



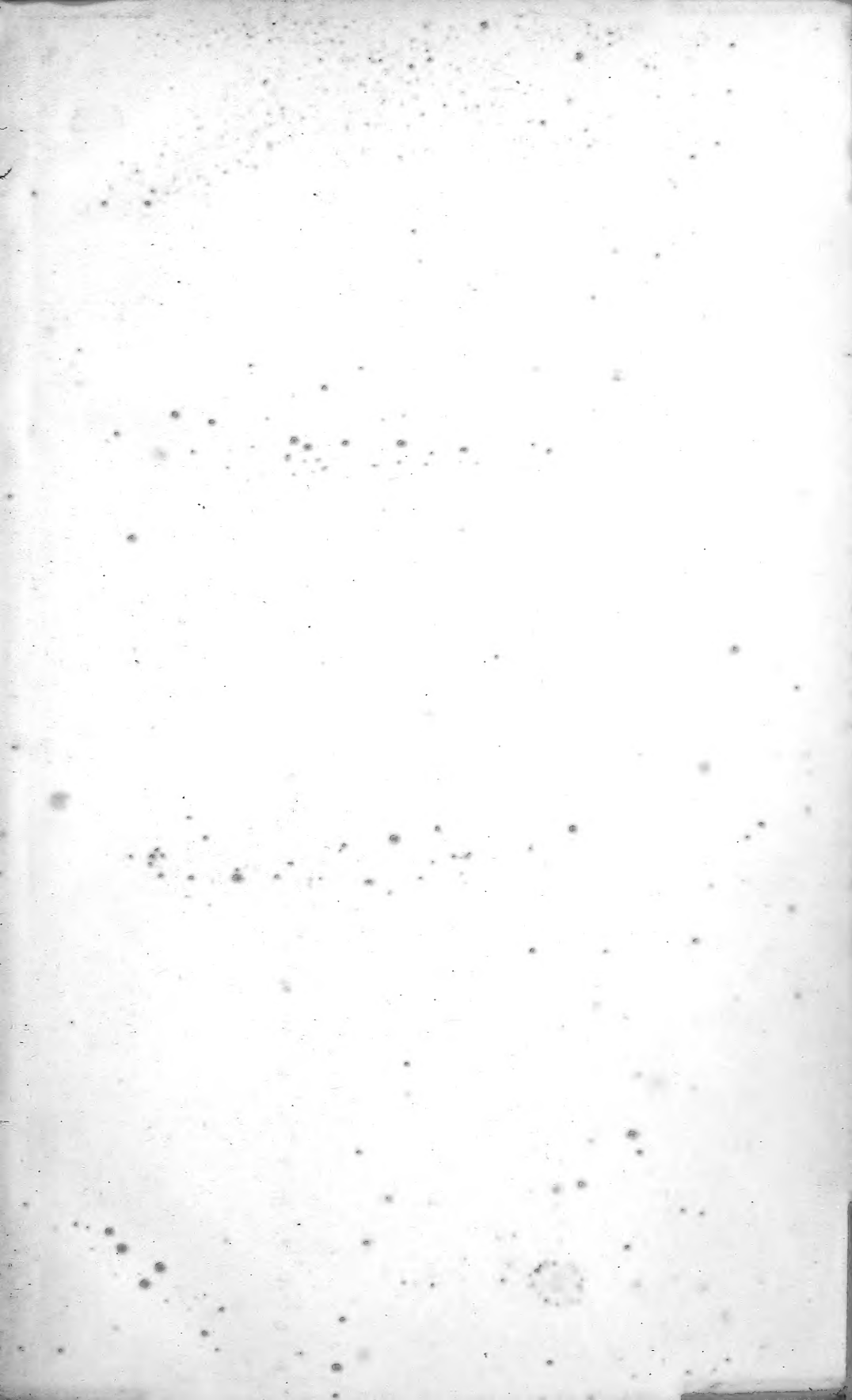
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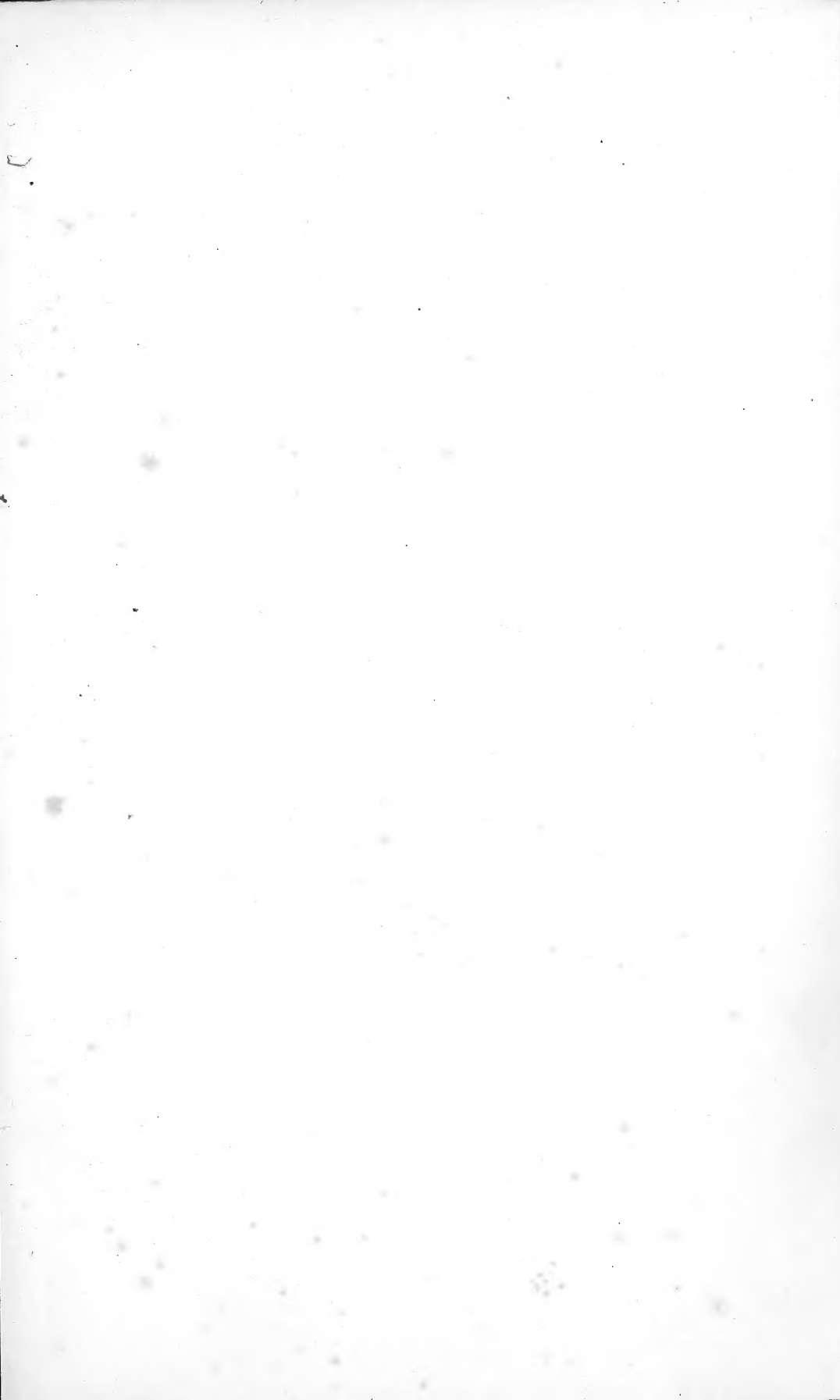
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# STUDIES

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# BIOLOGICAL LABORATORIES

OF

THE OWENS COLLEGE.



VOLUME I.

*PUBLISHED BY THE COUNCIL OF THE COLLEGE*

AND EDITED BY

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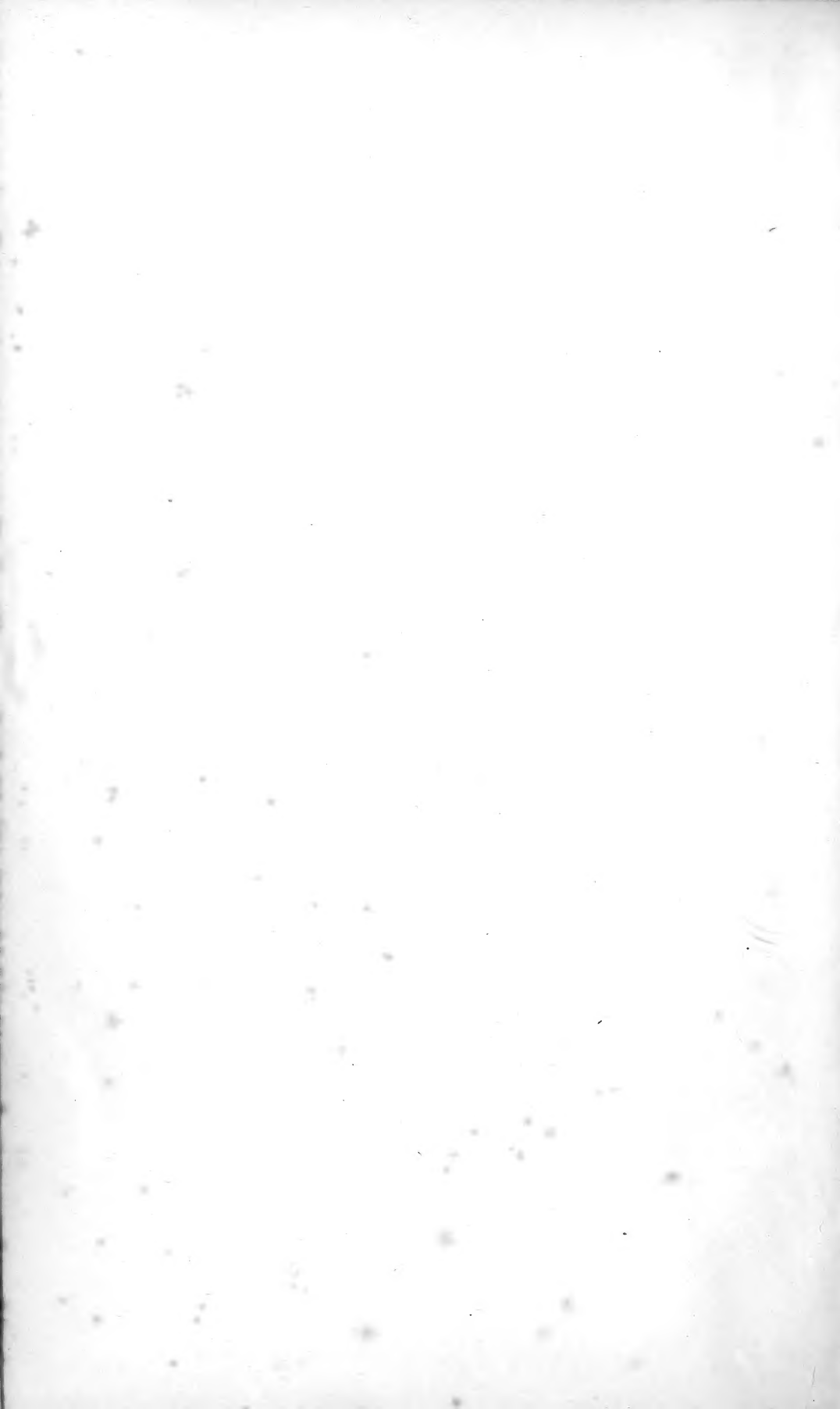
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SACRAS ESSE IUBEMUS  
CONSILIIUM SENATUSQUE COLLEGI  
MANCUNIENSIS.



## P R E F A C E .

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The present volume, the first of a series which the Council propose to issue from time to time, consists of papers containing the results of investigations conducted in the Biological Laboratories of the College during the past two or three years. Two papers of earlier date have been included because they deal with a problem that has attracted much attention of late years, and one of the latest phases of which is discussed in Dr. Beard's essay on the "Branchial Sense Organs of Vertebrates."

Mr. Marshall Ward's paper on "An Aquatic Myxomycete," and the contributions of Mr. Dendy and Mr. C. F. Marshall appear here for the first time. The remaining papers are reprinted from the Transactions of Scientific Societies, or from Scientific Journals, the editors of which have courteously granted permission to republish them and to reproduce the plates by which they are illustrated.

Special interest attaches to the two earlier papers of Mr. Marshall Ward, and to those of Dr. Beard and Mr. Fowler, as the investigations the results of which they record were carried on by these gentlemen during their tenure of Bishop Berkeley Research Fellowships. To the generous encouragement afforded by the founder of these fellowships is due the opportunity for the completion of these researches, and to him this volume is respectfully dedicated.

Much of the work here recorded was performed under the difficulties of cramped accommodation and limited appliances, and it may confidently be hoped that the material for future volumes will show corresponding improvement when, in the immediate future, the completion of the new Morphological Laboratories shall have placed the College in a position of exceptional advantage for the promotion of Biological research.

*Owens College,*

*March, 1886.*





## CONTENTS.

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	PAGE
Mr. MARSHALL WARD.—On the Morphology and the Development of MELIOLA, a genus of tropical epiphyllous Fungi. Plates I. and II. <i>Reprinted from the "Philosophical Transactions of the Royal Society," Part II., 1833</i> .....	1
Mr. MARSHALL WARD.—On the Sexuality of the Fungi. <i>Reprinted from the "Quarterly Journal of Microscopical Science," 1884</i> .....	23
Mr. MARSHALL WARD.—An Aquatic Myxomycete, Plates III. and IV. ....	64
Prof. MARSHALL and Mr. SPENCER.—Observations on the Cranial Nerves of Scyllium, Plates V. and VI. <i>Reprinted from the "Quarterly Journal of Microscopical Science," 1881</i> .....	87 ✓
Prof. MARSHALL.—The Segmental Value of the Cranial Nerves. <i>Reprinted from the "Journal of Anatomy and Physiology," 1882.</i>	125 ✓
Dr. JOHN BEARD.—The System of Branchial Sense Organs and their Associated Ganglia in Ichthyopsida. A contribution to the Ancestral History of Vertebrates. Plates VII, VIII., and IX. <i>Reprinted from the "Quarterly Journal of Microscopical Science," 1885</i> .....	170 ✓
Mr. B. MELLAND.—A simplified view of the Histology of the Striped Muscle-fibre. Plate X. <i>Reprinted from the "Quarterly Journal of Microscopical Science," 1885</i> .....	225
Mr. G. H. FOWLER.—The Anatomy of the Madreporaria. Part I. Plates XI., XII., and XIII. <i>Reprinted from the "Quarterly Journal of Microscopical Science," 1885</i> .....	243
Prof. MARSHALL.—On the Nervous System of Antedon rosaceus. Plate XIV. <i>Reprinted from the "Quarterly Journal of Microscopical Science," 1884</i> .....	263
Mr. A. DENDY.—On the Regeneration of the Visceral Mass in Antedon rosaceus .....	299
Mr. C. F. MARSHALL.—Some Investigations on the Physiology of the Nervous System of the Lobster .....	313
Prof. MARSHALL.—The Morphology of the Sexual Organs of Hydra. <i>Reprinted from the "Memoirs of the Manchester Literary and Philosophical Society," 1885</i> .....	324



ON THE MORPHOLOGY AND THE DEVELOPMENT OF THE  
PERITHECIUM OF *MELIOLA*, A GENUS OF TROPICAL  
EPIPHYLLOUS FUNGI.

By H. MARSHALL WARD, B.A., *Berkeley Fellow of Owens College,  
Victoria University; late Cryptogamist to the Ceylon Government.*

[PLATES I—II.]

During the course of recent researches into the nature of parasitic fungi, my attention was arrested for some time by several forms of epiphytal growths which occupy a sort of half-way position between the more pronounced endophyllous parasites and those fungi which cannot be looked upon as requiring more than a hold-fast or shelter from their hosts. Among these are the *Meliolas*, a group established by Fries in 1825 to receive certain tropical fungi.<sup>1</sup> In the 'Annales des Sciences Naturelles' for 1851<sup>2</sup> is a memoir by Bornet on the species constituting the genus *Meliola*, in which the characters of these remarkable epiphytes are enumerated and examined, and a classification of the known forms proposed: this paper is a standing authority on the subject, and I shall have occasion to refer to it at intervals subsequently, partly to confirm some of Bornet's work, partly to add new observations and correct older views as to the nature or significance of various points.

The *Meliolas* are minute epiphyllous fungi, belonging to the *Pyrenomyces*, the deep-brown or black mycelium of which appears as

<sup>1</sup> 'Systema Orbis Vegetabilium.'

<sup>2</sup> Ser. iii., Bot., t. xvi., pp. 257, &c.

sooty patches on many and various plants in the tropics, and presents, roughly, a similar appearance to the masses of *Capnodium* or *Fumago* sometimes observed in European woods on the leaves of living plants.

Though, according to Bornet, several species must have been known under different names to the earlier botanists, the name established by Fries, and published in his revised system, was accepted by Montagne and Léveillé and has persisted since: Berkeley, in England, has referred to the group in his 'Cryptogamic Botany,' and has described several species from the tropics in various papers.

The habit of these fungi, and the fact that no true *Erysiphe* had been found among the collections of travellers in the tropics, led Fries<sup>1</sup> to insist strongly on the known or supposed analogies between the two genera, and, Bornet following Fries, the *Meliolas* have thus come to be regarded as replacing the *Erysiphes* in tropical countries—as, in fact, "representative species." Bornet added several facts to those already known concerning the coarser anatomy of the group; but even his excellent and systematic memoir left large gaps in the knowledge of important details, and practically nothing was known of their development or of the formation of their "fruit-bodies." These and other gaps I hope to fill up to at least a large extent in the present essay.

The appearance of this fungus, as presented to the unaided eye, is much the same as that offered by *Asterina* and similar forms, and the reader may be referred to a recently published drawing of that fungus for a tolerably accurate idea of it.<sup>2</sup> The chief difference is that the black maculæ presented by well developed plants of *Meliola* are more decided and thicker than those of *Asterina*; all transitions are found, however, and, as with many other forms of epiphyllous *Pyrenomyces*, it is impossible to detect exactly what fungus is present by a superficial examination.

The fungus *Meliola* may be conveniently considered as composed of a mycelium, which supports appendages and perithecia, and which arises from spores developed within the asci of the latter. Bornet considered the "receptacle" as an equally important and distinct constituent, but this is perhaps unnecessary, since, as will be shown, the so-called receptacle can only be looked upon as a more or less acci-

<sup>1</sup> 'Summa Vegetabilium,' p. 406: "Genus in foliis tropicis vulgatissimum ut *Erysiphes* in terris temperatis."

<sup>2</sup> Quar. Journ. Micr. Sc., October, 1882, plate 27, figs. 1 and 2. See also Bornet's beautiful figures, Ann. des Sc. Nat., ser. iii., t. xvi., plates 21 and 22.

dental development, so to speak, depending and following upon the formation of the perithecium.

The mycelium, forming the chief part of the black patches found on the surface of the affected leaves, petioles, &c., spreads in an irregularly stellate manner from a common centre or centres (see fig. 1). It is detached with comparative ease from the epidermis of the leaf, and bristles with fine, simple or branched, pointed appendages, of a black colour, which spring from the main hyphæ, and from around the subglobular perithecia which are irregularly scattered over the surface.

The main hyphæ constituting this vegetative part of the fungus, are irregularly radiating, sinuous or zigzag filaments, closely appressed to the epidermis of the leaf, &c., and composed of cylindrical joints or cells placed end to end, and branching at angles of about 45 degrees (*cf.* fig. 2, and fig. 5). Their stiff and even brittle walls are deeply coloured brown or black, and thus obscure the view of their contents: sections and reagents prove these to be finely grained protoplasm, with or without oily drops in the interior. The diameter of the hypha is equal throughout, the apex being, as a rule, evenly rounded: the cross-septa dividing the hyphæ into cells are firmly marked, thick and dark-coloured like the outer walls.

The main branches of the mycelium all present the same general characters described above. In many cases, however, the blunt apices of the larger hyphæ, instead of being evenly rounded, become curiously deformed by an accumulation of abnormal growths, of the nature of caps (see 43, fig. 7) fitting roughly one over the other: these consist of swollen, more or less cuticularised thickenings of the cell-wall, with or without granular débris between the layers. They are evidently produced by irregularities in the forward growth of the hypha: in the moist intervals the growing apex, more delicate than the older portions of the hypha, creeps along the surface of the leaf in the normal manner; during recurrent dry and hot unfavourable periods, however, sudden hardening and stoppage of growth causes the accumulation of the caps. That unfavourable intervals in outward circumstances may produce such abnormalities is well seen in the *Saprolegniæ*, and I have in these observed the formation of successive shell-like caps of dense cellulose, more or less altered, and enclosing granular matter between the layers: the caps are coloured blue by solution of zinc-chloride and iodine, the granular débris yellow,

These phenomena were by no means uncommon with the hyphæ of *Achlya* and *Saprolegnia* grown, in summer, too long in the same water; of course the pathological changes are produced by different causes in the two cases.<sup>1</sup>

Besides the main branches of the mycelium, certain small pyriform or flask-shaped outgrowths are given off at pretty regular intervals from the cylindrical cells of the larger hyphæ (see figs. 2, 3, and 4): in some cases each cell or joint gives off such a short branchlet from each side, in others from alternate sides. More rarely they are absent altogether. In all cases examined the short lateral branchlet arises as a simple bulging out of the lateral wall of the cell: as this proceeds, the bud (as it may be considered) swells out, and its cavity finally becomes separated from that of the parent branch by a firm septum. The long axis of the bud-like protuberance is very generally, though not always directed at an angle of nearly 45 degrees to that of the portion of the main hypha lying nearer the growing point (see fig. 2, &c.): its walls are similarly dark coloured and firm, and it contains fine grained protoplasm much as the cells of the main hyphæ. Morphologically considered, the short lateral outgrowths are undoubtedly of the nature of arrested branches.

In one form of *Meliola*, growing on the leaves of a species of *Convolvulus*, I have observed a second form of the lateral branchlet (see fig. 4), co-existing with the commoner pyriform type. In this case the outgrowth was longer, narrowed into a sort of neck, and presented the general shape of a Florence flask, seated with its bulged out body on the parent branch. In some specimens, each cell of the latter supported two opposite flask-shaped branchlets: in others only one, with or without a pyriform body in addition. Sometimes one or the other type occurred singly and irregularly (fig. 4).

The flask-shaped body is sometimes open at the apex, though I have never succeeded in observing anything emitted from the pore. These flask-shaped appendages recall to mind the peculiar bodies figured by Woronin in another group of the *Pyrenomycetes* (*Sordaria*),<sup>2</sup> and although no grounds exist for correlating the two phenomena in detail, the fact is at least worth recording that the lateral pyriform bodies in *Meliola* are capable of subserving reproduction, as will be shown hereafter.

<sup>1</sup> There seems reason to believe that further investigation may throw light on this subject in connection with the apposition of the cell-wall.

<sup>2</sup> "Beiträge zur Morph. u. Phys. d. Pilze," De Bary and Woronin, ser. iii., plate 5.

When the hyphæ or branchlets of this fungus are looked upon from above, and a strong light passes through from below, one often observes a minute, circular, bright spot, which appears to shine through the upper wall like a very small oil-drop; on reversing the object, so that the lower side of the hypha comes uppermost, this brilliant pore-like spot appears much more evident, and is clearly due to a thinning in the wall of the under side of the hypha, at a spot where no colouring matter is deposited in the cell-walls, and where the contained protoplasm is placed more nearly in connection with the outside (see figs. 7, 21, and 40).

Bornet apparently refers to these bright spots when he speaks of oily globules in the interior of the hyphæ,<sup>1</sup> though he may have been speaking of actual oil-drops developed in the dried specimens with which he chiefly worked. If Bornet's remarks refer to the bright spots here described, the facts of their appearing only on the lower wall and not being altered by alcohol, &c., remain to be explained.

Taking all the facts into account, the view seems to recommend itself that these bright spots are the points of attachment of the hyphæ to the epidermis; if so, they are to be regarded as *haustoria* of a very rudimentary nature. The mycelium certainly is attached to the surface of the leaf, though but feebly, and it appears suggestive that alcohol specimens are more easily detached than fresh ones, possibly because the protoplasm becomes contracted and rendered brittle. No other anchoring bodies have been observed, and one notes that the position of these brilliant spots accords with that of the well-developed *haustoria* in *Asterina*,<sup>2</sup> a genus of fungi at least allied to the *Meliolas*. These bright points are not always present, and in some cases seem to be normally absent. They are very generally formed at once on germination, appearing on the first short tubes put forth by the spore (fig. 40), a condition of things which may again be compared to what occurs in *Asterina*,<sup>3</sup> and also in *Erysiphe* and allied forms.<sup>4</sup> Still another point reminding us of *Asterina* and the *Erysipheæ* is the function of the pyriform branchlets; in some cases at least, they become detached, and act as vegetative reproductive organs or *conidia*, each putting forth bud-like processes which develop into new hyphæ. Bornet remarked the separation of these buds in *Meliola amphitricha*,

<sup>1</sup> *Op. cit.*, p. 260, and plate 21, fig. 3.

<sup>2</sup> See my description in *Quar. Journ. Micr. Sc.*, October, 1832.

<sup>3</sup> Bornet, *op. cit.*, plate 28, fig. 5.

<sup>4</sup> De Bary, "Beiträge zur Morph. u. Phys. d. Pilze," 1870, ser. iii., plate 12, figs. 1 and 2.

and hints at their possibly serving as reproductive bodies much as the *Oidium* forms of *Erysipheæ*: since he worked with dried specimens, however, this question could not be decided.

Bornet remarks that the mycelium on the upper side of many leaves are sterile, while those below and protected from the direct rays of the sun alone support *perithecia*: this is certainly not true for the species examined by me, and, indeed, I cannot determine any difference between the upper and lower mycelia in this respect. Those on the upper surface seem quite as productive of spores, &c., as those below, and in many cases—*e. g.*, those *Meliolæ* so common on *Memecylon*—the mycelium vegetates almost exclusively on the upper surface, and is quite fertile there.

Besides the short pyriform and flask-shaped branchlets described above, the mycelium bears certain stiff, upright, appendages of the nature of *setæ* (see figs. 1, 41, and 8): these *setæ* spring from the cells of the hyphæ at various points in their course, and, from their position and mode of origin, are probably to be regarded, morphologically speaking, as lateral branchlets which become elongated in a direction more or less perpendicular to the plane of the leaf. Such a *seta* grows very rapidly and soon reaches its limit: the cylindrical cells composing it are relatively longer than those of the hyphæ, but resemble them in other respects (the walls being, perhaps, somewhat stiffer and more deeply coloured), and taper above, in the simple types, or become variously branched.

In most *Meliolas* the *setæ* are especially aggregated around the *perithecia*, forming circles of stiff radii springing from what Bornet terms the "receptacle": they are also developed, however, from various isolated points of the mycelium bearing no direct relation to the fruit-bodies.

The forms of the *setæ* vary from a simple, upright or curved filament, to structures branched like antlers, trifurcate, twisted, &c., at the tip (*cf.* fig. 8 and Bornet's figures<sup>1</sup>): Bornet has made use of these details in classifying the formal species, and although it is doubtful whether the more similar types are constant, there can be no objection to their use much in the same manner that the appendages of *Erysipheæ*, &c., are used to distinguish the forms of that group. Bornet regards the origin of the *setæ* at points on the mycelium as marking out places where new *perithecia* are to be developed: I cannot

<sup>1</sup> *Loc. cit.*, plates 21 and 22, figs. 6, 15, 16, &c.



say that this idea is altogether a false one, but investigation of the development of the fruit-bodies seems to show that at least no necessary connexion exists between the two phenomena.

