed 10 \( \mu \) in depth. That they are important is attested by experiments (Bullock, 1940), and by the strikingly uniform orientation of their constituent fibres. No plexus exists here, but a well-developed conduction tract. The correlated tendency to an alinement of radial fibres is well seen here.

It is significant in relation to Hanström's theory (1928) of the origin of longitudinal nerve-cords, to be discussed below, that no indications whatever of lateral nerve-cords have been
Nervous system of Enteropneusta

encountered in this study, nor have they been mentioned by earlier authors.

Epithelium of the gut.—A well-developed nerve-fibre layer exists in the lining of the buccal cavity. This is frequently thickened in the mid-dorsal and occasionally in the mid-ventral line. The figures for Saccoglossus pusillus are in the vicinity of 4–8 μ for these thickenings, 1–2 μ laterally. The buccal plexus is a direct continuation of the layer found over the outer surface of the collar. Van der Horst (1929) called the ventral thickening a ‘pharyngeal nerve’, but the term ‘nerve’ is probably better reserved for true internal bundles of fibres as opposed to medullated cords and thickenings of the epithelial plexus. The fibre layer can be followed back to the pharynx, where it disappears except in the epibranchial and hypobranchial strips. In some forms (Ptychodera) it may be detected in the pharyngeal face of the gills, but never on their anterior and posterior faces. Throughout the intestine the presence of nervous tissue is extremely dubious. In most regions nervous tissue is not visible; but in the terminal intestine of those forms which, like Balanoglossus, have a high, glandular epithelium, a very thin nerve-fibre layer is apparent.

The Internal Nervous System.—With the technics that have thus far succeeded in my hands I have found it impossible to identify with certainty as nervous any structure internal to the limiting membrane. There are, to be sure, many cells lining the coelom and scattered in it whose nature remains undetermined, and which, by their large nuclei, ample cytoplasm, and long, attenuated cell processes, could well be ganglion cells. But they are equally likely to be connective-tissue cells, peritoneal cells, or any of the strange and still unclassified types of mesodermal coelomic elements. A striking display of fibres in the coelom is seen in protargol preparations, such as impregnate selectively the nerve-fibres in the outer epithelium. Some of these may be followed across the limiting membrane into apparent continuity with fibres of the nerve layer of the epithelium. But that this elaborate plexus represents an internal nerve plexus, and the stellate cells whose processes contribute to it represent nerve-cells, is exceedingly dubious. Arguing
against such an interpretation are an extensive and too elaborately plexiform appearance, considering the musculature to be innervated, a difference in staining qualities from known nerve-fibres, and a ubiquitous intercellular disposition in compact muscle layers. These argentophilic fibres are perhaps better regarded as largely the processes of parenchymatous or 'connective-tissue' cells for the present. However, it is possible that true nerve-fibres are present in this plexus, but have not yet been distinguished from its other elements.

Spengel (1893) found structures in one species, Glossobalanus sarniensis, which he regarded as an internal nervous system. Two pairs of delicate cords passing longitudinally in the angles of the dorsal blood-vessel appeared to consist of punctate tissue and the surrounding cells resembled nerve-cells. He could find no connexion with the internal organs or with the superficial nervous system.

In no other species of enteropneust has such a strand been identified. Concerning Glossobalanus elongatus, Spengel (1904) specifically states that he could not confirm the existence of such a 'sympathetic' nerve. Nothing of the kind occurs in most of the species I have examined. In Balanoglossus occidentalis and Glossobalanus sp. (from La Jolla, California) structures suggestive of parts of Spengel's description have been seen. I am entirely uncertain whether the strands are indeed composed of nerve-fibres or that the adjacent cells are nerve-cells, but my observations do not extend or correct Spengel's description except in reporting the definite absence of such paravascular cords in most species examined.

The only other reference to internal nervous elements is that of Hess (1937). He reported a '... multipolar nerve network... in certain internal regions of the body...' in methylene blue preparations. This network is specifically mentioned only for the region of the proboscis near the heart. It is figured as a true anastomosing net whose cells are about 5 μ in diameter, multipolar, and protoplasmically continuous. 'From the ganglion cells... fibres, which are probably effectors, pass into the muscle fibres.'

It is impossible to deny the occurrence of nerve elements in
the coelom, but it is significant that there are no layers, concentrations, ganglia, tracts, or nerves (with the possible exception of Spengel's paravascular cords), that the elements must be diffuse and few, and are probably confined to direct or terminal neurons or parts thereof, to the exclusion of any considerable numbers of intermediate or connecting neurons.

Discussion.

There must now be undertaken a brief statement of (1) the bearing of the present results on previous anatomical descriptions, (2) correlations with the available information on the physiology of the nervous system, and (3) certain evolutionary considerations in which this system is involved.

Anatomical Concepts.

Sense Organs.—The literature contains no convincing reports of sense organs in balanoglossids. Spengel's (1893) account of a 'sensory pit' in the proboscis of *Stereobalanus canadensis* does not suggest a sense organ,¹ nor do Van der Horst's (1930) figures of an organ, supposedly sensory, in the dorsal epithelium of *Glossobalanus ruficollis*. Assheton (1908) found a structure in the collar epithelium of *Saccoglossus serpentinus* which he supposed to be sensory, but he gave no histological details.

