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Part I.—Original Articles.

NEUROGENESIS AND THE DEVELOPMENT OF "SYNAPSES", WITH PARTICULAR REFERENCE TO THE CONDITIONS IN *LEPIDOSIREN PARADOXA*.

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INTRODUCTION.

SINCE the appearance of von Baer's work on animal development in 1828 (1), the mode of origin and development of the nerves has been continuously under discussion. A vast number of observations has been made concerning the visible evidences presented by fixed and sectioned material, and very many experiments have been performed to elucidate the nature of the processes at work. Yet no account of nerve development has so far received general assent, and for some time the advantages ensuing from the adoption of an agreed opinion appear to have been allowed to work in favour of its acceptance. These advantages have been found to be academic rather than real. While the formulation of hypotheses is not a proceeding which can be stigmatized as unscientific or unsound, in the absence of useful developments their retention discourages inquiry. This discouragement is the more likely to occur when, as in neurology, more than a century of effort by many workers has not succeeded in establishing beyond question facts which are of fundamental importance. It is a reason why the central problems of neurogenesis have come to occupy a field not wholly favourable to fruitful discussion.

This is the more to be regretted because many of the highest practical interests of Man, as well as many theoretical interests, lie in the acquisition of greater knowledge of the nervous system, and any flaw in the foundations of neurological science shows itself in a retardation of the rate of advancement along all but a few isolated lines.

In these cicumstances no apology is needed for the publication of fresh

evidence bearing directly upon matters of fact, or upon accepted interpretations of accepted facts. One has only to link the names of Lugaro, Kraepelin and Freud to realize how discontinuous has been the approach to neurological and psychiatric problems during the present century. Associated with this lack of continuity has been not merely disregard of false and insufficient foundations, but impatience with the necessary work of finding right foundations.

The investigations recorded in the present communication were pursued in consequence of a chance observation that, in developing larvæ of the Salmonidæ, that part of the somatopleure which invests the ectodermal yolk-sac is richly supplied with nerves before the complex changes in the neural tube, leading to the formation of the " nerve-cells " of histology, can properly be said to have advanced beyond a purely preparatory stage of development, and while even the transient dorsal ganglionic cells of Rohon are themselves only in process of formation. The connection between these nerves and the permanent nervous structures formed later, and the general arrangement of these connections in relation to the developing myotomes, suggested that His (2) was right in attaching particular importance to that stage in the development of the peripheral nerves and their central connections in which " neuroblasts " become distinguishable in the general " spongioblastic " reticulum of the neural tube. This stage, which some writers omit altogether from consideration, supervenes upon that during which, as Graham Kerr (3) has described, neural tube and myotome, still closely applied to one another, are connected by protoplasmic bridges representative of the motor nerves of later development. If at this stage structures which are clearly identifiable as nerves are already present in those parts of the developing organism farthest removed from the neural tube, it cannot be gainsaid that such structures, however they come into being, are *not* the product of histological changes still to become apparent in the tube itself. In these altered circumstances, the changes themselves are deserving of re-examination to ascertain, if possible, what place may properly be accorded to them in the elaboration of the " neurone " of histology, in default of the neurogenetic role which they have been thought to play.

Thus the genesis of the histological " neurone " is to be sought in a comprehensive survey of the whole neural apparatus during a succession of stages, few in number, before, during and after what may be termed the " neuroblastic " stage of His. It is the described features of these stages which merit particular consideration. While the nervous system of vertebrates presents during development an appearance which differs from type to type, this difference is remarkably less in early than in later stages, and at all times is confined to points of minute detail which do not affect the order or the nature of the visible changes fundamentally. Thus there is great difference in the number and the size of the nuclei, and in the mass of the fibrillar material deposited in relation to the nuclei to form later the clearly differentiated neurofibrillæ which occupy most of the space commonly called the " cell-body ", and, in the peripheral

nerves, are closely packed together to form axis cylinders. The nameable parts of the "neurone" are uniformly present at all events in corresponding situations, and vary only slightly in form and appearance. Motor nerve-endings show great variation in shape if a comparative series is studied, but little or none in other respects; while similarly the great expansions of fibrillæ which occur in close relation to the voluminous "cell-bodies" of central and peripheral ganglia vary in pattern. But the order of events (by which is meant the occurrence of visible and recognizable details of minute structure) is the same in all vertebrates studied.

The early appearance of nervous structures at situations as far as possible removed from their supposed site of origin (i.e., the neural tube) was first observed in the salmon, which, although particularly suitable for study in the living condition stained by methylene-blue, is unsuitable for the conduct of close observation of its central neural histology during development, as its cellular elements are of very small size. The systematic examination of the embryos and larvæ of a large-celled creature stained by a modern silver method (Bielschowsky's) was therefore undertaken to ascertain whether the nerves observed in the somatopleure of the salmon could be demonstrated in it, and if so, what was the histological condition of the neural tube at the time and before the time of their appearance. Fortunately, *Lepidosiren paradoxa* responded very favourably indeed to the chosen technique. It possesses exceptionally large cellular elements, and it has the added advantage that it belongs to one of the two archaic groups (Elasmobranchii and Dipnoi) in which early continuity between the early myotome and spinal cord has been demonstrated. It was in *Lepidosiren* that Graham Kerr observed the protoplasmic bridges which unite these structures while they are still closely applied to one another. It is of importance to ascertain how the formation of neurofibrillæ is related to these simple structures. According to Held (4), the plasma bridges when first visible already form so extensive and intricate a network as to baffle any attempt to determine the cells from which they arise. The form in which this opinion is expressed draws attention to the plasma network as a morphological entity. The neurofibrillæ which traverse the network constitute another problem, and since it is the contention of the present writer that the recorded facts of neurogenesis are not so disharmonious as is supposed, but are capable of orderly presentation if due attention is paid to the order of events in the organism as a whole, it is essential that each of the processes known to be involved in neurogenesis should be studied in its entirety as far as possible, as well as locally in relation to other possibly more spectacular changes. This is possible in *Lepidosiren paradoxa*. It is not possible in the Salmonidæ or in avian or mammalian examples, although in the light of what is discoverable in *Lepidosiren*, many features of nerve development in other types, including Man, acquire intelligibility. For this reason, embryonic material of *Lepidosiren paradoxa* was chosen for the

purpose of systematic study. Several larvæ were prepared in bulk by Bielschowsky's method, and some by the ordinary methods of histology, and in addition the extensive resources of the Zoology Department of the University of Glasgow, in respect of embryonic material of *Lepidosiren*, *Polypterus* and *Ceratodus*, were made freely available through the kindness and generosity of Prof. Graham Kerr, affording greatly extended scope for observation and comparison.

The statement has been made above that the genesis of the histological " neurone " is to be sought in a survey of the whole neural apparatus during a brief succession of stages in development. It is of great importance, in the first place, to gain as complete and detailed knowledge as possible of the distribution of the plasma in which the neurofibrillæ appear. The most characteristic features of nerve-cells are those which are dependent upon the process of neurofibrillation, and it is a matter of considerable importance, but one of great difficulty, to distinguish the nerve-cell apart from these special features.

The significance of the protoplasmic bridges described by Graham Kerr lies in the fact that these " representatives " of the neuromuscular connection are *not* bundles of neurofibrillæ. Nor are they the processes of cells visible in the neural tube. In their earliest condition they are merely tiny cylinders of protoplasm uniting the protoplasm of the neural tube with that of the myotomes. How they come to be replaced by axis-cylinders or occupied by them is the central problem of neurogenesis. They deserve consideration apart from this process, and since they are not alone in being the site of the appearance of neurofibrillæ, they deserve consideration in relation to other parts with which they are connected, the tube and the myotomes. The bridges are, if not " pre-neural " in a functional sense, " pre-neural " in a histological sense. But they are not alone in this respect ; the structures which they connect are also in this " pre-neural " stage, and it is important that these structures should be considered as a whole before considering the changes which occur in the various parts. If this is done, features which possess great morphological significance reveal themselves unexpectedly. In setting out the results of the present inquiry this plan will be followed, and the " pre-neural " formation will be described before any attempt is made to describe the process of fibrillation. That process is confined to the protoplasm of the " pre-neural " formation. It will be shown later that the general morphological pattern of the nervous system, regarded in the light of neuro-fibrillar distribution, is *not* the same as that of the " pre-neural " formation. Although, spatially, the two systems coincide, the direction, extent and apparent independence of the neurofibrillæ lead us to regard the system to which they belong in a way which (however natural it may seem to the vertebrate morphologist) would have appeared highly artificial if we had knowledge only of the material in which the neurofibrillæ were formed. In the light of the functional significance of neurofibrillation, it is probable that of the two morphological interpretations

which are thus possible, that which may be placed upon the distribution of the " pre-neural " formation is the more fundamental. The implications of this statement as they affect both the " pre-neural " formation and the adult nerves will be clearer when some of the fruits of inquiry have been set out.

The Pre-neural Formation.

Coghill (5) remarks that " the embryo is perfectly integrated before it has a nervous system ". Between an earlier " non-nervous and the later nervous condition of the embryo there appears superficially to be a hiatus in development, a point where one condition ends abruptly and something entirely different takes its place ". But although the " pre-neural integrating forces " of the individual antedate the neural functions subserved by various conduction paths, this " is not true for the nervous system as a whole. . . . The pre-neural system of integration . . . overlaps the neural in the course of development ". Coghill believes the establishment of integrated neural function to be dependent upon the development of a patterned system of nerve-cells. " This pattern, in the nervous system, is established in its main outlines before nervous function, excitation or exercise begins ". Nevertheless, there is a stage in *Amblystoma* in which " the animal can contract its muscles but cannot do so in response to the stimulus of light touch upon the skin ". At this stage " about twelve of the anterior muscle segments " possess " motor nerve-roots " which are " naked protoplasmic threads that grow from nerve-cells in the spinal cord to the middle of the muscle segment ". The genesis of the " naked protoplasmic threads " may be omitted from consideration for the present. There are such threads, and they are present during the stage of development which precedes Coghill's stage of integrated neural function. Stewart Paton (6) also observed, in early larvæ of *Torpedo*, " definite movements of abduction and adduction " when the only possible paths for conduction were undifferentiated strands of protoplasm. Coghill, it is true, clearly regards these strands as the processes of cells, but assuming the strands he describes to be of the same nature as those described by Graham Kerr, Held and Paton (an assumption which is in conformity with the circumstantial evidence he supplies), it may be pointed out that his ascription of a cellular condition to the spinal cord at this stage is discordant. It is important to recognize that Coghill (whether rightly or wrongly is a matter for later discussion) is one of those writers who omit all consideration of the syncytialization on the neural tube to which His attached so much importance. While it so happens that these " threads " (Coghill) or " strands " (Paton) or " protoplasmic bridges " (Graham Kerr) are " pre-neural " in Coghill's sense (i.e., earlier in their appearance than integrated neuro-muscular action and the neurone patterns which subserve it), they are also pre-neural in the sense that in *Lepidosiren*, *Pristiurus*, *Scyllium*, *Torpedo*, and other examples—possibly in all vertebrates—neuro-fibrillation is a process

which occurs *within* them after their formation: they are not formed *by* the extension of neurofibrillæ. They are " pre-neurofibrillar " strands. It was Paton's opinion that neurofibrillation began about the same time in (1) Beard's cells, (2) the point of exit of the ventral root, (3) the strands, and (4) the myotomes. The situations in which neurofibrillation occurs thus constitute an integral part of the organism, widespread but interconnected; and they should be considered as a whole. In the past this has not been done. The event of neuro-fibrillation is so spectacular that it has captured the whole attention of the observer. But the systematized strands and larger masses of protoplasm in which neurofibrillation may be seen to occur may be considered without reference to this change of which they are the site, and if this is done, facts of great interest reassert their claim upon the attention. The cord, the myotome and the embryonic ectoderm (in relation to which important sensory structures are well known to arise) are all sites for the supervention of neurofibrillation. It is the writer's intention to treat them as a whole. First, however, it is necessary to examine this whole with reference to its appearance in different situations.

The Motor Nerve-trunk Rudiment.

Little can be gained from the study of stages of *Lepidosiren* earlier than Stage 24 (Keibel) (7). The demonstration of Graham Kerr's protoplasmic bridges at this stage is dependent upon the use of special methods to separate the myotomes from the cord to which they are closely applied, thus stretching the communications, the existence of which could not otherwise be detected. The result of this stretching is to tear the fragile nerve-rudiments more caudally placed—i.e., those less developed. In addition to there being thus a limitation to the use of the method, it discloses no more than the early presence of the bridges, which are studied more conveniently at later stages. At the same time it is worth while to quote some sentences from Graham Kerr's classical description of the conditions at this early stage (Stage 24): " The rudiments of the motor trunks already exist as soft, thin bridges metamerically arranged and connecting spinal cord rudiment and myotome. . . . The nerve rudiments at this early stage are formed of granular protoplasm either without yolk or containing only very minute granules, without obvious fibrillar structure. . . . The rudiment is quite naked. . . . Nor are there any nuclei in contact with [it] . . . The nerve rudiment . . . is at first extremely fragile, gradually becoming tougher as development proceeds. . . . A point . . . difficult to observe in the uninjured condition [is] that the protoplasmic mass forming the nerve rudiment spreads out over the inner face of the myotome. How far this expansion extends, whether—as is possible—it really covers the whole face of the myotome, is a point almost impossible to decide definitely by actual observation. Similarly I am deterred by the unreliability of observations made on a spinal cord so laden with yolk in its early stages from saying

anything as to the connections of nerve rudiments with neuroblasts or other cellular elements in the substance of the spinal cord. At this stage the spinal cord is without any obvious mantle of fibres ". Elsewhere in the same paper Graham Kerr writes of the bridges as connecting " the *substance* of the medullary tube with that of the myotome ", and while this is unexceptional, it is one of the matters for further elucidation.