As to the function of the *setæ*, little or nothing can be stated. The earlier suggestions of Sprengel and Fries (as quoted by Bornet) that they may be organs for the exit of the spores cannot be accepted: not only on the ground of the disproportion between the numbers, but also because the spores are too large to pass through the *setæ*, even supposing the cavity continuous and ending in an ostiolum, which is not the case. I have often tried to discover *conidia* or other bodies in connexion with the *setæ*, but have been forced to the belief that they have no function whatever connected with spore-production. One is not now impressed with the necessity for assigning any special function to such structures: if the *setæ* are merely free-growing branches of the otherwise appressed, creeping mycelium, there is nothing surprising in the fact that some differences in form and consistency are correlated with their sub-ærial habit. This is at least no more remarkable than that the looser branches of an alga, like *Coleochaete*, should have a facies slightly differing from that of the cell series comprising the lower, creeping, appressed parts of the thallus.

The collection of *setæ* immediately around the "fruit-body" simply results, immediately, from the vigorous development of hyphæ which accompanies the later stages of formation of the perithecium: this mass of setigerous hyphæ, which seems comparable with the formation of *haustoria* and such-like organs in other fungi during the fruit development, was called the "*réceptacle*" by Bornet. As to a possible protective influence of the circles of *setæ*, the question must be left open until we know more of the conditions: in some cases, at any rate, the *setæ* do not arise until the perithecium is completely formed, and the spores nearly ripe.

The perithecium, when completely developed, is a globular or sub-globular body, consisting of a shining black or brown external case, the outer thick walls of which appear regularly embossed, and an internal mass composed of asci and spores, &c. The embossed pattern on the outer walls results from the thick-walled cells, of which it is composed, projecting at their free surfaces: where the cells join each other, forming polygonal figures, they do not so project.<sup>1</sup> What may be termed the base of the perithecium is sessile on the mycelium:

<sup>1</sup> See Bornet's figures, *loc. cit.*, plates 21 and 22.

at the opposite pole, or apex, is frequently a slight papilla, not obviously pierced by any pore. Bornet, noting this fact, imagines that the dehiscence takes place below, the whole upper part of the perithecium becoming broken away by a circular rupture at the base. In some forms, at least, the spores escape through an opening at the apex: how far this is general I do not know (fig. 43), but facts exist to render it probable that a minute and dilatable pore occurs in others.

Vertical sections of the mature perithecium show that within the firm, deep-coloured, external wall is a lining of softer cells, with swollen envelopes and of a more or less flattened form: this inner lining of the perithecium extends two or three cell-series deep, and is slightly yellow or pale-brown in colour (see figs. 33 and 34). In the cavity thus enclosed are the groups of asci in various stages of development: these delicate, clavate sacs contain spores, or have emptied them into the semi-gelatinous, granular matrix around.

With these preliminaries, I may pass on to consider and describe the development of the perithecium, as followed step by step on a species of *Meliola* which I have investigated with no slight success:<sup>1</sup> this will be found to throw light on the morphology of these fungi from the best of sources—development—and aid in a more critical estimation of their proposed systematic position. After describing in detail the origin, mode of development and fate of the fruit and spores, I propose, therefore, to examine the relations of the *Meliolas* to *Erysiphe* and other fungi.

On examining portions of the epiphyllous mycelium bearing the short pyriform, lateral branchlets so often referred to above, one frequently discovers specimens presenting the appearances depicted at figs. 9, 10, 11, &c. The simple pyriform body, after becoming more swollen, has suffered division into two portions or cells by a septum, usually vertical to the plane of the mycelium and leaf, and passing diagonally across the cavity with a slight curve, so as to abut on the outer walls at right angles, or nearly so. The originally unicellular protuberance becomes in this manner divided into two more or less unequal cells, and it will be shown in the sequel that these two cells have, from the first, each a different destiny in the formation of the fruit. For this reason I have indicated in the drawings, by shading,

<sup>1</sup> I must take this opportunity of thanking Professor De Bary for kind suggestions with respect to this work.

a difference which does not present itself in the natural object at this stage. The more apical cell, which is smaller and shaded darker in fig. 9, may be indicated throughout by the letter A: it will be found that this cell produces the central ascogenous tissue of the young perithecium, while the other, which will be referred to as cell B, originates the outer portions of the case or perithecium wall.

Following close upon the preliminary division above described, a septum appears across the larger of the two cells, cutting the first-formed division wall at right angles, or nearly so: this is rapidly followed by another septum (fig. 10), and so the larger cell (B) becomes cut up into three. Following upon these, a number of further divisions in planes at right angles to the preceding are soon established (figs. 11 to 17), and at the same time, though much more slowly, one or two more division walls are formed in the cell A, thus cutting it up into a short series of about three cells (figs. 14, 15).

If the above description has been followed, it becomes clear that the division of the more rapidly growing cell, B, results in the production of a sheet of cells affixed, so to speak, to the few-celled mass resulting from the slow division of A: such being the case, and the sheet extending as new divisions are formed, the cells resulting from A become gradually enveloped more and more in those resulting from B. A comparison of the figs. 9 to 17 will facilitate matters here, and for convenience of description hereafter, and in consideration of its destiny, we may term the mass of cells produced from A the *ascogenous core*—or simply the core.

At a stage which may conveniently be considered the next one to fig. 11, the cells resulting from the division of B are observed to be extending as a curved layer over the "core" of cells formed by A. If, at this stage, the young fruit-body is cut off, and allowed to roll over in fluid under the microscope, the form and arrangement are found to be somewhat as sketched in fig. 12, where *a* represents the view from below, *b* that from the side, and *c* an end elevation of the structure. The cell A, in fact, is becoming gradually enfolded by the layer of cells derived from B, a process which results, at a later period (fig. 17), in the almost complete tucking in of the "core" as the centre of a sub-globular mass of cells.

As this process of "invagination by epibole" (as it would be termed in the case of an embryo) goes on, the "core" has been more slowly cut up into cells—at first by walls perpendicular to its long axis, and

then by septa in other planes at right angles—and the sub-globular body thus produced lies with the open part towards the epidermis.

After this period, two events occur: 1st, the cells of the "core," possessing very thin walls, acquire a different aspect from those of the outer shell; their finely granular protoplasm makes them appear denser and more opaque, shining through the latter until this becomes too thick to be transparent; 2nd, the open part of the growing perithecium becomes closed over, and the internal structures can no longer be made out without the aid of actual sections. At this point my observations have failed to decide which of two possible modes of growth take place: Is the covering in of the "core" completed simply by the extension and closing in of the edges of the outer layer; or are cells, cut off from the "core" below, intercalated, so to speak, into the open gap? One is led to expect by analogy that the former process takes place, but some events lead to the suspicion that such may not be the case.

At the stage corresponding to fig. 19 the young perithecium appears almost opaque, very little light passing through the dark-coloured and thick outer walls; from below, however, the larger cells composing the "core" can be readily seen in the optical section, shining by means of their dense, fine-grained contents through the shell. In the next stages, the "core" can only be seen dimly through the outer envelope (fig. 20), even after treatment with reagents, or, as in figs. 21 and 22, after cutting or tearing off some of the outer cells.

Nothing but a fortunate vertical section through the young fruit at or near this stage will decide finally whether the lower side is covered in by the meeting of the outer shell edges, or by partial "delamination" from the lower side of the "core," and this I have not succeeded in obtaining. The thick, dark outer walls have now become so opaque, that optical sections fail to determine the course of events; and treatment with reagents does not afford evidence sufficiently satisfactory to decide the questions, since it seems impossible to remove the colouring matter. Potassic hydrate or weak acids do, it is true, render the structures a little more translucent after some time; but even the extreme resort of heating in weak chromic acid has only yielded partial results, and with this slight information on the point I have reluctantly been compelled to content myself for the present. A comparison of figs. 17 to 21 certainly suggests that the process of envelopment is completed by the outer layer of cells derived

from the repeated and rapid division of the cell B, and this view may be recommended on the ground of analogies with the *Erysipheæ*, to be examined hereafter; but, while figs. 19 and 20 by no means decide the point, we shall find that in the perithecium of another species of *Meliola* (or an allied form) the construction almost certainly proceeds by continued cutting up and "delamination" of the results of division of one cell.

Be this as it may, the young perithecium now consists of the following parts:—A central "core" of delicate-walled colourless or yellowish cells, very rich in finely granular protoplasm, and, surrounding this completely, a single layer of cells with thick, hard, dark-coloured walls (especially those on the exterior surface); the whole mass is attached to the hypha from which it originated by a very short pedicle or joint (see figs. 19–24).

At a period slightly later than the above, the cells of the outer layer are becoming multiplied by tangential walls, and those of the inner core by radial and horizontal divisions: these processes go on for some time until the whole perithecium is a complex of many small cells, the outer of which become firmer and darker-coloured, the inner delicate and full of fine-grained protoplasm as described.

No trace of the internal structure is, however, visible now from the outside. On isolating a perithecium at this stage—a matter of no slight difficulty, but practicable with a slender knife used under a low power of the microscope—it presents the forms shown in fig. 25 on being rolled over. Above, the outer surface curves equally away from the centre, and the slightly projecting walls of the cells give it an appearance of being embossed (fig. 25, *x*). From below (fig. 25, *y*), the object looks very different; the surface is much flattened and nearly circular, and from many of the cells are processes developing as hyphæ in all directions. These radiating processes creep close along the surface of the leaf, to which the fruit-body is also appressed, and no doubt serve to give a much firmer hold for the fruit; at first their thin walls are only of a pale brown hue, but rapidly acquire the thickness and deep colour of the fruit and mycelium. Seen from the side, the young perithecium presents the appearance sketched at fig. 25, *z*. It is these radiating anchoring hyphæ which form collectively what Bornet terms the "*réceptacle*," and from them, at a later period, the bristling *setæ* found around the mature fruit are developed.

From the stage just described the development of the fruit-body

proceeds rapidly; but, since the objects now become of a more manageable size, I have been able, by actual sections through the perithecium embedded in spermaceti or gum, or, better still, in elder pith, to obtain some insight into the processes going on even in the centre of the mass of cells.

At stages just prior to the one last described, the central core of thin walled cells—which it will be remembered has been derived from continuous divisions of the cell A—is commencing to divide up by septa in several directions (figs. 23, 24), while the outer layers surrounding this—derived primitively from B, and, possibly, in part from A—are divided more regularly by tangential walls, followed by radial ones at right angles as the area enlarges. As the increasing small and delicate cells of the core become formed more rapidly, a certain tendency at least to a regular arrangement can be recognised in the later stages, as shown in such sections as figs. 28 and 29 and fig. 27: this regularity becomes interfered with by the mutual pressure of the cells, and the outer ones, of which the walls are especially soft and swollen, become flattened and pulled in the tangential direction, and only marked by the very granular yellowish protoplasm in their diminishing cavities. In the central lower part of the core, vertical sections at this, and slightly later stages, show that certain cells, with very delicate outlines and finely granular refractive contents, maintain their larger size and upright arrangement, and are by these peculiarities well distinguished as a special group or tuft of cells (see fig. 28, and fig. 31). In oblique (fig. 29) and horizontal (fig. 30) sections passing through the lower third of the developing perithecium, they can also be readily distinguished by their special peculiarities, and no question can be entertained as to their significance in the formation of the essential parts of the fruit-body. This group of cells is the forerunner of the young asci, and may be termed the *Ascogonium*.

As development proceeds continuously, the outermost layers acquiring thicker and more deeply coloured walls, the above named group of upright cells become relatively larger, increasing slowly in number by a few divisions, while the diffuent, compressed cells between them and the outermost layers slowly give up their contents, and become reduced to mere granular streaks embedded in a jelly-like mass of swollen and fused cell-walls (see fig. 31). This process is exactly comparable to what takes place in the developing embryo-sac

of certain phanerogams,<sup>1</sup> or of the pollen mother cells in the anther,<sup>2</sup> in so far as the larger cells clearly develop at the expense of material derived from those around.

The tuft of successful cells thus nourished is, in fact, the "ascogonium" of this fungus. At a slightly later stage than the one last figured, the space formerly occupied by the deliquescent remains of small cells is filled with an almost transparent semi-fluid mucus, in which a few bright granules are embedded; while the lower part of the perithecium contains a tuft of asci in various stages of development (see fig. 33), and which have evidently proceeded from the large cells of figs. 28 and 31, which have devoured all, or nearly all, the smaller soft cells surrounding them.

Sections of perithecia at a stage between those shown in figs. 31 and 33, have not been obtained, but enough evidence has been secured to enable me to conclude that the asci are the direct result of the transformation of the elongated upright cells of fig. 31, which are nourished at the expense of the cells of the inner layers. Partly from the brittle nature of the outer walls, enclosing a space filled with almost fluid contents, and partly from the extreme delicacy of the young asci, I have been unable to decide whether any distinct branching of the ascogenous cells precedes the formation of the definite asci: probably such is the case. We have now followed the development of the perithecium to the period when it may be considered ripe: a period of some duration, since the asci are continually and successively formed in the tuft for some time.

Fortunate sections of the perithecium wall at this stage have yielded the following information. In the centre of the apical wall, where a slight protuberance sometimes occurs, the cells of the inner wall are found to radiate towards a pale translucent spot or pore (see fig. 36), and although I have not been able to obtain sections exactly through this, and am therefore unable to affirm positively that it is an actual pore, there seems little doubt that this is at least the weak point through which the spores escape from the ripe perithecium, no doubt forced through by the swelling of the materials around. Bornet<sup>3</sup> believes that the perithecium opens by a circular rupture at the base: I have tried to confirm this, but failed, and am strongly persuaded that the apical spot figured is the point of exit for the spores. That a

<sup>1</sup> Cf., amongst others, Strasburger, 'Angiospermen und Gymnospermen.'

<sup>2</sup> Cf., Strasburger, 'Bau und Wachstum der Zellhäute,' 1882.

<sup>3</sup> *Op. cit.*, p. 261.

minute pore should escape observation from without is not remarkable: the reflection of the light from the black shining outer cells might easily obscure it. The general structure of these walls has already been described, and figure 34, drawn from an extremely fortunate and very thin section, shows the details.

The very young ascus presents no features of importance to distinguish it from that of many other pyrenomycetous fungi. In its earliest state it is recognisable as a single thin-walled, club-shaped cell, tapering to a point at the lower attached end, and filled with finely granular, yellowish protoplasm (see fig. 37 *a.*): sometimes a small pale, refractive nucleus-like point is seen in the protoplasm. As the young ascus grows longer, and its protoplasm increases in quantity, a fine, sharp division line makes its appearance somewhat oblique to the long axis of the whole (fig. 37, *c.*); this is soon followed by a second, similar longitudinal division, in a plane at right angles to the former (fig. 37, *d.*), and four well-defined masses are thus marked out. These, the young spores, do not include the whole of the protoplasm (fig. 37, *d* and *f.*), but lie in a scanty matrix of granular matter, closely apposed face to face, and following the curve of the wall of the enlarging ascus on their outer walls.

As the four, almost fusiform young spores increase in size, and acquire more distinct membranous envelopes, they come to lie somewhat more loosely in the cavity of the ascus, and may cross one another in accommodation to the space at disposal. Then appear cross-septa (fig. 37, *e.*, *f.*), dividing the material of the spore into a number of compartments varying from three to five—or, in one case, a single septum only is formed—and vacuoles and granules appear in the hitherto almost homogeneous contents. As the spores ripen, their cross-septa become more firmly marked, their outer walls thicker, and gradually brown or nearly black in colour, like the hyphæ of the developed mycelium; the side walls of the separate compartments also become bulged out slightly, giving the mature spore the appearance of a long oval body, constricted at intervals (see fig. 39). Very commonly one or two oily-looking drops accumulate in the compartments of the ripe spore.

Such is the typical mode of development of the perithecium, asci and spores. I have found no modifications of importance from a morphological point of view; it should be recorded, however, that the number of spores in the ascus varies from two to eight. Sometimes



in the same perithecium one finds asci in which one, two, or three spores develop at the expense of their presumably weaker neighbours (fig. 38), in other cases the number two appears constant, only one complete division occurring in the ascus (fig. 38), while in one case to be referred to later, the asci normally produce eight two-chambered spores (fig. 43).

On germination, which may take place soon after the emission from the ripe perithecium, the spores seem to behave generally in the same manner; one or several simple protuberances emerge from any of the partitioned chambers (see fig. 40), and proceed to develop into a typical mycelium, often with a preliminary formation of the rudimentary haustoria referred to in an earlier part of this paper. This mycelium grows rapidly in moist weather, forming branches, *setæ* and fruit-bodies as before. In some seasons the leaves of various plants may be seen covered with hundreds of these young mycelia, which dry up when the atmosphere does, only to renew their growth with the rains.

Before passing on to the consideration of the pathological influence of these fungi, and of their systematic position, I will record a few details concerning a form of *Meliola* which varies somewhat from the typical cases hitherto considered; at any rate, it seems to differ more from the six or eight forms to which the above description refers, than they do among themselves.

The species to be examined has only been found on the leaves of *Pavetta indica*, and its mycelium forms more spreading and less defined patches on the leaves of that plant than the easily recognisable sooty patches of the other *Meliolas*. The main features of its mycelium, &c., are shown in fig. 41, and differ chiefly in the delicate straggling hyphæ, with a paler brown colour and no trace of haustoria. The branching is very irregular, and somewhat like that of the form figured at fig. 3, but the short, lateral branchlets are not always ovoid, but often have sinuous, almost angular outlines, reminding one of the similar structures in *Asterina*, except that the latter bear distinct haustoria. The *setæ* are here quite simple, short, and not so hard and brittle as usual; they are also produced in smaller numbers than in the more typical species.

The greatest peculiarities, however, are offered by the fruit-bodies, or perithecia. Each of these arises as before by the successive dividing up of a short, lateral branchlet (plate 44, fig. 42), with this difference,

that the rapidly following septa permit no recognition of primitive cells destined to form the outer walls, ascogonium, &c., as before.

After a few radial, vertical, and horizontal walls have been formed, tangential septa (fig. 42, *d.*) make their appearance, cutting out series of cells which are to form the outer wall, and which become firmer and more deeply coloured, from an inner cell mass which gives rise to the ascogonium much as before. Only a few asci are formed, in each of which arise eight small oval uniseptate spores, which acquire a pale brown colour as they ripen (fig. 42, *f.*, and fig. 43).

The mature perithecium is shaped like a pear or top, the broad end attached to the hypha by a short pedicel, the narrow free end, or apex, becoming thin and diffuent in order to allow of the escape of the spores (fig. 43). Very few or no *setae* are formed around the perithecium, and these of the same simple type as those scattered on the mycelium (fig. 41). The whole structure of the fruit-body is, therefore, much simpler than that of the above described forms, and, from the semi-translucent characters of the thinner cell-walls, allows the main details to be made out by optical sections only. In some of the dark-coloured cells of freshly prepared specimens, a bluish tint is often observable; I have not seen this in any other similar form.

In no case have I succeeded in tracing a distinct alterative or destructive action of the *Meliolas* on the cells of leaves to which they are attached. In many instances, as, for example, thick leathery leaves like those of *Memecylon capitellatum*, &c., the haustoria seem to have no function beyond that of holdfasts; in others, such as *Pavetta*, *Triumfetta*, &c., attacked leaves certainly suffer from the presence of the fungus. Nevertheless, I cannot trace this to any direct action of the mycelium; the contents of the cells show no effects which can be regarded as due to the fungus mycelium directly. We must conclude, therefore, that where the life of the leaf is interfered with at all, it is indirectly; the dense crust of a well-developed *Meliola* no doubt obstructs the play of physiological functions in an obvious manner by obscuring it from light, blocking up stomata, &c.

It is now possible to consider the question of the systematic position of these remarkable and interesting fungi. Bornet,<sup>1</sup> following Fries and Lévillé, places *Meliola* near the old group of *Sphaerias* with especial reference to *Erysiphe*. I have already quoted the view of Fries that the *Meliolas* may be considered tropical representatives of

<sup>1</sup> *Op. cit.*, p. 266.

our *Erysipheæ*, and Berkeley<sup>1</sup> takes the same position. These opinions appear to have been based simply on the resemblance in habit and the more obvious anatomical characters, and on the fact that no *Erysiphe* is known in the tropics.

The details of structure, and especially of the development of the fruit-bodies above described, enable us to criticise these views from a somewhat firmer standpoint.

Apart from minor points of resemblance between *Meliola* and the typical *Erysipheæ*, such as the haustoria (not well developed in *Meliola*), the asci, &c., there can be no question as to certain points of agreement in the structure and development of the perithecia; nevertheless, the origin of the fruit-body in the two groups is not obviously similar, and at first sight the differences may seem greater than they really are.

In the typical simpler *Erysipheæ*, such as *Podosphaera*, as is well known from De Bary's classical researches,<sup>2</sup> the *carpogonium* and *antheridium* arise each as a short lateral branch from separate hyphæ, at the point where two hyphæ cross: each becomes cut off by a septum, which is formed close to the parent hypha in the case of the pyriform *carpogonium*, and about half way up the curved *antheridium* branch. The free end of the latter becomes closely applied to the top of the *carpogonium*, and fertilisation—possibly not complete in a physiological sense, however—is said to be complete. After this process numerous branchlets arise from the base of the *antheridium* filament (and also from the base of the *carpogonium*), grow rapidly and with numerous segments, and invest the *carpogonium*, which meanwhile begins to be (more slowly) cut up into cells.

In *Eurotium*<sup>3</sup> we have an essentially similar process, except in minute details, and the *antheridium* is a branch springing from the same hypha which bears the *carpogonium*, and arises just beneath the latter. Here, as before, the perithecium envelope is formed chiefly by the rapid overgrowth of cells derived from the *antheridium* branch. It is quite conceivable that a form allied to *Erysiphe* and *Eurotium*, &c., might have the unicellular *carpogonium* and *antheridium* arising quite in contact at their bases from the same branch.

If we now compare the above with the succession of events in the

<sup>1</sup> Introd. to 'Crypt. Bot.,' p. 275.

<sup>2</sup> 'Beitr. z. Morph. u. Phys. d. Pilze,' R. iii., 1870

<sup>3</sup> Cf., De Bary, *loc. cit.*

development of *Meliola*, the following points of analogy seem to me sound. The original pyriform branchlet—containing in itself, so to speak, the elements of the fruit-body—after the first division (fig. 9), may be considered as establishing morphologically an *archecarpium*<sup>1</sup> and an *antheridial branch*—or the latter may be considered as containing in itself the *antheridium*, plus the elements of the perithecium wall.

If the cells A and B (fig. 9) became further developed, and diverged at their apices, we should have no difficulty in seeing these points of homology.

Thus much cannot but be allowed. The cell A resembles a true *archecarpium* in so far that it slowly produces the ascogonium and asci; the homology will not be weakened, but the contrary, if further research shows that part of the perithecium wall results from cells derived from A. The cell B so far acts as an *antheridium* branch in that it is closely applied to A, divides up more rapidly, and thus produces most—*perhaps all*—of the perithecium wall.

The above may possibly suggest some difficulties to those who have not followed the recent progress in our knowledge of sexual organs and their homologies in the lower fungi. It has of late been shown to be not improbable, but on the contrary very likely, that we should view the *Erysipheæ* as a group connecting the higher *Ascomycetes*, on the one hand, and the *Phycomycetes*<sup>2</sup> (*Mucor*, *Peronosporæ*, and *Saprolegniæ*) on the other: the evolution of the latter group seems undoubtedly attended by a fusion of parts before separated—a withdrawal of the sexual organs, so to speak, into one another,—and De Bary has followed this out with marvellous skill and success in a number of forms passing from *Pythium*, through the *Peronosporæ*, to certain *Saprolegniæ*, in which the male sexual organ (*antheridium*, *pollinodium*) is normally suppressed. Whether or not we suppose, with De Bary, that the *Erysipheæ* took origin from some *Peronospora*-like form, it seems reasonable to look upon *Meliola* and its immediate allies as a branch group derived from the *Erysiphe* stem, either from the ancestor of *Erysiphe* itself or from ancestors which gave rise to *Eurotium* and *Erysiphe*, and that this group has become developed in tropical lands along lines more or less parallel to those along which the European forms have proceeded in temperate climates, being, in fact—though not in the strictest sense perhaps—“representative species.” Be this

<sup>1</sup> De Bary, Beiträge IV., proposes to use this word as denoting that part of the body which becomes the ascus and pedicel in *Podosphaera*.

<sup>2</sup> Vide De Bary, “Beitr. z. Morph. u. Phys. d. Pilze,” R. IV., 1881.

view entertained or rejected, I am strongly impressed with the necessity for further and closer investigation of the very remarkable group of fungi centering around or near the *Meliolæ*, since they will probably fill up yet more completely the gap—partially bridged over, it is true—between the lower and higher *Ascomycetes*.

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DESCRIPTION OF PLATES I & II.