I have found on the base of the proboscis in several species (*Saccoglossus pusillus*, *Saccoglossus kowalevskii*, *Glossobalanus minutus*, and *Ptychodera bahamensis*, see fig. 17, Pl. 6) the pre-oral ciliary organ first described by Brambell and Cole (1939), and homologized with the wheel organ of Amphioxus and the hypophysis of Craniota. This organ consists of two very low parallel ridges in the transverse plane. The cilia borne by the cells which form the ridges are somewhat longer than those found elsewhere, and the rootlet cones stain more deeply. Contrary to Brambell and Cole's findings, the nerve-fibre layer appears to have no consistent thickening related to these ridges. The evidence does

¹ This is the only published species of the genus. In an undescribed species of *Stereobalanus* from Central California I find no such pit.
not justify the assumption of much greater sensitivity of the ridges than the adjacent epithelium. Rather the base of the proboscis as a whole, and not just the region of the pre-oral ciliary organ, is to be regarded as highly sensitive (Bullock, 1940).

It may be concluded that the Enteropneusta lack true sense organs. This is confirmed by the absence of any clear-cut case in the literature, and by the complete failure to find any evidence of such organs in this investigation.

**Nerve-cell Types and the Constitution of the Epithelium.**—Bateson (1886a) regarded '. . . the skin of Balanoglossus . . . as a collection of sensory cells ending in long fibres, which may either be connected to the central nervous system, probably by the longitudinal fibres of the “punktsubstanz” (i.e. those passing parallel to the plane of the epithelium, which we now regard as comprising the entire nerve layer) or may pass directly through this as motor-fibres into the muscles'. These motor-fibres are evidently Bateson’s ‘ciliated, tailed’ epithelial cells which we recognize from his figures and description as the supporting fibres, or cell feet, of ordinary epithelial cells. Thus Bateson did not distinguish non-nervous, epithelial supporting cells and sensory nerve-cells, but conceived of the epithelium as consisting of (1) a very few ganglion cells (merely because ‘of the antecedent probability of their occurrence’; he did not find them) and, (2) of ciliated, sensory cells many of whose processes passed directly into the muscles. That is, a reflex ‘arc’ of one neuron would be the rule. Our evidence to-day forces us to abandon many aspects of this view, although no evidence can be cited that would preclude the existence of one-neuron reflex ‘arcs’. His concept of motor-fibres crossing the limiting membrane everywhere, diffusely, though very possibly correct in view of the evidence presented here, was an erroneous conception as he visualized it, and based on false histologic pictures. Spengel distinguished between the indifferent epithelial ‘Stützzellen’ and ‘Nervenzellen’. The former he associated with the elongate dark-staining nuclei, the latter with the more basal, round or oval light-staining nuclei. Only when he noted a great discrepancy in numbers between the
elongate nuclei and the 'fadenförmigen Zellenfüsse' (radial fibres) in favour of the former, in regions like the base of the proboscis, did he conclude that some of these nuclei represent sense-cells. He did 'not doubt that such cells occur also in other places', but evidently regarded them as less regular or numerous occupants of the epithelium than the 'Nervenzellen' or ganglion cells. Van der Horst believed that Spengel's conception of sensory elements corresponded to present-day 'true' or secondary sense-cells. The combined evidence from selective stains, epithelial counts, and the comparison of well-developed sensitive epithelia with simple non-glandular, non-nervous regions indicates that no secondary but only primary sense-cells occur in these organisms (as is true of all other invertebrates; see Hansström, 1928), that these cells are far more abundant than ganglion cells, and that in general they possess and account for the elongate nuclei. The ganglion cells do possess round or short oval nuclei, as Spengel supposed, but do not, as he thought, nearly account for these forms. I believe that in general the ciliated epithelial 'Stützzellen' have basal, round or short oval nuclei, as is especially evident in the simple epithelia where other cell-types are rare or absent. Spengel stated that elongate nuclei represent the indifferent epithelial cells even in the collar cord, but I have noted no such nuclei here, and a careful search through his plates shows that he figures none. The points mentioned, together with a cursory description of giant cells, are about the extent of Spengel's interest in the nervous system, aside from topographic anatomy of the cords.

Van der Horst (1927-39) goes no farther than to say that certain epidermal cells must, a priori, be sense-cells, and that he believes them to be primary sense-cells connecting with an asynaptic nerve set. Brambell and Cole (1939) describe and figure a type of cell in the epithelium which they believe to be a sensory nerve-cell. This is almost certainly a gland-cell.

Hess (1937, 1938) has offered the only picture of the cellular elements of the nervous system of an enteropneust supported by special nerve stains. His papers principally concern the existence and function of one cell type. This is a large, bipolar
cell lying horizontally, that is, parallel to the plane of the nerve-fibre layer. It is described and figured from methylene blue and protargol stains of fragments of Saccoglossus kowalevskii. According to the scale on his figures, these cells are in general 27–36 μ long, a truly giant length for balanoglossids. Their processes may be very long, some being said to extend throughout the length of the proboscis, about 30 mm. They are usually darker at one end, which end contains the nucleus. None of the fibres figured branch. They are indicated as much larger than ordinary fibres of the nervous plexus. The entire cell is said to be in the nerve-fibre layer, and where fibres run in both directions, as in the proboscis, those oriented longitudinally are internal to those passing transversely. Fibres running obliquely are not mentioned or figured. The bipolar cells are described as least numerous on the ventral side of the trunk, specifically 543 cells per square millimetre, more abundant on the dorsal and dorso-lateral sides of the trunk, where 801 cells were counted per square millimetre, and thickest on the proboscis (no figures). In the trunk they are said to be oriented exclusively antero-posteriorly, in the general plexus as well as in the cords; and they are found as abundantly in the interglandular areas as under the glandular islands, dorsally, but only between these islands on the ventral side. The fibres are just as numerous under the islands as between them.