At Stage 25 the bridges are visible as short straps. No artifice is necessary at this stage to separate the myotomes from the cord and so to reveal the presence of the minute connections, which, however, in the absence of stretching, show fine striation parallel with the long axis of each bridge. Very small granules of yolk are present therein, but there is still no sign of a sheath. At the outer end the bridges are seen in sections to spread out fan-wise, with corresponding splaying-out of the striæ, which are lost to view among the yolk-granules and septa of the myotomes. Since scepticism is still entertained concerning the nervous nature of the bridges, the following facts may be given to assist the reader to come to a conclusion on this point :

(1) The bridges occur bilaterally and regularly in series.

(2) Each bridge is attached to corresponding situations of both cord and myotome, the ventro-lateral angle of the former and a ventro-medial prominence of the latter, constantly present, jutting out towards the surface of the cord from a constant position near the junction of the anterior three-eighths with the posterior five-eighths of each myotome.

(3) Where the dorsal root-rudiments can be detected (see p. 460), they are like the ventral roots in structure and alternate with them in position.

(4) Both cord and myotome exhibit cytological peculiarities in places which correspond regularly with the site of attachment of the bridges. Each myotome has roughly the form of a cylinder closed at each end, the nuclei being congregated nearer to the collapsed, slit-like lumen than to the periphery, which is free from nuclei. This is more obvious in the thick inner layer than in the thin outer, or " dermal " layer. Regularly, however, the nuclei approach the surface of the myotome in the neighbourhood of the bridge attachment. The bridges form a definite series, then, corresponding to metameric segmentation. The conditions are illustrated in the accompanying diagram drawn from alternate sections of a larva at Stage 25. The dorsal attachment there shown is not necessarily representative of dorsal bridges. Such approximations of cord and myotome, which are common, make identification of the dorsal nerve-rudiments difficult.

In the spinal cord a corresponding peculiarity in the arrangement of the nuclei is to be made out. The central canal is surrounded at regular intervals by a cuff of nuclei. Karyokinetic figures, when present, occupy a narrow inner band of this cuff, and between one cuff and the next, extending to the periphery, is a nuclear-free zone, from the greatest expansion of which the bridges arise. Graham Kerr (7) gives the number of metameric segments at Stage 25 as "about 47 ". The counting of the bridges at this stage is attended with some difficulty because of the tail curvature. In this region it is not easy to be quite sure that some of the bridges (which here are relatively shorter and thicker than elsewhere) have not been counted twice. The figures obtained by the present writer for

one larva were 51 on the right side and 54 on the left. When due allowance is made for errors of working and individual variation this correspondence is sufficiently close to be conclusive. It is to be noted that the first spontaneous movements have been observed two days before hatching, which occurs at Stage 27.

The gradual acquisition of root characters in the situation of the bridges is, of course, additional evidence of the significance of these structures; but in the

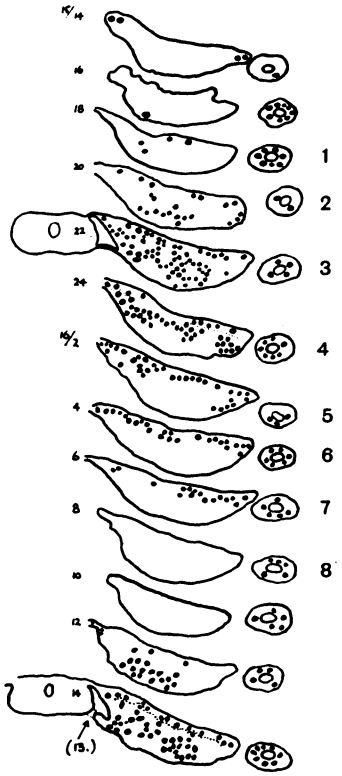

DIAGRAM 1.—Arrangement of nuclei in alternate myotomes of *Lepidosiren* at Stage 25. The spinal cord is shown where the neural bridges join it to myotomes. The archinephric duct lies to the right. The large figures, 1–8, cover one segment.

light of what has been said it does not seem possible any longer to set them aside as artefacts or the results of chance adhesion of myotome to spinal cord.

If it can be shown that at these (or later) stages of development the bridges are not constituted from " the processes of cells which have their location in the spinal cord ", the question of the precise time of origin of the bridges is irrelevant so far as the problem of neurogenesis is concerned, although the answer to it may be of interest for other reasons. It is important to realize

what the material point of evidence is. It is *not* the *ab initio* presence of a connection. It is the existence, before the differentiation of a histological nerve, of a connection between cord and myotome, which is the *site* of the development of the adult nerve.

At the same time fresh evidence is forthcoming from two sources concerning the time and mode of appearance of the bridges : (1) All parts of the embryonic axis do not develop at the same rate, retardation of the rate being marked at the tail end and also at the head end, where, however, it is subject to developmental features incidental to the special characteristics of this region. Since the bridges may be detected even in the most retarded region (the tail), it is to be presumed that their presence antedates the demonstration of them in the mid-region of the body. This is relatively a small point. (2) Systematic examination of larvæ at Stage 25 reveals that there are protoplasmic strands at the head end which closely resemble those elsewhere. *There is as yet no visible head myotome for the attachment of the lateral end of these bridges.* This is clearly a matter of importance in view of the characteristics peculiar to the mesoderm of the head-region, the complexity and importance of the cranial series of nerves and the special features of the bridges themselves.

It is impossible to elucidate the meaning of these bridges without first recalling the peculiarities of the head mesoderm.

In *Lepidosiren* (9) compact mesoderm in continuity with the lateral mesoderm enclosing the anterior end of the splanchnocœle (pericardial cavity) and the occipital myotomes appears in the head region. There is, according to Agar, no trace of segmentation in the head mesoderm in *Lepidosiren* at any stage, and at Stage 20 the mass is already becoming converted into loose mesenchyme. This process continues for the next three stages, the compact mesoderm disappearing with the formation of mesenchyme at its expense. Some fragments remain, presumably, and to these the later development of the ocular muscles is, in Agar's opinion, attributable. There is this reason for believing that regions corresponding with the somatic portions of the first three head somites of Balfour and van Wijhe are differentiated from one another. Posteriorly to the otocyst there are three occipital myotomes (*x*, *y*, and *z*), as in *Protopterus*. The anterior of these (*x*) disappears entirely, and its nerve, according to Agar, has " not been found in any specimen ", the hypoglossal plexus being formed from *NN y.z.* 1 (ventral roots only).

Not only does the anterior end of the neural tube at Stage 25 show the variations in the diameter of the lumen which have been supposed to indicate " neuromery "—this view will be subject to discussion later—but a characteristic distribution of nuclei and mitotic figures can be made out as in the mid-body region and, preserving the series of relationships described for the bridges elsewhere, *three pre-otic* bridges can be seen to originate from the neural tube. The first arises from the ventro-lateral aspect of the brain-stem immediately behind the optic rudiment, the third in the cleft between the otocyst and the

brain, passing in front of the expanded part of the former structure, while the second is intermediate.

Since myotome x is transitory, it is not possible to identify the members of the series of nerves, x, y, and z, 1, 2, 3, etc., from their situations alone. In the preparations studied the fourth bridge may belong to myotome x or to myotome y. If $x = 4$, then $z = 6$; and since the sixth bridge is accompanied on the left side by evident traces of a dorsal root (which should not appear, according to Agar, until later in the series), it seems that the arrangement of the rudiments of the nerves (the writer would prefer to say, "rudiment of the nervous system") are more "phylogenetic" than in the adult. Moreover, the "bridge" immediately posterior to the otocyst is very close to it, and corresponds to an expansion of the lumen of the neural tube placed regularly in position in series with the rest. It is probable, therefore, that bridge No. 4 represents the motor root of $N\,x$. Owing to the flexure of the brain in this region, the plane of section is not favourable to the identification of dorsal rudiments in front of z (the sixth bridge). The peripheral ends of these bridges are lost sight of in the loose mesenchyme which has resulted from the proliferation of the compact mesoderm of the head. This process is completed at Stage 23. The earliest stage at which bridges have been demonstrated is a stage later. We must go back at least three stages to find conditions comparable, in respect of the mesoderm of the head, with those of the mid-body region and the complete bridges connecting cord and myotomes of Stage 24. All these facts point to the early existence of the bridges, and it is of particular interest to notice that their existence is not inseparable from the presence of compact mesoderm. They are to be found in positions of morphological significance where there has been compact mesoderm at an earlier stage, and they remain in a diffuse form in the mesenchyme to await the reconstitution of the myogenic centres in the head.* It is unnecessary to suggest the inferences which might be drawn from these facts. The appearances described are figured in Figs. 1–3, the accompanying diagram affording a key to the interpretation of the drawings.

The Dorsal Root Rudiment.

Identification of the ventral nerve rudiment is relatively uncomplicated. Each rudiment has to bridge the small intervening space between cord and myotome, and although this space increases and comes to be occupied by other structures (e.g., blood-vessels) besides the rudiments, it is relatively clear and well defined by its boundaries.

The conditions in which the dorsal rudiments have to be sought and found and their connections made out are totally different. During the stages under consideration the spinal cord is still continuous with the ectoderm, and the

* From the point of view of the interpretation of visible evidences provided by sections of higher vertebrates, it is very desirable that the significance of this observation should be recognized.

FIG. 1.

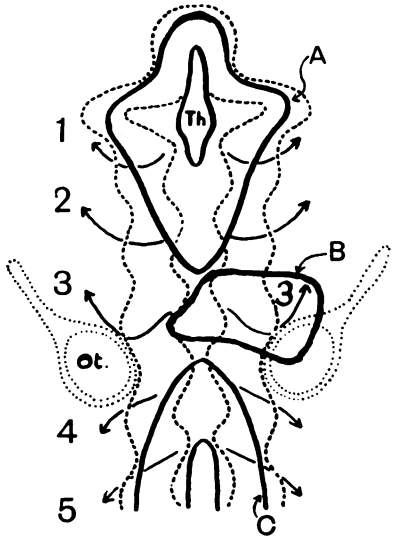

DIAGRAM.

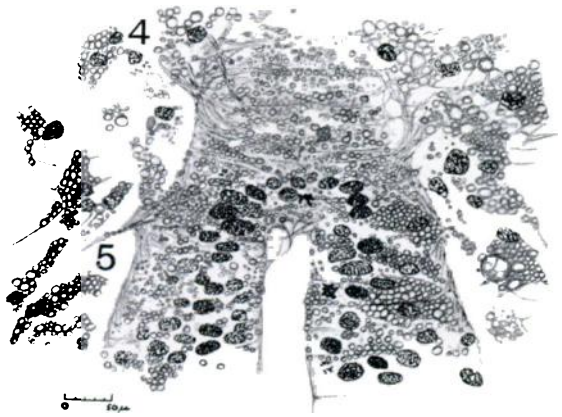

FIG. 3.

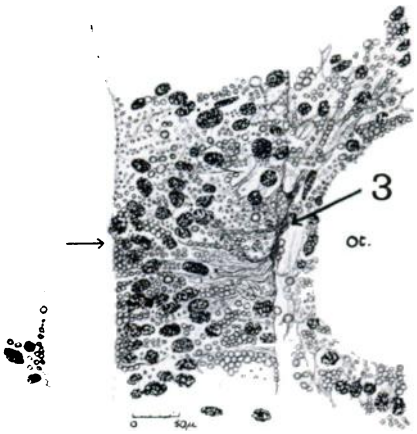

FIG. 2.

FIGS. 1–3 AND DIAGRAM.—Brain of *Lepidosiren paradoxa* at Stage 25, stained hæmatoxylin and eosin. Protoplasmic bridges uniting the brain with the mesoderm of the head in front of and behind the otocyst. The diagram shows the formation of the neural tube at this stage, and the relationship of the neural bridges, numbered 1–5, the continuous black lines indicating the regions covered by the sections illustrated.

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dorsal edges of the myotomes encroach upon the line of union of the two. Tissue-shrinkage is particularly liable to occur in this situation, and results in fissuring and deformation of the parts, with rupture of the slender nerve rudiments. Moreover, the course of the dorsal nerve rudiment is interrupted by the presence of a root ganglion, and the whole apparatus, as Miss Ballantyne has pointed out, is of such a length as to make it almost impossible to find it displayed in its entirety in a single section. But there is still another source of difficulty. The dorsal ganglion itself is not easily recognizable in early stages. Thus Ballantyne (10) writes that " at Stage 30 the ganglion is much less definite, being represented by a somewhat scattered mass of cells among which there is a lot of yolky material. At this stage it is already possible to trace a connection betweeen the ganglion and the motor nerve ". At earlier stages this connection is represented only by " an aggregation of yolky material ". The ganglia are then " even more diffuse ". It was Balfour's view that the two roots of each spinal nerve were " at first quite unconnected with each other " (*Torpedo*).

Why is it not possible to observe the connection with the ventral nerve rudiment in early *Lepidosiren ?* I suggest that so far from the reason being the initial separation of these structures, it is because we entertain an erroneous opinion concerning the location of the dorsal ganglion during early stages. To observe the early form and appearance of the dorsal ganglia is admittedly so difficult that the origin of the cells constituting it was long in dispute. Even if this matter is held to be satisfactorily settled (11), the intimate association of the two roots in a common trunk in a constant position relatively to other structures (e.g., the *ramus superficialis lateralis vagi*) is a matter still unexplained. Indeed it does not seem to have occurred to morphologists that explanation is necessary. Yet a fundamental fact of morphology which is best illustrated by reference to the mode of development of the dorsal root rudiment underlies the phenomenon. An excellent starting-point from which to approach this problem is Balfour's description of the conditions under which the lateral line nerve develops in *Scyllium canicula*. The diagnostic features upon which Balfour relied for the identification of this nerve are clear from his statement that the lateral line nerve " rapidly thins out posteriorly and also approaches closer and closer to the lateral line. At the front end of the trunk it is quite in contact with it, and a short way behind this region the cells of the lateral line arrange themselves in a gable-like form, in the angle of which the nerve is situated. In this position the nerve, though small, is still very distinct in all good sections, and is formed of a rod of protoplasm, with scattered nuclei, in which I could not detect a distinct indication of cell areas. The hinder part of the nerve becomes continually smaller and smaller, without, however, presenting any indication of becoming fused with the epiblast, and eventually ceases to be visible some considerable distance in front of the posterior end of the lateral line ".