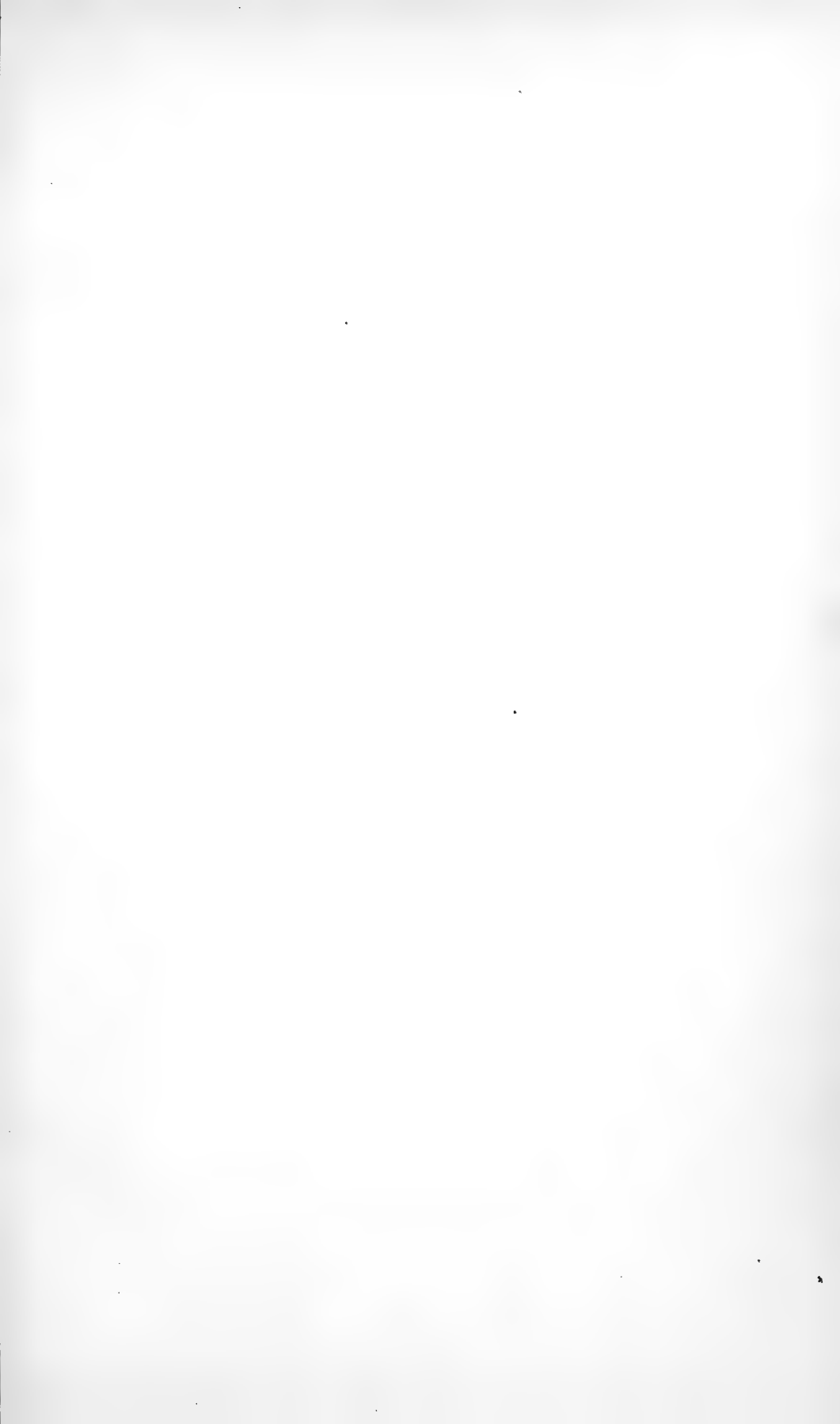
- Fig. 1. *Meliola* sp. with portion of epidermis of *Memecylon*. On the mycelium are *setæ*, branchlets, and fruit-bodies in various stages of development.—Zeiss D.
- Fig. 2. Mycelium of another species of the same, found on the leaves of *Schutereia* (*Conv.*), with portion more highly magnified.—Gundl.  $\frac{1}{3}$  and Zeiss D.
- Fig. 3. Portion of mycelium of a species of *Meliola* on *Triumfetta* (*Tiliaceæ*).—Zeiss D.
- Fig. 4. Portions of more advanced mycelium of fig. 2 more highly magnified, and showing various forms of lateral branchlets.—Zeiss J.
- Fig. 5. Portions of mycelium on *Memecylon* showing mode of branching and young fruit-bodies.—Gundl.  $\frac{1}{3}$  and Zeiss D.
- Fig. 6. Vertical section through portion of mycelium where fruit-body is being formed. The section is not median.—Zeiss J.
- Fig. 7. End of hypha with three cap-like thickenings and pore-like spot (*haustorium?*) seen from below.—Zeiss J.
- Fig. 8. Various forms of *setæ* in plan and elevation.—Zeiss D. and J.
- Fig. 9. End of hypha (with one cap-like thickening) bearing lateral pyriform branchlet which is to become a Perithecium. The first oblique septum has already appeared, the smaller cell (A) represents the ascogonium, &c., and is shaded darker; the larger one (B) will divide up more rapidly, and enclose the cell A and its progeny.
- Figs. 10 and 11. Further stages in the development of the young Perithecium. The cell B is becoming divided.—Zeiss J.

- Fig. 12. Young Perithecium seen from below (*a*), from the side (*b*), and from one end (*c*). In all, the dark cell is the one marked (A) in fig. 9; the remainder have resulted from the growth and division of the cell (B).—Zeiss J.
- Figs. 13 and 14. Slightly later stages seen from below. The cell (A) has become divided by a cross septum.—Zeiss J.
- Figs. 15 and 16. Similar preparations seen from above and below.—Zeiss J.
- Fig. 17. Somewhat more advanced Perithecium seen from the side. The cells resulting from the division of A (“ascogenous core”) are seen through those formed by B, which are growing over them.—Zeiss J.
- Fig. 18. Somewhat more advanced stage.—Zeiss J.
- Fig. 19. Slightly later stage. The upper figure is seen from above, the lower from below: the latter shows the “ascogenous core.”—Zeiss J.
- Fig. 20. Similar preparations seen from above (lower figure) and below (upper figure).—Zeiss J.
- Figs. 21 and 22. Slightly advanced Perithecia cut by the razor. The “ascogenous core” is exposed at the cut parts.—Zeiss E.
- Figs. 23 and 24. Similar preparations treated with chromic acid. The “ascogenous core” is seen enveloped by the cells forming the Perithecium-wall: all much swollen, and fig. 23 slightly crushed.—Zeiss J.
- Fig. 25. More advanced Perithecium seen from outside and above (*x*), below (*y*), and from the side (*z*). The radiating hyphæ (*réceptacle*) spring from the external walls below.—Zeiss E.
- Fig. 26. Portion of mycelium with young Perithecium seen from above and below.—Zeiss E.
- Fig. 27. Somewhat older Perithecium. The razor has cut off one side obliquely.—Zeiss J. (camera).
- Fig. 28. Vertical section through young Perithecium about this stage. The ascogenous cells in the middle are distinguished by their larger size and arrangement.—Zeiss J.
- Fig. 29. Oblique (nearly horizontal and median) section through the same.—Zeiss J.

- Fig. 30. Horizontal section above the base of same.—Zeiss J.
- Fig. 31. Somewhat older stage in vertical section. The ascogenous cells in the centre are enlarging at the expense of those around.—Zeiss J.
- Fig. 32. Portion of outer wall with disorganised cells lining it.—Zeiss J.
- Fig. 33. Vertical section through nearly ripe Perithecium, showing asci and spores embedded in the gelatinous mass produced by the disorganisation of the unemployed cells.—Zeiss D.
- Fig. 34. Portion of outer wall of latter in vertical section.—Zeiss J.
- Fig. 35. Vertical—not median—section through ripe Perithecium (and portion of epidermis of host-plant), showing crowds of spores.
- Fig. 36. Thin slice from top of similar Perithecium. A pore-like spot is seen in the centre of the radial marking.
- Figs. 37, 38 and 39. Various stages in the development of the asci and spores.—Zeiss J.
- Fig. 40. Germinating spores.—Zeiss D. and E.
- Fig. 41. Portion of mycelium of a species of *Meliola* found on *Pavetta*, showing mycelium, *setae*, and young Perithecia.—Zeiss J. and D
- Figs. 42 and 43. Development of Perithecia and extrusion of spores.
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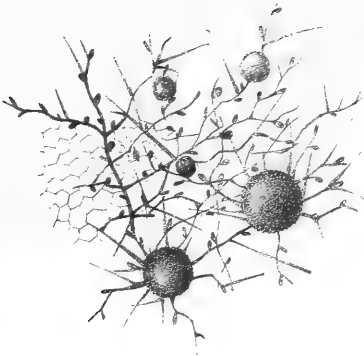


Fig. 1.

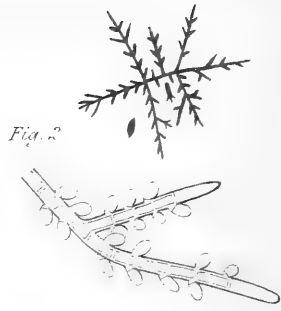


Fig. 2.



Fig. 6.



Fig. 3.

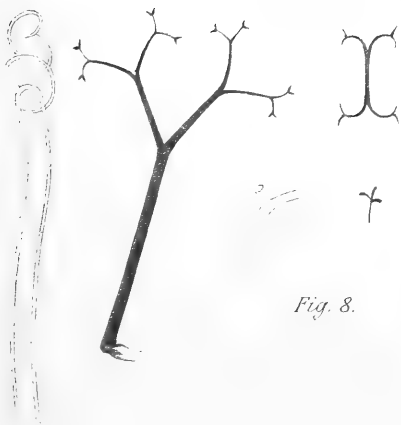


Fig. 8.

Fig. 9.



Fig. 17.



Fig. 18.

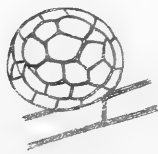


Fig. 19.

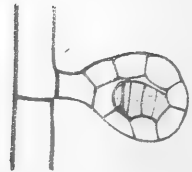


Fig. 20.

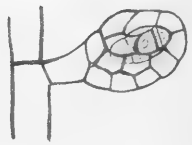


Fig. 21.



Fig. 23.





Fig. 4.

Fig. 5.



Fig. 13.

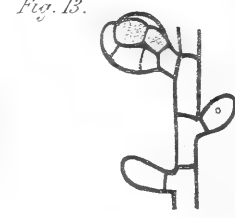


Fig. 11.

Fig. 12.

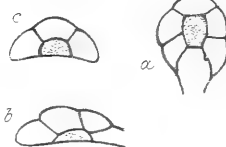
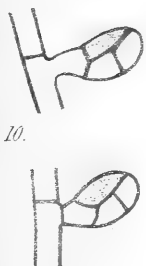


Fig. 10.

Fig. 14.

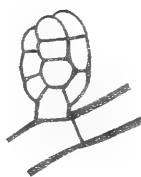
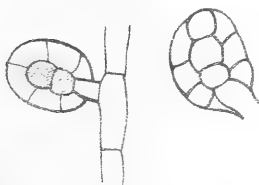


Fig. 15.

Fig. 16.



Fig. 22.

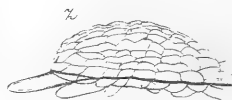
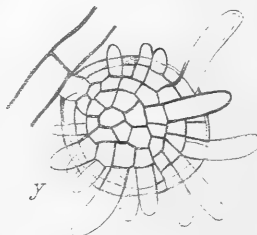
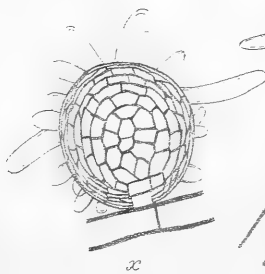
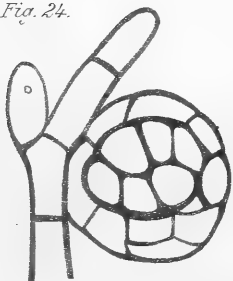


Fig. 24.

Fig. 25.







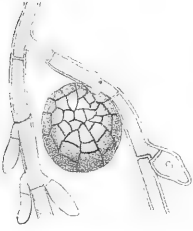


Fig. 26.



Fig. 27.

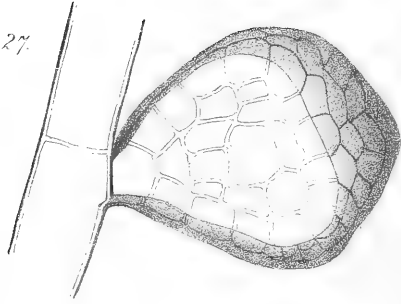


Fig. 28.



Fig. 29.



Fig. 34.



Fig. 33.



Fig. 35.



Fig. 39.

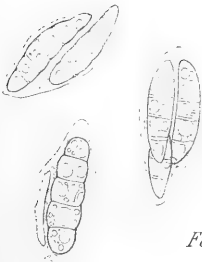


Fig. 38.



Fig. 41.

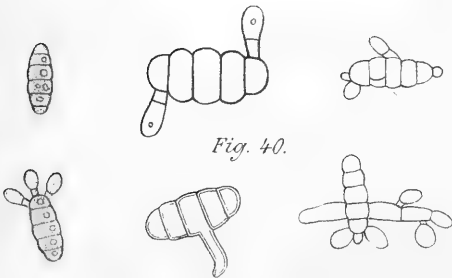


Fig. 40.

Fig. 30.

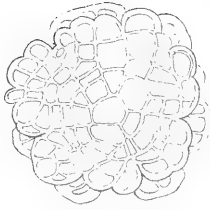


Fig. 31.

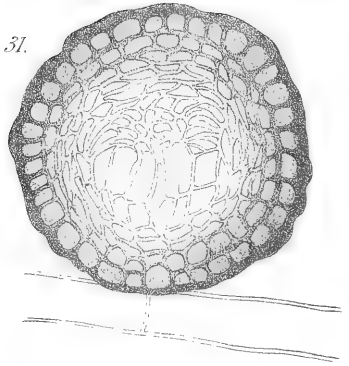


Fig. 32.

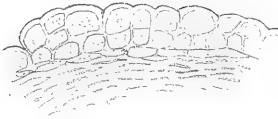


Fig. 36.

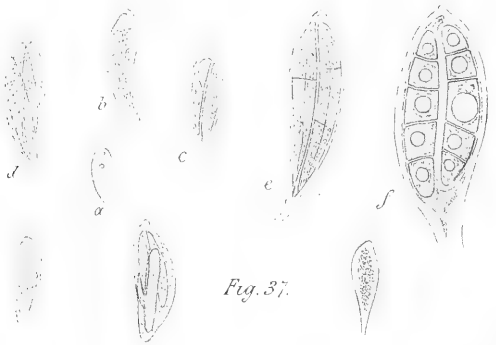


Fig. 37.

Fig. 43.

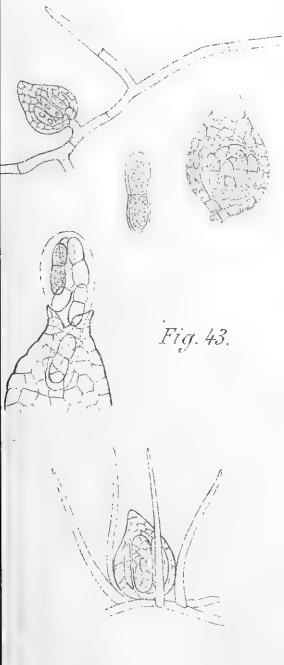
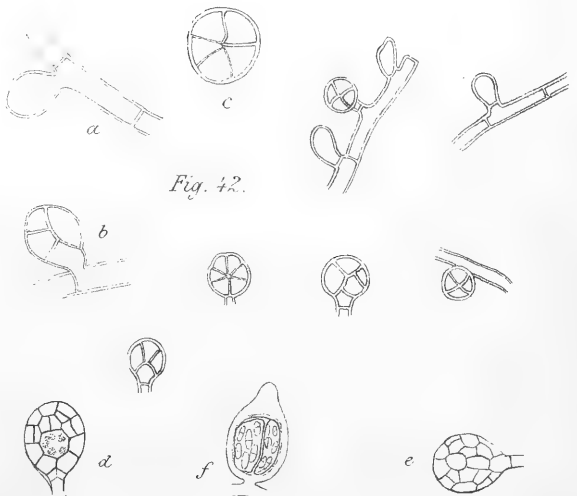
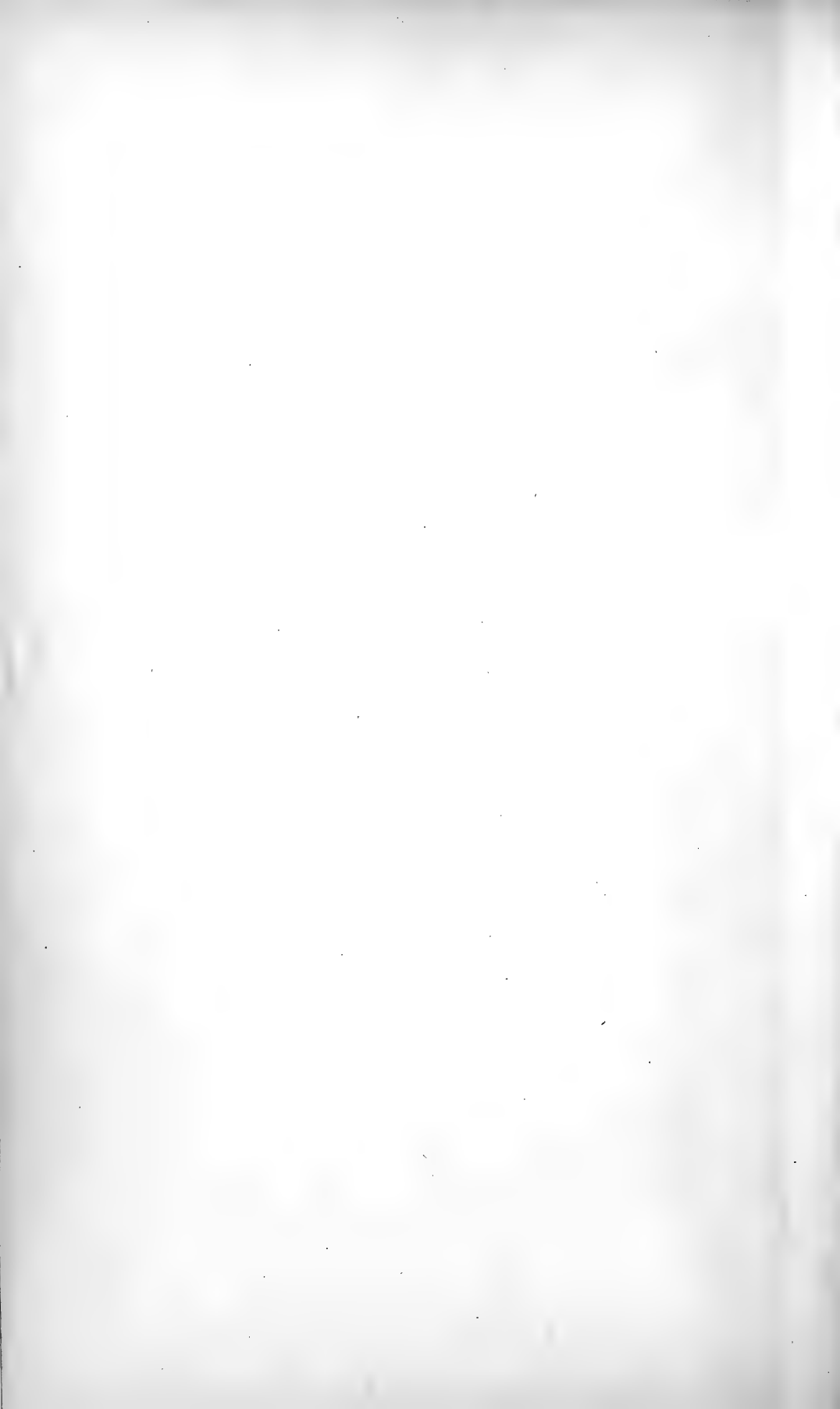


Fig. 42.







## ON THE SEXUALITY OF THE FUNGI.

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The fruitfulness and stimulus of the theory of descent have probably been felt in no province of biology with more effect than in the investigation of the more minute forms of plants; and the results obtained from the study of microscopic fungi have absorbed attention and interest of late years to an extent which, whether commensurate or not with their importance, promises even more in the future than has been attained in the past.

Not only with respect to the economic aspect of a thorough knowledge of Fungi inimical to the animal and vegetable world, but also as regards the real position of these remarkable organisms in nature, it is of the greatest importance that investigations should proceed and multiply. For we have learned in this as in other departments of science, that the results of thorough and accurate knowledge cannot really be foreseen, and that new side lights are thrown on other matters by every acquisition of facts and principles.

Apart from their interests more directly affecting mankind, the fungi have seemed to present problems of life in some respects simpler than other forms, and have thus in a manner promised a solution of phylogenetic and physiological questions more nearly approaching the ideal of the evolutionist. As research progressed, however, and the methods of observation were improved, experience showed that the study of the Fungi—though yielding results much beyond rather

than below what was expected—is attended with unlooked for difficulties. Not only is the isolation and cultivation of any given fungus an extremely difficult matter, but the following it through all the phases of its life-history brings the observer face to face with problems of quite a special nature.

As time progressed and observations multiplied, it became clear that the fungi are by no means so simple as they perhaps appeared. Apart from practical difficulties of manipulation, consequent on their minuteness, number and intermixture with other forms, it soon became evident that special conditions of various kinds affect their development, and that the complete life-cycle of any one fungus—and evidence based on a thorough knowledge of this is alone admissible for the purposes of science—may present various forms of complexity.

Even to-day, notwithstanding the considerable additions to our knowledge derived from the study of development, and notwithstanding that we possess several comprehensive generalisations as to the curious changes undergone by typical forms in their development, we are far from possessing sufficient knowledge of these matters to enable us to group the fungi satisfactorily from a phylogenetic point of view. This, however, is a distinct aim of biology, and every addition to knowledge in this direction is to be welcomed.

In the present essay it is proposed to describe some of the more recent and most suggestive observations on fungi; and especially on their reproductive organs, since it is in these that the most important phenomena (from the phylogenetic point of view) are centred. We shall have occasion to refer to, and in part to trace certain processes connected with their development; and finally to see how far it may be possible to generalise from the facts now known.

In so far as this paper simply recounts observations—for the most part made by others—it cannot claim scientific merit; but if, after condensing and arranging the facts, and stating the condition of our present knowledge of the subject, the attempt to bring this knowledge under a more general statement succeeds, it may be that we have helped to advance matters after all.

If, however, further criticism results in the overthrow of the hypothesis brought forward at the conclusion, we may nevertheless hope that some service is rendered in arranging the facts, and drawing attention to the necessity of employing physiological as well as morphological considerations in the attempt to construct a phylogenetic

system of the fungi. In the last case we may at least succeed in attracting more attention to the direction in which modern research in this region is impelling the more thoughtful biologists, and so call forth confirmatory evidence or criticism of unseen fallacies.

If we neglect a few isolated observations as having led to no general views on the subject, we may regard Pringsheim's discovery of the sexual organs in the *Saprolegniæ* in 1858<sup>1</sup> as the starting point of our knowledge of the sexuality of the fungi. This observation was made at a time when attention was being drawn particularly to the sexes of the Algæ by the researches of Thuret, De Bary, Cohn, Nägeli and others; and the *Saprolegniæ* were then, and for a long time afterwards, regarded as Algæ.

Since that time numerous cases of the occurrence of sexual organs have been described among other fungi, chiefly by the labours of De Bary and the school of cryptogamic morphology practically established by him and his pupils.

With De Bary's brilliant researches on the *Ascomycetes*,<sup>2</sup> and especially the *Erysiphææ*,<sup>3</sup> a point was reached where a definite opinion on the sexuality of the fungi became accepted; and the conclusions drawn from the study of *Sphærotheca*, *Eurotium*, and *Peziza* led to the view that the fruit-body of a higher fungus results from a process of fertilisation preceding the development of asci, and that Hofmeister's supposition that the asci are sexual organs was to be abandoned.

In 1871 Janczewski<sup>4</sup> described the sexual organs in *Ascobolus furfuraceus*. Other researches by Baranetski, Gilkinet, Woronin, Van Tieghem, and Brefeld were considered to support the then generally received opinion that the fungi, while differing considerably as to their forms and mode of producing spores and fructification, probably all develop their chief reproductive bodies as the consequence of a sexual process.

When in 1874 Stahl demonstrated the sexuality of a Lichen,<sup>5</sup> the matter seemed to be placed beyond doubt; and it was freely admitted that in the cases—now somewhat numerous—where a definite union of sexual organs could not be established, that the failure was largely due to the extraordinary difficulties attending the investigation. In

<sup>1</sup> Sachs, 'Geschichte der Botanik.'

<sup>2</sup> 'Ueber die Fruchtentwicklung der Ascomyceten,' 1863.

<sup>3</sup> 'Beiträge zur Morph., &c., der Pilze,' R. iii., 1870.

<sup>4</sup> 'Bot. Zeitg.' 1871.

<sup>5</sup> 'Bot. Zeitg.' 1874.

this manner, apparently, it came to be widely believed that in such cases as *Sordaria*,<sup>1</sup> *Penicillium*,<sup>2</sup> *Sphaeria lemaneæ*,<sup>3</sup> and *Chaetomium* the sexual process is essentially the same as that described for the simpler *Erysiphææ*.

All the observers agreed in the main that the asci are either parts of the *Ascogonium*, or female sexual organ (*Sphaerotheca*, *Podospheera*), or are developed by budding from it, in each case no doubt after the *Ascogonium* had received something from the male organ (*Pollinodium*) attached on its surface. Meanwhile, investigation was not confined to the *Ascomycetes*.

The researches of Brefeld,<sup>4</sup> Van Tieghem,<sup>5</sup> and others<sup>6</sup> demonstrated a simple form of sexual reproduction in the *Mucorini*, now so well known that we need not dwell upon it. It is interesting to note in passing, however, that Ehrenberg had discovered the conjugation of *Syzigites* so long ago as 1820.<sup>7</sup>

We now pass to the discovery of the true nature and sexual organs of the *Peronosporæ* by De Bary, who, in a series of masterly memoirs,<sup>8</sup> has made this group and its allies a special study. De Bary showed that in certain members of this group an antheridium bores into the oogonium, sending a "fertilising tube" into the oosphere contained therein; the oosphere then becomes an oospore, and capable of germination.

A considerable amount of labour had been devoted to the study of the *Saprolegniæ* since Pringsheim's first publication, much of it by this investigator himself,<sup>9</sup> and among other remarkable discoveries his observation that, in certain cases, the oospores become normally developed and capable of germination without any male organs being formed at all, is to be noted. Pringsheim himself termed these oospores parthenogenetic.<sup>10</sup> We may pass over the controversy between

<sup>1</sup> Gilkinet, "Recherches Morphologiques," 'Bull. Acad. r. de Belg.,' ser. 2, 1874. Woronin, 'Beitr. z. Morph., &c.,' R. iii.

<sup>2</sup> Brefeld, 'Schimmelpilze,' ii.

<sup>3</sup> Woronin, 'Beitr. zur Morph., &c.,' R. iii.

<sup>4</sup> 'Schimmelpilze,' H. i.

<sup>5</sup> 'Ann. des Sc. Nat.,' ser. 6, t. i.

<sup>6</sup> De Bary, 'Beitr. z. Morph.,' i.

<sup>7</sup> This is the date in Sachs' 'Geschichte der Botanik,' p. 473. De Bary, 'Beitr.,' i., p. 74, gives the date 1829.

<sup>8</sup> 'Ann. des Sc. Nat.,' ser. 4, vol. xx, and later, 'Beiträge zur Morph., R. ii; ditto, R. iv. 'Bot. Zeit.,' 1881.

<sup>9</sup> 'Jahrb. für wiss. Bot.,'

<sup>10</sup> 'Jahrb. für wiss. Bot.,' ix.

Cornu<sup>1</sup> and Pringsheim as to certain details in the manner of fecundation of the oospheres of *Saprolegnia*. It is sufficient to note that in 1880, or thereabouts, the matter appeared to stand thus: While the typical *Saprolegnia* possess oospheres in an oogonium, and antheridia as simple or branched structures which send "fertilising tubes" through the walls of the oogonia as far as the oospheres, which they appeared to fertilise; there are others in which the oospheres develop into fertile oospores without contact of the antheridia.

If we now turn aside from the fungi referred to in the preceding sketch, we find a vast number of forms comprehended under the *Ustilagineae*, *Uredineae* (*Æcidiumycetes*), and the larger *Basidiomycetes*. The parasitic *Ustilagineae* have received much attention since Tulasne<sup>2</sup> and De Bary<sup>3</sup> brought them together and led the way to a more scientific knowledge of their nature. Much has been done since, and much opinion has been expressed as to the signification of the cross unions made by the "sporidia" developed from the promycelium of the germinating spores<sup>4</sup> in some cases. We must regard the view as to its supposed sexual character with grave suspicion.