Now, this picture is a remarkable one. The results of the present study indicate that nuclei and cell-bodies are rare in the fibre layer, and that the plexus is characterized by fibres running in all directions, and unstratified. The plexus in the abdomen shows a conspicuous lack of longitudinal orientation, and experiments have shown (Bullock, 1940) the great difficulty of longitudinal conduction in the general plexus of the trunk, and the ease of transverse or circular conduction. Cells and fibres are said to be at least as abundant between glandular islands as under them, more abundant ventrally, whereas the nerve-fibre layer in the interglandular areas in sections is so thin as to be invisible, and no excess of nuclei over ciliary rootlet bundles occurs. The glandular islands, on the other hand, have a distinct fibre layer and a definite excess of nuclei over ciliated
and gland-cells. Although considerable numbers of huge, basal, horizontal bipolar cells are described, no other types, especially vertical, primary sense-cells (bipolar, but with one process very short) are mentioned. (In fig. 2, 1938, a diagram of the epithelium, a single vertical 'sense-cell' is drawn, but no mention of such cells is made in the text; in fact, it is to be inferred that none exist.)

I do not doubt the existence of horizontal bipolar cells throughout the superficial plexus. I have seen and described such elements. But I have not encountered any such giant cells and fibres as those Hess represents, although such neurons should be easily found in ordinary sections without special stains. Further, I feel that the very regular distribution is doubtful. In my own methylene blue preparations appearances suggestive of uniformly longitudinal fibres were encountered in the trunk, but these were readily shown to be sub-epithelial muscle-fibres. (In this region there are no circular muscles, whereas in the proboscis there are outer circular and inner longitudinal muscles.)

But, most importantly, I question the representativeness of this concept. Hess apparently regards these bipolar cells as the only element, or at least the principal constituent, of the peripheral plexus (see quotations below). He does not recognize a division of nerve-cells in the general plexus into sensory, associative, and motor, although all three are indubitably present. The absence of the primary sense-cell (except for the single cell diagrammed) is remarkable considering its ubiquity in every other group from the coelenterates to the vertebrates. I do not believe that 1 per cent. of the fibres making up the nerve-fibre layer, even in the trunk where that layer is not very thick, could be accounted for by 501 cells per square millimetre. This means one neuron in a 35·4 μ square or in about 320 μ of a 10 μ section!

Finally, the interpretation which Hess has placed upon these cells seems open to question. He has assumed them to be the receptor organs of these animals, '... since the subepithelial (sic) nerve plexus of Dolichoglossus [Saccoglossus] is a bipolar plexus which is connected directly with the central
nervous system, it is probably composed of sensory neurons which are concerned with the reception of stimuli and the conduction of impulses to the co-ordinating centre in the proboscis stalk and collar'. (Hess regards the collar cord and peduncle as a central nervous organ.) He specifically designated the horizontal bipolar cells as photoreceptors: 'Hence, because the elongated nerve-cell bodies described above are larger and more numerous in the more photosensitive regions, because they stained with the selective nerve stains used, and are the only cells that so stained that they could be the photoreceptor cells, because they lie at the base of the outer body epithelium and consequently resemble in their general location that of the photoreceptor cells in the earthworm, . . . and since they resemble in their morphology the retinal cells of vertebrates these elongate nerve-cell bodies have been designated the photoreceptors of Dolichoglossus.'

Since in all groups of animals with a nervous system, including the earthworm, the peripheral sensory cells (aside from secondary sense-cells, which are peculiar to vertebrates) are essentially vertical elements with a single nerve-fibre and a short distal process projecting towards the surface (see Hanström, 1928), it seems improbable that a horizontal cell lying in the basi-epithelial plexus and giving off a long nerve-fibre from each end is a sensory cell. It is more likely an intermediate or associative 'ganglion' cell.

**Functional Correlations.**

The general plexus of balanoglossids conducts diffusely and with apparent decrement, in the manner of a classical nerve-net (Bullock, 1940). Pieces of the body-wall show a high degree of autonomy, as is true with primitive net systems elsewhere. These properties are in agreement with and find their anatomical bases in the diffuse distribution of sensory cells, ganglion cells, interneuronal junctions, and motor innervation. But, whereas the nerve-net has generally been assumed to depend on anatomical continuity between neurons (see, for example, Parker, 1919), no evidence for such continuity was found in this study. The balance of evidence is in favour of discontinuity although
this cannot be said to be demonstrated. These findings agree with other recent evidence (e.g. Bozler, 1927) to the effect that nerve-nets are not necessarily protoplasmically continuous. Especially significant in this connexion is the evidence of Pantin (1935 et seq.) that the property of ‘decremental’ conduction is probably an indication of a chain of neurons separated by synapses, each of which requires the ‘facilitation’ provided by two or more impulses to break down its resistance. The existence of diffuse conduction and the probability of synapses suggest, as the simplest explanation, that the synapses are unpolarized. Most textbook definitions of the synapse included the word polarized, but it is becoming questionable whether the property is necessarily or even actually true of the individual junction in higher animals. It may be instead an effective polarization due to numerical or anatomical relations. In any case, it is entirely conceivable that in primitive nervous systems the synapse is a potential barrier to conduction with no polarization, and that effective polarization developed gradually through various degrees as is seen to-day in many nerve-nets, for example, that of the balanoglossids.