It seems fair to assume from this passage that Balfour was looking for the

detachment of a *chain of cells*, or at all events a nucleated rod of protoplasm, from the ectoderm before he was willing to assent to the view that the lateral line nerve originated directly from the ectoderm. He admits that the position of the nerve affords a presumption in favour of this origin, but proceeds to give three reasons against it. The first of these is that the front part of the line is undoubtedly supplied by the vagus, "and we should not expect to find part of the lateral line supplied by nerves which originate in one way, and the remainder supplied by a nerve having a completely different and abnormal mode of origin". It is hard to understand what Balfour had in his mind in presenting this argument. The second reason is that the nerve arises subsequently to the lateral line, "so far as is shown by the inconclusive observations of my earliest stage", and never presents "any indubitable indication of becoming split off from this, or of fusing with it." The third reason begs the question by asserting that the cranial representatives of the lateral line are supplied with nerves which "originate in the normal way". It is now well known that neither the dorsal nor the ventral rudiments are nucleated, or "cellular" in their earlier stages. But nevertheless Balfour's researches are of interest because of certain features which he brought to light. In his view, the dorsal roots were cellular outgrowths from the dorsal angle of the neural tube. In the first place, "there arises from the spinal cord", he said, "a continuous outgrowth from which discontinuous processes (the rudiments of the posterior roots) grow out." Again, "before the close of Stage I each posterior root has a separate junction with the spinal cord. What then becomes of the originally continuous outgrowth? It has not been possible for me to trace the fate of this step by step; but the discovery that at a slightly later period (stage *K*) there is present a continuous commissure independent of the spinal cord connecting the dorsal and central extremities of all the spinal nerves renders it very probable that the original continuous outgrowth becomes converted into this commissure". Balfour points out that a similar commissure connects the vagus branches, and says that "both its appearance and history are very remarkable and deserve the careful investigation of future investigators. There can be little doubt that it is some sort of remnant of an ancestral structure in the nervous system". The structure, cellular at first, disappears at a later stage.

Allis (13) gives assent to the view that in Elasmobranchii the dorsal root of a cranial nerve grows forward and downward from the neural crest, towards a local thickening of the ectoderm to form the branchial sense-organs. At the level of the notochord, this nerve "fuses with the epiblastic thickening, a part of it, however, passing on as the future post-branchial nerve to the lateral muscle-plate of the segment. At the point of fusion cells are rapidly proliferated. The deeper part of the mass which thus arises is the rudiment of the ganglion of the dorsal root, and the superficial portion the rudiment of a branchial sense-organ. The deeper portion soon separates from the rest of the

mass, leaving a nerve strand connecting it with the sensory portion, and, lying deeper in the mesoblast on the root of the nerve, apparently at first distal to the point of separation of the post-branchial branch, becomes the ganglion of the nerve ".

This observation is valuable because it defines the relative position of the dorsal ganglionic rudiment in terms of the lateral line rudiment. The terms " fusion " and " mass " are worthy of notice in conjunction with Balfour's statement that the young nerve in *Scyllium* is a nucleated rod of protoplasm " in which I could not detect a distinct indication of cell-areas ". The difficulty of assigning boundaries to the cells of animals is notorious, but it is not a reason for assuming that cell walls are always present. The simple explanation that a wall which cannot be seen may be absent is worthy of consideration.

Miss Ballantyne (10), who worked out the early development of the dorsal rudiments and lateral line nerves in *Lepidosiren*, concluded that the developing dorsal ganglion " in passing ventrally to its adult position never loses continuity with the cord. The at first protoplasmic bridge, which is a continuous strand, not a chain of discrete cells, becomes fibrillated and lengthens out as the embryo grows in size, but it is quite distinct at each stage ". In the case of the lateral line organ, " as far back as Stage 23 the rudiment appears to be already present as a thickening of the ectoderm in the region where the otocyst is developing, and in some sections this thickening appears to be united to the brain by a nucleated mass of protoplasm, which represents the ganglion of the nerve. . . . At Stage 24 the connection between the organ and the brain is recognizably a nerve ".

Pinkus (14) described another nerve, the *ramus lateralis superficialis inferior vagi*, in *Protopterus*, which is present in *Lepidosiren*. It runs along the medial margin of the lateral muscle masses or is embedded in its edge. In the writer's opinion it is represented by the plexus uniting deep and superficial branches of the segmental nerves along the ventral edge of the myotomes in teleostean fishes, and the plexus of nerves within the anterior abdominal wall of mammals. It is this plexus which gives off the early yolk-sac nerves in the salmon.

It has been pointed out that in the mid-body region the conditions are unfavourable for the observation of the peripheral nerve-rudiments in their entirety. The ventral rudiment can be identified in practically every case on both sides (Stage 25), and in many cases the whole extent of the bridges can be seen in single sections. The dorsal rudiments are obscured by the myotomes. Nevertheless more favourable conditions are found slightly more anteriorly. There is a danger here that the rudiments discoverable may be those of cranial nerves and thus present some atypical feature. This cannot be the case in sections which contain the fifteenth or sixteenth ventral rudiments, even if the whole cranial series were held to be accounted for, and as stated above, this series at Stage 25 is represented by 3 plus 3 or 4 rudiments on each side.

Observations made at this level over a range of successive sections are illustrated in Fig. 4—a composite drawing of fourteen sections. The areas between the dotted lines indicate the corresponding areas of the sections (6μ) chosen for representation, *a–n*. A myotome fragment appears on section *c–d*; but elsewhere the sections are free from muscle, which shrinks from *x'* to *x* before the advance of the nerve. Thus only yolk-granules and some loose mesenchymatous filaments intervene between neural and embryonic ectoderm dorsally, while laterally the protoplasmic bridge becomes indistinguishable from the deep layer of the ectoderm. In the area *f–g*, a dorsally directed branch filament can be detected. Others can be seen directed dorso-medially from the " trunk " of the bridge in the area *a–b*. At *L*, the nerve terminates in the lateral line, and only minute fragments of the rudiment can be found more ventrally. They might belong to longitudinally directed parts of the complex, or to a branch proceeding to the situation of the rudiment (not identifiable at this stage) of the ***ramus inferior***. The more deeply placed and more massive branch running downwards across areas *b–c–d–e–f* undoubtedly re-establishes connection with a ventral protoplasmic bridge originating in the sections corresponding to the areas *f–g–h*. Obviously it is impossible to demonstrate the whole of this apparatus in a single section. But the extensive communication I have described is unquestionable if due heed be paid to the constancy of the relationships present. If the rudiment of what later on must become the " anterior " (ventral) primary division of the nerve-trunk is taken into account, the whole apparatus has roughly the form of a letter H placed upon its side. Not only in this region but in other regions every part of this pattern can be found in close association. Later their continuity is unquestionable, and constitutes a well-known fact of vertebrate anatomy. The striation rendered by pencil lines in the drawing is not indicative of neurofibrillation of the kind stainable by silver; nor, as can be seen from close examination of the spinal cord in the neighbourhood of the root, is it a sign of the attenuation of cells in the cord as they pass out into the root. The lines are present within the cord, and although the cell boundaries there are ill-defined peripherally, the lines are within the areas marked out by these septa, which are relatively coarse. Further, the lines are more numerous than would be the case were they the outlines of attenuated cells. This matter will be more fully treated when the cord comes to be considered.

The union of the portions considered takes place either on the surface of a myotome or in the cleft between neighbouring segments. It is to be observed that there is no identifiable dorsal root ganglion, and that the region of the apparatus in which it will appear is at this stage far dorsal and rather lateral in the right upper limb of the I. The vertical limb of this figure is the future connection between the dorsal and ventral roots, the left lower limb the " anterior primary division " of anatomy.

Not only are the lateral line organs at first represented by a continuous band

of thickening in the deep layer of the ectoderm, extending backwards no farther than the heart at Stage 29 (Ballantyne), but the backward development of the rudiment and its fragmentation into separate neuromast organs is associated with a corresponding liberation of the lateral line nerve from contact with it. In terms of the ⊥, this great longitudinal connection lies in relation to the left upper limb, while the dorsal ganglion (also a structure tending to continuity in a longitudinal direction) is first seen as an expansion of the right upper limb.

The myotomes during these early stages occupy situations internal to the lateral line nerve and its connections, including the dorsal root and its ganglion. *Without interruption of this formation*, the lateral line nerve comes gradually to occupy a position internal to the myotomes in immediate relationship with the notochord, the ventral primary divisions alone intervening ; while the dorsal root ganglion and its connections are displaced to their well-known positions between the myotomes and the spinal cord. It is of particular interest to observe how this mass movement of the whole system of nerve-rudiments occurs relatively to the myotomes. Wherever the conditions require it, the nerve-rudiments are drawn through the myotomes. The occurrence can be followed most clearly in the case of the lateral line-nerve itself. Even as late as Stage 33, the dorsal and ventral parts of the myotome are still connected together, the nerve lying laterally to the connection between them. But at this stage the myotomes are so deeply grooved on their outer side as to be almost severed, and at later stages they have been divided by the nerve on its relative change of position inwards to the side of the notochord. At the same time the attached parts of the ⊥ are drawn through the dorsal segments. It must be emphasized that before this severance the myotomes are complete and homogeneous structures with no sign of any longitudinal partition. They bend inwards against the resistant longitudinal nerve, retain their fibrillation to the last, and only break finally into two parts as the nerve cuts through them. The dorsal primary division arises from the nerve-root before it reaches the lateral line-nerve, which at later stages receives neuro-fibrillar communications from the root, and lies between its own connections with the surface organs and the lateral sensory branches.

There has been mentioned a ventral extension of the early nerve-rudiment in apposition to the deep layer of the ectoderm. It is impossible for many reasons to work out the development of this portion of the peripheral nerve-rudiment in *Lepidosiren*. In the salmon, however, owing to the ease with which the nerves related to the margin of the lateral mass may be stained by the introduction of methylene-blue into the body-cavity of the living animal(15), the nervous connections in this region are easily made visible. The accompanying diagram will serve to illustrate the conditions :

The line *M–M* represents the edge of the muscle sheet advancing to muscularize the abdomen as the yolk-sac is absorbed. Dorsally the mesoderm

of the somatopleure is differentiated into segments and these again into striped muscle. The edge is thin, being in places not more than one or two muscle-fibres thick. Below the shaded portion the mesoderm is not differentiated, and the mesenchyme of the somatopleure is not separated into segments. Here the layer is sparsely nucleated, and forms a mesoglœa rather than a true mesoderm. While some of the branches of the segmental nerves correspond accurately with septa between the myotomes, and run deeply to the continuous mesoderm, others cross the myotomes diagonally and are superficial in position. *The two sets join with one another to form the plexus along M–M.* This plexus is marginal in position in relation to the lateral mesoderm, and forms a longitudinal connection which approaches nearer and nearer to the mid-line as the muscle edge advances and the yolk-sac becomes absorbed. In other words, its position and

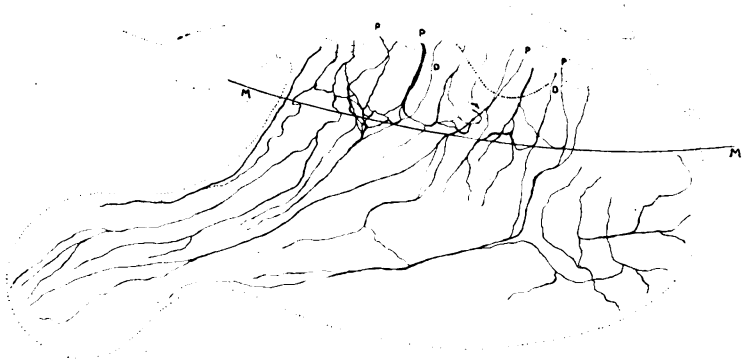

DIAGRAM II.—Nerves of somatopleure of salmon larva. MM = line of undifferentiated mesoderm advancing to enfold yolk-sac.

connections are identical with those of the *ramus lateralis inferior vagi* in *Lepidosiren* and *Protopterus*. A similar plexus is to be found even in man in the substance of the rectus abdominis muscle. In mammals it is a continuation of the phrenic nerve, which gives branches to the *vena cava inferior* and to the situation of the sino-atrial node of the heart. In *Lepidosiren* corresponding structures are connected with the nervous system through the inferior branch of the lateral vagus.(16) There are thus cogent reasons for the view that the rudimentary pattern of these nerves, whatever they may be called in different creatures, is fundamentally the same and has the form of a continuum, displaced and interpenetrated by the myotomes.

One may approach more closely to the heart of this matter by regarding it in the light of the phenomena of metameric segmentation as they reveal themselves in comparative anatomy. Metameric segmentation " is probably to be associated primarily with the cœlome and its lining the mesoderm "(17). As in *Polypterus*, (18) the opening of the archinephric duct at its hinder end into the alimentary canal is well shown in *Lepidosiren* at Stage 25. Graham Kerr

remarks upon this striking resemblance to the primitive communication of mesoderm segment with enteron. Opinion is divided only concerning the site of origin of the mesoderm from entoderm, whether this occurs dorsally (19) or ventrally. While it may be pointed out that the dorsal site of origin (Diagram III, Fig. 1), considered in relation to parts of the complex nerve rudiment which remain to be described (the sympathetic chain and its connections), is broadly inconsistent with their evolutions within the developing vertebrate body, acceptance or rejection of the arguments (20) in favour of a ventral origin are not under discussion at the moment. Whichever view is held, it will be seen that the metameric segmentation which occurs in all vertebrates may be regarded very simply as merely a phenomenon associated with the re-distribution of the nervous connections within the vertebrate body. There is not necessarily any other " problem " of metameric segmentation. The regularly occurring dilatations of the neural tube, concentrations of nuclei within it, nerve bridges, septa, etc., are simple and inevitable expressions of innervation. There is only one situation in which the nerve-rudiment fails to traverse the mesoderm—the region which in the salmon is occupied by a continuous strip of undifferentiated mesoderm, the original site (Diagram III, Fig. 3) of the separation of the mesoderm from entoderm according to the theory of ventral origin. Here the lateral plexus in the salmon or the inferior lateral branch of the vagus in *Lepidosiren* rests. It is evident that it has been transported to this situation after the separation of the myocœle from the cœlome by the ventral extension of the edge of the muscle mass on the superficial aspect of the body-cavity, all its primary connections being preserved.