The *Uredineae*, apart from their interest as parasites on economic and other plants, have absorbed much attention from the point of view we are concerned with. It was natural to look for sexual organs in them, especially after the successes met with elsewhere. Nevertheless, from Tulasne's<sup>5</sup> and De Bary's<sup>6</sup> earlier investigations, more than thirty years ago, down to the present time, no one has succeeded in demonstrating even a trace of any intelligible sexual process or organs. This is the more remarkable since many of the *Æcidiumycetes* produce no less than four forms of reproductive bodies. Moreover, the group has been studied with extraordinary success, and our knowledge of the nature of parasitism and heteroecism is largely if not chiefly due to this success. The best views as to the reproduction of these fungi held up to 1880 may be fairly stated thus. They form at most two kinds of asexual spores (*Uredospores* and *Teleutospores*) and *Æcidia* and *Spermogonia*; the latter were regarded as probably the bodies concerned in sexual reproduction, the *Spermatia* emitted by the *Spermo-*

<sup>1</sup> 'Ann. des Sc. Nat.,' ser. 5, t. xv, &c.

<sup>2</sup> 'Ann. des Sc. Nat.,' ser. 3, t. vii, and ser. 4, t. ii.

<sup>3</sup> 'Die Brandpilze,' 1853.

<sup>4</sup> Tulasne, op. cit.

<sup>5</sup> Op. cit.

<sup>6</sup> Op. cit.

*gonia* being the male organs, and the "æcidium fruit" probably resulting from a fertilised body equivalent to the ascogonium of the Ascomycetes. This view was strengthened and supported by Stahl's discovery of the sexual process in Lichens; but no organs like the ascogonium or trichogyne have yet been discovered in spite of much labour. Finally, we may dismiss the larger *Basidiomycetes* by referring to Brefeld's magnificent research<sup>1</sup> on certain types, and particularly on *Coprinus*.

Brefeld placed beyond all reasonable doubt that the stalked pileus arises from the mycelium, and completes its development without the intervention of any sexual process, or the appearance of any sexual organs; and since no one has succeeded in rendering it probable that sexual organs occur later, we may probably accept Brefeld's view that no sexes exist in the Agarics as we know them, but that they are large aggregations of hyphæ producing asexual spores. Whether we really know the whole life history of any of these forms is a question which cannot be raised with much advantage just now.

It thus appears that while the discoveries of Pringsheim, Tulasne, and De Bary led, on the one hand, to numerous other observations of sexual organs in the fungi, and seemed to show that a sexual process is nearly universal with them as with other groups of living beings equally complex in organisation; on the other hand, there were numerous cases where room for serious doubts existed—doubts not dispelled by the recognition of the difficulty of the research. As time passed, moreover, the suspicion that certain groups of fungi are really devoid of sexual organs (although analogy would lead us to expect them) increased, and in some cases reached conviction. Of course, we are not referring to the very obscure lower groups—the *Schizomycetes*, *Saccharomycetes*, and *Myxomycetes*, &c.—which we shall leave out of account altogether in this survey.

It is not to be forgotten that much more was known about the physiology of the fungi by this time, and that the recognition of saprophytic and parasitic forms implied considerable advance in our knowledge of their modes of life, changes of habit, and so forth. The progress made in the study of fermentation, moreover, had its effect on the study of mycology generally; and the progress of biology as a whole—so particularly active during this period—had, in 1880, left its mark on this specialised branch of research.

<sup>1</sup> 'Schimmelpilze,' H. iii.

It is not necessary to enter into all the systems of classification proposed for the fungi during this period. The well-known grouping of Sachs and Cohn, presented to English readers in the "Text-book" of the former, was admitted to be in great measure artificial, and those proposed by Van Tieghem<sup>1</sup> and by Winter<sup>2</sup> appear to answer their purpose only temporarily, and certainly need not occupy us here. The same is true for other classifications up to 1880.

It was just prior to this period that the very important memoir by Brefeld<sup>3</sup> was published, in which he detailed the results of his investigations into the nature of the *Basidiomycetes*.

By cultivation in nutritive media, Brefeld succeeded in tracing the whole cycle of *Coprinus* from the basidio-spore to the formation of a mycelium and fructification. He shows that the latter arises by a purely vegetative process from the dense mass of interwoven hyphæ (*Sclerotium*) budded off from the mycelium, and that no trace of a sexual process or of the formation of sexual organs can be detected either previously to the development of the *Sclerotium* or afterwards. The pileus with its hymenium are produced simply by a budding off of numerous hyphæ growing up together, either directly from the mycelium, or with the intervention of the *Sclerotium*. Brefeld regards it as certain that these fungi are entirely without sexual organs.

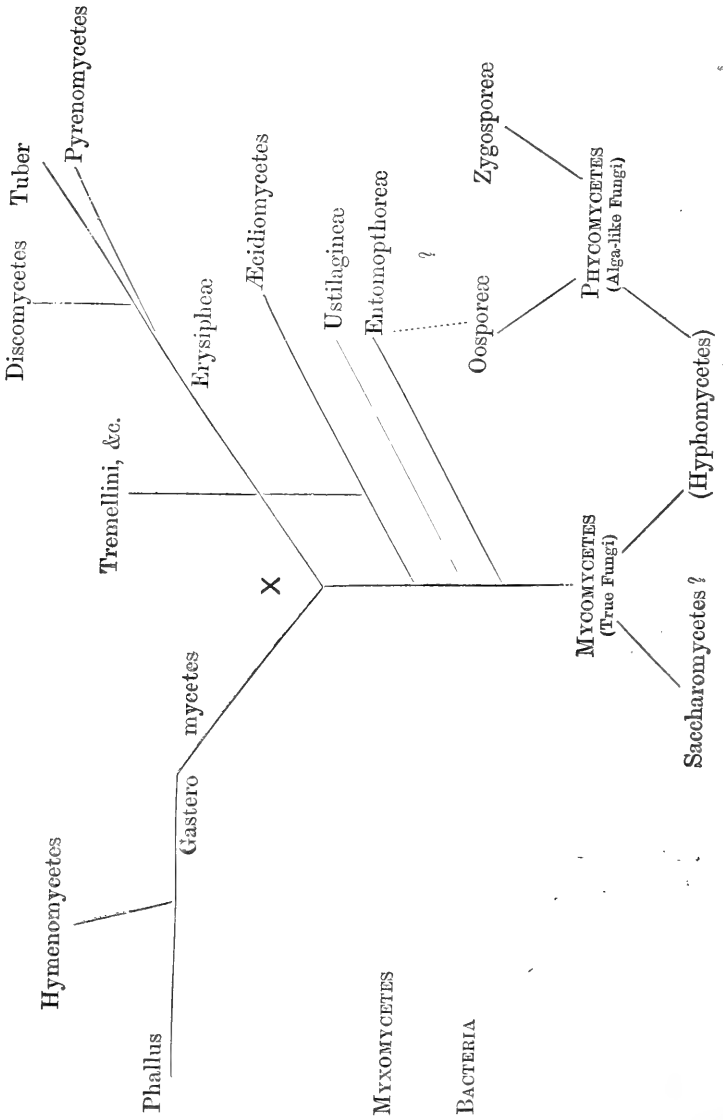
It is impossible to go into the details of this voluminous memoir; but it is important to notice the results embodied in a scheme of a proposed classification of the fungi which Brefeld tabulates at the end of his valuable paper, since we have here a comprehensive view of the direction in which modern speculations in mycology were tending.

In the accompanying diagram I have slightly condensed Brefeld's scheme, since the original contains details of little importance for our present purpose.

<sup>1</sup> 'Ann. des Sc. Nat.,' ser. 6, t. iv, 1878.

<sup>2</sup> 'Hedwigia,' 1879—see also Rabenhorst's 'Kryptogamen Flora.'

<sup>3</sup> 'Schimmelpilze,' Heft iii, 1877.





There is little need to dwell on this scheme, since its chief interest for us is in its being an intelligible attempt to classify the fungi from the point of view of the theory of descent. A point of some importance, however, may be referred to, as we shall have occasion to speak of it later. Brefeld indicates the possibility that the *Oosporeæ* (typified by *Peronosporæ*) may be allied to the "true fungi" otherwise than by a common descent from some Alga-like ancestor. He also recognises a common origin for the *Oosporeæ* and the *Zygomycetes*. In other respects the system is chiefly remarkable for the peculiar views taken of the descent of the two great groups, the typical *Basidiomycetes* (*Gasteromycetes* and *Hymenomycetes*, &c.) and the *Ascomycetes*, which he regards as having long ago diverged from a common point, at a time when the ancestral forms commenced to specialise their reproductive organs. While on the one hand asci arose as specialised forms of sporangia—complications resulting from the development of perithecia, &c., being considered unimportant—on the other hand, the sporangia became degraded to conidia, and the *Basidiomycetes* came to be merely highly developed tufts of conidiophores.

In a later memoir<sup>1</sup> Brefeld insists on regarding the so-called *Pollinodia* of the *Ascomycetes* as simply tubes for enveloping the ascogenous cell or filament; and it is interesting to note that he quotes *Melanospora* as a case where the non-sexual relation of the ascogenous cell and the filaments which envelop it may be clearly observed. Brefeld also points out that in the *Ascomycetes* we can trace gradual degradations of the various forms of fructification, with a disappearance of sexuality at the same time. He supposes that all the fungi arose from an ancestral form containing chlorophyll and possessing sporangia, and that the variations met with are derived by modifications of this sporangium, as already indicated.

It seems unnecessary to criticise these views in detail, since it is obvious that no decision can be arrived at apart from the consideration of numerous facts. It will be noticed, however, that Brefeld's hypothesis assumes that, in addition to purely vegetative modes of multiplication (*e. g.* the breaking up of filaments, &c.), certain fungi must have acquired other forms of reproduction than those inherited and specialised—some *Æcidiomycetes*, for instance, with their four kinds of spores or spore-like bodies (æcidiospores, spermatia, uredospores, and teleutospores) must have acquired at least one of these spores.

<sup>1</sup> 'Schimmelpilze,' iv, 1881.

At this point we may leave this short survey of Brefeld's important work, and turn to the consideration of a memoir<sup>1</sup> published by De Bary about the same time as the last one quoted. In this—probably the most important contribution to mycology yet made—the author describes his observations on the *Peronosporæ* and *Saprolegniæ*; and bases upon these and other previous observations a classification of the fungi which is in large measure new, and certainly promises to be more fruitful than any yet proposed.

De Bary finds that, passing from the typical *Peronosporæ* (*Pythium*, &c.) to the *Erysiphææ* on the one hand, and to the *Saprolegniæ* on the other, the sexual process is gradually eliminated, and the sexual organs become at first functionless and then disappear altogether. In *Pythium* itself, the antheridium pierces the oogonium wall and fertilises the oosphere by pouring protoplasm into it.<sup>2</sup> In *Phytophthora* and *Peronospora* the process is essentially similar, but the quantity of protoplasm passed over from the antheridium is smaller.

In the *Saprolegniæ*—which differ from *Pythium* and other *Peronosporæ* in forming several oospheres in each oogonium—the fertilising tubes do not open, and no protoplasm can be observed to pass over from the antheridium to the oospheres.<sup>3</sup> Or, in some forms, no antheridia are present at all—a fact already recognised by Pringsheim—and the parthenogenetic spores are nevertheless capable of germinating.

Now if the typical and thoroughly investigated case of *Podospheera*<sup>4</sup> be compared with the *Peronosporæ*, it is, as De Bary states, evident that the antheridia correspond in both cases; while the "archecarpium" (*i. e.* the cell which produces the ascus, and to which the antheridium applies itself) of *Podospheera* is homologous with the oogonium of the *Peronosporæ*. It is a remarkable fact that, as De Bary noticed long ago,<sup>5</sup> the antheridium of *Podospheera* only applies itself closely to the archecarpium, and does not pierce it; it appears highly probable, moreover, that no passage of substance from one to the other takes place—that the ascus, in fact, arises without a sexual process, though the sexual organs are present.

<sup>1</sup> 'Beiträge zur Morph. and Phys. der Pilze, &c.' R. iv, 1881.

<sup>2</sup> Cf. 'Quart. Journ. Mic. Sc.,' October, 1883.

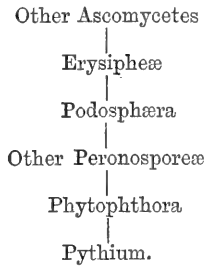
<sup>3</sup> Cf. also Quart. Journ. Mic. Sc., July, 1883.

<sup>4</sup> De Bary, 'Beitr. zur Morph. und Phys.,' R. iii.

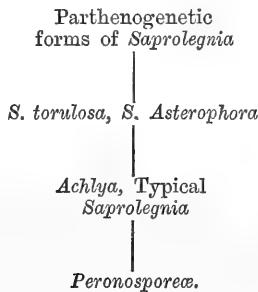
<sup>5</sup> De Bary, *op. cit.*

Now a whole series of forms are known leading us up from *Podosphæra* and the other *Erysiphæe* through the *Pyrenomycetes* and *Discomycetes*, and it is remarkable that (apart from some peculiar forms to be referred to shortly) the best investigations lead us to conclude that while the sexual organs are present, *but functionless*, in the lowest forms, they *disappear entirely in the higher Ascomycetes*.

These facts may be put shortly in the form of a diagram as annexed, where attention is only paid to the points referred to.



If we now turn to the *Saprolegnia*, we may note that De Bary finds that, between the typical cases where the antheridia pierce the oogonium wall, *but do not empty any protoplasm into the oospheres* (*Achlya* and some *Saprolegnias*) and the extreme parthogenetic forms of *Saprolegnia* where no antheridia are formed at all, there exist cases where the antheridia apply themselves to the outside of the oogonia, but either form *no antheridial tubes at all* or only rudimentary ones (*S. torulosa*, *S. asterophora*). These facts may also be diagrammatically represented as follows.



If now the various cases are duly considered, De Bary thinks that we may probably regard the *Peronosporæ* as phylogenetically important in two senses :

1. Their general biology strongly suggests that they are derived from Algal ancestors, possibly not very unlike *Vaucheria* and its allies. !

2. That they are the progenitors (or the near relations of progenitors) of a few chief series of true fungi—on the one hand the main series of *Ascomycetes* and allies; on the other the *Saprolegniæ* and forms derived from them, and allied to them.

If we now regard these forms more closely, it is not difficult to agree with many of De Bary's conclusions. It will be clear, in fact, that some of them are not new, though they are stated in a much clearer form than by Brefeld and others who have helped to systematise the chief groups already. We will first shortly consider the main subdivisions themselves.

The *Zygomycetes* are regarded as branching off from the *Peronosporæ*. In this group De Bary arranges the *Mucorini* and the *Entomophthoræ*, basing the conclusion that *Entomophthora* is a *Zygomycete* chiefly on Nowakowski's description of the zygospores.<sup>1</sup> It should be remembered that Brefeld considers the resting spores of this genus as arising asexually; but that he, too, indicates the possible alliance of the *Entomophthoræ* with the *Oosporeæ*, and therefore, indirectly, with *Zygomycetes*.

*Pythium* seems closely allied to the *Ancylistecæ* of Pfitzer,<sup>2</sup> which lead us on to the *Chytrideæ*, in which we meet with forms which conjugate (and are therefore sexually simpler) as well as purely apogamous genera. It is not improbable that among these latter the asexually produced resting spores are really oogonia, a view already held by Brefeld in other cases.

De Bary raises the point of the possible alliance of these simple parasitic *Chytridiaceæ* with the lower Algæ (*e. g.* *Protococcaceæ*), and decides that it is, on the whole, more probable that they have been derived from the higher fungi, as indicated, by degeneration.

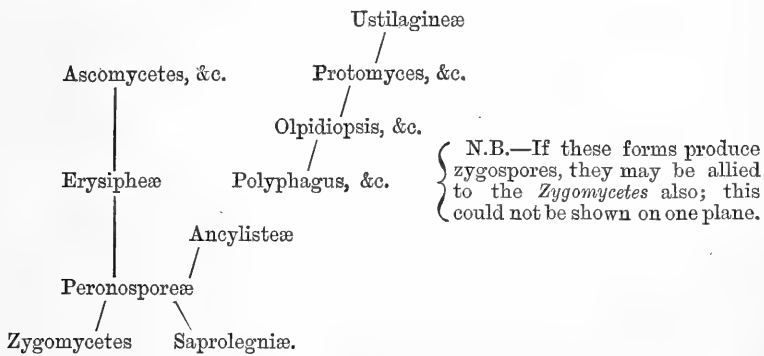
The *Ustilagineæ* are next dealt with. The author expresses himself cautiously, but points out that this important and very natural group may be looked upon as a series, beginning with the simpler *Entyloma*, *Tilletia*, &c., and rising to more complex forms, such as *Sorisporium* and *Urocystis*, on the one hand, and *Ustilago* on the other.

There are many difficult points to consider in classifying the *Ustilagineæ*. Their asexually produced resting-spores generally form a "promycelium" on germination, from which "sporidia" arise; and, as is known, these "sporidia" commonly become united by cross-

<sup>1</sup> 'Bot. Zeitung,' 1877.

<sup>2</sup> 'Monatsber. der Berlin. Akad.,' May, 1872.]

unions. But whether this is to be regarded as a "copulation" or not, the sporidia often germinate without it. It should be noted that Woronin<sup>1</sup> points out this significant fact also, and it is all but certain that the so-called "copulation" is not of a sexual nature at all. If the lower *Ustilagineæ* are allied to the *Chytrideæ* by means of *Entyloma* and *Protomyces*, we seem to have a satisfactory position for the former group. Of course, if this view be accepted, the resting spores of the *Ustilagineæ* are the homologues of oogonia, which become developed apogamously. The preceding facts may be summarised in the following diagram :



Continuing our survey of De Bary's memoir, we may pass over the opinion respecting the *Saccharomyces*, and proceed to the part dealing with a much more difficult and important series of forms. As the author showed in 1879,<sup>2</sup> the *Tremellini* may well be looked upon as derived from *Uredineæ* and allies; while those *Uredineæ* which form æcidia resemble the *Ascomycetes* in so many points of structure and development, that we may regard them as closely allied. The formation of three forms of conidia (*Uredospores*, *Teleutospores*, and *Sporidia*) may be in part due to specialisation; but it must be remembered that the *Ascomycetes* are also in the habit of forming many and various conidia. It is, however, in the many points of resemblance between the æcidia and perithecia, and the spermagonia and spermatia of both groups, that the alliance appears most clearly. True, no observer has found a trace of sexual organs in the young æcidia; but the same is certainly true for the perithecia of many *Ascomycetes*. In structure, apart from peculiarities in the development of the spores in

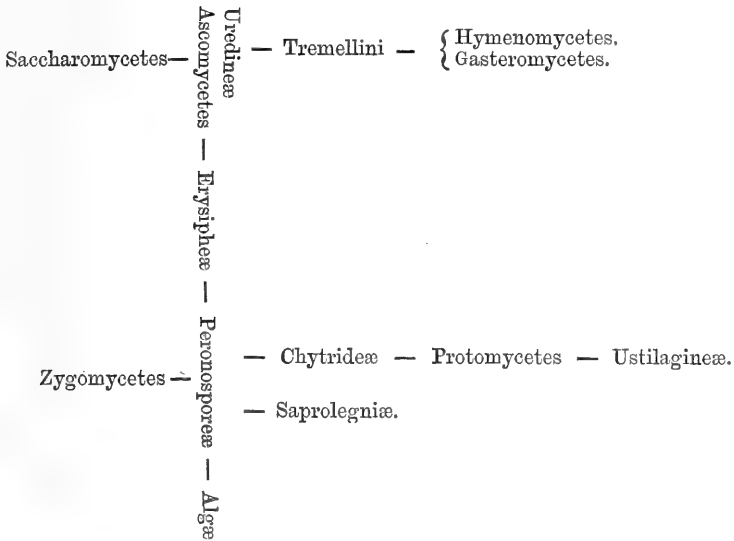
<sup>1</sup> 'Beitr zur Morph.' R. v., 1882

<sup>2</sup> 'Bot. Zeit., 1879, p 825 &c.

the two cases—æcidia and perithecia present many points of agreement, while the spermogonia and spermatia of both groups are quite alike. It cannot be overlooked, however, that the peculiar development of the æcidiospores affects the question of this alliance. De Bary does not even allude to the similarity between these and the "conidia" of *Cystopus*; and it is of course obvious that the asexually-produced spores of the latter are rather to be regarded as homologous with one of the conidial forms of the *Æcidiomycetes*; for if the æcidium fruit is homologous with the perithecium of *Podosphaera*, its further homologies are with the oogonium, &c., of *Cystopus*. It has been suggested, however, that a solution of these difficulties should be sought in the direction hinted at here.

De Bary thus considers the *Æcidiomycetes* as a group allied to the *Ascomycetes* genetically, though we have not sufficient knowledge as yet to enable us to place them at any particular spot in the scheme of that series. The *Tremellini* are *Basidiomycetes*, with basidiospores so suggestive of the teleutospores of *Uredineæ* that De Bary does not hesitate to place them as derived—with considerable reduction and simplification—from those of *Uredineæ* which possess no æcidia (e. g. *Chrysomyxa*). This is regarded as no more extraordinary than the peculiar simplification of phanerogamous water-plants, &c., or of *Saccharomycetes*, if they are reduced *Ascomycetes*. The *Tremellini* would then lead us to the *Hymenomycetes* and *Gastromycetes*, though it is by no means clear how this came about. We are here plunged into the greatest difficulties, because the development and life-history of these groups are so little known; and we may thus leave the discussion of their phylogeny for the present. It must suffice to add that De Bary believes it possible that the *Tremellini* having arisen by degeneration of uredinous forms, the other *Basidiomycetes* developed anew progressively as forms adapted to special modes of life.

The annexed scheme sums up the whole of the preceding.



The details may be filled in according to what has been said.

On comparing this diagram with that offered by Brefeld, it will be noticed that there is some agreement in general between them; it is chiefly in points of detail that the differences appear. Both the authorities agree as to the serial arrangements (on the whole) of the main groups; but Brefeld, while also placing the *Tremellini* as derived from Uredineæ, seeks another origin for the other *Basidiomycetes*. When we notice De Bary's caution in not deriving the *Uredineæ* from any particular point in the huge ascomycetous series, we may allow that he and Brefeld do not differ much in opinion as to their origin—the latter simply places their origin more definitely lower down in the main series, a fact which would possibly be of significance if we were inclined, after all, to regard the similarities between *Acidimycetes* and *Cystopus*.

The chief motive in Brefeld's scheme is afforded by his peculiar views on Pycnidia, and on the relationships of the *Entomophthorææ*. He regards the point where the main series of higher fungi developed pycnidia, as the point whence the *Basidiomycetes* (excepting *Tremellini* and allies) and *Ascomycetes* diverge; the former then became specialised as conidium-bearing fungi, the latter as modified sporangium-bearing forms—*i. e.* as ascus-bearing fungi.

The great motive of De Bary's views, as already shown, depends

upon the value of the new knowledge obtained of the *Peronosporæ*, and the meaning of the oogonia (or ascogonia) and antheridial filaments respectively.

If, in Brefeld's scheme, we tack the remaining *Basidiomycetes* on to the *Tremellini*, &c., and bring his "*Oosporeæ*" to the base of the main trunk of the phylogenetic tree, slight though not unimportant alterations in detail are necessary to make the two diagrams agree.

If we now turn our attention to the investigations of the last two or three years, it is suggestive that the best results have been won among the *Ascomycetes*. The influence of De Bary's memoir shows itself repeatedly in these, and they must be regarded as tending on the whole to strengthen his conclusions. No object is to be served by taking the various memoirs in strict chronological order, and I may therefore commence by reviewing an important contribution by Kihlmann.<sup>1</sup> It may first be stated that *Pyronema confluens*, a *Peziza*, is one of the *Ascomycetes* which has been particularly well studied; and is classical in that De Bary<sup>2</sup> first described the sexual organs in it in 1863, and that Tulasne's celebrated figure<sup>3</sup> of these organs has been so much copied. In 1866 De Bary, having devoted much attention to the development of the fructification of this fungus, wrote as follows concerning the pairs of peculiar organs assumed to be sexual: "Ob und wie sie einer Befruchtung dienen ist eine durchaus unentschiedene Frage."<sup>4</sup>

These sexual organs consist of pairs of swollen branchlets arranged in groups. Each pair consists of a macrocyst and a so-called paracyst. The macrocyst is an ovoid vesicle, filled with protoplasm, &c., and provided with a hook-like tubular prolongation. The paracyst is a club-shaped branchlet close to the macrocyst; the apex of the paracyst and the hook-like prolongation become united. After this—the "process of fertilisation"—branches spring from below, envelope the sexual organs, and form the perithecium, in which the asci arise.

Tulasne<sup>5</sup> described an open communication through the hook-like process, placing the protoplasm of the macrocyst and paracyst in connection. De Bary, much later,<sup>6</sup> speaks with great caution, and

<sup>1</sup> 'Acta Soc. Scient. Fenn.,' t. xiii.

<sup>2</sup> 'Ueber the Fruchtentw. der Ascomyceten.'

<sup>3</sup> 'Ann. des Sc. Nat., S. 5, t. vi; cf. Sachs' 'Textbook, &c.'

<sup>4</sup> 'Morph. und Phys. der Pilze, &c.,' p. 164.

<sup>5</sup> Op. cit.

<sup>6</sup> 'Beitr. zur Morph., &c.,' R. iv.



considers it undecided that a true sexual process occurs. Fisch<sup>1</sup> doubts whether these organs have anything to do with the formation of the asci. Sachs<sup>2</sup> and Goebel<sup>3</sup> describe the organs and the sexual process in terms which Kihlmann thinks too confident and premature at the time.

I now pass to the observations made by Kihlmann himself. The macrocysts and paracysts arise together in pairs as terminal swellings of the branches. The hook-like prolongation of the macrocyst becomes fused to the apex of the paracyst, much as described by previous writers; but before the apex of the hook-like process meets the paracyst, a solid septum cuts off its communication with the macrocyst at the point where it leaves the latter.

Hence a mingling of the protoplasm in the two "sexual" organs is impossible, and the open communication described by Tulasne never occurs. From certain changes in the appearance of the protoplasm of the paracyst, at the time when the hook-like prolongation fuses with it, and from the peculiar refractive appearance of the septum, the author is compelled to ask, May not diffusion occur? But, as a matter of observation, the protoplasm in the paracyst does not perceptibly diminish in quantity, and soon regains its former appearance, like that of the macrocyst.

The paracyst and macrocyst both become enlarged, and hyphæ bud out from below, enclosing them as described before. Meanwhile, buds appear on the paracyst, which are from the first much thicker than the paraphyses, and are evidently the ascogenous hyphæ.

The macrocyst is therefore an *Ascogonium*, and the paracyst is morphologically an *Antheridium*. Whether these sexual organs are so physiologically is very doubtful.

Comparison with the *Collema*<sup>4</sup> suggests that the hook-like prolongation from the macrocyst is really a *Trichogyne*; and it is not impossible that here, as in the *Collema*, an extremely small quantity of material may pass into the ascogonium, and the sexual act be physiologically complete also.

Proceeding to compare the foregoing with what we know of other *Discomycetes*, Kihlmann thinks that the sexual process in *Ascobolus*

<sup>1</sup> 'Bot. Zeit.,' 1882; 'Beitr. zur Entw. einiger Ascomyceten.'

<sup>2</sup> 'Text-book,' p. 309.

<sup>3</sup> 'Grundzüge der Systematik, &c.,' p. 123.

<sup>4</sup> Stahl, 'Beiträge zur Entw. der Flechten,' H. i.