The fact that the ventral nerve-cord is physiologically more important (less dispensable) than the dorsal agrees with the anatomical demonstration that the former is larger. It is to be noted also that most of the musculature of the trunk is ventral. Both lines of evidence have emphasized that the cords, including the collar cord, are probably chiefly conducting tracts and only to a lesser extent ganglionic. Evidence from both approaches indicates that this is truer of the dorsal cord of the trunk than of the ventral. The importance of the circular connectives, despite the continuous communication of dorsal and ventral cords through the general plexus, was shown by the considerable effect of cutting these connectives.

The anatomical picture in general is that of a very primitive and simply organized nervous system, with a small ratio of associative or connective neurons to sensory neurons. The functional picture agrees with the anatomical, being characterized by simple, undifferentiated and largely local responses. These considerations bring up the historically interesting
question of whether the collar cord, or that cord together with some or all of the other nervous concentrations, should be regarded as a central nervous system. It seems clear now that in so far as a central nervous system is a region involved in all ordinary reflexes, which contains all the intermediate neurons, and to which pass all the sensory nerve-fibres, the cords of balanoglossids are not a central nervous system. Only in so far as they represent specialized conduction tracts and may have a modifying effect on such reflexes as do traverse them are they ‘central’ organs. Their organization and dispensability emphasize the gulf which separates them from the central nervous systems not only of vertebrates but of most invertebrates. In view of the generally accepted connotations of the term, I prefer to regard the enteropneusts as lacking a central nervous system. Spengel (1893) denied the collar cord the status of a central nervous system, but on morphologic not neurologic grounds. Hess (1937, 1938) speaks of the central nervous system of these animals, and goes so far as to call the peduncle a ‘centre’ (for light reception). I do not believe the facts available justify the assumption of ‘centres’ in the ordinary neurologic meaning of that term.

Phylogenetic Speculations.

A number of problems of morphologic interpretation demand at least cursory treatment. One of these arises from the existence of either a continuous canal or blind lacunae in the collar cord. These structures have for long challenged speculation and it may be said of them, as of most others of the morphological features prominent in phylogenetic discussions, that the authors of forty years ago were in as favourable a position for their interpretation as we are to-day. The essential facts became known when Spengel published his monograph (1893), and no additions or alterations significantly changing their totality for morphological purposes have been made since. The problem, therefore, is simply one of evaluating the facts and applying changing points of view. The present paper proposes no original phylogenetic argument. My objective in this connexion is to make available a summary statement of the data and of the
principal homologies that have been suggested relating to the nervous system.

The general facts of the comparative anatomy of cavities in the collar cord have been presented above in their place. A few unusual cases, considered by their describers to be significant, may be mentioned here. And the known embryological facts, incomplete as they are, should be indicated. Bateson (1886a) described an anterior axial canal which reached only part way into the cord in Saccoglossus kowalevskii, and which was followed by blind cavities of the kind we have called lacunae. He wrote: 'It is possible that the spaces [lacunae]... may in some indirect manner communicate with the neural tube [anterior canal].' But Spengel (1893) denied this possibility, and every subsequent worker has furthered the evidence against any such intercommunication. My observation on Saccoglossus kowalevskii and other species have also failed to confirm Bateson's belief in any way. Spengel (1893) mentions some instructive cases of anomalous individuals. A specimen of Glandiceps talaboti, a species which normally has scattered lacunae only, was encountered whose collar cord was traversed by a continuous axial canal through at least the anterior half. Schizocardium brasiliense likewise usually exhibits isolated cavities only; but one animal possessed not only lacunae (lateral, as is usual) but a median axial canal. Finally, young individuals of Glossobalanus minutus exhibited axial canals, quite unlike older members of the species. Later (1904b) Spengel described a species, Glossobalanus elongatus, whose cavities he regarded as an interrupted axial canal. In several species lacunae are present in the dorsal nervous concentration of the peduncle. All these cases he considered as evidence that the 'Axencanal' and the 'Markhöhlen' are not to be confused and that they had, if not a different, at least an independent origin.

The development of the parts in question should offer most illuminating clues, but, unfortunately, the appropriate stages have been described for only three species, and only incompletely. Bateson (1884, 1885) describes the collar nerve-cord of Saccoglossus kowalevskii as delaminated from the
medial dorsal ectoderm and at first solid. The description of cavities goes no farther than to say that at the anterior end of the collar, by simultaneous forward growth of the organ and backward (horizontal) invagination at the point of junction of the internal nerve-cord with the superficial nervous layer of the peduncle, a lumen is formed which extends a 'short distance into the cord'. Bateson's stages do not reveal whether the definitive lacunae arise from this lumen by a process of tissue invasion, which would not account for their occurrence throughout the cord, or de novo in the solid rod of nervous tissue, which would seem unlikely in view of their epithelial character and ciliary apparatus. The situation is simpler in the other two forms, both having indirect development by means of Tornaria larvae (whereas Saccoglossus kowalevskii develops 'directly', without such a larva). In the Tornaria of New England Morgan (1891) showed a modified form of invagination. The median dorsal plate sinks bodily below the general level and the edges roll over it, finally meeting and fusing to leave a cord, solid in some regions, provided with a lumen in others—whether clearly an enclosed bit of outside space or not is uncertain. Again, the numerous definitive lacunae (for this species, probably Balanoglossus aurantiacus, has many isolated lacunae in the adult) cannot be exactly related to the one, or few, embryonic cavities, in the present state of our knowledge. In Tornaria morgani, considered by Stiasny-Wijnhoff and Stiasny (1927) to be the larva of Ptychodera bahamensis, Morgan (1894) demonstrated a simple invagination producing a continuous lumen, from which doubtless is derived the continuous axial canal of the adult Ptychodera. We may say, therefore, that certainly in some species, and possibly in others, the cavities of the collar nerve-cord represent portions of outside space, either enclosed or still continuous therewith. This statement, however, must be considered as extremely tentative, based as it is on but one clear and two incomplete accounts of the embryology of the Enteropneusta. The scattered lacunae of the majority of species are not embryologically obvious derivatives of a continuous axial canal by fragmentation or tissue invasion, and no transitions
between the two conditions are known. Nevertheless, the latter is commonly regarded as the primitive enteropneust condition and the former as derived from it.