Segmentation is so striking a feature of the arrangement of structures in the somatopleure that it is not remarkable that it has dominated both anatomical description and morphology. But if the nerve-rudiments described above are reconstructed in their entirety, it becomes apparent that their arrangement in accordance with a segmental pattern is a less conspicuous feature than the presence of great longitudinal connections which unite the " peripheral nerve " rudiments with one another and are transported with them to deeper levels than they at first traverse. The greatest longitudinal connection of all is the spinal cord itself. A natural longitudinal division has long been recognized in the furrow between the alar and basal laminæ, each with its distinctive columns of nerve-cells in the adult, its own nerve-root and specialization of function. The mass constituted by these two laminæ forms in *Lepidosiren* a posterior boundary to the blastopore, instead of an anterior as in many types, and definitely encircles the opening. At the head end the continuity of each side with the other is as definite, and the formation of a central canal does not interfere with it. Quite irrespective, then, of the " closure " of the neural tube, in its middle part, by the approximation and fusion of neural crests, there is actually, in *Lepidosiren* as in *Peripatus* (21), a band of neural tissue encircling the blastopore, and from this band both the alar and basal laminæ are

developed. In the accompanying diagram these laminæ are represented by the lines cutting M and D'.

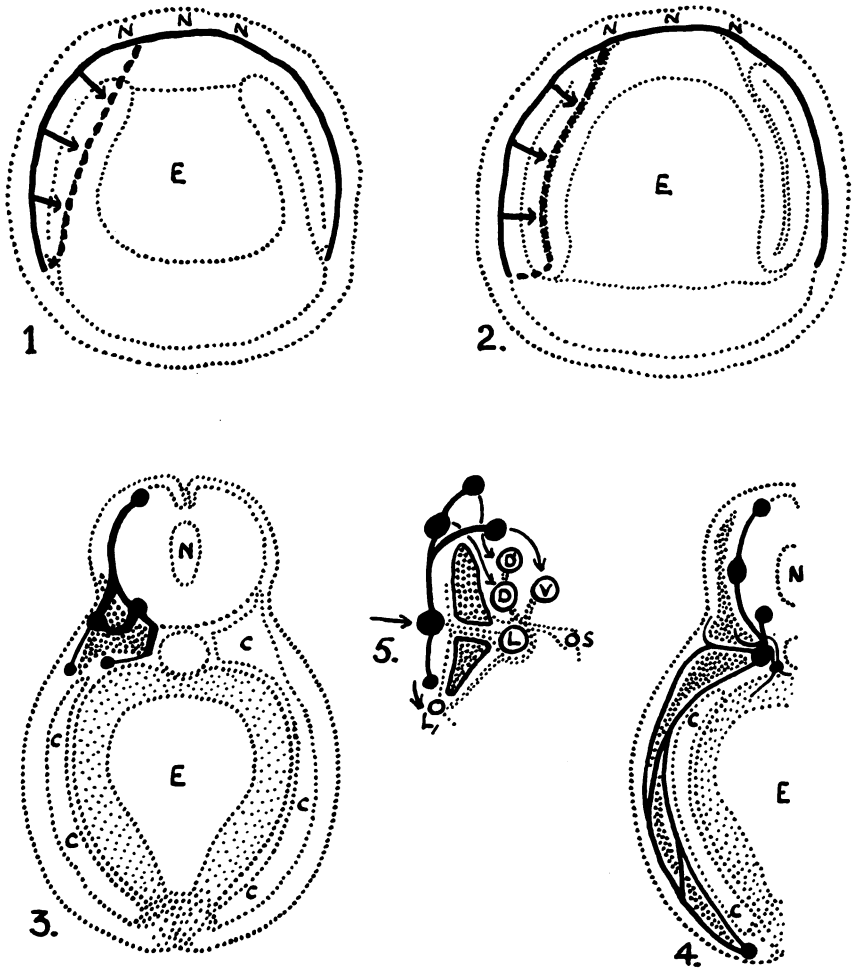

DIAGRAM III.—Neural rudiment referred to its situation in relation to the mesoderm at various stages in its early development: 1. In accordance with O. Hertwig's view of the origin of the mesoderm in an amphibian. 2. In accordance with Graham Kerr's view. 3. Early form of the neural rudiment referred to its situation in a posterior section of *Raja alba* with twenty somites. 4. Position and connections of the peripheral nerves typical of vertebrates. 5. Position as in (3)—heavy black—and as in (4)—circles and dotted line—referred to myotome to show relative displacement. N. = neural ectoderm; E = archenteron; C = cœlome; D D′ = dorsal ganglia; L L′ = lateral line nerves; V = ventral ganglia; S = sympathetic chain.

The other great longitudinal connections may at first sight not appear to possess a like circular character. Thus, despite the longitudinal flange-like structure mentioned by Balfour (12) as uniting the dorsal rudiments of the

nerves in *Scyllium* and the well-known continuity of the ganglia, particularly during early stages and towards the head-end of many types (22), the dorsal ganglia do not form anything like a complete circle in later stages. But even in man during the third week the rudiments of the ganglia are connected together by a continuous band extending from the auditory vesicle to the extreme tip of the neural tube. The lateral line nerves, with their central connections anteriorly, form a circle broken (?) only posteriorly. The inferior superficial ramus is similar, and in the case of the sympathetic chain, not only is there longitudinal continuity, but there is *posterior* continuity which persists even in man in the fusion of the two chains in front of the coccyx. Anteriorly, the sympathetic systems of the two sides are united by plexuses in relation to the blood-vessels supplying the brain. In this case, therefore, the circle is complete. So far from there being few other instances of nerve anastomoses across the mid-line in regions relatively anterior and posterior, there are many.

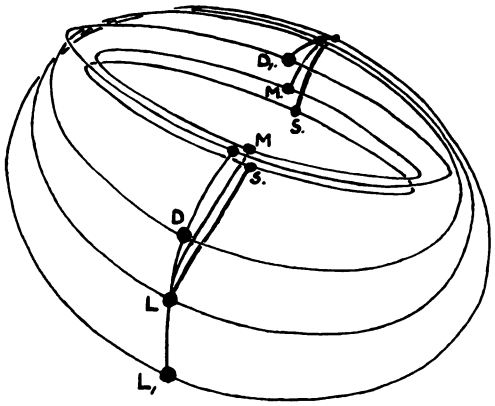

DIAGRAM IV.—Relationship between transverse (segmental) and longitudinal nervous connections in most vertebrates.

The cardiac and visceral ganglia and their nerves provide several instances; the hypoglossal nerves of the two sides not infrequently anastomose in man, and indeed all over the body there exist minor instances of exception to the strictly unilateral arrangement of the nerves. In view of the recognized specialization which has occurred at the two ends of the vertebrate body, and the marked bilateral symmetry associated with functional independence of the two sides, it is surprising that so many indications remain to suggest that the great longitudinal nervous connections are arranged so as to encircle the neurenteric canal. Consideration of the fact that the functions which these circular connections subserve can be fulfilled in vertebrates by the many decussations within the nervous system itself only makes this anatomical persistence the more significant. Further discussion of this feature is the subject of a brief note at the close of this paper. The point which it is desired to make clear

here is the continuity of the rudiment in which the " neurones " appear, and its arrangement in accordance with a plan which, in some respects at least, is very markedly different from that commonly held to govern the development of the peripheral nerves.

Cytology of the Neural Rudiment.

Stewart Paton (6) remarks that " the whole question connected with the origin and growth of these bridges is so involved that certain phases of it deserve far more attention than they have yet received ". He thought the bridges too large to be monocellular in origin. " The protoplasmic extensions seem to be so extensive that the idea of considering them to be the products of a single cell is scarcely tenable." Graham Kerr (3) refers consistently to the bridges as connections between myotome and *spinal cord*—i.e., not " cells " *in* the spinal cord. It is clear that the point to be settled is whether these protoplasmic masses, at first granular but otherwise homogeneous and later longitudinally striated (not necessarily fibrillated), are cellular processes or parts of a protoplasmic syncytium.

It is not possible with the technical methods at present available to settle this question finally by reference to cytology alone. But it is essential for the proper understanding of the process of fibrillation that the visible features of the sectioned rudiment should be described, and some of these are suggestively if not conclusively in favour of the view that the fibrillation process occurs in a syncytial matrix.

At first granular (3), the cytoplasm of the bridges is unmistakably continuous with the cytoplasm immediately within the limiting membrane of the spinal cord. This, however, is very densely strewn with yolk-granules which obscure the cell boundaries. The large nuclei are still at a considerable distance from the periphery of the cord, and in their neighbourhood are to be seen, whatever the method of staining, radiating lines which must be considered to represent the cut edges of partitions dividing one cell territory from another. These lines can never be traced to the periphery ; nor do they approach each other peripherally, as they should if converging to form the slender " tail " of a " neuroblast ". On the contrary, the earliest sign of neurofibrillation in the marginal zone is the appearance of rods or globules of denser cytoplasm in some cases closely applied to the partition membranes as though adherent to them. In other cases they lie between the membranes. A fine reticulum connects one rod with another. The arrangement is only consistent with the view that the dividing membranes have broken down peripherally, and the marginal fibrillæ are intra-protoplasmic structures connected together by a reticulum. Continuity of the reticulum is maintained around the dissolving outer edges of the old cellular walls.

With the appearance of striation in the bridges, a similar appearance becomes visible in the reticulum (Figs. 6–10 and 12), and in place of the very

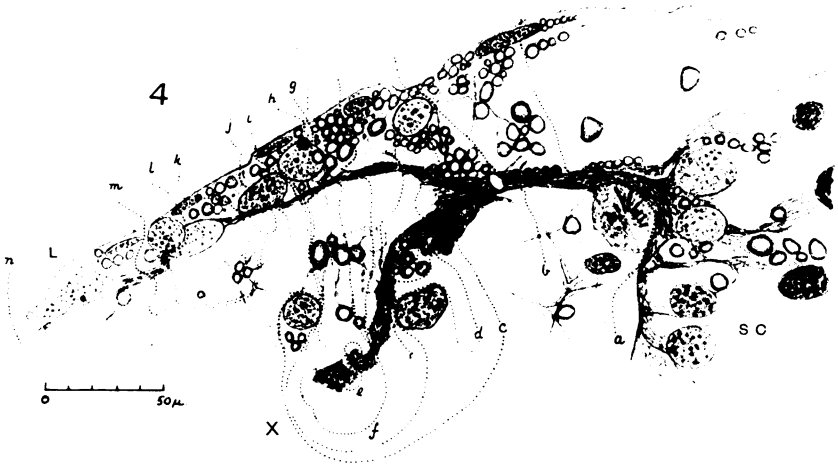

FIG. 4.

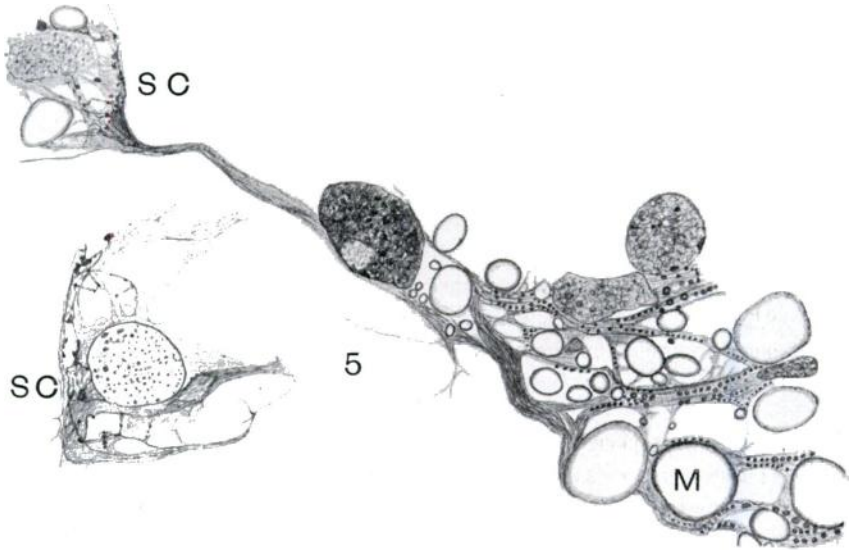

FIG. 5.

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large columnar cells there is a vacuolated syncytium in which the nuclei lie embedded. Some of the nuclei appear to be free to move about within the strands of the syncytium. They may often be seen near the funnel-like central ends of the neural bridges, and in this situation acquire, invariably, characteristics which distinguish them from the common nuclei of the cord, being larger, rounder and much more finely granular in respect of their karyoplasm. Invariably they deflect the striæ, which pass close to them to invest other

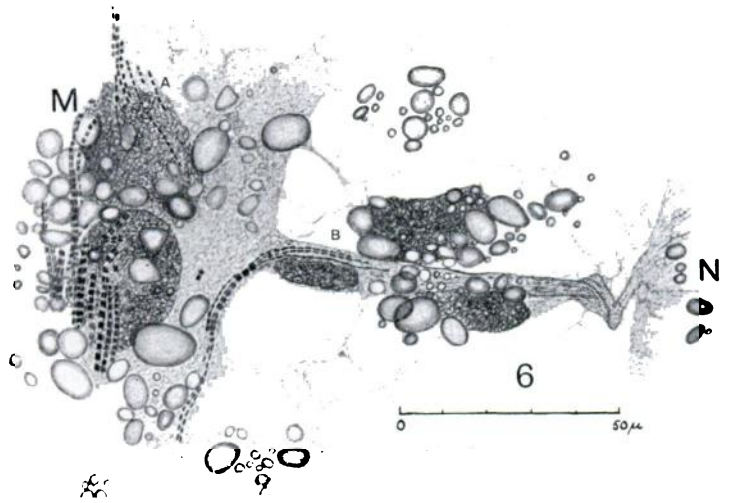

FIG. 6.