*furfuraceus*<sup>1</sup> is still less established than in *Pyronema*. The ascogonium in *Ascobolus* is large and well marked, it is true, but the so-called pollinodium is very little, if at all, different from the ordinary mycelium. Probably in this, as in other *Ascoboli*,<sup>2</sup> the male organ is degenerated. Reference is then made to recent researches on other *Discomycetes*. Mattirollo's<sup>3</sup> and Brefeld's<sup>4</sup> investigations of *Peziza sclerotiorum* show that the process of reduction has gone still further in this case; even the ascogonium seems to have disappeared. Other *Pezizae*,<sup>5</sup> so far as the researches allow us to judge, seem to present similar degenerations.

Kihlmann thinks we may probably say that *Pyronema confluens* possesses sharply distinguished sexual organs—at any rate, morphologically. *Ascobolus furfuraceus* probably produces its fructification parthenogenetically, while *Peziza sclerotiorum* forms its asci and paraphyses in a purely vegetative manner. At any rate, apogamy must be regarded as occurring in these *Discomycetes*, and as being attained gradually through a series of forms.

Before referring to other work of Kihlmann's I wish to review an important paper by Fisch,<sup>6</sup> published in 1882. In this are detailed the development of the fructification of several *Ascomycetes* which form a stroma, in which the proper perithecia are buried, more or less. He is clearly acquainted with the recent researches and speculations of De Bary, and, in fact, worked in his laboratory. Very little has been done with regard to the Fungi mentioned, and so careful a paper as this is especially welcome. The Fungi examined by Fisch are *Polystigma*, *Xylaria*, *Claviceps*, and *Cordiceps*.

*Polystigma* occurs in the leaves of *Prunus*,<sup>7</sup> forming swollen masses. The formation of the ascospores takes place some months after the fall of the leaf. The ascospores, sown in water, produce secondary spores. These send hyphæ through the epidermis of the living leaf, and a mycelium is formed in the intercellular spaces. This breaks down the cells in part, or stimulates them to hypertrophy, and thus the stroma is formed, partly of mycelium, partly of hypertrophied leaf-tissue.<sup>8</sup> Eight weeks after infection, the young spermagonia

<sup>1</sup> Janczewski, 'Bot. Zeit.,' 1871.

<sup>2</sup> E. g. *A. pulcherrimus*; Woronin, 'Beitr. zur Morph.,' R. ii.

<sup>3</sup> 'Nuovo Giorn. Bot. Ital.,' vol. xiv.

<sup>4</sup> 'Schimmelpilze,' iv.

<sup>5</sup> Cf. Woronin, op. cit., and Tulasne, 'Ann. des Sc. Nat.,' ser. 5, t. vi.

<sup>6</sup> "Entw. einiger Ascomyceten," 'Bot. Zeit.,' No. 49, 1882.

<sup>7</sup> Cf. Frank, 'Krankheiten der Pflanzen,' p. 632.

<sup>8</sup> Cf. Tulasne, 'Selecta Fung. Carp.,' iii, and De Bary, 'Morph. und Phys. der Pilze,' &c., p. 8; also Frank, loc. cit.

appear as knots of hyphæ, which become hollow and abstrict the spermatia.

The young perithecia now arise as small clumps of fine hyphæ, which soon form a sub-globular mass, and in the interior of which a spirally-coiled group of cells represents the *Ascogonium*, and reminds the observer of the ascogonium of the *Collema*.<sup>1</sup> This body is somewhat irregular, not evidently attached to a particular part of the mass enveloping it, and it slowly grows as the surrounding perithecium cells multiply.

One end of the spiral grows out straight, passes through a stoma, and is clearly of the nature of a "trichogyne." This was frequently seen, and is figured several times. Though spermatia were seen to adhere firmly to the end of the trichogyne, the author could not convince himself that fertilisation took place.

It requires two or three months to complete these processes and the formation of the ripe perithecia. Meanwhile, the trichogyne begins to be disorganised from its free apex inwards. This was confirmed in both the species examined, and the author thinks it is a more pronounced degeneration than the change induced in the trichogyne of *Collema* on fertilisation.

The paraphyses now bud from the base of the perithecium—not from the ascogonium—and soon fill up the space formerly occupied by the dense tissue surrounding the coiled portion of the *Ascogonium*. This tissue meanwhile becomes resorbed, and the few remaining basal cells of the ascogonium—the trichogyne and upper part have disappeared—give rise to asci by budding. All the stages of development are clearly described.

With *Xylaria polymorpha* Fisch was able to clear up the points left undecided by De Bary<sup>2</sup> and Fuisting.<sup>3</sup> The young perithecia arise in the dense stroma as clumps of interwoven hyphæ, in the midst of which a mass of paler cell-rows arises, which are coiled and interwoven into a core or "nucleus." These are the "Woronin's hyphæ" of Fuisting. While these are developing, the outer walls of the perithecium become differentiated. The "Woronin's hyphæ" now break up, first into pieces of one or two cells, and then into a disorganised mass, which soon becomes gelatinous and amorphous.

<sup>1</sup> Stahl, op. cit.

<sup>2</sup> 'Morph. und Phys.,' pp. 97—99.

<sup>3</sup> 'Bot. Zeit.,' 1867, pp. 303—310.

The paraphyses now spring from the dense mass forming the inner wall of the perithecium. The asci arise from among these, and have therefore nothing to do with the "Woronin's hyphæ," which have disappeared in a slimy mass. Further details offer nothing new.

In *Claviceps*, the young perithecium arises as a mass of small cells, which rapidly divide and form a parenchyma-like mass. A hollow then appears in the interior (reminding one in many respects of the cleavage cavity in some animal embryos) by the separation of the cells. The mass now consists of a thick basal portion, above which is a hollow space roofed over in a dome-like manner by the upper cells. There is no trace of an ascogonium or of "Woronin's hyphæ" at any time; the asci arise by budding from the cells forming the floor of the cavity.

*Cordiceps* and other species of both genera agree in the main with what has been described.

In summing up the foregoing, the author points out that while in *Polystigma* we have morphologically the same organs as occur in the Collemaceæ (viz. spermatia, trichogyne and ascogonium), no sexual process could be demonstrated. In *Xylaria* the sexual organ—at any rate the ascogonium—is represented morphologically, but has become functionless—it deliquesces and is absorbed before the asci arise; these spring in a purely vegetative manner from the lining walls of the perithecium. It is possible that certain facts observed in *Cucurbitaria* point to the same end.

In *Claviceps* the perithecium is purely apogamous—no trace of an ascogonium occurs, and the asci are produced by a vegetative process of budding from the floor of the perithecium cavity.

Putting together the foregoing facts, and what is known otherwise of the allies of these Fungi, Fisch shows that the compound *Pyrenomycetes* present a series of forms which commence with a complete differentiation of sexual organs (ascogyne, trichogyne, and spermatia), and end in forms which are quite apogamous, and with no trace of sexuality. We may go further than this.

Beginning with *Podosphaera*, in which a sexual process is possibly still recognisable, we trace a series through the simple *Pyrenomycetes* and the *Discomycetes* branching off from these, ending with the completely apogamous *Chetomium*<sup>1</sup> and *Pleospora*.<sup>2</sup> In this series there is no place for the composite Ascomycetes and Lichens—for although

<sup>1</sup> Van Tieghem, 'Compt. rend.,' 1875; and 'Bull. Soc. Bot. Fr.,' 1876.

<sup>2</sup> Zopf, 'Bot. Zeit.,' 1879, p. 73. Bauke, 'Bot. Zeit.,' 1879.

the sexual process by means of spermatia is only an adaptive form, the difference is too great to fit into the main series. How then do these forms abut on to the main series? Has a sexual process arisen in the Ascomycetes a second time; or did these forms branch off early, and evolve and specialise their peculiar mode of fertilisation from the original type? The first hypothesis cannot be maintained; the second seems highly probable.

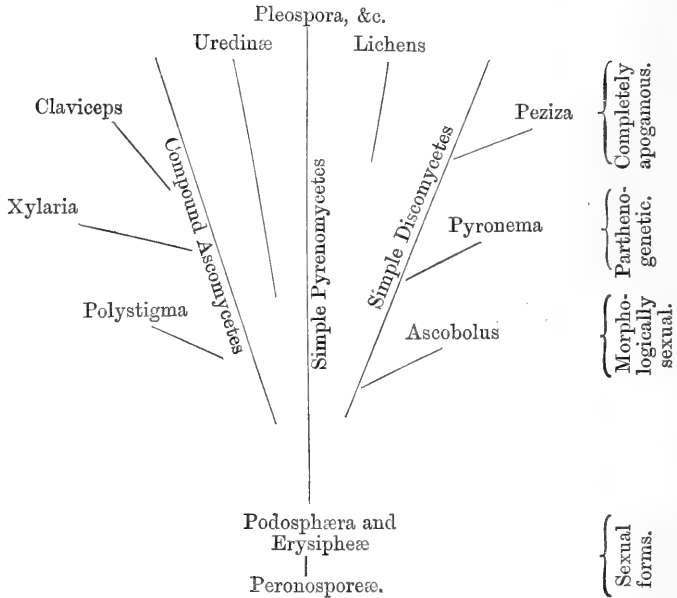
We must regard the separation of the sexual organs in the composite Ascomycetes and Lichens as adaptation, though we cannot say how it came about or served the organisms concerned. It is remarkable that this only occurs in forms which develop a stroma.

The composite Ascomycetes, therefore, branched off before the sexual process was lost; whether the Lichens came off at the same time is not clear—the latter possibly form more than one series, moreover.

The *Discomycetes* must also have branched off early from the main series; they form a series in the following forms, gradually culminating in apogamy—*Ascobolus furfuraceus*, *A. pulcherrimus*, &c., *Pyronema confluens*, *Peziza tuberosa*, *Fuckeliana Willkommii* (the latter examined by Fisch).

The Uredineæ must also have come off very early from forms in which sexuality still existed.

As I understand the foregoing, the following scheme fairly expresses the views; it being borne in mind, however, that the lines are not intended to indicate more than the general directions in which descent may be traced. Of course, such a diagram suffers from being drawn on a plane surface. No doubt more than one line should be drawn towards the Lichens.



We may now shortly consider the chief results obtained by Kihlmann from cultivations of *Melanospora parasitica*,<sup>1</sup> a pyrenomycetous fungus found associated with, and parasitic upon *Isaria*.

*Isaria* grows upon and destroys insect larvæ, and Kihlmann observed that large quantities of the perithecia of *Melanospora* soon appear with it; the same is true for *Botrytis*—another insect killing fungus. In both cases the sowing of *Melanospora* spores on these fungi soon resulted in the formation of abundant perithecia.

This, of course, only suggests, but does not prove, the parasitism of the *Melanospora* on the *Isaria*, or *Botrytis*.

Spores of *Melanospora*—whether conidia or ascospores—if sown in water only swell and throw out short tubes, which invariably die off soon. The same happened with all the numerous nutritive solutions tried. These solutions were varied not only as to composition, but also as to concentration, &c.

If a spore germinates in the neighbourhood of a living hypha of *Isaria*, however, the germinal tube fixes upon the *Isaria* hypha and at once emits more tubes, which are thicker and more vigorous than before. If the germinal tube from the *Melanospora* spore comes within a certain maximum distance from a branch of *Isaria* its apex grows directly

<sup>1</sup> Op. cit.

towards the latter until a union is effected.<sup>1</sup> This was observed and confirmed several times. All the above facts are generally true of the conidia also; and *Botrytis* may be substituted for *Isaria* as the host plant.

After about eight days the above processes have resulted in the formation of a vigorous mycelium and the formation of young perithecia. The perithecium commences by the development of a lateral branchlet, which becomes coiled two or three times, and divided by a few septa; this is the ascogonium. It frequently resembles that of *Ascobolus*.

Thinner hyphal branches now spring from below the ascogonium, and envelope it by applying themselves closely to it, and branching and dividing; although one of these may grow out more rapidly at first, it does not seem to more than hint at an antheridial branch. But very often two or more arise together, and others soon follow in all cases.

None of these branches copulate with the ascogonium. Although *Isaria* branches may be close to and serve to nourish the hyphæ producing the fructification, there is no doubt whatever that only the hyphæ from the *Melanospora* enter into the constitution of the fructification.

The details of the development of the perithecium wall from the enveloping hyphæ are interesting, but present nothing essentially new, and need not be described here.

Of the four or five cells into which the coiled ascogonium is divided, the cell below the apex forms the ascogenous tissue. The terminal cell above it becomes disorganised; it is sterile, and soon disappears. Its immediate neighbour (*i. e.* the cell below the apex) becomes cut up by numerous septa in all planes, and forms an ascogenous core of parenchymatous tissue. As this occurs, the internal layers of the now dense envelope—produced by repeated ramifications and divisions of the interweaving hyphæ of the enveloping branches—become disorganised, deliquescent, and evidently then serve as nourishment for the cells of the ascogenous core.<sup>2</sup> The proximal cells (*i. e.* those cells between the ascogenous cells and the mycelium) of the ascogonium also disappear, and the enveloping layers around them become elon-

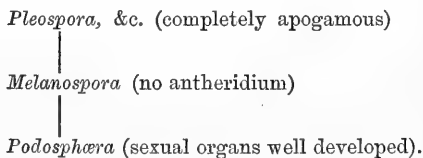
<sup>1</sup> Cf. my description of the behaviour of a hypha of *Pythium gracile*, 'Quart. Journ. Mic. Sc.,' October, 1883, p. 504; and also the remarks below on the behaviour of *Spirogyra* in conjugation.

<sup>2</sup> Cf. my description of the development of the perithecium in *Meliola*, 'Phil. Trans.,' 1883, p. 592, &c.

gated to form the long neck of the perithecium. It thus comes about that the free apex of the ripe perithecium corresponds to what was the attached end of the ascogonium.

The walls meanwhile become coloured deep brown, and rudimentary paraphyses spring from their internal layers. The asci arise from the colourless cells of the ascogenous core, in which a cavity is produced by the tangential growth of its peripheral cells.

In the concluding remarks stress is again laid upon the fact that we cannot speak of an antheridium here; the antheridia have degenerated to mere vegetative hyphæ, and the ascogenous core produces its asci without any sexual process whatever. A single cell produces the asci. In most respects this agrees with Gilkinet's description of the analogous processes in *Sordaria fimicola*.<sup>1</sup> But in *Sordaria*, Gilkinet finds one enveloping hypha apply itself to the ascogonium before the rest, though he could not decide that copulation took place.<sup>2</sup> Kihlmann denies that this can be termed an "antheridium." He regards *Melanospora* as somewhat midway between those *Erysiphææ* which, like *Eurotium* and *Podosphæra*, have the sexual organs at least morphologically present, and the truly apogamous *Pyrenomyces*, *Chaetomium*<sup>3</sup> and *Pleospora*.<sup>4</sup> We may no doubt fairly represent these views in such a diagram as the following:



I now proceed to notice a further contribution to our knowledge of the Ascomycetes, by Eidam.<sup>5</sup> Passing over his description of *Eremascus albus*, a new species and genus (in which the process of conjugation is, however, strongly suggestive of the *Zygosporææ*), we may briefly notice the general absence of any recognisable process of fertilisation, though an ascogonium is always present, and Eidam seems to regard one of the enveloping branches as an antheridium—morphologically at least.

This observer has studied the development of the perithecium in

<sup>1</sup> 'Bull. Acad. r. de Belgique,' ser. 2, t. xxxvii.

<sup>2</sup> Cf. also De Bary and Woronin, 'Beitr. zur Morph. und Phys. der Pilze, &c.,' 1870, R. iii

<sup>3</sup> Zopf, 'Nova Acta, Leop. Car. Akad.,' B. xlii.

<sup>4</sup> Bauke, 'Bot. Zeit.,' 1877.

<sup>5</sup> Cohn's 'Beitr. zur Biologie, &c.,' B. iii, H. iii.



*Chaetomium*. The ascogonium arises as an isolated coiled branch; fine branched hyphæ then envelope it. The tufts of fine hyphæ may, however, arise independently of ascogonia also. Other cases occur where the ascogonia show no traces of anything but vegetative budding from the hyphæ. He regards it as possible that a sexual process occurs in the cases first described, and that all stages of degeneration to complete apogamy occur. Questions of nutrition seem to affect this matter.

It must be allowed that the figures do not establish this, however, and it seems very questionable if any antheridial branch whatever can be distinguished.

*Sterigmatocystis nidulans* is a new species of fungi allied to *Aspergillus* and *Eurotium*. An interesting description of its capability of producing pathological changes when injected into the blood of animals can only be adverted to here. The fructification occurs embedded in a sort of stroma of hyphæ, interwoven into dense cushions, the peculiarities of which need not be detailed.

The simple ascogonium is enveloped by a hypha ("antheridial branch"), which soon becomes septate and branched, and forms the perithecium wall. The ascogonium forms a multicellular core, from which the asci arise. No fertilisation is shown to occur.

*Helicosporangium parasiticum* was also thoroughly studied. Here, again, we have simple ascogonia enveloped gradually by so-called antheridial branches. The author does not make quite clear, however, what are the ultimate fates of the several parts. One central cell becomes filled with spores, but Eidam differs from Karsten as to the meaning of this. He also denies that *Helicosporangium* is parasitic.

A closely-allied form is *Papulospora*, which agrees with the latter in forming the peculiar masses of cells which seem to represent young perithecia. It is difficult to avoid the conclusion that Eidam has either figured ill-nourished specimens—which appears unlikely—or that some unknown conditions would have caused the bulbil-like bodies to form perithecia.<sup>1</sup> Be this as it may, the bodies in question form no asci, but "germinate" like compound spores. The great variability in the formation of the spores and fructification in these fungi supports the suggestion ventured above, and there can be little

<sup>1</sup> I have drawings of somewhat similar bodies from an unknown fungus, which cannot as yet be made to develop further: they appear to be young perithecia, but they germinate directly, like gemmæ, when the conditions are favourable.

doubt that Eidam has opened a question of great importance, and succeeded in showing that variability occurs in these processes—whether due to conditions of temperature, nutrition, moisture, &c., or not, cannot yet be determined. There are facts to support this, and indeed Eidam has shown this in some examples.

Concerning *Eremascus*, where a true conjugation takes place between the apices of two similar hyphæ coiled round one another like a double screw, it is not easy to see why the product of the sexual act (a globular body situated between the conjugating apices) should not rather be termed a zygospore than an ascus. The fact of its containing eight "spores" instead of one, is no more peculiar than in the case of the *Saprolegniæ*, where an oogonium may contain one to twelve or many more oospheres. Eidam recognises the general similarity to *Zygosporææ*, but gives no adequate reasons for choosing the name "ascus" in preference to "zygospore." The eight-spored body would be an extremely anomalous ascus; but it is impossible to decide the matter until the asexual spores are discovered. It is interesting to note, however, that the so-called "asci" arise parthenogenetically in rare cases.

The main results of Eidam's observations go to prove that in apogamous forms there may be more or less indications of certain rudimentary organs—antheridial branches (?)—but they do not seem to establish his conclusions that sexuality exists in these forms. Of course it is open to imagine that the sexual act comes in now and again, as Eidam suggests, but no one acquainted with the facts will lay stress on this supposition.

If we now turn from the Ascomycetes to the other groups of fungi, the chief papers published lately are not very numerous.

The most important, probably, is Woronin's memoir on the *Ustilagineæ*,<sup>1</sup> and his description of the hitherto little-understood *Tubercinia trientalis*. Woronin devoted much time to this investigation, commenced sixteen years ago. We may shortly summarise the life-history as follows:

In May and June the under side of *Trientalis* leaves are apt to be covered with white patches. These consist of the colourless conidia,<sup>2</sup> supported on long hyphæ, much like those of *Ramularia*, *Peronospora*,

<sup>1</sup> 'Abhandl. Senk. Nat. f. Gesellschaft,' B. xii, H. iv, 1881.

<sup>2</sup> These are true conidia, homologous with those of *Ascomycetes*, and have nothing to do with the ordinary spores and "sporidia."

and others. These conidia-bearing hyphæ spring from a mycelium in the leaves. In the mesophyll are abundance of the usual brown ustilaginous spores—very like those of *Sorosporium*, &c.—in dense clusters.

In the autumn the *Torientalis* plants are found spotted with black patches. These are due to the densely-clustered brown compacted spores, as before, but no conidia occur now. The pyriform colourless conidium germinates on the leaf surface; the germinal tube bores its way in, grows to a mycelium which ramifies between the cells, and sends branched haustoria into their cavities.

At certain points on the mycelium arise lateral branchlets, which superficially resemble ascogonia, at least in some cases; these—single or several together—become enveloped by fine hyphæ, and soon present the appearance of a dense grape-like cluster of spores embedded in the interwoven mass of fine hyphæ. The investment becomes disorganised as the clusters of spores turn yellow, and then brown, and acquire thick coats. The cluster of spores germinates as a whole, putting out tubes (“promycelia”) at the end of which the crown of “sporidia” appear according to the type of *Tilletia*. These oval sporidia may also “copulate” in pairs in the well-known manner; but this often does not occur, and it seems to be an unimportant point, not affecting the future of the sporidia or their progeny at all. Secondary and tertiary sporidia may arise from the primary sporidia by budding.

After sowing the brown spores on young plants of *Torientalis*, still level with the ground or nearly so, the mycelium arises in the seedling, and, as soon as the leaves unfold, produces the white conidia externally and the brown compound spores internally. This is no doubt the best established case of the existence of two generations (producing conidia and spores respectively) that we as yet know of in this group; it is true, it is not the only case, for we have the same thing in *Entyloma*.<sup>1</sup> *Tubercinia* also agrees with the others in being antœcious, *i.e.* in passing its whole life on the same host plant. Woronin is of opinion, however, that “a whole series of heterœcious forms” will be discovered among *Ustilagineæ*; whether this remark is inspired by facts not yet published does not appear.

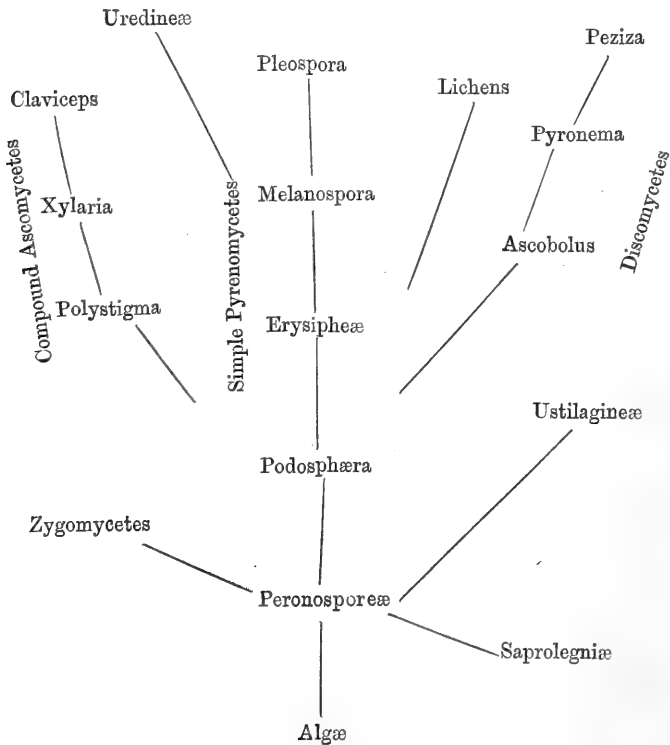
Of the remainder of Woronin’s magnificent paper nothing need be said here; and space does not admit of our referring more in detail

<sup>1</sup> De Bary, ‘Bot. Zeit.’, 1874 p 81

to the other papers lately published on the *Ustilagineæ* by Brefeld<sup>1</sup> and Max Cornu,<sup>2</sup> which, moreover, contain little of importance for our present purpose.

In the foregoing part of this essay I have collected a mass of evidence tending to support the view definitely stated by De Bary, to the effect that, as we proceed along the main lines from the lower to the higher fungi, the sexual process and sexual organs gradually become less and less evident, and at length disappear altogether, and the fructification arises by apogamy.

If we try to follow the various groups of fungi phylogenetically, there can be no doubt that they may be placed, on morphological grounds, much as De Bary has grouped them; and if, taking into account what has been said above, we attempt to arrange the smaller groups as branches of the larger ones, we shall, I think, arrive at a scheme not very different from that annexed.



<sup>1</sup> 'Schimmelpilze,' iv.

<sup>2</sup> 'Ann. des Sc. Nat.'

It must be borne in mind that we confine ourselves strictly to the evidence derived from the study of living forms—it may or may not be that numerous primary or ancestral forms, long since disappeared, would lead us to different conclusions, as the imagination of such led Brefeld to different views; but the only true method is to adhere to what we know as the basis of our plans for knowledge to come.

It must be allowed at the outset that we know very few forms accurately or thoroughly, and there are therefore almost endless possibilities in the future. Keeping these cautions in mind, however, we need not fear to point out whatever points of general significance can be obtained from our present knowledge.

The first and most important fact with regard to the scheme is that if we pay regard to the terminal members of most of the main branches, we notice that they are all, or nearly all, parasitic forms, or, at any rate, include such forms.

The higher Ascomycetes offer us the following examples from different branches, the Lichens, *Pleospora*, &c., *Claviceps*, &c., and *Peiza sclerotioides*.

Then come the *Uredineæ* (we need not necessarily imagine the Tremellas and Basidiomycetes as derived from the highest *Uredineæ*; the evidence does not decide this), all strongly parasitic.

The *Ustilagineæ* of course are parasites *par excellence*, and they terminate a side series.

We have still two main groups to deal with—the *Saprolegniæ* (which, so far as known, are mostly saprophytes) and the *Zygosporææ*, which are also generally saprophytes. Our very imperfect knowledge of the *Basidiomycetes* will be cited as an excuse for putting them aside in what follows: I do not for a moment under-value what we do know of them, but, as the sequel will show, their present position becomes more and more anomalous, if we really know the entire life-history. Of course we have no right to quarrel with the evidence, but the story of these fungi, as told at present, completely negatives their being included in the scheme to follow, and we must therefore neglect them for the moment, merely reminding the reader that some of them are parasitic.

Neglecting the *Basidiomycetes*, then, we may proceed to note that not only are the terminal groups of the series named usually parasites, but that it is just in those groups which are most intensely parasitic that least hope of our discovering sexual organs exists. In the *Zygo-*

*mycetes*, on the other hand, we have the sexual process and typical saprophytic habits together, while in the *Saprolegnia* the case seems doubtful.

Looking still more closely into the matter, it appears as if the absence or presence of sexual organs (or their rudiments) rises or falls with the nature of the parasitism or saprophytism displayed. In the *Saprolegnia*, for instance, the Fungi may probably be looked upon as very highly nourished by the decomposing proteids of animals.<sup>1</sup> Their sexual organs seem to be present in most cases, but functionless.

In the *Zygomycetes*, which are essentially saprophytes on decaying vegetable matter, &c., or parasitic on one another—and may probably be regarded as not so highly nourished—we find the sexual organs functionally perfect, though very simple in character.

In the *Ustilagineæ* we meet with parasitism of a peculiarly high order, so to speak. The fungus not only robs its host, but has in most cases curiously adapted its life to the habits of the latter, using it rather as a slave than as a victim to be destroyed forthwith.

The same is true for the highly organised *Uredineæ* (*Æcidiumycetes*), and we here meet with the highest adaptation of all, heterœcism. But in these two groups the search for sexual organs has proved utterly futile (if we except the so-called “copulation” of the “sporidia” in *Ustilagineæ*, which cannot be regarded as an essential process, or as sexual in the above meaning).