Similar difficulties attend the interpretation of the dorsal unpaired strands which connect the collar cord and the outer epithelium. From the taxonomic distribution we may regard the possession of a dorsal root or crest as the primitive condition. The significance of these structures for the individual can only be conjectured. They certainly make possible an exchange of fibres between cord and integument; but the fewness of these fibres in most roots and all crests, and the complete absence of these structures in all spengeliids and in occasional members of species which normally have them, argue for their very slight functional importance. Their cells, furthermore, do not appear to be nerve-cells. The morphologic significance of these dorsal, unpaired connecting strands is even more difficult to ascertain. Their embryology is not known. The simplest assumption would seem to be that they represent rests of the embryonic connexion with the epithelium which exists before delamination or invagination of the collar cord. Spengel believed them to be rests left by the anterior and posterior margins of the collar as these grew forward and backward, respectively.

Regarding homologies it is difficult to make general conclusions without considering simultaneously the morphologic features of all the other organ systems. This is beyond the scope of this paper. But it is instructive to examine the specific homologies that have been applied to the nervous system, apart from others, because that system has represented one of the classical arguments for the most important phylogenetic theory relating to enteropneusts. This theory identifies the enteropneusts with the chordates, as is well known. It is, to my knowledge, the only important positive suggestion of the relationships of the balanoglossids that has been advanced in fifty years. Moreover, earlier alternative theories either lost tenability with increasing knowledge of the animals concerned (Agassiz, 1873; Eschscholtz, 1825) or never gained any considerable support (Spengel, 1893). The obvious and frequently proposed homology with respect to the nervous system relates
the collar cord of enteropneusts with the neural tube of chordates. The basis for this homology is the dorsal position and hollow character of the collar cord. The former feature depends very largely for its significance on the fact that in this particular region of the body no ventral cord exists, whereas in the greater part of the body a ventral as well as a dorsal cord occurs and is indeed larger and more important. The hollowness of the collar cord is pronounced in only a few species; in most it is only suggested; but it may well represent the primitive condition. Koehler (1886) has compared the tissue which interrupts the continuity of the axial canal to those grey commissures of the vertebrate brain which cross the ventricles, but it is still doubtful whether the discrete lacunae of balanoglossids arise by invasion of a once continuous cavity. Hanström (1928) has voiced the obvious corollary of the general homology of axial canal and neural tube by comparing the indifferent epithelial cells which line the former with the ependymal cells which line the latter. It is plain that if one homologizes the collar cord and the neural tube he must be willing to limit the correspondence to the anterior end of the latter tube and to accept the superficial, mid-dorsal nerve-cord of the trunk as corresponding with the rest of the vertebrate neural tube. He must further, of course, hypothecate a complete loss of the ventral cord and circular connective. Willey (1899) not only did this, specifically delimiting the extent of neural tube anterior to the trunk, but went farther and found the unfused medullary folds of the trunk:

'The medullary tube of the collar of Enteropneusta is the homologue of the cerebral vesicle only of Amphioxus and of the Ascidian tadpole and probably represents no more than the primary fore-brain (thalamencephalon)[sic] of Craniota.... Just as the medullary tube of the collar is admittedly an invaginated portion of the dorsal nerve-trunk, so the medullary folds which arise and fuse to form the medullary tube are to be regarded as specializations of the anterior portion of pleural folds which are retained in
the Ptychoderidae as the genital pleura.' (Italics Willey’s.)

These genital pleurae are great, wing-like folds of the body-wall of ptychoderids, ventral to the gill-slits and occupied by gonads. In the same connexion, he states farther on:

‘... the genital folds of Enteropneusta, the atrial folds of Amphioxus and the medullary folds of Vertebrata belong to the [same] system of pleural folds of the body-wall, and are differentiations from a common primordium.’ (Italics Willey’s.)

Assuredly, then, if phylogenetic precursors of the vertebrate neural tube are so clearly represented in the balanoglossid, the dorsal roots must have some profound significance. Bateson (1886b) found their homologues in the paired dorsal roots of vertebrates, and Willey (1899) in the epiphysis of the Craniata! Hess (1938) carries the detailed homology still farther, comparing his bipolar ‘photoreceptor’ cells with rods and cones of the vertebrate eye; some resemble rods, some cones. And, according to him, all ‘are chordate-like in being embedded in the tissue of the central nervous system as in Amphioxus’, though they represent an earlier stage in that they are also found throughout the general plexus. It would seem doubtful how characteristic of chordates are sense-cells which are in their most primitive position—scattered throughout the integumentary epithelium. These topographical relations are true of both Hess’s bipolar cells and the probable actual photoreceptors, the undifferentiated primary sense-cells. But it is, unfortunately, not true that these latter occur in the collar cord which was most of Hess’s ‘central nervous system’. (He included the proboscis stalk, wherein photoreceptors do occur.)