FIG. 4.—Composite drawing of fourteen successive sections of *Lepidosiren* at Stage 25 (hæmatoxylin and eosin). *a–n* = lines limiting the areas of different sections chosen for representation. A myotome fragment appears on area *c*, *d*. × = Ventral limit of myotome. S C = Spinal cord. L = Lateral line.

FIG. 5.—Neurofibrillation and myofibrillation. *Lepidosiren paradoxa*, Stage 28. The fibrillar systems of the cord, neural bridge and myotomes develop in continuity in a common cytoplasm.

FIG. 6.—Spinal cord, neural bridge and myotome of *Lepidosiren paradoxa* at Stage 29. Several of the myofibrillæ have been ruptured, and their cut ends, free to move through the common cytoplasm of the myotome and bridge, have been displaced (A–B) until they have come to lie in the bridge. This displacement, which could not have occurred in the presence of an obstructing membrane, demonstrates the continuity of the neuromuscular cytoplasm.

nuclei. Near the peripheral pole of each nucleus, when fairly hemisected, there is thus often a clear tapering zone which is free from striation. It is this, studied in small-celled tissues, which has, in the writer's opinion, given rise to the conviction that the " neuroblasts " are tailed cellular bodies " growing out " towards the periphery.

The similarity of the peripheral end to the central end of the bridge does not seem to have excited comment. It is, nevertheless, very close, and extends even to the relationship between the striæ of the rudiment and the myofibrillæ.

In the myotome, however, the syncytial character is generally admitted. Membranous septa, comparable with those within the cord, persist for some time ; the myofibrillæ develop in close relationship to them ; and the striæ of the neural element pass definitely in large numbers to the regions separating one septum from another. Where the bridge expands on the surface of the myotome (Stage 27) the striæ diverge slightly, but are visible in much larger numbers than in the bridges. This is another reason for believing that they do not indicate the presence of the thread-like tails of individual cells. That there is no membranous barrier between the bridge and the myotome is strikingly demonstrated by the preparations shown in Figs. 5 and 6 (Stages 28 and 29). Owing probably to contraction during fixation, several of the myofibrillæ shown in Fig. 6 have been ruptured, and their cut ends, free to move through the common cytoplasm of myotome and bridge, have been displaced until they lie within the cytoplasm of the neural bridge. This displacement, which could not have occurred had it been obstructed by a membranous barrier between the cytoplasm of the myotome and that of the nerve, demonstrates the continuity of the neuro-muscular cytoplasm.

That extension of the rudiment which is associated with the sympathetic nervous system, the enteron and the heart and blood-vessels has not been demonstrated in early stages (before Stage 34) by the usual histological methods. The use of Bielschowsky's method reveals it after the stage mentioned and probably much earlier ; but it has not yet been possible to prepare whole embryos of *Lepidosiren* at earlier stages by Bielschowsky's method. Since the method is specifically for the display of neurofibrillæ, the description of the cytoplasmic features of this part of the rudimentary nervous system will be postponed until the next section, dealing with neurofibrillation.

An account of the nuclei of this part may nevertheless be given here.

In the thousands of sections studied (Stages 24–37), mitotic figures, when present, were almost invariably in or in close relation to the ependymal layer, which retains its cellular character. The exceptions are so few as to be very remarkable. Between each ventral rudiment and the next, dividing nuclei are common forming the core of a cylinder of nuclei surrounding the central canal. Either the more peripherally situated of the nuclei are earlier products of the central nuclear division than those more deeply placed within the spinal cord, and have migrated towards the surface ; or amitotic division supervenes upon the primary, " germinal " division. In the spinal cord the number of divisions which occur cannot be very large ; but it is clearly impossible to count them. The number of nuclei in the mantle layer in the brain is very much larger than in the cord ; but here again the rule holds that karyokinetic figures are not found within the nuclear mass but only on its deep surface. The freedom from mitotic figures of the thicker regions of the grey matter at stages when proliferation of the nuclei is proceeding rapidly, considered in conjunction with the fact that mitotic figures in the ependymal layer are no more richly scattered

than in the cord, where the grey matter is relatively thin, suggests that the karyokinetic divisions do not give rise to all the nuclei, or the rule would hold : the more nuclei, the more karyokinetic figures. This rule does not hold.

On the other hand, many of the nuclei stained by Bielschowsky's method present forms and arrangements—contiguity and confluence, frequently with obvious continuity of the pattern assumed by the chromatic material—which suggests that the amitotic mode of division is the rule in the outer layers of the grey matter—i.e., all those but the ependymal layer. This is in harmony with the suggestion that mitotic division is rarely associated with syncytia. Bouin (23) says only that the amitotic mode is the more frequent in syncytia. Agduhr (24) found that the division of nuclei in spinal ganglia, at first mitotic, was later amitotic.

There is a slight visible difference between the nuclei more peripherally placed and those more deeply placed (omitting from consideration the ependymal layer) in the cord. The more deeply placed nuclei are more deeply stained by all methods tried. Outside the normal range of the nuclei there are occasionally to be found nuclei of altogether different appearance, larger, rounder, pale and finely granular. Dart and Shellshear (25) interpreted such nuclei figured by His, Hensen and Sterzi as migrants from the ventral roots. But in *Acanthias vulgaris* of 6 mm. the nerve-roots are nucleated. The peculiar nuclei mentioned as occurring in *Lepidosiren* have, it is true, a distinct relationship to the site of attachment of the roots, both ventral and dorsal ; but they are present long before the sheath nuclei appear. The suggestion of Dart and Shellshear that the central (neuroblastic) nuclei of the ventral horn are " inclusions " of peripheral origin is contradicted by this fact. Similarly in the case of the dorsal roots (Fig. 4) the ganglionic nuclei are of central origin. While the nuclei designated by Graham Kerr " sheath nuclei " (or sheath-cells) appear to result from approximation to the naked root of mesenchymatous elements, there is probably a third element associated with the peripheral nerves which is neither the ganglionic nucleus nor the mesenchymatous sheath of the nerve as a whole. When the nerve has increased greatly in size, mesenchymatous nuclei are distinguishable owing to their property of staining darkly with silver by Bielschowsky's method, while the nuclei which come to be embedded within the nerve do not. It is possible that the cytoplasmic environment of the deeper nuclei might induce some change in them, or even alter their subsequent development, even assuming that they have sunk into the nerve from the surface envelop of mesenchyme. However, it will be seen that a peculiarity of the nucleation of the visceral part of the nerve makes it possible to say more on this point when the process of neuro-fibrillation there has been described.

It is certain that in *Lepidosiren* the nuclei which become involved in the series of changes giving rise to the appearance of nerve-" cells " are at first indistinguishable from the rest of the nuclei present in the mantle layer of the spinal cord. They are the most superficial of the layer, and in some important

instances (the nuclei of Mauthner's and of Müller's cells) are closely invested by branches of the developing vascular system before any apparent change can be observed in them (Figs. 7 and 8). While it cannot be asserted dogmatically that the appearance of a blood-vessel in close proximity to the nucleus itself is the cause of the changes which ensue either in the nucleus or in the surrounding cytoplasm (through which the vessel passes), it is a fact of observation that these changes succeed the appearance of the vessel, and not vice versa. The elaboration of the vacuolar-space system, interpenetrating the syncytial plasma of the cord, into definite blood-containing channels, leads to the rapid formation of a blood-vascular system of great anatomical constancy, and the same vessels are recognizable from their form and relations in the *Salmonidæ* as in *Lepidosiren*.

The visible changes affecting the nuclei are (*a*) changes in the quantity and distribution of contained material colourable by hæmatoxylin, orange G, eosin, gold chloride, methylene-blue and silver nitrate by Bielschowsky's method; and (*b*) changes in size. Changes in size and in internal state as indicated by these stains accompany one another, and are both related to the density of neuro-fibrillation in the immediate vicinity of the nucleus concerned. This relationship between events inside and outside the nucleus is very definite. It is not peculiar to *Lepidosiren*, but occurs in all types examined.

Nuclear size.—The nuclei of the spinal cord of *Lepidosiren* at Stage 23 have an average diameter of about 20 μ. The outlines are fairly constant in size, as seen in section, showing that the greatest expansion of one or the other half of most nuclei is represented in the outline. At Stage 25 there is no measurable increase in the size of the nuclei generally; but composite outlines of twenty or thirty of the nuclei of different types show that those with coarse, densely-staining granules are more elongated than those with coarse and less densely-staining granules; while the finely-granular nuclei seen near the dorsal and (less frequently) the ventral roots are nearly spherical in form and definitely larger than the majority of cord nuclei.

The first measurable increase in nuclear size occurs at about Stage 30 in the nuclei which are destined to become the nuclei of Mauthner's " cells ". These expand rapidly, and reach their maximum about Stage 32. They are no larger at Stage 34, although the neurofibrillar investment is then more elaborate. No sooner does expansion begin than the granulation becomes much finer and the nuclei as a whole appear paler than their neighbours, whatever the method of staining. The nucleolus at the same time increases in size both relatively and absolutely, until it is a large globule of homogeneous content. This is in keeping with the increase in the size of Mauthner's " cell " in other types and of nerve-cells generally. Both in respect of size and pallor, associated with a sparse distribution of finely-divided chromatic material, the nuclei of nerve-cells assume rapidly, at a definite stage in the process of neurofibrillation, an appearance very closely resembling that of the mature ovum or of spermatocytes of

the first and second generations. The spermatocytes are subject to further changes of a nature which removes them from the field of comparison. The nucleus of the mature ovum of *Lepidosiren* has not yet been measured satisfactorily. The nucleus of the segmenting egg in an early stage of mitosis is over 50 μ in diameter (26). The nucleus of Mauthner's cell after expansion in this species is ovoid and 40 μ long. The egg nucleus of *Polypterus* is 30 μ (27), and the nucleus of Mauthner's cell after expansion is 28 μ long. Measurements of the nuclei of *Cavia* (total number measured about 300) give the following results:

TABLE.—*Nuclei of various tissues of* Cavia *in micromillimetres* (μ).

Liver.	Epididymis.	Spermatocytes 1 and 2.	Dorsal horn-cells.	Mature ova.	Ventral horn-cells.
5 **6** 7	5 **6** **7** 8	8 **9** **10**		11 **12–14**	
80%	80%	90%	80%	95%	90%
			7 8 **9–13**		11 **12–17** 18

EXPLANATION OF PLATE.

FIG. 7.—Mauthner's cell of *Polypterus*, Stage 36 (H. and E.). The cytoplasmic continuity cannot be made out with the same certainty as in large-celled types. The non-cellular character of the perikaryon of Mauthner's "cell" is, however, shown conclusively by the capillary blood-vessels traversing it (B). The arrangement of these is indicated in the Text Diagram. The scale is the same as that of Fig. 8.

FIG. 8.—*Lepidosiren paradoxa*, Stage 30. The large centrally-placed nucleus is that of the future Mauthner's cell. Note the capillary blood-vessel closely investing it. A nucleated red blood-corpuscle lies in one limb. The difficulty of tracing early blood-vessels is indicated by the presence of the transversely-cut nucleus of another erythrocyte above the Mauthner's cell nucleus. Note the cytoplasmic continuity and the absence at this stage of perikaryon. The blood-vessel and the expansion of the nucleus precede this development.

FIG. 9.—*Lepidosiren paradoxa*, Stage 36 (Bielschowsky). Junction of the marginal and mantle layers in the anterior horn region of the grey matter of the cord. The same features as in Fig. 5 are observable. Note the matted neuro-fibrillæ of the white matter (marginal layer).

FIG. 10.—*Lepidosiren paradoxa*, Stage 36½ (Bielschowsky). Developing neurofibrillar reticulum in a dorsal ganglion. The formation extends continuously to invest seven neighbouring nuclei, and the continuity of individual neurofibrillæ can be made out across five planes of separation. The mass is, therefore, a syncytium.

FIG. 11.—Mauthner's cell in a salmon larva of 10 mm. Note the large nucleus still partially exposed to the general neurofibrillar reticulum. The space above the nucleus is a capillary blood-vessel. (Bielschowsky.)

The above table illustrates very strikingly the relationship between the nuclei of the nerve-elements and those of the sex-cells in respect of size. The only nuclei larger than those of mature ova are nuclei of the ventral horn-cells ; while the range of nuclear sizes covered by the dorsal horn-cells places them in a position between spermatocytes and ova. It is commonly asserted that nuclear size may be expressed as a constant function of cell size. While it is practically impossible to estimate the volume of cytoplasm associated with a dorsal or ventral horn nucleus, the grouping indicated above does not suggest a direct relationship between nuclear size and cytoplasmic volume. It suggests that the nuclei of nerve-cells share a peculiarity, whatever it is, common to the nuclei of germ-cells. If it is considered that the nucleus of the mature ovum is, of all nuclei, the most certainly inactive, and that nothing will provoke activity within it except fertilization (and some totally abiological conditions), its close resemblance to the reputably most active of nuclei is remarkable. This resemblance extends to other features than size, viz., the large globular nucleolus, and the attenuated, finely-granular condition of the basichromatin. Whatever staining method is employed, the pallor of the nerve-cell nucleus is one of its most distinctive features.