Again, if we proceed upwards from the *Erysiphaceæ*, which are epiphytes—adapting themselves to parasitic habits of that special kind which leads to life in the interior of temporary organs like leaves—through the *Ascomycetes*, we find, speaking generally, more and more tendency towards close and specially adapted parasitism, ending in the Lichens, the parasitic *Pezizas*, forms like the *Pleosporas*, &c., and especially *Claviceps*.

Now it is at least remarkable that no trace of sexual organs has yet been found in the higher Lichens—*i.e.* in those forms in which the fungus makes a particularly well-regulated use of its slave-like host, which is an Alga containing chlorophyll. Krabbe<sup>2</sup> considers that in *Sphyridium* the fructification arises independently of any process of fertilisation, and my own observations on *Strigula complanata*<sup>3</sup> lead to the same conclusion. It will be noted that in the beautiful case de-

<sup>1</sup> If not, indeed, by living flesh. Cf. Prof. Huxley, 'Quart. Journ. Mic. Sc.,' 1882.

<sup>2</sup> 'Bot. Zeitg.,' February, 1882, No. 5.

<sup>3</sup> 'Linn. Trans., ser. 2, 'Bot.,' vol ii, 1884.

monstrated by Stahl,<sup>1</sup> the host is a blue-green Alga, and the parasitism may well be considered as lower in many respects. Moreover, it is by no means certain that the Lichens represent one group.

In *Claviceps purpurea* we have an excellent example of the highly-developed parasitism referred to. The ravages of the parasitic mycelium seem to be confined to one organ of the host—the young fruit—and we have seen from Fisch's researches that the asci arise in the stromata, developed later, in a purely vegetative manner.

Our knowledge of the large group of the simpler Pyrenomycetes does not enable us to make a generalisation of very much value; but it is significant for our present purpose that the apogamous *Pleospora*, for instance, is parasitic during the early stages of its life, and, like so many of its allies, adapts its cycles to those of its host, producing a large stock of asexual conidia on the living leaves, and using up their contents before falling, to complete the development of the asci, &c., on the ground. It is scarcely necessary to remind the reader how great an advantage accrues to these higher parasites, when they scatter immense quantities of spores from leaf to leaf of the living tree. That their perfect "fruits" should be formed later, when the mycelium has gathered up all the material possible, is quite in accordance with what occurs in the formation of stromata, sclerotia, and masses of hyphæ (often with haustoria) around the young perithecia in other cases.

The same is generally true for such Discomycetes as *Peziza sclerotoides*,<sup>2</sup> *P. Fuckeliana*, and other parasitic *Pezizæ*; and it will be remembered that it is in these forms that De Bary and others failed to find any traces of sexuality, thus placing them in strong contrast to such as *Ascobolus* (according to Janckzewski's researches), unless intermediate forms like *Pyronema* and the saprophytic *Pezizas* are compared also.

Enough has now been said to show that there is at least strong reason for believing that a connection exists between the mode of life of a given fungus and the extent to which it is apogamous. It will no doubt be suggested that there are still cases where this view seems at variance with the facts. Without wishing in any way to strain matters at this point, it may be noted that we really know very little of the mode of life of very many fungi, and that the terms saprophyte

<sup>1</sup> Op.cit

<sup>2</sup> Frank, 'Krankheiten der Pflanzen,' p. 531, &c.

and parasite are used somewhat loosely. This being admitted, it may happen that further knowledge will strengthen the connection spoken of.

We are at least assured that profound differences exist—in degree, at any rate—between the saprophytism of a *Mucor* growing in a solution of horse-dung, and of a *Pythium* developing its fructification in the rotting parenchyma of a plant which it has previously killed.

There is also an equally striking difference between the parasitism of an epiphyte like *Erysiphe* and that of a highly-specialised *Æcidio*-mycete like *Puccinia*. But I would insist upon more than this. It is not only in the mode of attacking or living upon the substratum that one fungus differs from another; differences as to the kinds and quantities of the various matters absorbed must also exist, and a Uredine in a leaf no doubt obtains different food (and in a different way) from that taken by *Claviceps* in a grain of rye, or *Ustilago* in a hypertrophied swollen stem of *Zea Mays*. That these differences may be very important—though we do not know exactly in what they consist—is fully demonstrated in cases of heterœcism.

I have already pointed out that the coexistence of apogamy (or the total suppression of sexual organs) and parasitism is noticeable especially in the highly specialised parasites. In forms which, like the majority of the parasitic *Peronosporæ* and *Zygomycetes* (e.g. *Peptoccephalis*), are nevertheless provided with sexual organs, which, so far as we can see, are quite like those found in the saprophytic forms, we have two points to notice. First, these forms are close to the parent stock in phylogeny—i.e. they are not much modified from the type of *Pythium* itself, which (as a comparison with *Vaucheria* shows), is no doubt derived from algal ancestors, and with strongly inherited sexuality. Secondly, such forms are probably not so highly parasitic as is commonly supposed. I do not mean to say that their living hosts are not robbed by them; but it is significant that the *Peronosporæ* are often saprophytes, and that even the most parasitic forms break down the parenchyma of the hosts to a rotting, fetid mass, on which they then flourish. Moreover they are aided by bacteria in this process. In addition to this they are apt to be omnivorous. I have cultivated *Pythium De Baryanum*<sup>1</sup> on the most various substances, as well as on more than a dozen widely different living plants.

In all these cases the parasite appears to flourish in a variety of

<sup>1</sup> Cf. also De Bary op. cit., and 'Quart. Journ. Mic. Sc.,' 1883.



substrata, and it has not got over the clumsy habit of destroying its host forthwith. If we compare the highly developed, almost intelligent, parasitism of a higher *Ascomycete* or *Uredine* with this, it will be understood what I mean by specialised parasitism. Instead of clumsily destroying its host (like *Phytophthora infestans* does the potato), a *Puccinia* is adapted to live in isolated patches of carefully-sheltered leaf tissue, ramifying in the lacunæ filled with oxygenated air and aqueous vapour. Here it taps the cells as they manufacture organised substances in the sunlight, taxing them not too much for their strength,<sup>1</sup> and its mycelium keeping near the stomata. Its spores are then protruded in centrifugal succession, and shaken off from their advantageously high position on to other leaves, &c. All such adaptations must imply long periods of descent (and the fungus is therefore much further from the parent stock in the phylogenetic scheme), during which even the strong hereditary tendency to produce sexual organs, &c., might become lost, if such organs for any reason became superfluous.

This, however, brings us at once to the last object of the present essay; and I propose to show that it is probable that the sexuality of the higher fungi has disappeared, because its purpose has been equally well or better attained otherwise than by means of sexual organs.

Preliminary to this it will be necessary to be quite clear as to what sexual organs and the sexual process essentially are.

The two points common to all the cases of sexual reproduction which have been directly observed are the following:

1. A larger or smaller quantity of protoplasmic material passes from one portion (the male organ) of the same or another individual, into the protoplasm contained in another portion (the female organ).

2. The protoplasm contained in the female organ therefore becomes capable of further development; either at once, or, more generally, after undergoing a period of rest.

It is not necessary to quote the numerous cases of observed analogies between the sexual reproduction of animals and plants; but will suffice to note that the essential in the sexual process is always the addition of a portion of protoplasm from the male, to the protoplasm of the female.

But this is not all. It is now well established in embryology that

<sup>1</sup> Many *Uredinæ* appear to do no injury at all, unless in large amount and for a long time—*i.e.* the host can pay the tax easily.

the normal ovum, or female mass of protoplasm, is incapable of further development until it has received the protoplasm of the male; that the latter, in fact, incites the former to further development. In many cases, indeed, the protoplasm of the egg or ovum gets rid of a small portion of its substance, as the "polar bodies," as if to make room (so to speak) for the substance coming to it from the male.<sup>1</sup>

While in the higher organisms we can distinguish the male elements—spermatozoa, antherozoids, &c., only in so far that they are much smaller and more numerous than those of the female organs; we find that in the lower forms of life even this difference in size is absent, and there is absolutely no safe criterion to determine which of the two conjugating masses of protoplasm is to be regarded as male and which as female.

Nevertheless, if we consider cases such as are afforded by the fungi, we are certainly on safe ground when we call the antheridium of *Pythium* a male organ, and the oogonium of the same a female organ. The protoplasm contained in the former is itself incapable of further development, but normally passes over into the protoplasm (oosphere) contained in the latter; the oosphere is then—*i.e.* after fertilisation—capable of further development.

This "further development," however, is nothing more than growth; and, what is more, growth according to the same laws as affected the parent plant which produced the sexual organs. In cases where the plant is divided into cells, this growth or germination of the oospore commences with division into a number of cells.

The outcome of all we know of these matters leads to the conviction that we have in the germination or development of an oospore—and the same is true for an egg, &c., the terms being different—simply a *renewal* of the growth of the organism; and from this and other convictions follows the result that the formation of an oosphere, although it may take place after an accumulation of large quantities of food, implies a condition of weariness—if the term may be allowed—on the part of the protoplasm for the time being. No doubt the molecular energy of the protoplasm forming the oosphere, is less than that of the rest of the plant for the time being; the access of the antherozoid or male protoplasm, however, reinvigorates the sluggish mass, and renewed life ensues. This may require some time, however, and we

<sup>1</sup> That something of the same kind takes place in the *Saprolegniae* is suggested in my paper on this group, 'Quarterly Journ. Mic. Sc.,' 1883.

may possibly not be far wrong if we imagine that interval to be occupied in molecular rearrangements in the mass.

But, although we can sum up the foregoing by saying that, after a time, protoplasm requires re-invigorating by the addition of fresh protoplasm from another source, it is extremely improbable that the protoplasm of the male and female organs is at all similar.

While we have reasons for believing that the mass of an oosphere consists in the main of protoplasm such as occurs in any cell capable of growth, it would be absurd to suppose that the protoplasm of the male element is of the same nature. There is, moreover, strong evidence to support the opposite view, that the protoplasm of the male and the essential protoplasm of the female differ extremely.

Anyone who reads Strasburger's description of the process of fertilisation in the ferns,<sup>1</sup> cannot fail to be struck with the peculiar behaviour of the antherozoids as soon as they come within a certain distance of the oosphere. It seems impossible to avoid the inference that the oosphere in some way attracts the spermatozoids. A similar phenomenon is described by Juranyi in the fertilisation of *Ædogonium*,<sup>2</sup> where the relatively large antherozoid *forces* its way through an aperture too small for it, in order to reach the attracting oosphere.

With such phenomena may be compared the case of *Spirogyra* and other Conjugatæ, where, as is well known, the cells of filaments which are laid parallel to one another, and within a certain distance of one another, put forth conjugating tubes which meet in the middle; or neighbouring cells conjugate.

In the Peronosporæ, again, the oosphere appears not only to attract the antheridium, but even to induce its formation from a neighbouring hypha;<sup>3</sup> and other cases may be cited, all tending to show that some important difference exists between the protoplasm of the two sexual organs.

It does not concern us here to give any opinion on De Bary's suggestion that profound chemical differences exist, and affect the environment; or on Sachs' recently expressed view<sup>4</sup> as to the analogies between ferment actions and fertilisation.

Enough for our purpose that the knowledge we possess goes to show that sexual reproduction essentially consists in the reinvigoration of a

<sup>1</sup> 'Jahrb. f. wiss. Bot., vii.

<sup>2</sup> 'Jahrb. f. wiss. Bot., ix.

<sup>3</sup> De Bary, 'Beitr. zur Morph. und Phys.,' iv.

<sup>4</sup> 'Vorlesungen über Pflanzenphysiologie,' p. 491.

sluggish mass of protoplasm, by the addition of another and different mass of protoplasm. That an advantage is often attained by the latter mass coming from a distant source, is sufficiently evident from what we know of cross fertilisation generally.

It now remains to be seen if we can throw any light on the curious disappearance of sexual organs and sexuality in the fungi—curious, because the sexual process appears to be all but universal in all organisms excepting the very lowest.

A hypothesis which suggests itself, and which Eidam favours, and which is certainly supported by some analogies, is to the effect that the apogamous fungi are not always apogamous. We know that many forms only produce their sexual organs at comparatively long and rare intervals. The *Mucors*, for instance, may be propagated through numerous generations by means of the asexual spores; the sexual organs only arising now and again under favourable conditions.

Accepting that the sexual process consists essentially in a re-invigoration of the protoplasm of the organism, may it not be that one sexual act is effective through long periods and many generations? Such a view is supported by the known cases of parthenogenesis in other plants, and would explain such cases as the *Saprolegnia*, if it were placed beyond doubt that protoplasm does occasionally pass through the "fertilising tubes" to the oospheres.

Moreover, the cases of polyembryony—where several embryos arise in an embryo sac, although only one oosphere is fertilised—favour the view that the effect of fertilisation may be extensive; and we cannot doubt that such is the case where adventitious covering branches arise after the conjugation of certain *Mucorini* (e.g. *Mortierella*), and in the *Orchideae*, where fertilisation or even the mere growth of the pollen tube affects the whole flower.

In other cases, however, great difficulties are experienced. It is not easy to conceive how fertilisation in a distant past has transmitted its effects through countless generations to the individual plants of *Chara crinita* which now reproduce without any sexual act at all. And the same is true for other cases.

There is one fact apparently universal in sexual reproduction; it does not take place until a large quantity of material is either accumulated, or is in some way placed at the disposal of the sexual organs. If these sexual organs are to be looked upon as specialised to secrete

the sexual elements, or to sort the substances of which they consist, as it were, this may be of importance.

It must be allowed that no satisfactory theory exists, however, to account for the gradual disappearance, first of sexuality, and then of even the morphologically represented sexual organs in the fungi; and any attempt to explain the matter seems to involve more than one vicious assumption.

The sexual act, however, consisting simply or mainly in the re-invigoration of protoplasm by the addition of protoplasm of a different nature (though we do not know the kind or limit of difference) from a distance, it may be that an explanation of what occurs in the fungi is afforded by their mode of life. I have already pointed out that the fungi in which sexual organs seem to be most certainly absent are those which are most highly specialised as parasites. Now, we have every reason to believe, first that parasitism is a matter of degree, and secondly that the most highly specialised form of parasitism consists in directly obtaining those contents of the cells of the host which are chemically most complex, and therefore contain most energy.

I need not dwell on the degrees of parasitism exemplified by plants which merely rob their hosts of space or moisture, or which have obtained a hold so intimate that they break it up and feed on the rotting *débris*, but may at once pass on to consider a few consequences which follow from the mode of life of those highly specialised parasites which have become so closely adapted to their host, that they exist for a time as all but an organic part of its tissues and substance.

It can scarcely be doubted that the protoplasm of a higher plant, such as a phanerogam, differs from that of a lower cryptogam in being capable of doing more work; and that the great advantage derived by a parasitic fungus which has its life so adapted that it can tax the cells of a phanerogamous host plant, is that it obtains its food materials in a condition more nearly approaching that of its own substance, than would be the case if it had to work these materials up from inorganic matters.

Now it seems not improbable that the protoplasmic substance of a higher phanerogam may contain so much energy, that it can not only supply the vegetative mycelium of a parasitic fungus with all that it requires for its *immediate* growth, but also suffices to enable that fungus to store up enough energy in its asexual or apogamous spores

to last until the next generation of the fungus gains its hold-fast on another (and it may be distant) source of life-giving substance.

Let us take the case of a *Uredinous* fungus parasitic in the leaves of a phanerogam. We know that the substances necessary for the whole growth of the phanerogam are formed in the cells of the leaf; not only so, the matters which eventually find their place in the reproductive organs must be formed there also, potentially at least. The leaf of a phanerogam so attacked, moreover, is able to support the parasitic fungus for a long time uninjured, as I have convinced myself by experiment, and there can be no doubt that substances pass into the fungus which would normally have passed into other parts of the host plant itself. Since these substances serve to support the comparatively enormous display of energy evinced in the growth, &c., of the phanerogam; we need not be surprised if they can also provide in addition for the parasite for the time being.

But we may imagine even this to fail after a time. Without speculating as to the possible differences effective to a mycelium which obtains enough to produce spores on one leaf, which, germinating on another, produce a mycelium which derives an advantage corresponding to that obtained by plants when cross fertilised—we may suppose that at length the fungus derives too little benefit to be able to go on, or the season during which the host plant flourishes is drawing to an end.

No doubt we have in heterœcism the salvation of such a fungus. Not only is it carried through a dangerous period, by seeking relief at the hands of a second host, but—and which I believe to be far more important—it obtains re-invigoration by the new protoplasm with which it comes in contact. We may not inaptly compare the sojourn of the fungus on its second host, to a trip to the sea-side, where the weary and enfeebled organism enjoys fresh diet and associations for a time, which in their turn pall and prepare the recipients to renew old modes of life.

We have seen that the disappearance of the sexual organs, leading to apogamy, commences especially in the lower *Ascomycetes*, and it may be more than a coincidence that epiphytic forms, which show a tendency to produce one kind of spore while on the living leaf and develop their asci on the fallen leaf are common here; such forms suggest how the parasitism and heterœcism of higher forms may have

begun, and it is remarkable that the apogamy becomes more and more complete as we ascend through the latter.

It is not pretended that the hypothesis embodied above at once explains all the cases possible, and it will be well to state a few of the greater difficulties at once. The *Basidiomycetes* I shall not dwell upon, since our knowledge of them is still very imperfect. The few cases of parasitic *Basidiomycetes* known can hardly be cited as supporting the view adduced; and if it turns out that all the forms are as utterly devoid of sexuality as Brefeld's *Coprinus*, and that no other generation exists than the one now known, it will be difficult or impossible to reconcile the facts, and the coincidences referred to in this essay may have to be accepted as coincidences only.

Apart from this, the difficulty must suggest itself to many that there are parasitic fungi—such as the *Peronosporæ*—which nevertheless develop the sexual organs in the condition typical and perfect for the group to which they belong. I have already referred to the fact that many of these forms are really saprophytes, and that others break down and destroy the tissues of their hosts—clumsily killing their prey and then feeding on the rotten mass—and have pointed out that this is a much less specialised form of parasitism than that of the higher fungi and *Ustilagineæ*. It is true we do not know much about the nature of the food which these fungi take from the host; but there is evidence to show that it is of the nature of fermenting sap, and therefore possibly contains far less energy than the substances absorbed by the higher parasitic fungi. There are two other points which may also be of importance.

The *Peronosporæ* are almost certainly descended directly from Algae which had already won and established strongly marked sexuality. This would probably be lost only after a long time; for we have every reason to suppose that inherited sexual tendencies are among the last to disappear in the modified descendants of organisms.

Nevertheless, and this is the second point, the sexuality shows signs of disappearance in extreme members, even within the groups of the *Peronosporæ*. De Bary<sup>1</sup> shows that in *Phytophthora* and *Peronospora* there is a less evident passage over of protoplasm from the antheridium to the oosphere than in *Pythium*; and that in some cases, indeed, the quantity passing over is too small to be observed. I will not attempt to lay stress on the coincidence that in *Phytophthora infestans* (the

<sup>1</sup> 'Beitr.,' iv, p. 72.

fungus of the potato disease) no sexual act has as yet been discovered.

Another obvious objection may be raised as follows: The *Saprolegniæ* are in the main saprophytes, and yet they are said to be advanced towards apogamy—parthenogenetic, at any rate. The answer may be that they are saprophytic chiefly on animal protoplasm which contains more potential energy than does vegetable protoplasm. At the same time, some *Saprolegniæ* are parasitic on plants, and *S. ferax* now appears to be parasitic on fish.<sup>1</sup>

Among the *Zygomycetes*, again, we meet with parasitic forms in which the very simple sexual organs and process are, so far as we know, as typically perfect as in the other members of the group. The reply here is the same as in the case of the *Peronosporæ*. The *Mucors* must be an older group than *Piptocephalis* and others which are parasitic upon them. Hence we may assume that the inherited sexuality is too strong to have been replaced by the effects of admixture of the protoplasm of the *Mucor*, which, moreover, is probably not very different, and can scarcely be considered as provided with more energy. A similar argument may apply to the Lichens. The higher forms are specialised parasites on green Algæ, which must be able to supply substances containing great potential energy, and no traces of sexuality are found in them. In the *Collemaceæ*, however, where sexual organs occur, the fungus is associated with a very low form of Alga, one of the *Cyanophyceæ*, and appears rather to be feeding upon the diffluent matters around the algal cells, than strictly parasitic on the Alga proper. This is so much the case that, as is well known, some lichenologists have doubted whether to rank the *Collemaceæ* with Lichens at all; and all observers must agree that it is difficult to decide when a mass of *Nostoc* is to be regarded as all Alga or passing into the state of *Collema*. I remember cases in my time at Cambridge, when I observed patches of *Nostoc* on the roadside at Shelford, and patches of *Collema* some distance away. At points between, there were patches of *Nostoc* in various stages of transition between the two. In Ceylon, again, I have observed masses of *Rivularia* with fungoid hyphæ associated at least as definitely as in these cases, and the same occurs in masses of *Glæocapsa* in greenhouses. I do not attempt or wish to cast a doubt on the lichen character of the *Collemaceæ*; I merely point out that, as in the case of other parasitic Fungi, the *Ascomycetes* of the Lichens exhibit gradations of parasitism from mere

<sup>1</sup> Prof. Huxley, loc. cit.



association to highly adaptive parasitism, where the fungus has learned (so to speak) to use its host as a slave.

The most serious objections to the above hypothesis will probably occur to those who draw conclusions from the life-history of imperfectly known forms. Without wishing to disarm any criticism whatever, I would mention two points to be borne in mind in this connection.

Many fungi are known to be capable of adapting themselves to widely different modes of life, and it is extremely difficult to say how far they are parasitic or saprophytic. Leaving the *Bacteria* alone, I need only mention Koch's experiments with species of *Mucor* and *Aspergillus*, and Eidam's observations on *Sterigmatocystis*:<sup>1</sup> these fungi were found to be pathogenic to a disastrous extent when injected into the blood-vessels of living animals. Again, Kihlmann's brilliant research on *Melanospora*, embodied in an earlier portion of this essay, brings to light an extraordinary case of parasitism and adaptation.

Secondly, we really know very little of the mode of life of many fungi in their earlier stages; we *assume*, rather than *know*, that many forms of *Pyrenomycetes*, for instance, are saprophytes. However, less is to be gained by dwelling upon these doubtful matters than by court-  
ing criticism of the main point at issue.

I may say, in conclusion, that it was during the study of the parasitic fungus of the Coffee disease (*Hemileia vastatrix*) in Ceylon that I was first led to speculate on the enormous amount of energy displayed by an organism which shows not the remotest satisfactory trace of sexuality, but which reproduces itself through many generations exclusively by means of asexual spores. That this energy of reproduction is derived from the Coffee tree there can be no doubt, and that it is at the cost of the reproduction of the host is sadly evident; the clear inference from the fact that the Coffee leaf supplies substance for the reproduction, &c., of a fungus at the expense of its own fruit, is that the fungus takes matters which are very rich in energy, so rich, indeed, that the fungus is not necessitated to sort these substances in special reproductive organs, and to secrete sexual elements, one of which would then re-invigorate the other, but may employ them forthwith for the purposes of its own relatively simpler existence and reproduction.

<sup>1</sup> Cohn's 'Beiträge,' B. iii., H. iii., p. 397 ff

THE MORPHOLOGY AND PHYSIOLOGY OF AN AQUATIC  
MYXOMYCETE.

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[PLATES III—IV.]

During the progress of certain experiments which I instituted during the course of the past winter and spring, in order to obtain information as to the feasibility of employing the electric light in the botanical laboratory, so that demonstrations on the assimilation of green plants might be made independently of the sunlight, of which we obtain so little in Manchester, it became necessary to employ among other plants actively growing Hyacinths; in order the better to study the effect of the electric light on these, I grew them in nutritive solutions consisting of minerals dissolved in pure water, according to a method devised some years ago by Sachs, and now much used in my laboratory course for teaching purposes. A few fractions of a gram of phosphates, sulphates, and nitrates of calcium, magnesium, and potassium are dissolved in one litre of distilled water, and a trace of common salt is added. All the salts should be chemically pure, and plants may be easily cultivated in the solution for weeks and months, as is now abundantly proved (fig. 1).

The Hyacinths in question were grown with their bulbs resting on the necks of tall glass jars, cylindrical in shape, and the roots of the

plant hung perpendicularly into the solution mentioned above; in this they developed vigorously, and were to all appearance quite normal. During the course of the experiments, and while the plants were exposed to the influence of the electric light emitted from incandescent Swann's lamps, certain minute black spots were several times observed on the white roots hanging down into the liquid; these spots were all very small, but varied in size, and evidently increased in number day after day and week after week, and beyond making a few notes as to their occurrence and increase I paid little attention to them at first, my interest being aroused in the progress of the more special investigation to which my experiments were directed. Very soon, however, the time came when it was necessary to examine the roots, and their tiny black spots, and I then discovered that the little specks were really the sporangia of a microscopic Myxomycete. Fig. 1 shows the appearance to the unaided eye of the roots and the black spots referred to, drawn to their natural size.

In the first place their order of appearance showed no obvious regularity with respect to either space or time. Sometimes one of the tiny sporangia would arise at or near the tip of the root, at others it would appear near the basal portion, or irregularly at any spot between the base and apex. Again, while three or four might be formed during a given 24 hours, none or several might make their appearance during the next day, and so on. Moreover, I could detect no obvious relationship between the order (or want of order) of succession, and any changes in the light (electric light) and temperature which were noted with reference to my other investigations. All that I was able to establish in this connection was that the black sporangia became developed on the roots, both in the glare of the electric light used, and in the diffuse daylight of January and February; subsequent investigation showed that they also became developed on the roots when kept in the dark. The temperatures varied somewhat considerably, and the sporangia were formed in some cases at 18° C., during the periods when the electric light was turned on, and even at 14° or 15° C. during the night, and at those periods when the roots were in the dark.

Another point of interest is that by far the majority of the sporangia referred to were developed in the nutritive solution—*i.e.* on parts of the roots entirely submerged—though, as I shall show later, they may also be formed on parts of the roots which are in the damp

atmosphere outside the liquid. On the sides of the bulbs, or on the leaves, and generally on any part of the Hyacinth exposed to the dry air outside, the sporangia are not developed, and every attempt to cultivate them there has so far failed utterly.

There can be no doubt that the life-history of this Myxomycete then is passed through in the aqueous solution, and it seems to me a point of no small interest to have found what may justly be described as an aquatic Myxomycete.

There is still another point of general importance to be mentioned. The sporangia arise on what are to all intents and purposes perfectly healthy, living, and intact roots, and there are no observations to show that the organism causes any injury whatever to the plant on which it is found. It is not intended to deny that this Myxomycete may produce its sporangia on dead or dying roots—a point which I have not directly investigated, though some experiments to be described later show that it may well be so—but all investigation places beyond doubt that no interference with the normal relations of the root are necessary for the well-being of the Myxomycete.

The black sporangia referred to contain spores; these spores germinate readily and emit "myxamœbæ," which pass rapidly into "myxozoospores"; after a varied term of life, during which nutrition is carried on, and various phenomena connected with reproduction are observed, these "myxamœbæ" unite into plasmodia; which, in their turn, again form sporangia. The above may be regarded as a cursory sketch of the life-history which I have observed point for point, and partly because the above headings agree broadly with the order of investigation, and partly for other reasons, I shall adopt the simple plan of describing the subsequent details in the following order. (1) The anatomy of the sporangium and its contents; (2) the germination of the spore, and the production of myxamœbæ; (3) the changes undergone by the myxamœbæ—their nutrition, changes of form, encystment and division; and (4) the formation of the plasmodium, and the changes which it undergoes. Other remarks, on the physiology of the myxamœbæ chiefly, may find place under one or more of the above headings.