All these corollary homologies stem from, and probably depend on, the primary comparison of the cavities of the internal portion of the enteropneusts’ dorsal cord and the cavity of the chordate central nervous system. If it were not for this hollowness it is very doubtful how vigorously these homologies would be pursued. And yet, the existence of a roofed-over nerve-cord, or of invaginated, even hollow ‘ganglia’, is not new with
the chordates. Hanström (1928) has reminded us of the cerebral ganglion of rotifers like Asplanchna which is formed by invagination, and of the cavity in the cerebral ganglia of many ectoproct bryozoans which persists in the adult. Finally, I should like to call attention to the cross-section of the arm of ophiuroid echinoderms. The relations are very like those in the collar cord of enteropneusts—an internal tube with the nervous tissue concentrated on the inner side, cellular portion nearest the cavity, and fibre matter on the periphery. These instances may mean nothing for the present problem, but are mentioned to suggest that the mere existence of cavities in a portion of the nervous system should not be taken as prima facie evidence of a chordate-like central nervous tube.

The argument for chordate affinities from the condition of the nervous system I regard as weak, considered by itself. But it is important to say that the general concept of the nervous system of enteropneusts to which we are brought, emphasizing as it does the primitive level of organization achieved, does not argue at all against the theory of chordate affinities. And the reservations made above as to the classical morphological homologies do not in the least add up to negative arguments. My own position is one of concurrence in the thesis of chordate affinities, based primarily on other grounds than that of the nervous system. Of these the chief, to my mind, are the series of embryological characters in which the two groups agree. ‘Chordate affinities’ does not necessarily mean inclusion in the phylum Chordata. I am not prepared to deliver an opinion on this question depending as it does not only on the morphologic facts but on the concept of the phylum as well.

Homologies with lower groups are even more difficult. The problem essentially reduces itself to the question of what bases can be found for identifying the nerve-cords of the present group with those of a second group, but not of a third. Or, must we place the nervous system in a position unique among the organ systems by declaring homologous the principal nerve-cords of all animals? No definite suggestions of the former type are known to me, involving Enteropneusta. But the latter alternative is just what Hanström (1928) has taken in his orthogon
NERVOUS SYSTEM OF ENTEROPNEUSTA

theory. All the principal longitudinal nerve-cords are derived, according to this theory, from an original series of, probably, eight paired longitudinal condensations of the diffuse plexus of coelenterates, connected by commissures. Such an orthogonal pattern is still found in ctenophores and certain platyhelminths. Further concentration of the nervous system has resulted in the fusion, loss, or hypertrophy of the members of the original orthogon. Commissures for the most part cannot be traced phylogenetically, but Hanström believes the longitudinal cords of most groups can be. Thus the two median cords of balanoglossids are supposed on this view to represent fusions in each case of two lateral cords, although no evidence remains of an originally paired condition. This group would resemble in this feature the nematodes (differing, however, in many other ways). But the dorsal cord and the ventral cord would separately be comparable directly to all median dorsal and median ventral or dorso-lateral paired and ventro-lateral paired cords, respectively, throughout the animal kingdom. They, moreover, would be homologous with all longitudinal cords as derivatives of the same original series. Many groups show traces of other members of the orthogon than those principally developed in that group. Thus the nematodes have small lateral cords in addition to the median dorsal and ventral cords. I have failed to discover in the balanoglossids any trace of lateral cords, and no previous author mentions them.

A comparison of the nervous system of enteropneusts with that of other invertebrates on the basis of the level of organization or the complexity achieved, quite apart from phylogenetic identification, is significant. The present group is immediately seen to be far below all those with internal ganglionic systems. Detailed consideration of the degree of histologic elaboration and physiologic complexity as we now see it shows it to be on the same general level as the lowest of those with diffuse, superficial, aganglionic systems. Specifically, the balanoglossid does not show nearly the degree of nervous organization found in the annelid, nematode, nemertean, platyhelminth, or echinoderm and is more closely comparable in this respect to the ctenophores and coelenterates, though it lacks the sensory
discrimination and diversity of response even of some members of these groups.

**Summary.**

1. Results of a detailed study of the nervous system of *Saccoglossus pusillus*, with comparative material of about two dozen other species of Enteropneusta, are presented.

2. The primary feature of the enteropneust nervous system is its position within the superficial epithelium. Pertinent relations with non-nervous elements of the epithelium are described. The indifferent, ciliated cells elaborate supporting fibres in those regions where the epithelium is well developed and nervous tissue is concentrated. Such cells are considered to represent neuroglia in its most primitive form. The fibres appear in places to be continuous with the ciliary rootlet cones.

3. Nerve-cells are distributed diffusely in all the epithelia of the body, with certain exceptions such as the intestine, gills, coelomoducts, and the non-glandular areas of the abdomen of some forms. Both sensory and connecting and possibly also motor neurons occur here; but the sensory cells greatly predominate, often outnumbering all other epithelial cell-types combined. However, but one morphologic type of sensory cell—a true primary sense-cell—and no sense organs seem to be present. The thesis of Hanström is borne out that the low order of complexity of the nervous system as a whole is correlated with a low order of development of sensory structures. This in turn is correlated with a sluggish bottom living habit of life.

4. The nervous tissue is shown to be conspicuously undifferentiated. All nerve-cell processes are alike and resemble the most primitive nerve-fibres. A single exception is formed by the giant nerve-cell fibres, of which a few dozen exist in the nerve-cords. The absence of strata, tracts, and special neuropile-like regions as well as of elaborate nerve endings, ganglia, nerves, and 'nuclei', is impressive. Following the neurologic principle that complexity of function is reflected in complexity of structure, this is taken to mean a low degree of functional specialization.