The suggestion that these exceptional characteristics are directly related to neural activity is gratuitous. Without seeking to dethrone the nucleus—the " brain of the cell "—from the high position it holds in the estimation of biologists, it may be said that the evidence available concerning it deserves examination on its own account. This consideration acquires greater force in the light of the fact that nuclear division, once the requisite number of nuclei has been attained, can have no significance in regard to the fundamental properties of nerves. *It is not nuclear division but the inhibition of nuclear division that is the most necessary condition for the permanence of structure which neural function is commonly held to require ;* and it is suggested that the peculiarities exhibited by the nuclei are more likely to be related to this necessary absence of proliferative function than to exceptional potency in respect of neural function. How this condition is secured and what other purpose it may serve can be discussed only when the process of neurofibrillation has been described, for, as will be seen, this process is coincidental with the apparent nuclear changes.

Neurofibrillation.

Neurofibrillation is essentially the elaboration in the neural cytoplasm of a substance which possesses distinctive staining reactions both before and after death. A substance which is not distinguishable by its staining reactions alone but is subject to a different spatial distribution is elaborated at the same time in the muscles and in the cytoplasm of sensory epithelia as well as in the neural cytoplasm. In the muscles this substance is arranged with remarkable geometrical regularity, and gives rise to the appearance of longitudinal and

transverse striation and to the optical properties associated therewith. That these do not arise from any peculiarity, physical or chemical, in the substance itself is demonstrated by the fact that if a cast is made of the surface of muscle-fibres (e.g., in celloidin impressed and hardened at the same time by the application of fibres soaked in chloroform), the cast, stained to show the presence or absence of adherent myofibrillæ, has birefringent properties (28). The neurofibrillæ, also, stained by Bielschowsky's method with silver, have birefringent properties (29). During life, this substance stains with methylene-blue in a rapid succession of stages, each of which gives rise to an appearance characteristically different from the rest, details made visible at one stage sometimes disappearing in later stages. Post-mortem staining tends to produce a picture which is the summation of the stages of staining by methylene-blue. The stains which produce this picture are better or worse according to whether they commonly reveal clearly details in all situations. Among them are hæmatoxylin and its various modifications, gold chloride, methylene-blue, and silver nitrate and its modifications in use. A suitable technique will reveal the fibrillar structure with all these stains in sharp contrast to other stainable material excepting yolk-granules. The chromatic material (basichromatin) of the nuclei is stainable by the same substances, but not to the same density during later stages of development. In very early stages, non-fibrillar elements in *Lepidosiren* tend to stain deeply when prepared by Bielschowsky's method; but this tendency diminishes rapidly as development advances. Since neurofibrillation involves only a small minority of the nuclei of the spinal cord, and of the dorsal and peripheral ganglia as late as Stage 37, it is not necessary to use very young embryos in order to study most of the characteristic features of the process.

The first spontaneous movements occur in *Lepidosiren* two days before hatching (Stage 27). The next ten stages (Keibel) bring the larva to metamorphosis and occupy forty days. The whole larval period is passed, therefore, with only what may be called a skeleton organization so far as neurofibrillation is concerned. Before the close of this period, "axis-cylinders" which are in some cases distinctly separated from one another constitute the motor and sensory nerves and their branches to their terminations throughout the larval body. The peripheral ganglia of the heart and gut are also differentiated. Yet the characteristic histological features of adult "nerve-cells" have yet to be acquired.

In small-celled types, including man, masses of neurofibrillar material occur in the immediate neighbourhood of the neuroblastic nuclei and elsewhere throughout the nervous system. The distribution and density of this material when it is stained are characteristic. In the spinal cord the substance appears in largest amount in such close apposition to the peripheral pole of the nucleus that Cameron (30) considered it to be intranuclear. In the dorsal ganglia this "polar-cap", still peripheral in position, is often seen to be contained along one

side of the nucleus and to expand again in relation to the centrally-directed pole, or it may be placed on the side of the nucleus. Invariably it is gathered up into strands, frequently matted together, forming a tailpiece stouter on the peripheral than on the central side. Held's monograph (4) abundantly illustrates these appearances. Held refers to the substance as " neuroreticulum," and many of his figures show the " polar-cap " of one nucleus continuous with another one or more. This anastomosis between the neurofibrillar reticulum of one nuclear territory with others is conclusive demonstration that no cellular wall intervenes to prevent it (Fig. 10). In sectioned material most of the " polar-caps " are cut in a plane which avoids these connections, and very commonly the " polar-cap " appears as a densely-staining tangle of intercommunicating varicose fibrillæ, not unlike an egg-cup with a long twisted stem. In the concavity the nucleus is lodged. That the twisted stem should have been interpreted as an outgrowing axon is understandable, particularly if regard is paid to its density, its branched character, and the difficulty of tracing it peripherally in many cases. It will be apparent, too, that in small-celled types, the stages preparatory to the development of this very characteristic appearance must be difficult to make out, and that, since these stages involve the periphery as much as the centre, very striking features of the central development tend unduly to attract attention, to the exclusion of less striking features, both centrally and peripherally.

In *Lepidosiren*, many of the most characteristic appearances associated in other types with the formation and distribution of the neurofibrillar material are inconspicuous, while details impossible of precise observation in them can be studied with relative ease. Thus the perspective is adjusted.

The closest approximation to the features which so strongly attract attention in small-celled types is met with in the dorsal root ganglia in *Lepidosiren*. For a long time the nuclei of these masses are closely crowded together. When stained by Bielschowsky's method from Stage 24 onwards (earlier stages have not been stained successfully by this method), some of the nuclei on the surface of the mass are seen to be invested by a thin coating of densely stained fibrillar material which has all the appearance, in section, of a thickened nuclear membrane. This change spreads, however, to adjacent nuclei, until at Stages 36–37 it is frequently possible to see, spreading from a single focus, a reticulum enclosing from three to six or seven nuclei in a common net. In favourable situations this network may be seen to be continuous from nucleus to nucleus (Fig. 10). In the large nuclear masses of the ganglia of the cranial nerves, the process starts from more than one place ; but if the nerves related to these masses are traced, reasons will in every case be found for ascribing each " focus " of neurofibrillation to a different morphological part of the ganglion. Throughout the whole dorsal ganglionic system the process is the same. Extending both centrally and peripherally from the reticulum in each ganglion are bundles of neurofibrillæ, the separate fibres of which can be traced for short distances as

independent units ; but in every case the individual fibres are merged sooner or later in others. The fibrillæ thus intercommunicate, and may either pass through the ganglion between the unaffected nuclei, or become involved in the peri-nuclear reticulum, in which case there is a marked tendency for them to become matted together to form the two poles of a bipolar " cell ". In many such cases it is possible to observe the detachment of a fibril from the matted polar axon and its independent re-connection with the general reticulum. But during these stages it is strictly impossible to regard either axon or dendrites as being other than parts of a reticulum common to many nuclei at once. The " anastomotic " nerve-cells frequently reported are thus explainable as cases in which the close developmental relationship between the neurofibrillar systems of adjacent nuclei has continued into adult life. It is not an accident of nuclear division that has determined their existence. Not only does the elaboration of the neurofibrillar material ignore the planes of separation of different nuclear territories ; but it ignores the planes of morphological separation such as that between the two dorsal halves of the spinal cord. It is true that in *Lepidosiren* this plane is potential rather than actual, since the neural tube cavitates by the appearance of a centrally-placed lumen in a solid rod of cells derived from the surface ectoderm ; but the plane of separation between the right and left halves of the dorsal portion of the spinal cord is a morphological plane.

Within the spinal cord the process of neurofibrillation leads to appearances which are *superficially* very different from those common in other types and from those described as occurring in the dorsal ganglia of *Lepidosiren*. This difference concerns the immediate neighbourhood of the neuroblastic nuclei only. It does not concern the marginal layer of the cord, where the process is essentially the same as in other types. The remarkable feature of the spinal cord is the absence of " polar-caps " in relation to the nuclei, and their replacement by an investment of the neurofibrillar material which, instead of appearing in coarse masses applied to the nuclear membrane, appears from the first in the form of fine fibrillæ which extend from nucleus to nucleus, gradually congregating more closely together to form bundles in relation to two or three aspects of a single nucleus. Thus the formation of the neurofibrillæ of the " cell-body " from the scattered neurofibrillar material of the cytoplasm of the syncytial cord is not obscured in *Lepidosiren* by the apical condensations characteristic of other types. The appearances visible in *Lepidosiren* thus throw a flood of light upon the meaning of this characteristic but misleading feature.

At the same time it should be pointed out that the feature under discussion (the appearance of the " polar-cap " in the medulla spinalis) is much more easily demonstrable in some types than in others. It approximates most closely to its counterpart in dorsal and sympathetic ganglia in the domestic fowl, the duck and the pig. In these creatures, too, the dorsal ganglionic " polar-caps " resemble most closely those in man (24-mm. embryos). " Polar-caps " are also present in the spinal cord of *Petromyzon*, *Salmo*, *Emys*, *Lacerta*, and

Cavia ; but the anterior (ventral) horn of the grey matter (mantle layer) of the spinal cord in man (24 mm.) contains no such appearances, although at a much earlier stage (4·2 mm.) quite dense accretions of fibrillar material surround some of the most peripherally placed of the nuclei. A marginal layer then begins to appear, the cord being syncytial. Between the two stages the nuclei proliferate enormously ; but even at the 24-mm. stage collocations of nuclei closely packed together in irregular masses of cytoplasm staining more densely than elsewhere are found in the neighbourhood of the emergent anterior root-fibres, which are well formed, and can be traced even to the small muscles of the extremities. The fibrillar material lies in irregular interlacing threads, here and there becoming reticular and expanding to fill small areas of cytoplasm, and in other situations being gathered up again into fine, irregular fibres with varicosities. These fibres extend to the outer surface of the ependymal layer. Some, fine as they are, can be traced for considerable distances without alteration in average thickness except towards the peripheral nerve-root, where they join up with others and become a compact and much thicker bundle, the constituent fibres of which are liable again to detach themselves from the composite " fibre " over and over again before reaching their terminations.

These facts may be summed up by saying that the fibrillar material in the human cord seems to condense to form the neurofibrillæ of " axons " and " dendrites " before condensing around the neuroblastic nuclei. In the Salmonidæ there is still another variant to the uniform massive elaboration of neurofibrillar material affecting all parts of the neuronal apparatus. The essential feature seems to be the very finely-divided state of the neurofibrillar material, which, condensing to form typical " polar-caps " in the dorsal ganglia and fine but clearly distinguishable fibres in the peripheral nerves, appears in the cord, after the expansion of a minority of the nuclei of the mantle layer, as a fine, diffuse cloud surrounding sometimes one, sometimes two or three of these nuclei. This cloud has no definite margin, and although it condenses later to take the form of a nerve-" cell ", does so while retaining continuity with the general fibrillar reticulum.

So far as the peripheral nerves are concerned, the conditions in *Lepidosiren* are best illustrated from the study of the main division of the lateral line of the vagus. It is relatively straight, and runs practically the whole length of the body, and can thus be studied at levels very wide apart, affording valid standards of comparison. While there is scarcely any perceptible difference, except in size, between the nerve at one level and the same nerve at another level, the appearance of sections of the nerve at one stage of development is widely different from their appearance at other stages. Between Stages 32 and 37 the appearance of the nerve undergoes a complete transformation characteristic of the changes in the appearance of all the peripheral nerves and, to a higher degree, of the marginal layer of the cord, the peripheral ganglia and their connections. These changes involve (*a*) the size of the nerve, (*b*) the distribution

of the neurofibrillar material, and (*c*) the density of the stainable material within the nuclei. The nuclei do not become more scattered with the increase in size of the nerve ; and since the nerve does not taper greatly in the individual, there is presumably an increase in the number of nuclei from one stage to another. Their colourability, however, changes from a state in which it is greater than that of the somatic nuclei to one in which it is so much less as to make the nuclei invisible on the photographic plate, their forms being represented by spaces between the " axons " of the nerve. This change is accompanied by a great increase in the density of the neurofibrillar material, and its condensation into thicker masses as seen in transverse section. At Stage 32 the material closely invests the nuclei, and may be found also clinging to the trabeculæ of the cytoplasmic reticulum. It is itself an irregular, flocculent reticulum, the thicker parts being connected together by thinner processes. This lack of separateness of the fibrillæ is still apparent at Stage 34½, although there is then an increase in the quantity of the material as compared with Stage 32. At Stage 37 this increase has reached its maximum, and it is necessary to study the nerve in longitudinal section to make out any connection between the fibrillæ which have condensed together to form the massive " axon " fibres.

It must be emphasized that the nuclear changes here described are closely similar to those to which the neuroblastic nuclei themselves are subjected. No such pronounced and sudden loss of staining power affects the somatic nuclei, and the appearance of the nuclei of the nerve-trunks is thus in sharp contrast with that of the nuclei immediately adjacent to the nerve. The latter, the " sheath " cells of Graham Kerr, are of mesenchymatous origin. Lying within the nerve are nuclei of rounded form, unstainable by silver nitrate by Bielschowsky's method after the neurofibrillæ are well formed. Up to Stage 37 there is no further development of these nuclei in the lateral vagus of *Lepidosiren*. They do not enlarge. Two kinds of nuclei are thus associated with the nerve-trunks. One is of mesenchymatous origin (Graham Kerr). The other shares with the nuclei of ganglia and neuroblastic nuclei at least one striking peculiarity.