#### THE SPORANGIUM.

As shown in fig. 1, the sporangium is a minute black body, of globular or ovoid form, or occasionally less regular and shaped like a

slug or planarian : it varies in diameter from  $\frac{1}{2}$  a millimeter to  $1\frac{1}{2}$  millimeters or more, and stands up from the epidermis of the root, to which it is attached by a slightly flattened base. As shown both by sections and by the ease with which the sporangium can be detached from the root by means of a razor or even the point of a needle, the sporangium is only fixed on to the outside of the epidermal layer, or to the outer cells of the root-cap, and is in no way connected anatomically with the interior of the cells or tissues : in some cases, however, it can be shown that the base of the sporangium is actually in contact with the external cell-walls of the root, and not merely adherent to the slime, &c., found on the surface.

When detached, examination fails to show any aperture in the sporangium, or any special organs of adhesion, except in so far as the thin network to be referred to shortly, and which passes off from the surface of the sporangium to that of the root may be looked upon as a hold-fast.

On slightly magnifying the sporangium (figs. 2, 3, and 4) it is at once noticed that the smooth hard black appearance is due chiefly to two causes. In the first place the thin outer covering or shell reflects the light in various ways from its rounded surfaces, which are, moreover, wet and bright, and evidently coloured with a dark pigment ; and secondly the densely packed, minute, purplish-brown spores in the interior render the whole mass perfectly opaque, except at the edges where they may be seen shining through with more or less distinctness (figs. 3 and 4).

Closer examination now shows also that the deep black mass of sporangium and contents proper is fringed as it were (at any rate in most cases) with a bright canary yellow border, and, as will be shown below, this extends over the sporangium proper as a sort of outer coat or network.

The whole sporangium, then, is composed of two coats—an inner coat composing the wall of the sporangium proper, is a thin, tough, very elastic and almost horny homogeneous membrane, which is quite smooth or nearly so, being occasionally marked with almost imperceptible rugosities. Its colour is, as already stated, dark, and evidently due to a purple-brown pigment usually evenly distributed throughout its substance. In comparatively rare cases the pigment is nearly absent in places, and the depth of its hue varies somewhat : these variations

seem to depend on the age and relative thickness of the membrane, but may be in part due to other causes.

External to this, the proper shell of the sporangium, is a yellow network or coarser membrane somewhat less constant in character. In some cases, at any rate, it can be traced all over the surface of the sporangium, but in other cases it appears to be incomplete at places. The meshes of this layer vary considerably in size, and the whole network is thicker or thinner at some spots than at others: to these peculiarities may be referred the different shades of the yellow colour. It is where this yellow meshwork passes off from the surface of the sporangium to become extended irregularly on to the surface of the substratum that the yellow fringe or border (figs. 3 and 4) is observed, and in a certain sense this "outer peridium," as the network might be termed, may be looked upon as fixing the sporangium which it imprisons to the substratum. The relative positions of the two structures are no doubt due to the fact that the network is a mass of coarse granular material excreted by the protoplasm of the plasmodium when it is forming the spores and sporangium.

In the large number of cases where the sporangia present the typical bright black colour to the unaided eye, or when but slightly magnified, this thin yellow network offers nothing further worthy of note. It occasionally happens, however, that certain of the sporangia referred to above as being developed not in the nutritive solution but on parts of the roots which are in the damp atmosphere just above the surface of the liquid, present a dull greyish appearance, in place of the bright black one described in detail above: in these cases the thin outer network is infiltrated with carbonate of lime in varying degrees and may be closer in texture accordingly. This seems to mask the yellow colour, and the network becomes a coarsely granular and, it may be, brittle greyish membrane. There is much variability in this connection however.

Two points of interest present themselves here. First, as already said, the external granular membrane or network is evidently a remnant or excretion from the plasmodium, left over in the process of formation of the sporangium proper from its protoplasm; and secondly, it depends on circumstances whether it contains much or little calcium carbonate. My observations on the subject lead to the following conclusions.

The bright black sporangia developed on the completely submerged roots have no perceptible amount of lime on their surfaces; they are smooth and bright, simply because the yellow network which passes over them is too fine and translucent to materially affect the appearance of the pigmented proper membrane of the sporangium (*c. f.* figs. 3—6). But in the case of the dull greyish sporangia developed on the roots outside the liquid, the dullness is clearly due to the large quantities of calcium carbonate deposited as fine granules and crystalline masses in the outer coat. In some cases the infiltration with lime goes so far that the membrane is quite brittle and cracks into angular pieces (fig. 5), and on the addition of dilute acetic acid the crystalline needles (fig. 9) or granules rapidly dissolve, copious effervescence of carbon dioxide taking place at the same time. These facts suggest the question whether calcium carbonate is not perhaps formed in abundance on all the developing sporangia, but in the case of those submerged in the water it is possibly carried off dissolved in the carbonic acid diffused throughout the solution, whereas in the case of those sporangia developed out of the solution, the surplus carbon dioxide escapes into the damp air around leaving the crystalline solid calcium carbonate behind.

That calcium carbonate is actually produced in the interior of the submerged sporangia also is abundantly proved by the excreted nodules of that mineral which are contained in the nodes of the capillitium to be referred to shortly; of course the conditions under which clusters of needles or granules of calcium carbonate are excreted in the cavity of the sporangium might be very different from those referred to above. The capillitium, as shown in fig. 8, is constituted simply of a loose irregular network of delicate horny fibres, which may be either colourless and nearly transparent, or tinged with the purple-brown pigment so common in other parts of the sporangium (fig. 5). What may be termed the nodes of this irregular meshwork are usually enlarged, and in the triangular or irregularly angular enlargements are deposited the concretions of granules or crystals of calcium carbonate referred to above (fig. 8). The fibres or rods forming the capillitium are apparently solid, and spring from the inside of the wall of the sporangium, their bases being dilated at the areas of attachment (figs. 5 and 8). The fibres pass directly across the cavity of the sporangium in some cases, while in others they seem to terminate freely in it; or they are connected together, at various angles, with other fibres which cross

them. The meshwork of this rudimentary capillitium reminds the observer of the ceratose skeleton of some sponges, a similarity which is perhaps not lessened on examining the texture and composition of the fibres.

In concluding these remarks on the sporangium, a few words may be added as to the action of reagents on the structures described. Even in extreme cases it is possible, after removing all the calcium carbonate by means of dilute acetic acid, to separate more or less completely the outer network or membrane from the thin pigmented proper wall of the sporangium (fig. 5): the latter retains its even colour and tough elastic character, but the external layer, if much hardened by calcium carbonate, may be cracked in all directions under the pressure of the cover slip.

Solutions of iodine alone colour both the decalcified outer membrane and the horny internal one yellow or brown, and the addition of sulphuric acid enabled me to detect the blue colouration indicative of cellulose in some parts at least of the inner membrane. This reaction is by no means satisfactory however, and the rule is that when it does make its appearance the blue colour is only in patches here and there. The same is true of the reaction with carefully prepared chlor. zinc iodine (Schulze's solution): very often the only indication of cellulose is a pale bluish-green patch here and there in the membrane. Sulphuric acid causes partial solution of the test and swelling of the fibres of the capillitium; and the latter are coloured bright golden brown by iodine, the colour only being rendered deeper on the addition of sulphuric acid.

Certain variability in these reactions of the walls and capillitium is no doubt due, in part at least, to the age of the sporangium, as is the depth of colour of the natural pigment: of course the depth of colour is affected by the thickness of the membrane also, but this could scarcely be a serious difficulty. In all the cases referred to the spores, the only remaining contents of the sporangium, were ripe; and I now proceed to describe them.

The ripe spore (figs. 5, 6, 7, and 10) in sporangia which have become normally mature on roots in the solution, is a spherical cell,  $\frac{1}{1000}$  in. in diameter, with a tough homogeneous cell wall, which is coloured purple-brown, and appears to resemble the proper membrane of the sporangium in all essential respects. With the usual reagents the spore membrane yields better cellulose reactions, however, though



even here it evidently does not consist entirely of that substance, but appears to contain a body (possibly approaching cutin in composition) of some more resistant nature. In most cases the spores are so nearly smooth that the extremely fine punctations on their exterior may easily be overlooked or neglected; but in other examples the fine punctation is quite noticeable, and it seemed to me that these more evidently marked spores were smaller than the average size, though no differences could be detected in their behaviour. They certainly germinate as readily as do the smoother spores, and behave in exactly the same manner during the process. But, as I shall show directly, there are much greater variations in the size of the spores than these, none of which seem to affect the purpose or behaviour of the spore in the slightest degree.

Each normally ripe spore contains a quantity of very pale translucent protoplasm in or near the centre of which is a paler and brighter round spot: this is the nucleus shining through the hyaline protoplasm. The nucleus is particularly clear at the moment when germination commences (fig. 11, 14), but can always be detected in the resting conditions, and also, as will appear subsequently, in other conditions of the contents of the spore.

With respect to the other varieties of spore referred to above, these are much rarer than what I have described as the normal form. The specimens drawn in fig. 13, however, all came from the same sporangium: they are clearly of the nature of double or triple, &c., giant spores, or are malformed specimens, the ripening of which is perhaps completed before the protoplasm has finished dividing up in the young sporangium. In some cases it is almost certain that they simply result from an imperfect separation of the formative masses set apart for the production of spores; an assumption which is supported by the fact that these malformed or giant spores (or complexes of spores) germinate quite normally, simply giving rise to a larger number of individual myxamœbæ than do the perfectly isolated spherical spores, selected, on account of their being the more common, as the typical spores. Such abnormal spores are by no means confined to this Myxomycete<sup>1</sup>; apart from the variations in the sizes of the spores of numerous species,<sup>2</sup> I have myself observed equally striking differences in the spores of a Myxomycete in the tropics. There can be no doubt, I think,

<sup>1</sup> Cf. De Bary, *Vergl. Morphol., der Pilze, &c.*, p. 452.

<sup>2</sup> Zopf, *Die Pilz-Thiere*, p. 52.

that the differences referred to are of no morphological importance, and only depend on obscure conditions which make themselves felt during the development of the spores in the sporangium.

In the case of the typical spores formed in the sporangia on the submerged roots, the spherical shape is maintained until the moment of germination; but in those sporangia which ripen outside the liquid, it often happens that the ripe spores present the appearance represented in fig. 12. This, however, is simply due to an infolding of the spore membrane, consequent on the loss of water, a fact which may easily be proved by partially drying any of the spores slowly and at a low temperature; or a similar result follows if the water is extracted by means of alcohol or glycerine. In both cases the plump spherical shape is immediately restored on the addition of water, but of course the spores long treated with the reagents are killed. Spores contracted in the former manner, however, germinate at once and normally exactly as do those taken from the submerged sporangia: if dried very thoroughly, however, it may require several days before the swollen spore germinates in water, and of course it is possible to carry the desiccation too far unless very great care is taken.

#### GERMINATION.

The normally ripened spore is at once capable of germination, and the process is usually completed in from 12 to 24 hours, at ordinary temperatures.

The germination is easily observed as follows. A sporangium ripened in or out of the water (there are no further differences to be noted, and the following description applies to spores obtained from both sources) is broken open by means of a sharp clean needle, and the point of the instrument is then immersed in a small drop of water on a perfectly clean glass slide: some of the spores attached to the needle are left behind in the drop of water, and the whole is then placed in a moist chamber, and kept at the ordinary temperature of the laboratory. In a few hours germination commences, and the escape of the contents of the spores as myxamœbæ is completed in from 12—24 hours, even when the temperature has varied between 20° C. and 15° C.

In order to study the details of this process, and to cultivate the myxamœbæ further for even long periods (several weeks) I have successfully employed a method well known in Germany but still far

too little used in England, and it may be worth while to give a description of the process, which can of course be made use of for the cultivation of almost any minute organisms.

A moist chamber is made by cutting a piece of thick cardboard, or several thicknesses of filter paper, to the size of an ordinary glass slide (3in. by 1in.) and cutting a hole through the centre: this hole may be about  $\frac{3}{4}$ in. in diameter, large enough to let all necessary light pass through, but not too large to be efficiently covered by means of a square or circular thin cover-slip, which will be held close down to the perforated board or paper when the latter is saturated with distilled water by the capillary attraction at the edges. The cardboard or paper pad is then saturated with water, and can obviously be boiled or heated if necessary, for a short time; its wet surface adheres firmly to the glass slip on which it is now placed, and which supplies a transparent floor to the small cylindrical chamber, the walls of which are formed by the saturated board or paper, and the roof by the thin cover-slip. It is obvious that a small drop of water suspended from the under side of the thin cover-slip, will evaporate very slowly, so long as the cardboard or filter-paper is kept thoroughly wet, because the air in the chamber will be nearly saturated with water; hence any spores &c. suspended in the drop will run no risk of drying up for a considerable number of hours. Moreover, it is clear that light can pass through the whole chamber, and its suspended drop, in sufficient quantity to enable us to examine what is going on in the drop even with fairly high powers, provided the cover-slip is sufficiently thin, and the drop of water not so large that objects falling to the lower surface of it are out of focus. At any rate it is easy to work with Zeiss D and E in this way. Another great advantage to be claimed for these damp cells is the ease with which atmospheric oxygen can gain access to the interior: the comparatively trivial practical difficulties need not be entered upon here.

It is, of course, difficult to keep Bacteria, &c., out of such damp cells—heated needles, pure water, treating all the glass parts with acids, absolute alcohol or heat, &c., are all without avail unless the water saturating the parts is kept clean, and, of course, unless spores and foreign bodies are absent from the material sown, &c. Some remarks on this subject will be made shortly, however, in connection with certain experiments of physiological importance.

Observed in such a suspended drop of water as that described above, the first changes noticed in the germinating spore are as follows. The spore slowly swells, the nucleus being very distinct, and a slight protuberance makes its appearance at one side: over this protuberance it is easy to see that the cell wall is distinctly thinner, and hence this portion of the spore looks paler, from the two facts that the same amount of pigment is distributed over a larger area of thinner membrane, and the translucent refractive protoplasm shines through with a sort of pearl-like lustre.

Meanwhile the protoplasm in the interior begins to move very slowly, and in some cases it is certain that a vacuole is already formed which begins to pulsate feebly at long intervals. As the protuberance becomes more pronounced, the movements become a little more active, and the vacuole pulsates at intervals of from one to two minutes: minute brilliant granules can now be distinctly observed to change their positions in the hyaline protoplasm. Very soon after this the lateral protuberance ruptures, the crack slowly extending (figs. 11 and 14) and the contents becoming exposed: all these phenomena may occur in from 4—12 hours after sowing the spores.

In favorably situated spores the contents may now be seen to divide into two, and two nuclei (in one case two vacuoles also were seen) make their appearance. In some examples the following phenomenon was observed: the protoplasm thus exposed to direct contact with the water suddenly retreated into the spore again, then slowly commenced to emerge as before and shrank back suddenly again. This was repeated several times before the protoplasm escaped as two myxamœbæ.

In other cases, it may be observed that, in a few minutes after the appearance of the two nuclei, there are two amœboid masses of protoplasm in the spore, one of which slowly emerges, followed soon afterwards by the second one. Thus, in fig. 15 the spore had developed the protuberance at 11.40: this ruptured at 12.10, and the naked protoplasm rapidly withdrew as if it had sustained some shock on contact with the environment: at 12.22 one of the myxamœbæ was seen slowly emerging (the other one of the two having escaped about one minute previously was now slowly creeping about on the surface of the spore) and at 12.24, when nearly completely emerged, it suddenly withdrew into the spore, as if some shock had irritated it. At 12.26 it protruded again, and in the course of the next minute escaped as a free myxamœba. During the course of the next few minutes both

the myxamœbæ slowly moved about the field, each with a clearly marked nucleus and a slowly pulsating vacuole, and at 12.32 each of the active myxamœbæ began to come slowly to rest, and rounded itself off as a cyst. At 1.37 the two spherical cysts were lying in the same places and their vacuoles only making extremely feeble pulsations at very long intervals: shortly afterwards they had completely come to rest—a condition I shall have more to say about shortly.

It occasionally happens that the process of germination is even less normal than in the case mentioned above. The drawings in fig. 16 will illustrate this, and I may describe these before proceeding to offer some explanation of both sets of phenomena. In this case the spores were sown at 4 p.m., and left to germinate during the night: at 10-30 next morning many of them were germinating, and the figures were drawn from one that was watched without intermission during the next two hours. At 10.46 one of the spores (fig. 16, *a*) emitted its contents bodily as a spherical mass of hyaline naked protoplasm, in which were numerous very fine bright granules, and a large well-formed nucleus: during the course of the next quarter of an hour several (2 to 4) small vacuoles kept making their appearance and very slowly pulsating (fig. 16, *b*), and a feeble "frothing," so to speak, of the protoplasm went on (fig. 16, *c*) until about 11.20, the granules and nucleus slowly changing their positions at the same time. At 11.26 (compare the figures bracketted fig. 16, *d*) three faint lines made their appearance in the mass of protoplasm, and these disappeared in about half a minute as quickly as they had come, the slow movements and "frothing" still continuing: at 11.33 a feebly marked line appeared half way across the protoplasm, and this, too, only lasted for about half a minute. Five minutes later (*e*, fig. 16) not a trace of these lines could be detected; but at 11.45, in place of the one not very conspicuous nucleus seen shortly before, there appeared two nuclei imperfectly separated by a line passing about half way across the protoplasm (fig. 16, *f*): this line also disappeared during the next three minutes, but at 12.1 (fig. 16, *g*) the sphere of protoplasm was unmistakably divided across by a line passing between the two nuclei, and two minutes later these two nucleated portions commenced to separate. It now became evident that the protoplasmic mass had formed a very thin membranous envelope on its exterior, and, in fact, the two masses of nucleated protoplasm escaped one after the other (through separate

apertures) from their envelope, and moved away very slowly as myxamœbæ (fig. 16, *i*).

In the drop of water containing the spores, the germination of which has just been described, there were relatively large quantities of organic debris, as well as Bacteria and numerous Infusoria and other oxygen consumers, and I have very little doubt that what was abnormal in the process was due to the want of oxygen caused by these organisms; for although it was impossible to show this directly, subsequent experiments proved that an additional supply of fresh water caused the dormant myxamœbæ to become active again, and I found that the more free the cultivations were from Infusoria and Monads especially, the longer the myxamœbæ tended to be active. Bacteria need not be absent, though of course there may be too many of them: there are even facts pointing to the conclusion that within certain limits the Bacteria not only do no harm, but even serve as food. The deprivation of oxygen, then, no doubt accounts for the tardiness of some of the processes described; while it seems not improbable that the curious phenomena which took place during the germination of the specimen to which fig. 16 refers, were connected with nuclear division, however difficult it may be to follow the connection in detail.

It now remains to describe what may be termed the normal process of germination which occurs when sufficient precautions are taken to avoid an accumulation of oxygen-consuming objects in the cultivation and, it may also be added, when the temperature is a little higher than before; I have satisfied myself, however, that the spores can germinate normally when the thermometer in the laboratory stands at 18° C, and even lower. I will describe the germination first, and the methods employed afterwards.

On the rupture of the thin-walled papilla (*c. f.* figs. 11 and 14) the two myxamœbæ escape very rapidly, and at once begin to move about actively, and close examination now shows that each possesses a single flagellum—a long, very thin, but tapering, stiff cilium, the free end of which is exceedingly flexible—in addition to the evident nucleus, and now actively pulsating vacuole. The vacuole slowly expands, taking about  $\frac{3}{4}$  of a minute to fill, and then closes with a rapid wink-like action, to repeat the process almost immediately. The nucleus offers nothing remarkable, so far as I could determine; it and the fine granules are in the more internal portions, and are constantly changing their positions as the amœboid movements proceed. The flagellum stands out

straight and stiff from the scarcely distinguishable ectoplasm, its very fine flexible tip swaying to and fro with an irregular swinging movement. The pseudopodia are small, numerous, and very irregular (fig. 17*a* and fig. 18). Very often the myxamœba may be seen to creep forward, the flagellum in front, for a short time, and then rather quickly to leave go, as it were, of the glass and subside in the drop, and at once assume an elongated shape, like that of a club, and steadily glide forward, suspended in the water as a "zoospore" (fig. 17*b*). This zoospore moves with the flagellum projecting stiffly from the front pointed end, where the nucleus also is situated; the pulsating vacuole appears to be always at the rounded dilated hinder end. The free swimming zoospore-form may then come in contact with the glass surface, and either assume the amœboid form and movement at once, or wriggle about with quick jerking movements (fig. 17*cd*) for a time, and then pass again into either the zoospore or the amœboid form. The outlines figured at *d* for instance are sketches of the forms assumed within 3 minutes by the same zoospore; while those shown in fig. 18 sufficiently illustrate the passage to the amœboid condition.

After a certain time, however, it is noticed that a number of perfectly spherical colourless bodies (fig. 17*e*) are scattered about the field, and as these increase in number the amœbæ and zoospores diminish: these spheres are encysted myxamœbæ, and indeed fig. 17*e* was drawn from a specimen observed to pass over from the zoospore to the amœboid conditions (*d*), the latter of which then slowly rounded off and assumed the encysted state.

Before describing this and other phenomena, however, I may say a few words as to the methods of culture referred to above. It is obvious that absolute immunity from foreign organisms cannot be expected when the sowing is made from sporangia on the submerged roots of Hyacinth, and moreover it could not be supposed that the results of germination could be cultivated for any length of time in distilled water: nor was such the case in the examples cited above, for small quantities of organic matter from the roots and of minerals from the solution in which they grew were certainly added during the process of sowing.

In order to obtain as few foreign organisms as possible, and to be at least fairly certain whence they were derived, I employed the following precautions. A nutritive solution (that used for growing the Hyacinths in) was prepared in a large test-tube, and a piece of fresh Hya-

cinth root added: the whole was then boiled. A new close fitting cork was fitted and provided with a short bent tube and a long one as for an ordinary wash-bottle, but with the following differences. The long exit tube dipping below into the solution in the test-tube was drawn to a fine capillary end, and the free end of the same tube was drawn out very fine indeed and its end hermetically sealed in a flame. The short tube had the limb which is placed in the mouth constricted in the middle to a narrow capillary, between which and the mouth a plug of cotton wool was fitted. The fused tip was then broken off and the whole was ready for use. On blowing gently down the mouthpiece, the cotton-wool prevents the passage of spores into the liquid, and the fusion of the exit end, after a minute drop of the nutritive fluid has been expelled, effectually prevents spores obtaining access that way. The other advantages are obvious, and the highly putrescible nutritive solution can be employed day after day for the supply of minute drops of nutriment to the cultures. While it is not intended to imply that such a vessel is absolutely proof against bacteria, it is certainly useful in preventing their being added in serious quantities, and can easily be quite freed from other spores.

The next precaution was to sow as few spores of the *Myxomycete* as possible at a time, and this I accomplished by the well known method of dipping the needle point, on which the spores to be sown adhered, into a drop of pure water, a drop from which was then placed in the culture drop suspended in the damp-cell. Certain other more obvious precautions may be passed over. The result was that in a fair proportion of sowings germination proceeded normally and rapidly, and very few bacteria (and no larger organisms) made their appearance during the earlier stages; hence the objectionable turbidity was obviated, and the struggle for existence was considerably in favour of the *Myxomycete*.

A number of experiments were next made in which the nature of the fluid was varied: the following sums up the chief results. Germination takes place normally in pure water (unless the temperature is too low, or other unfavourable conditions prevail), but the *myxamœbæ* rapidly pass over into the dormant condition and encyst. In a purely mineral nutritive solution the same results are obtained. If an infusion of Hyacinth roots is added to the mineral solution, however, the *myxamœbæ* most evidently flourish, and I have kept them and their progeny alive for as long as 16 days in such a solution. Bacteria



always make their appearance from spores, &c., introduced with those of the culture; but, as already said, it is not difficult to exclude Infusoria and other relatively large oxygen-consumers, and the myxamœbæ seem to flourish so well in the presence of a certain quantity of bacteria, that the question naturally suggests itself whether the latter do not favour their growth and nutrition, either by breaking up the organic matter, or by serving directly as food. This point is not easy to decide: in cases where the myxamœbæ pass over rapidly into a dormant condition in a solution of nutritive substances, it is difficult to determine whether a want of oxygen (due to the long-continued boiling, &c.) or some other cause is acting, and any manipulation introduces risks which only increase the final difficulty.

For instance, having satisfied myself that something other than pure water and minerals was absolutely necessary for the nutrition of the myxamœbæ, the next question was, is organic matter (derived from boiled Hyacinth roots, *e.g.*) in solution the only other ingredient necessary? and in the successful cultures one always felt that the answer was obscured because there were always bacteria present, and three obvious possibilities suggested themselves: (1) the myxamœbæ might be feeding simply and directly on the dissolved substances in the original solution; (2) they might be absorbing substances that only arose after the action of the bacteria on the original solution; and (3) they might be chiefly nourished by the bacteria themselves, since they certainly envelope bacteria after the usual manner of amœbæ. It thus follows that my experiments only prove that organic matter in some form is necessary for the nutrition and growth of the myxamœbæ, but do not decide in what form it is absorbed. I regard the last question as important with respect to any hypothesis as to the animal or vegetable characteristics of the amœboid stage, though a positive answer even to this question could by no means be regarded as conclusive of the animal nature of the organism, in view of the other facts to hand. It may be shortly remarked, in this connection, that the conditions of cultivation in my damp-cells must have been remarkably similar to those which the myxamœbæ meet with in the roots on the glass vessels. I may now pass on to the other phenomena observed in the life of the myxamœbæ.

DIVISION.<sup>1</sup>

It is easy to convince one's self of the fact that the myxamœbæ divide. I have several times observed this process directly and in detail; but it might also be safely concluded from the fact that in sowings of from 8 or 9 to 20 or 30 spores, which yielded twice that number of myxamœbæ on germination, it was frequently observed that the number of myxamœbæ increased several fold in the course of two or three days. In figs. 19 and 20, however, are drawings of the process as actually observed in successful cultures. A myxamœba was detected in the condition at *a* (fig. 19) at 10.48, having become extended and slightly constricted in the middle, where it was relatively at rest, though several small pseudopodia were being protruded and withdrawn at the ends. No nucleus was detected, but two pulsating vacuoles were clearly visible. Two minutes later the constriction had deepened considerably, and at 10.52 (two minutes later still) it had cut the mass in two (fig. 19*b* and *c*), and a nucleus as well as a contractile vacuole was observed in each. The two daughter amœbæ remained quiescent and in contact for a few minutes, and at 11 o'clock were again active and moving away. In some cases (fig. 20) the process was varied to the following unimportant extent. The dividing myxamœba (fig. 20*a*) was extremely sluggish, and then elongated very slowly as in the figure: two nuclei were now observed in it. This was at 1.17 o'clock. Division then followed quickly, the nuclei not being noticed during the process. The stage *b* was attained at 1.20, *c* at 1.22. The two separated daughter amœbæ remained quiescent for several minutes, then slowly moved apart (*d*) to a very little distance, and then encysted. Here again I have reasons for thinking that deficient oxygen had to do with the sluggishness noticed in some cases.