5. Indications of several kinds agree in suggesting that the relations between neurons are something other than proto-
plasmic continuity. In the sense that nerve-fibres from different neurons are discontinuous the enteropneust nervous system is tentatively to be regarded as synaptic. Experimentally, however, the plexus has been shown to function as a nerve-net. It is proposed that such physiologic behaviour be taken to indicate a net in the sense of diffuse conduction, but that it does not predicate anatomical continuity of the fibres of the net. Such a picture requires the assumption of unpolarized synapses and the facts derived from the present organisms are taken to be evidence for this assumption.

6. Other primitive characters are described. The synapses are unlocalized, being scattered throughout the plexus. No special structural modifications have been developed at the synaptic endings. Connexions with the interior across the limiting membrane, heretofore unknown, are astonishingly difficult to demonstrate, but they must be assumed to exist and evidence is accumulated that they are diffuse. The widely scattered sense-cells, synapses, ganglion cells, and connexions with the interior are correlated with, and account for, the experimentally demonstrated autonomy of small pieces of the body-wall.

7. The general plexus is locally thickened and modified (1) in the cords of the mid-dorsal and mid-ventral lines of the trunk, (2) circularly around the junction of the collar and trunk, (3) through the dorsal collar coelom as an internal, primitively hollow, medullary strand, and (4) on the dorsal side of the peduncle. These are primarily conduction paths and are only secondarily important as ganglionic or modifying regions. The ventral cord in the trunk is shown to be larger and more important than the dorsal. In the sense of an organ which is involved in all reflexes, which contains all the intermediate neurons, and to which pass all sensory nerve-fibres, the balanoglossid has no central nervous system.

8. Internal to the limiting membrane no concentrations of nervous tissue are known with certainty to occur. No nerves, ganglia, or layers have been developed. As yet inadequately demonstrated, the internal nervous system can at most be only a sparse and diffuse system of cells and fibres communicating
across the limiting membrane with the superficial plexus, at the least nothing but motor axons passing from cell-bodies in the integument inwards to the muscles.

9. The histologic evidence supports the previously demonstrated physiologic picture placing the Hemichordata in respect to the level of complexity of the nervous system below all other groups of animals with nervous systems except the coelenterates and ctenophores. No evidence is adduced that this primitiveness is secondary rather than original. In numerous histologic respects the enteropneust nervous system resembles that of Echinodermata and Phoronidea, but is simpler than either.

10. The chordate affinities of the balanoglossids are here accepted. But the strength of the argument from the nervous system is considered to have been overdrawn. No aspect of the general picture of primitiveness now demonstrated is, however, considered to argue against these affinities.

LITERATURE CITED.


—— 1885.—“Later stages in the development of Balanoglossus Kowalevskii, with a suggestion as to the affinities of the Enteropneusta”, ibid., 25 (Suppl.).

—— 1886a.—“Later stages in the development of Balanoglossus Kowalevskii, and morphology of the Enteropneusta”, ibid., 26.


Semi-diagrammatic representation of an enteropneust of the genus *Balanoglossus*.

Fig. 1.—Anterior end extending into the branchio-genital region.

Fig. 2.—Posterior end of genital region and transition to hepatic region.

Fig. 3.—Post-hepatic abdomen. This is swollen, as shown, in some species, but in many it tapers and exhibits glandular islands separated by non-glandular epithelium.
nerve, abdominal region; b, buccal cavity; bc, branchial chamber; bd, 'branchialdarm', upper portion of pharynx; c, collar; cc, coelom of collar; enc, collar nerve-cord; cv, cardiac vesicle; dnc, dorsal nerve-cord; dr, dorsal root; dv, dorsal vessel; g, gonads; gl, glomerulus; go, genital opening; gp, gill-pore; gr, genital ridge; gs, gill slit; gw, genital wing (pleura or ala); h, heart; hc, hepatic coecum; hr, hepatic region; lm, longitudinal musculature of trunk; m, mouth; nd, 'Nahrungsdarm', ventral portion of pharynx; ns, nuchal skeleton; p, proboscis; pc, perhaemal coelomic pouch of trunk coelom; pm, proboscis musculature; pp, proboscis pore (a coelomoduct); s, stomochord ('notochord') with cavity opening into buccal cavity; t, trunk; te, coelom of trunk; vnc, ventral nerve-cord; wi, wall of intestine.

Plate 3.
Photomicrographs of sections prepared by the Bouin's-nitrocellulose-Masson technique.

Fig. 4.—Mid-sagittal section through anterior end of trunk, collar, and base of proboscis of Saccoglossus pusillus, a species without continuous axial canal. Dorsal to the right, anterior end up. ×33.

Fig. 5.—Dorsal part of similar section of Ptychodera bahamensis, a species with continuous axial canal (interrupted in this section near the posterior neuropore by a bend of the body which makes the lower part of the figure slightly parasagittal). Dorsal to the left, anterior end up. ×30.

Fig. 5a on overleaf, mid-sagittal section of anterior end of Ptychodera showing portion drawn in Fig. 5.

Fig. 6.—Transverse section through the collar of Balanoglossus occidentalis. ×12.

Fig. 7.—Transverse section through the anterior (branchiogenital) region of the trunk of Balanoglossus occidentalis. ×12.

Plate 4.

Figs. 8, 9, 10.—Bouin's-nitrocellulose-Masson; fig. 11, Zenker-nitrocellulose-Koneff.