Now exactly the same features occur in certain positions in relation to the heart and gut ; but to understand their significance it is necessary to review briefly the development of the nerves in such a region as the heart. It has been pointed out (31) that there exists in the heart of *Lepidosiren* at Stage 36½ a continuous nervous structure in continuity with the central nervous system at several points. To this structure may be referred all the described conducting mechanisms of vertebrates, whether they are " nodal ", atypical muscular structures, accompanied or unaccompanied by nerves, or nervous structures—nerves, ganglia and plexuses. The form of this rudiment is complicated ; but it may be divided into sinus, sino-atrial and atrio-ventricular parts, with extensions into the ventricles corresponding to the bundle of His. At Stage 36½ the whole of this apparatus is in a form indistinguishable from that of the

marginal layer of the early spinal cord, presenting the characteristic appearance of the close reticulum—the " flocculent " appearance of the vagus at Stage 32 but (like the early cord) *without nuclei*. Scarcely later (Stage 37), in a heart of which the main anatomical features were less well developed, this complex of attentuated strands, far removed from the central nervous system along all lines of communication with it (the two vagi and segmental communications at two or three levels), was found to be composed of distinct neurofibrillæ coalescing into " axons ", a few nuclei of the sheath type and many of the expanding ganglionic type, already pale like the lateral vagus nuclei at Stage 37, with an investment of beaded neurofibrillæ. These last-mentioned nuclei occupied definite anatomical situations.

Whence come these nuclei ? There are no such nuclei at Stage 36½ ; but at Stage 37 they are present in their most developed form. In the work cited (op. cit., p. 238), it is stated that the part of the vagus nerve associated with the heart merges by its dorsal root insensibly with the intermedio-lateral region of the medulla oblongata, and that on both the central and the peripheral side are congregated nuclei identical with each other in appearance. In the case of the ventral root, similarly, many nuclei can be seen separated from the main mass of neuroblasts by the great ventral conducting systems of fibres. " Thus the nuclei of the dorsal ganglion and its root, those of the ventral root and those of the margin of the medulla may be said to mix. In both ventral and dorsal regions the arrangement might almost be mistaken for an extension of the grey matter through the chondrocranium."

Ross Harrison (32) records observations of the nerves in the fin fold of living anuran larvæ which show that the sheath-cells are migratory. (The present writer would say that what is demonstrated is the movement of the nuclei.) Their movement, Harrison says, is sporadic, cells remaining often for a long time stationary, but at other times moving at the rate of 35 microns in thirty minutes. Cutting experiments devised to ascertain their source point to the conclusion that these cells have nothing to do with the formation of the axon, although they originate " for the most part from the ganglion crest in connection with the spinal ganglia ". When this source is rendered unavailable by experimental interference, the appearance of sheath-cells is delayed. But " a few medullary cells migrate from the cord by way of the ventral roots ". Ross Harrison therefore regards the sheath-cells as " the neuroglia of the peripheral nerves ". In keeping with his very strong opinion in favour of the integrity of the histological neurone, he insists that the elements are marginal to the axons and separate from them. " While they have but a small amount of cytoplasm, they are in no sense mere nuclei and part of the axon itself."

The sheath-cells of the early motor roots of *Lepidosiren* have an appearance which warrants similar description, and Graham Kerr was induced to come to the conclusion that they were of mesodermal origin : cells applied to the surface of the protoplasmic bridges and essentially separate from them.

Now, if reference be made again to Fig. 4, showing the appearance of the dorsal protoplasmic bridge at Stage 25, it will be seen that large nuclei of the pale-staining type are found (*a*) separating the striæ within the spinal cord, (*b*) within the funnel-shaped attachment of the dorsal root to the spinal cord, and (*c*) lying in the angle between the cord and the root. These features are in complete harmony with Ross Harrison's view that just as the indifferent nuclei of the cord may become the nuclei of either neuroblasts or neuroglia, so may they become the nuclei of ganglionic or sheath elements, and it is hard to place any other interpretation upon the appearance in the figures referred to but that they are the static expression of migration such as Ross Harrison has witnessed. How else could the ganglionic elements of the dorsal root reach the situation in which they are observed after the relative displacement of the bridge apparatus from the outer to the inner side of the myotome? They are not present at Stage 25, when the part of the nerve which they will occupy lies deeply in the cleft between adjacent myotomes. In the case of small nerves (and the roots are small nerves in their early stages) the peripheral situation is the only one which could conveniently be occupied by nuclei of any description. With increase in size, however, nuclei come to occupy more central positions, and, in the case of the lateral vagus, the whole cross-section of the nerve is crowded with nuclei, while nuclei of a different type (the " sheath " nuclei of Graham Kerr) remain alongside the nerve-trunk. The flocculent fibrillar material at Stage 32 spreads from nucleus to nucleus, closely investing each. It is only later that this substance becomes, as it were, polarized into longitudinal threads, and they themselves for a long time connected with one another. In other words the nerve is still syncytial and the nuclei are embedded in this syncytium.

Further evidence in favour of this conclusion is forthcoming in the fact that the nuclear changes which are so marked a feature of the period occupied by neurofibrillation are shared by (*a*) nuclei contained within the nerve (the neurilemma nuclei proper), (*b*) neuroblastic nuclei of the cord, and (*c*) nuclei of peripheral ganglia. The cardiac ganglia, for example, which appear so suddenly, differ from the transversely-cut vagus only in the greater expansion of the nuclei, and in the slightly different arrangement of the neurofibrillæ in relation to them.

In the light of these observations, it is noteworthy that where fine plexuses of non-myelinated fibres exist in mammalian tissues, the neurilemma nuclei usually appear as excrescences on the axons, invested in common with them by a thin film of cytoplasm. The nuclei have special staining reactions, which they share with the nuclei of the neurilemma of myelinated fibres, and in both cases, in suitably stained preparations small polar-tufts of very fine fibres connected with the axon can be made out. It is these which have given rise to the legend that " nerve-cells " are to be found in the human iris: the polar tufts are the " cell-bodies " of the " nerve-cells ".

The nuclei strewn so lavishly throughout the lateral vagus are not the foci of

changes leading to the acquisition of ganglionic appearances within the nerve up to Stage 37; but small clusters of them in the line of the vagus itself in relation to the gut have this appearance in respect of the investment by a skein of neurofibrillæ. They thus appear exactly as in the heart. The only feature distinguishing the sheath nuclei from ganglionic nuclei is the absence of expansion of the nuclei themselves in the case of the former. The sheath nuclei proper or nuclei of the perineurium are quite clearly connective-tissue elements, and have all the features of somatic nuclei. It is the so-called neurilemma nuclei that fall so definitely into the same class as the neuroblastic nuclei. Ultimately what distinguishes the members of this class from one another is only the *amount* of neurofibrillar material in their neighbourhood. As the amount increases, so the nucleus increases in size and appears to empty itself of colourable material. In the case of the neurilemma, the neurofibrillæ form either polar-tufts, sometimes reduced to dimensions not very much larger (though definitely larger) than centrosomes, or a less regular fibrillar investment as in the peripheral nerves of *Lepidosiren*. In the case of peripheral ganglia this investment is more abundant; and in the central and dorsal root ganglia it reaches so great an amount as to give these elements their characteristic appearance.

The neurilemma is one extreme instance and the large nerve-cells another of the same process. It is therefore of importance that the features made out for the least developed elements should be studied in the most developed.

Neurofibrillation in Relation to Mauthner's Cells.

One nerve-cell in fishes offers unique opportunities for the study of neurofibrillation in relation to a single isolated nucleus. That is Mauthner's cell. There is normally but one of these elements on each side of the brain, so that it is possible to be certain that its appearance in different individuals at successive stages reflects developmental change. It is of enormous size, and neurofibrillation in the neighbourhood of its nucleus is very far advanced before there are signs of change in relation to neighbouring nuclei.

While reference will be made to some of the features described by Bartelmez (33), the later paper by Holmgren and Horst (34) may be cited on account of some curious features which these authors have brought to light. "This enormous cell," they say, "is situated just caudally to the motor facialis fibres where they pass from the fasc. long. post. to their emergence. According to Bartelmez, the cell lies just rostral to the root-fibres in teleosts. *It is difficult to make a distinction between the cell body and the main dendrites.* In other cases the cell body is well defined as a large perinuclear mass from where the much thinner dendrites run in a lateral or ventral direction. . . . Also in *Amblystoma*, described by Herrick, the cell body is well defined. In *Ceratodus*, on the contrary, the cell body is formed only by a slight swelling around the nucleus, not much thicker than the two main dendrites; especially the lateral

dendrite is very thick. . . . The end branches [of the lateral dendrite] themselves are very fine, forming a sharp contrast with the dendrite. They have not the club-like expansions that Bartelmez found in siluroid fishes but form a simple neuropil as in *Salmo*." The lateral dendrite receives stimuli from the eighth cranial nerve. " The axon that joins Mauthner's fibre first of all could easily be traced to its cell of origin, a large reticular cell lying just behind Mauthner's cell. This reminds us of what has been described by Beccary in *Salmo*. This author found at one side two typical Mauthner's cells, at the other side also two cells, but only one of them having a lateral dendrite, the chief characteristic of Mauthner's cell. So it is an ordinary reticular cell. Also the axons of these cells lie close together." The authors go on to comment upon this feature which they consider to illustrate the tendency of fibres conducting in the same direction to join together in bundles, and remark upon the way in which the periphery of such bundles contains all the myelin. In *Ceratodus* the large tractus olfacto-tegmentalis consists of a number of such bundles, the non-myelinated being surrounded by myelinated.

Mauthner's cell has received attention from the point of view of the multiplicity and variety of the connections established through its agency as much as from the opportunity it affords for cytological study. The paper by Bartelmez deals with both these aspects. Two points may be mentioned. Bartelmez figures a space present on the dorso-medial aspect of the cell, which he attributes to " cell-shrinkage ". This space is constantly present in preparations of salmon larvæ made by the present writer. Setting aside the suggestion that " cell " shrinkage might account for a space constantly present in the same part of the cell, the space is bounded by minute beaded fibrillæ, and can itself be traced both dorsally and ventrally among the neuroblasts. It is a minute blood-vessel traversing the " cell " (Fig. 11).

Lepidosiren hatches at Stage 27. Spontaneous movements have been observed two days earlier, and at Stage 32 the individuals have been active larvæ for 24 days. It is clearly possible, therefore, that a relatively unfinished development, estimated by histological appearance, is quite consistent with function. How many of the features to which functional and anatomical significance is attributed may be merely age changes of no biological significance at all ?

The observation of Beccary, referred to by Holmgren and Horst, is of interest because it clearly indicates that the nucleus brought into relation with the fibrillar system has so little specific function that two may share it, one becoming associated with the neurofibrillæ subserving half the total function of the cell, the other with those subserving the remainder. The appearance in *Ceratodus*, too, of a " slight swelling " around the nucleus which extends into two thick dendrites, suddenly breaking up into very fine end-branches, deserves comment. Frequently the " cell-body " and " dendrites " of large nerve-cells stained by Bielschowsky's silver method, in all the species examined by the

writer and in innumerable figures published by other workers, present the appearance of a branched cylindrical shell filled with homogeneous substance which stains brown, orange or yellow or not at all. This appearance is the result of inequality of staining due to the deposition of a membrane of silver impermeable to the reagents used. Thus an artificial surface is formed where the neurofibrillæ become more congested. As is shown by its optical properties (35), the compound which gives rise to the dark appearance, by transmitted light, of Bielschowsky-stained neurofibrillæ is not a surface deposit but a compound with the neurofibrillar substance which shares its birefringent properties. In successful preparations the "cell-body" is shown to be a space transmitting continuous fibrillæ, as Miss Ballantyne (10) has described. The sudden "breaking up" of dendrites into fine end-branches would be better described as the sudden cessation of the staining process at a situation where physical conditions become unfavourable to the proper staining of them throughout the rest of their course. As the tissue ages, the tendency of the fibrillar bundles and of the massed fibrillæ in the neighbourhood of the neuroblastic nuclei (the nuclei of the "nerve-cells") to appear as hollow cylinders becomes more pronounced. Careful inspection of the concavities will nevertheless often reveal the fibrillar texture less clearly than well-stained preparations. The Mauthner's cells of *Lepidosiren* may usefully be studied in preparations stained by hæmatoxylin and eosin as well as by silver methods.

Stained by the more usual method, the element can be recognized by its large nucleus in close relation to that of a future Müller's fibre (the reticular element of Holmgren and Horst). There is no sign of the neurofibrillar investment. The cytoplasmic strands investing the nuclei can be seen to be striated, as though under longitudinal tension; but they are confluent, with large vacuolar spaces in which an occasional erythrocyte can be found. Such isolated blood elements, separated from one another by great distances, precede the appearance of vascular walls, as might be expected from Graham Kerr's description of the origin of blood-vessels in *Lepidosiren* and *Polypterus*. In an example studied, the relationship of the small blood-vessel to the nucleus of the future Mauthner's cell is of great interest. The nucleus is invested on two sides so closely as almost to be in contact with the vessel wall; while on a third side the darkly-stained nucleus of an erythrocyte is seen to lie in cross-section in relation to a strand of the syncytial cytoplasm. No vessel-wall is here visible. Such conditions make it virtually impossible to reconstruct the vascular pattern until much later stages.

At Stage 32 the hæmatoxylin stained nucleus is relatively much paler, more finely granulated than the surrounding nuclei and expanded to its full size. There is nothing to suggest that the cytoplasm has lost its syncytial character. It forms a web with larger vacuolar spaces than at the earlier stage. On its dorsal aspect the nucleus of Mauthner's cell is attached by radiating strands confluent with the cytoplasmic strands investing the other nuclei; but on its

ventral aspect a marked increase in the density of the cytoplasm represents the neurofibrillar formation, while the pink-staining strands of the reticulum are orientated to give the appearance of direction to them in this part of the field. Nevertheless, all along the margin the reticulum blends with the common reticulum of the marginal layer of the brain. A study of the next section shows the " bed " in which the nucleus lay. In this shaving from the surface of the " cell " the continuity between the denser cytoplasm of the " cell-body " of Mauthner's cell and the common reticulum is clearly shown. This appearance is quite inconsistent with the view that the mass is an enclosed structure invested by a " cell-wall ". It is a fibrillar knot of great size and density in a common matrix.