Fig. 8.—The ventral nerve-cord in a transverse section of the branchiogenital region of Balanoglossus occidentalis. Dorsal to the right. ×114.

Fig. 9.—The dorsal nerve-cord in the same section. ×114.

Fig. 10.—The dorsal portion of the peduncle of the same animal in transverse section. ×110. Fig. 10a on overleaf, transverse section of peduncle.

Fig. 11.—Dorsal portion of transverse section through the proboscis of Saccoglossus pusillus, showing the dorsal cord of that region. The proboscis complex is also shown. Dorsal upwards. ×130.

Plate 5.

Figs. 12, 13.—Bouin's-nitrocellulose-Masson; fig. 14, Flemming's strong with acetic-nitrocellulose-Masson.
Fig. 12.—The collar nerve-cord in a transverse section of Balanoglossus occidentalis. The dorsal mesentery of the collar coelom, with small blood-vessels separating its two lamellae, is attached asymmetrically in this section. The great dorsal vessel is just ventral to the nerve-cord. Longitudinal muscles occupying the perihemal extensions of the trunk coelom are seen at right and left. A giant nerve-cell fibre is emerging from the cellular portion of the cord at the left to run medially in the fibre layer. The lacunae are distinguished from other white spaces in the cellular portion (glands) by their dark-stained lining (basal granules) and the cytoplasmic fibres radiating from them. ×150.

Fig. 13.—A dorsal root in a similar section of the same animal at another level. The section passes through the centre (antero-posteriorly) of the root only ventrally, near the nerve-cord. Here it can be seen that most of the root consists of ‘cellular’ material, only a thin nerve-fibre layer occupies the periphery. Dorsally the root communicates with the superficial epithelium. Many small and only virtual lacunae can be seen in the cellular region. ×80.

Fig. 14.—Giant nerve-cell in the collar cord of Saccoglossus pusillus, transverse section. The single process dichotomizes and the two fibres pass into the fibre layer. A dorsal ‘crest’ is also shown connecting the cord and the overlying epithelium. Flemmings’ strong with acetic-Masson. ×570.

Plate 6.

All figs.—Bouin’s-nitrocellulose-Masson.

Fig. 15.—Transverse section of the body-wall in the floor of the hepatic region of Ptychodera bahamensis. The outer epithelium above, the gut below. The trunk coelom is occupied by muscle-fibres. Ciliary rootlet cones are suggested in outer epithelium. The zones of the epithelium, the segregation of nuclei, the supporting fibres and, in the gut epithelium, the absence of a visible nerve layer are shown. ×400.

Fig. 16.—An oblique section through the proboscis epithelium of Ptychodera bahamensis to show how the limiting membrane loses the appearances of a sharp boundary. The short, heavy, dark-staining fibres immediately inside the limiting membrane are the circular muscles. ×450.

Fig. 17.—Mid-ventral epithelium at the base of the proboscis, in sagittal section, Ptychodera bahamensis. Anterior to the left. Note the sudden transition from a highly nervous epithelium to the left (extends beyond the margin of the figure for some distance, slowly thinning to the general average of the proboscis epithelium) to a glandular and less nervous epithelium to the right. The same region is shown at lower magnification in the upper right corner of fig. 5. The horizontal fibre layer at the right and the sharp limiting membrane separating the nerve-fibre layer from the coelomic lining are well seen. The figure also shows the ‘pre-oral ciliary organ’ of Brambell and Cole, represented by the region of longer cilia. ×415.
NERVOUS SYSTEM OF ENTEROPNEUSTA

PLATE 7.

All figs.—Susa-nitrocellulose-protargol.

Fig. 18.—Portion of the peduncular nervous concentration of Saccoglossus pusillus stained for nerve fibres. The surface of the epithelium is at the right. Note sensory fibres leaving the nuclear zone to enter the plexus, and the negative images of radial supporting fibres. ×750.

Fig. 19.—The same at higher magnification. Epithelial surface up, ×2,000.

Fig. 20.—The nerve-fibre layer in the proboscis epithelium. Nuclei of epithelium above; below, the proboscis coelom occupied by muscle-fibres (dark-staining bodies about the size of nuclei) and an argentophilic network of uncertain nature. The limiting membrane is unstained but lies immediately below the nerve-fibre plexus. In this field of view could be seen numerous thornlike nerve-fibres apparently penetrating the limiting membrane, but they cannot be traced with certainty to the muscles or into the argentophilic network of the coelom. This is the only anatomical evidence for communication between the nervous system on one side of the limiting membrane and the muscles on the other. The limitation imposed by the narrow focal plane of the photograph is particularly acute in this situation. A swollen goblet gland in the epithelium. ×2,000.

Fig. 21.—Another and more typical field of view in the same section, showing no apparent crossing of the limiting membrane. ×2,000.

PLATE 8.

Figs. 22-31.—Sketched from still living specimens of Saccoglossus pusillus stained with methylene blue. All ca.×1,500.

Figs. 22-5.—Primary sense-cells which stand perpendicular to the surface of the epithelium and reach or nearly reach that surface. The thick distal process may be two or three times as long as shown or apparently absent (fig. 24).

Figs. 26 and 27.—Indifferent ciliated epithelial cells which are occasionally stained. Note multiple and branched end feet, corresponding to the radial supporting fibres.

Figs. 28-30.—Various sorts of bipolar neurons seen deep in the epithelium parallel to its surface. Note intracellular fibrillae.

Fig. 31.—A multipolar neuron, likewise found at the base of the epithelium. Note that all processes are alike and are not dendritic.