The consolidation of the " dendrites " of this giant element is well shown at Stage 34. The " margins " of both cell-body and dendrite are nevertheless quite clearly in continuity with the reticular cytoplasm surrounding the undeveloped nuclei in the neighbourhood.

Bielschowsky preparations covering the same stages in the development of *Lepidosiren* reveal other features than those described above. Firstly the blood-vessels penetrating the cell-body of both Mauthner's and Müller's cells are clearer, and the neurofibrillæ passing in their neighbourhood are more definitely individualized. Secondly, it is quite evident that at Stages 32 and 36 there is not only reticular continuity, but neurofibrillar continuity between contiguous nuclear territories. In one instance this could be made out in seven places, the observations being in each case checked by the use of the binocular microscope. In one place the progressive nature of the differentiation of the neurofibrillar substance into clear-cut cylindrical fibres of large size—the kind of fibres called " axons ", whether they occur in the central nervous system or in the peripheral nerves—is well shown. In the large lateral dendrite of Mauthner's cell at Stage 36, for example, it is clear that the fibres assume their form gradually as they pass peripherally, while nearer to the nucleus they are progressively more difficult to recognize as separate structures. Here, too, the " axon cap ", regarded as a synaptic ending, can be seen in its partially developed form to be a region of greater density of the neurofibrillar substance involving most of the thickness of the dendrite, and the neurofibrillæ here have continuity with those of the dendrite.

The appearances suggest a process of polarization extending from different points within the common neurofibrillar reticulum, and a simple simile which comes readily to mind is that of a " coherer " dependent for its action upon the polarization of individual small particles of iron under the influence of an electromagnetic field. It must not be forgotten that at these stages the central nervous system of *Lepidosiren* is functional, and it must therefore be inferred that such conditions as have been described suffice for functional activity. Much that is seen through the microscope is demonstrably an age change and biologically irrelevant.

The Inter-fibrillar Capillaries.

Golgi (36) asserted the existence of capillary blood-vessels penetrating the large multi-polar ventral horn-cells of *Lophius;* but this interesting feature—itself sufficient to dispel the illusion of the cellular nature of the nerve-elements—has not since been studied. Noel (37) has described a special arrangement of capillaries in close relation to, if not actually entering the eminences of Doyère. Occasionally capillary blood-vessels may be observed to pass through the perikaryon of the larger nerve-cells of mammals. This is a constant feature of Mauthner's cell, and in Figs. 18 and 19 are representations of the conditions visible in *Lepidosiren* and in *Polypterus* at Stage 36. In the latter no fewer than six capillary loops pass through the "cell-body", and rejoin in accordance with the plan indicated in the accompanying diagram:

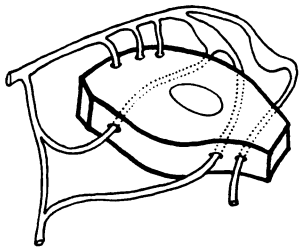

Diagram V.—Diagram illustrating the course of vessels among the neurofibrillæ of the Mauthner's cell of *Polypterus* shown in Fig. 7.

A capillary is related to each of the several afferent systems of fibrillæ and to the axon cone.

This fact is not seen, however, in its proper perspective unless it is clearly apprehended that the neurofibrillar condensations in the immediate neighbourhood of nuclei do not define by any means the boundaries of a cell. That capillaries approach so closely to the nucleus as is demonstrated is merely a proof that even the dense mass commonly regarded as a cell-body is subject to invasion by them. But a vessel which avoids the situations of greatest density is not on that account any less in close relationship with the neurofibrillæ. The arrangement in *Polypterus* suggests that the vessels may have a profound functional importance. These small vessels are themselves innervated, as may be shown in suitable preparations, and may well be the cause of that functional discontinuity which is the basis of synaptic action within the nervous system. There is no anatomical discontinuity at the synaptic end.

Summary and Conclusions.

The syncytialization of the developing neural tube described by His occurs in *Lepidosiren paradoxa*, in which it affects not only the neural tube, but its primary connections with the myotomes and with the integument and their connections with one another.

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An early condition of the nervous system as a whole is thus a widespread system of neural communications, forming a single structure divisible into different parts, central and peripheral. This structure has definite anatomical form, but a changing relationship to the myotomes.

Its own continuity remains uninterrupted throughout its evolutions within the body. These, however, involve interruption of the continuity of the myotomes, both longitudinally and transversely.

The cord, ventral and dorsal roots and lateral line nerves constitute the main central-peripheral system; but subordinate parts connected with the main system are found represented by rudimentary and adult structures in relation to the heart and other organs.

The morphology of the system as a whole is discussed in a separate note.

The histological changes to which the rudiment as a whole is subject during neurogenesis are described. They are:

(1) The appearance of a general neurofibrillar reticulum.

(2) The condensation of this reticulum into definite pathways within the conducting systems of the cord and peripheral nerves on the one hand, and massive "nodes" in relation to the neuroblastic nuclei of the ganglionic centres, central and peripheral, on the other. These nodes, recognized as cell-bodies in histology, are in continuity with each other.

Myofibrillation and neurofibrillation are similar and contemporaneous processes, the first occurring in the syncytialized myotome and the second in the syncytialized nervous rudiment. Neurofibrillæ and myofibrillæ are in continuity. The most definite and important distinguishing feature is the arrangement of the fibrillar material—precisely and uniformly geometrical in the form of a tri-dimensional network with varicosities at the points of intersection in the muscle, but in long fibres tending to complete separation throughout the greater part of their course in the nerve.

The development of the very large Mauthner's cells of the medulla oblongata has been studied for verification of general conclusions by reference to an individual unit of unmistakable appearance at different stages of development.

The small blood-vessels of the developing central nervous system show great anatomical constancy of position and relationship. They are interfibrillar. A greater functional significance than is usually attributed to them is suggested.

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Note on the Morphology of the Neural Rudiment.

The term "neural rudiment" has been used in this paper for the whole of that continuous syncytial formation in which the processes of neurofibrillation and the subsequent condensation of th neuroreticulum into definite pathways and "nerve-cells" occur.

Emphasis has been laid upon the fact that no part of this formation actually disappears in the course of development. Although there is in some situations a further subdivision, or splitting, of component parts of the rudiment, primary connections are represented by fibre connections when development is completed. The nature and direction of these greatly obscures the pattern of the formation in which they occur, and has led to a view of the nervous system derived from a consideration of the fibre-pathways to the neglect of the whole in which these occur.

A diagram of the rudimentary nervous system as a whole (p. 470) is sufficient to show that there is an alternative and wholly different way in which the rudiment may be regarded. Despite their number, the peripheral segmental nerves are seen to form only a system of transverse communications between the members of a series of longitudinal nervous structures. The largest of these is constituted of the right and left halves of the neural tube, terminating behind, in *Peripatus* and in *Lepidosiren* (38), in a uniting band behind the blastopore. The fact that in less primitive types this union occurs in front of the blastopore supports rather than otherwise the inference drawn by Graham Kerr that the neural groove is a "reminiscence" of the slit-like mouth of cœlenterates. The two neural ridges unite in front as well as behind. The ring thus formed therefore, *surrounds* the blastopore in the primitive creatures mentioned. The closure of the canal leading from the blastopore to the anterior neuropore by dorsal union of the ridges does not affect the issue, nor does the presence of a floor to the neural tube thus constituted, for it may be regarded as a backward extension of the anterior margin of the blastopore. What is true of the neural tube is true also of any ring-like or longitudinal neural structure situated farther from the embryonic axis. Of such structures there are traces, more or less complete, of at least four: the line of the dorsal ganglia, the sympathetic chain, and the two (or three) lateral branches of the vagus. By the connections which it establishes with the cord, the ganglionic line forms a complete ring; the sympathetic behind is completed independently of its cord connections and in front through its connections, and thus forms another complete ring; while the various vagus elements are complete in front but not ascertainably so behind. In addition the profound difference functionally between the alar and basal laminæ of the tube suggests that it may be regarded properly as a double instead of a single ring, each being furnished with separate longitudinal systems of fibres in the adult, and by characteristically different columns of cells. When this system is diagrammatized, its resemblance to the circular and radially anastomotic nervous structures of a cœlenterate is so striking as not to require description. Graham Kerr comments upon the fact that the Protostoma theory *alone* affords explanation for several "different but equally puzzling bodies of facts". There is one body of facts which he does not mention. They are major facts of vertebrate anatomy, and have hitherto

completely eluded the ingenuity of morphologists to explain—the facts of decussation in the brain and cord. For this phenomenon no even superficially satisfactory explanation has been advanced.

Consider that while there is a commissure posterior to the blastopore there is, upon the formation of fibre tracts corresponding to the neural ring, provision for circular impulses. But, with the suppression of this feature (which in any case is only vestigial), and the great backward growth of the anterior lip of the blastopore, there is extended facility for the construction of paths and the passage of impulses *in front* of the blastopore, but none behind. Hence, the passage of impulses from one side of the cord or brain to the other for discharge peripherally upon that side is merely a "reminiscence" of the passage of impulses for some distance *around* the protostoma slit before discharge radially. It is suggested that herein lies a complete explanation of this feature of the vertebrate central nervous system, which is of such fundamental importance physiologically.

References.

(1) von Baer, K. E.—*Ueber Entwicklungsgeschichte der Tiere*, i, Königsberg, 1828.
(2) His, W.—*Die Neuroblasten und deren Entstehung im Embryonalen Mark*, Leipzig, 1889.
(3) Kerr, J. Graham—"On Some Points in the Early Development of Motor Nerve Trunks and Myotomes in *Lepidosiren paradoxa* (Fitz.)," *Trans. Roy. Soc. Edin.*, xli, pp. 119–127.
(4) Held, H.—*Die Entwicklung des Nervengeweber bei den Wirbeltieren*, Leipzig, 1909.
(5) Coghill, G. E.—*Anatomy and the Problem of Behaviour*, Cambridge, 1929.
(6) Paton, Stewart.—"The Reactions of the Vertebrate Embryo to Stimulation and the Associated Changes in the Nervous System," *Mitteilungen a. d. Zoologisc. Sta. Neapel.*, 1907, xviii, pp. 2 and 3.
(7) Kerr, J. Graham.—*Normentafeln* (*Keibel*), 1909, p. 10.
(8) *Idem.—Text-book of Embryology*, ii, London, 1919.
(9) Agar, W. E.—"The Development of the Anterior Mesoderm and Paired Fins with their Nerves, in *Lepidosiren* and *Protopterus*," *Trans. Roy. Soc. Edin.*, xlv, III (23), p. 611.
(10) Ballantyne, F. M.—"The Continuity of the Vertebrate Nervous System: Studies on *Lepidosiren paradoxa*," *ibid.*, liii, III (32), p. 663.
(11) Harrison, Ross G.—"Neuroblast versus Sheath Cell in the Development of Peripheral Nerves," *J. Comp. Neurol.*, xxxvii, 1, p. 123.
(12) Balfour, F. M.—*Monograph on the Development of Elasmobranch Fishes*, London, 1878.
(13) Allis, E. P.—*J. Morph.*, 1889, ii.
(14) Pinkus.—"Die Hirnnerven des Protopterus annectens," *Morph. Arbeit*, 1895.
(15) Jones, Tudor.—"The Structure and Mode of Innervation of Capillary Blood Vessels," *Amer. Journ. Anat.*, 1936, i, p. 58.
(16) *Idem.*—"The Primitive Conducting Mechanisms of the Vertebrate Heart," *Trans. Roy. Soc. Edin.*, lvii, 1 (6), p. 225.
(17) Kerr, J. Graham.—See (8), p. 500.
(18) *Idem.—The Work of John Samuel Budgett*, Cambridge, 1907.
(19) Hertwig.—*Handbuch d. Entwicklungslehre*, Jena, 1906.
(20) Kerr, J. Graham.—See (8), p. 56.
(21) Sedgwick, A.—*Quart. Journ. Micr. Sci.*, 1884, xxiv.
(22) Streeter, G. L.—*Amer. Journ. Anat.*, 1905, iv.
(23) Bouin, P.—*Elements d'histologie*, Paris, 1929.

(24) AGDUHR, E.—"Is the Post-embryonal Growth of the Nervous System Due only to an Increase in Size, or also to the Increase in the Number of Neurones?" *Kon. Akad. v. Wet. te Amsterdam*, 1919, xxvii.

(25) DART, R. A., and SHELLSHEAR, J. L.—"The Origin of the Motor Neuroblasts," *Journ. Anat.*, 1922, lvi (II), p. 77.

(26) MILLER, A. E.—"The Cleavage of the Egg of *Lepidosiren paradoxa*," *Quart. Journ. Micr. Sci.*, 1923, lxvii, (IV), p. 497.

(27) *Op. cit.* (18), p. 218.

(28) JONES, TUDOR.—"A Note on the Evidence concerning the Minute Structure of Striped Muscle," *Journ. Anat.*, 1929, xiii, (1), p. 168.

(29) *Idem.*—"Behaviour of Bielschowsky-stained Neurofibrillæ between Crossed Nicols," *Nature*, Apirl 2, 1932.

(30) CAMERON, JOHN.—"The Development of the Vertebrate Nerve Cell," *Brain*, 1906, cxv, p. 332.

(31) JONES, TUDOR.—See (16).

(32) *Op. cit.* (11).

(33) BARTELMEZ, G. W.—"Mauthner's Cell and the Nucleus Motorius Tegmenti," *Journ. Comp. Neurol.*, 1915, xxv, p. 87.

(34) HOLMGREN and HORST.—"Brain of Ceratodus," *Acta Zoolog.*, 1925, vi, p. 61.

(35) JONES, TUDOR.—See (29).

(36) DA FANO, C.—*J. Path.*, 1927, xxix, p. 500.

(37) NOEL, R.—"Sur un disposition vasculaire spécial observé au niveau des plaques motrices," *Bull. d'Hist.*, app. 3, (2), 1926.

(38) *Op. cit.* (8).