ENCYSTED STAGE.<sup>2</sup>

As already stated, the myxamœbæ become dormant, round off into a sphere, and form a delicate colourless membrane when oxygen is deficient in the culture, or the temperature is very low, or from other causes. This deficiency of oxygen may arise simply from the fact that the myxamœbæ have themselves increased so rapidly by division that the supply of oxygen is unequal to their demand; or it may be due to other causes, among which the presence of Infusoria and other

<sup>1</sup> Cf. De Bary, *Vergleichende Morphol. und Biol. der Pilze, &c.*, p. 455.—Zopf, *Die Pilz-Thiere*, p. 10.

<sup>2</sup> Cf. De Bary, *op. cit.*, p. 46, and Zopf, p. 90

oxygen-consumers is the chief. While not denying that the myxamœbæ may be impelled to encyst from lack of food, or when the temperature is too low, there is reason for ascribing these dormant states chiefly to a lack of oxygen, since the myxamœbæ may be re-awakened to activity on the mere addition of pure water containing oxygen, though the temperature (not too low) remains the same. Beyond the fact that such cysts may lie unchanged for 12 or 14 days, and then be restored to activity, I do not know how long they retain their vitality.

In fig. 21 (*a*) is a cyst kept under observation (with seven others) for some time: after resting for two days, a minute drop of fresh nutritive solution was added, and three hours later it and its companions were beginning to germinate. The process (*cf.* fig. 21 *c* and *d*) is very simple, the extremely thin membrane opens by a minute pore, evidently formed by the myxamœba itself, through which the whole of the protoplasmic contents pass out as an extremely active myxamœba, which soon shows the presence of a cilium. The pulsating vacuole is formed before its escape; the nucleus is evident all through the dormant state: the flagellum seems to be protruded after the escape of the myxamœba. In some cases the extremely fine colourless envelope was dragged for a short distance by the myxamœba, but it soon fell off, and the tiny round hole through which the contents had escaped could then be detected. Such empty cysts were often to be seen in certain cultures which had been allowed to dry up and were then wetted, and as they increased the number of encysted myxamœbæ decreased. Moreover, it was by no means difficult to see the process of escape of the contents, as in the specimen figured; I have watched the process from beginning to end at least five times. It only remains to add that the free myxamœba may feed, grow and divide, or may again encyst, according to circumstances; or, finally, under certain conditions, which I have failed to refer to anything external, the myxamœbæ may commence to congregate in masses and subsequently form plasmodia. I have failed to obtain satisfactory evidence as to the constitution of the extremely thin wall of the cyst: it is, presumably, cellulose, but its delicacy is such that reagents destroy every trace of it before one can be assured of a colour reaction. It is not improbable that this encysted stage is far commoner in myxamœbæ than has hitherto been supposed, for the very delicate transparent empty cases are almost sure to be overlooked at first.

THE PLASMODIUM.<sup>1</sup>

On the roots of the Hyacinths on which the sporangia were situated, it was not difficult to find larger and more complex amœboid masses of protoplasm (fig. 24*a*) and among the sediment at the bottom of the glass cylinder occurred very similar specimens (fig. 24*b*) which, however, seemed as a rule less active. Since those on the roots were mingled with myxamœbæ, bacteria, spores of the myxomycete, and sporangia in all stages, it may no doubt be inferred that they are the plasmodium produced by the fusion of the myxamœbæ. The inference becomes very near certainty after watching the specimens under cultivation. In fig. 22 are drawings of myxamœbæ from a much larger aggregate which were found in a culture kept under observation for 11 days. These myxamœbæ were cultivated from the spores, and had passed through the stages of division and encystment, and by this time outnumbered the original spores at least eighty or a hundred to one. They were now moving sluggishly about on the glass, and also on the lower surface of the suspended drop of nutrient fluid: the nuclei were quite distinct, the vacuoles pulsating slowly and at long intervals, and the flagellum appeared to have been withdrawn in all cases. It was not until I had seen such cases, where the sluggish myxamœbæ glide slowly over one another for some time, that it became clear that this is a preparatory stage to the formation of plasmodia. I then had opportunities of examining the process more in detail: as the myxamœbæ approach more closely to one another, they slowly glide over one another, in undoubted contact, but without sticking or blending together in the least. It appeared at times as if an extremely delicate investment separated them—if such an investment exists, however, it must follow every movement of the protoplasm. As fig. 25 shows, some of them may still retain the flagellum, but the majority have lost it, and all do so eventually: the conditions figured at *a* to *c* (fig. 25) were drawn at intervals of 2 minutes, and *d* 5 minutes after *c*, to show this slow "swarming" process, as it might be termed.

Later on, after from 12 to 30 hours, several relatively large plasmodia were found on the slide, and that these were formed by the fusion of the myxamœbæ was evident from the fact that the process was still going on. As shown in fig. 23, larger masses of plasmodium were being added to by smaller ones and by myxamœbæ, which now,

<sup>1</sup> Cf. De Bary (op. cit., p. 455), and Zopf, p. 22, for a general description of Plasmodia.

instead of gliding over the surface stuck to it and were either at once drawn in, as it were, or partially separated off and then again became part of the larger mass. In some cultures I had as many as eight or nine separate plasmodia slowly moving on the glass for two or three days.

One of the largest (more than a millimeter in length) is drawn at fig. 26, and a description of it will serve once for all. Its changing outlines were very irregular: short and long pseudopodia would be put forth together or separately, and withdrawn or extended, the fine granules (as well as fewer larger ones and nuclei) flowing quickly down the central portion. The general hue was a pale dirty yellow, the very clear ectoplasm being colourless, in thin parts at any rate. There were several or even many contractile vacuoles varying in size and activity. Bacteria, spores, empty cysts and other objects were frequently observed in the plasmodium, and were also seen to be enveloped by its pseudopodia; some to be carried into the interior, others to become free again as the pseudopodia withdrew. The following curious phenomenon was observed more than once. The actively moving plasmodium would come to rest in irregular or rounded clumps during the night (fig. 27), possibly on account of the lowered temperature, and would then spread out again next morning, and move as before: this would be repeated—even several times. In other cases this dormant condition would last a longer time, and a distinct investment (the character of which was not made out exactly) would be excreted (fig. 27*a* and *b*) suggesting an effort on the part of the plasmodium to produce a sporangium.<sup>1</sup> No undoubted sporangia were formed in the suspended drops, however.

There can be little doubt that external conditions have to do with these changes; but although I have notes on the subject which show that at certain stages the myxamœbæ collect at the lighted side or on the free surface of the drop, while at others they choose the surface of the glass generally, and that the plasmodia seem to favour the upper regions of the drop, they are too scanty and not sufficiently decisive for the purpose of determining the questions which arise.

The failure to produce sporangia in the above cultures, and the observation that the plasmodia tend to the upper regions of the drop, however, led me to try the following experiment, which was at any

<sup>1</sup> Or they may be the "macro-cysts" described by Cienkowski in *Perichænia*.—Cf. Zopf, p. 92.

rate partially successful. Having cultivated several fairly large plasmodia, I inverted the cover-slip (fig. 28c) and suspended vertically from a glass filament (*f*) supported by a cork (*e*) a piece of fresh clean Hyacinth root (*h*) so that its tip plunged into the drop (*d*) containing the plasmodia. The whole was then placed within a larger damp chamber, and the root kept thoroughly wet: in several cases the plasmodia crept on to the root, and in one example I believe that a plasmodium commenced to form a sporangium. Further than this I was not able to go, and so the matter rests for a time, my investigations being brought to an end by the pressure of other business.

It now remains to say what can be decided as to the systematic position of this aquatic myxomycete.

#### CLASSIFICATION.

Rostafinski<sup>1</sup> divides the more typical Myxomycetes ("Endosporeæ") into seven groups or "orders," characterised chiefly by the presence or absence of lime in the fructification, the colour of the spores, presence or absence of a capillitium and columella, &c. According to this classification the present Myxomycete would be included under Rostafinski's order VI. *Calcareæ*, and of the four "tribes" into which these are divided, we may at once discard the fourth, *Spumariaceæ*, on account of the columella there present. If the nature of the deposits of calcium carbonate—in crystals or amorphous—is really to be regarded as important, which may well be doubted, then the tribes *Cienkowskiaceæ* and *Physaraceæ* may be also put aside as not including the present species, and we are limited to the *Didymiaceæ*. But here there is also a columella, and so on; the fact being that the aquatic myxomycete I have described would, if certain characters were insisted on, impel us to connect two or more of Rostafinski's "tribes."

If we now follow the classification proposed by Zopf,<sup>2</sup> we may at once discard the first group of his extended system—the *Monadineæ*—for, although they are usually aquatic, they form no true plasmodia. Turning to the *Eumycetozoa*, which Zopf characterises as "*Luftbewohner*" however, there are three sub-groups to notice; the first (*Sorophoreæ*) have no zoospore stage, the plasmodia are not typical, and there are other characters which at once exclude the present Myxomycete: the third group, comprising the one genus *Ceratium*, may also be forthwith

<sup>1</sup> *Versuch eines Systems der Mycetozoa.* (Inaug. diss., Strassburg, 1873.)

<sup>2</sup> Zopf, *op. cit.*, p. 95.

discarded. Under the second group (*Endosporeæ*) it is not difficult to refer our species to the *Endotricheæ*, characterised by possessing a capillitium which traverses the lumen of the sporangium, and consists of solid fibres ("*stereonemata*"). Here we are limited to two of Rostafinski's "tribes"—the *Calcariaceæ* and the *Amaurochætaceæ*, and a comparison of their characters at once places the present form among the *Calcariaceæ*. Of the three "families" into which Zopf distributes these genera, the second (*Didymiaceæ*) seems to embrace the characters of our form, and it seems very likely to belong to the genus *Diderma*, having several of the characters of that genus well marked. It is, indeed, not improbable that we have here an aquatic form of *D. difforme*, one of the commonest of our Myxomycetes, and if so, we have another proof of the all but uselessness of attempting to classify the lower organisms, until we know more of their habits under varying conditions. In any case, some of the facts here described show that characters for the description of "genera" and "species" of Myxomycetes should be chosen very carefully.

## DESCRIPTION OF PLATES III—IV.

- Fig. 1. Sporangia of Myxomycete on the roots of Hyacinth. Natural size.
- Figs. 2, 3 & 4. Sporangia magnified. *a*, the yellow border. Figs. 3 & 4 are attached to the epidermis of the root: the spores can be seen within.
- Fig. 5. Portion of wall of sporangium, seen from within, and showing places of origin of capillitium.
- Fig. 6. Portion of wall seen from outside, with looser yellow network.
- Fig. 7. Sporangium bursting, and liberating spores.
- Fig. 8. Burst sporangia, showing capillitium *in situ*.
- Fig. 9. Spicules of calcium carbonate from walls of sporangium.
- Figs. 10 & 11. Spores commencing to germinate in water.
- Fig. 12. Spores after desiccation, after lying in alcohol.
- Fig. 13. Abnormal and giant spores, drawn in outline only.
- Figs. 14, 15 & 16. Spores germinating, in various stages (*vide pp. 74 seq.*)
- Fig. 17. Myxamœbæ and zoospores which have emerged from the spores. Each has a cilium, contractile vacuole, and nucleus.
- Fig. 18. A myxamœba sketched at short intervals.
- Figs. 19—20. Division of myxamœba: stages at short intervals.
- Fig. 21. Germination of an encysted myxamœba.
- Fig. 22. A group of myxamœbæ.
- Figs. 23—24. Plasmodia resulting from the fusion of myxamœbæ.
- Fig. 25. Myxamœbæ prior to their fusion into plasmodia.
- Fig. 26. A large plasmodium.
- Fig. 27. Resting condition of plasmodia previous to the formation of sporangia.
- Fig. 28. Apparatus used for cultivation of plasmodia (*vide p. 84*). *a*, glass slip; *b*, fibulous paper cell; *c*, cover slip; *d*, drop of water containing myxamœbæ and plasmodia; *e*, a cork, supporting (*h*) a piece of Hyacinth root by means of the glass rod *f*.
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Fig. 1.



Fig. 2.

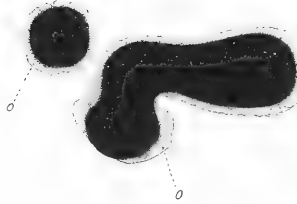


Fig. 4.

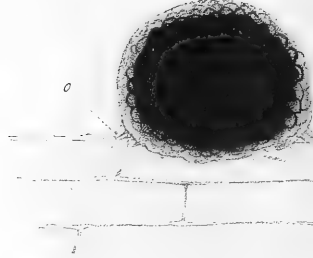


Fig. 3.

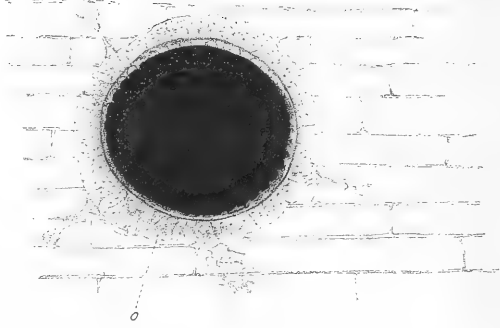


Fig. 7.

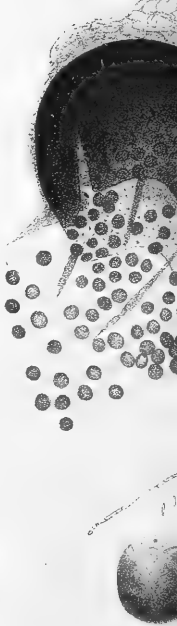


Fig. 9.



Fig. 10.

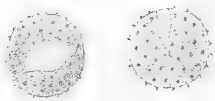


Fig. 13.

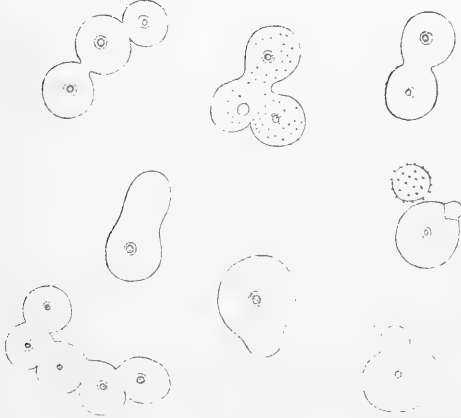


Fig. 11.



Fig. 12.



Fig. 5.

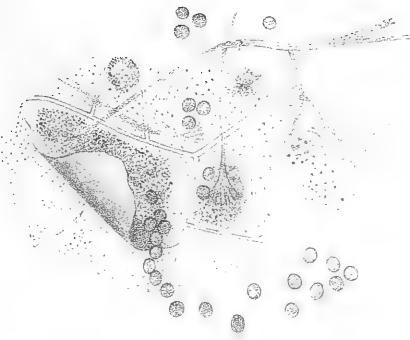


Fig. 8.

Fig. 6.

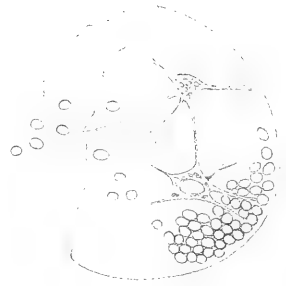
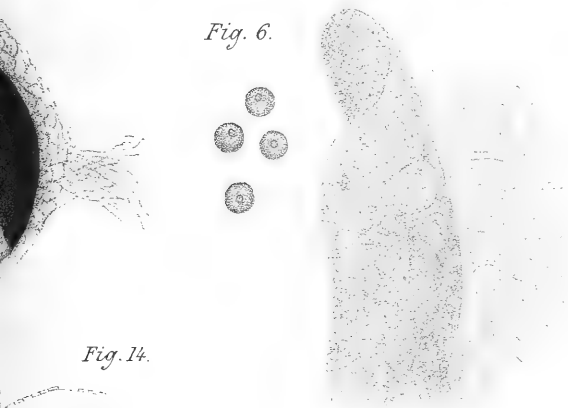


Fig. 14.

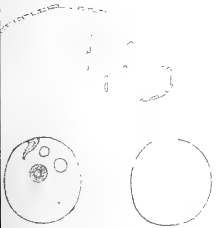
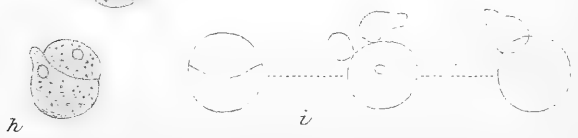
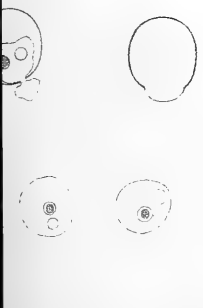


Fig. 16.



Fig. 15.



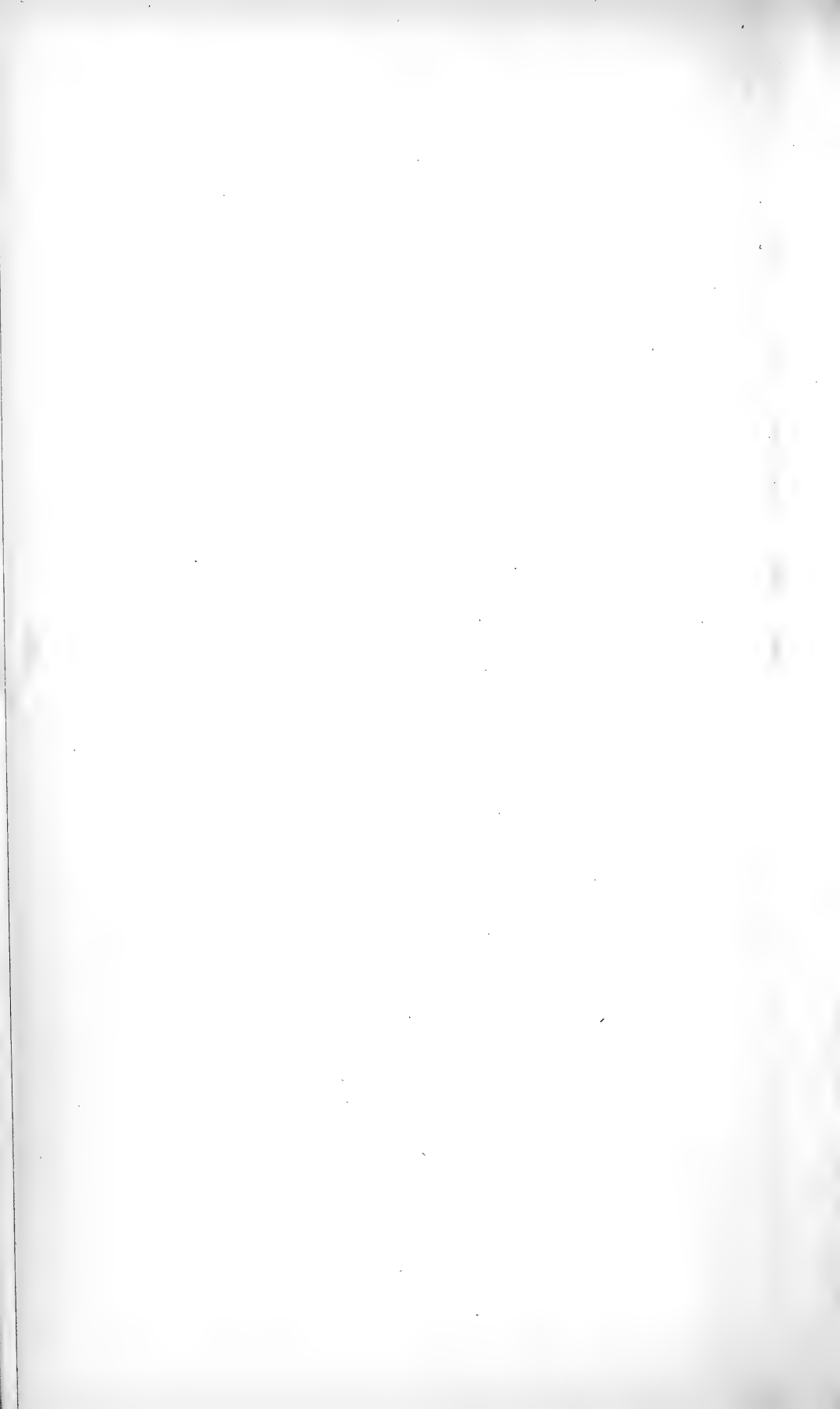




Fig. 17.



Fig. 22.



Fig. 23.

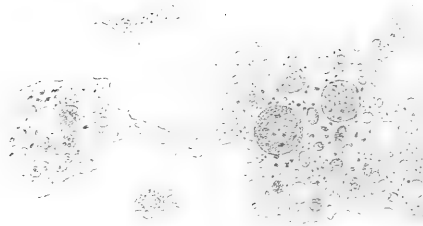


Fig. 25.



Fig. 27.

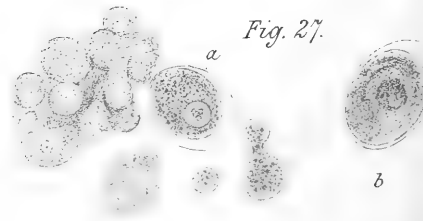


Fig. 26.



Fig. 18.



Fig. 19.



Fig. 20.

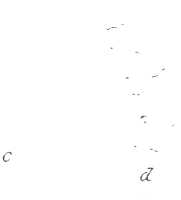


Fig. 21.

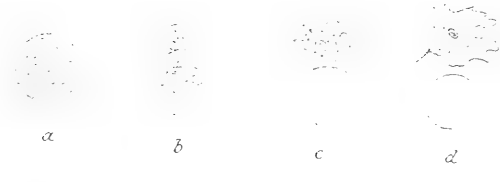
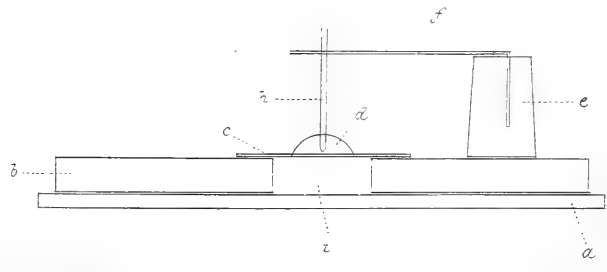
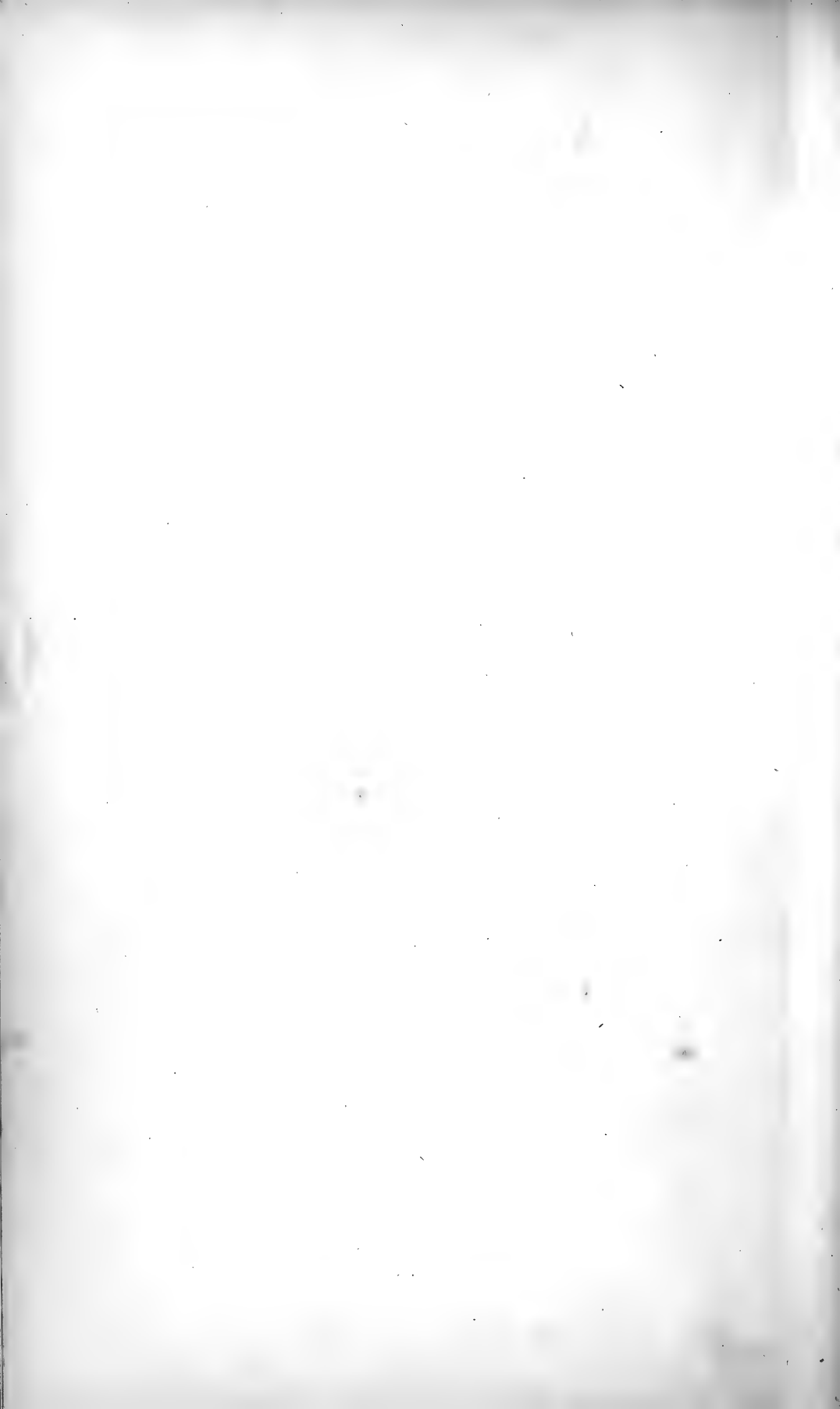


Fig. 24.



Fig. 28.







## OBSERVATIONS ON THE CRANIAL NERVES OF SCYLLIUM.

By A. MILNES MARSHALL, M.A., D.Sc., *Professor of Zoology in Owens College*; and W. BALDWIN SPENCER, *of Owens College*.

[PLATES V—VI.]

The investigations recorded here were undertaken in the first instance for the purpose of controlling certain determinations published by one of us in a previous number of this journal<sup>1</sup> concerning the cranial nerves of Elasmobranchs. To this end we have carefully re-examined the specimens upon the investigation of which the former account was based, and have, in addition, made a large number of new preparations, illustrating more especially the later stages of development—stages m to q of Balfour's nomenclature.<sup>2</sup>

During the course of our work so many altogether new and unexpected points were brought to light, that we soon found it necessary to widen considerably the scope and limits of our investigations, and have finally been led to attempt a complete account of the development of the cranial nerves from stage κ to the adult form, and to endeavour in this way to connect together directly the accounts previously given of the early stages<sup>3</sup> with the descriptions of the nerves of adult Elasmobranchs published by Stannius,<sup>4</sup> Gegenbaur,<sup>5</sup> and other anatomists.<sup>6</sup>

<sup>1</sup> Marshall, "On the Head Cavities and Associated Nerves of Elasmobranchs," *Quart. Journ. Micr. Sc.*, Jan., 1881, pp. 71 *seq.* Future references will be to this paper unless otherwise specified.

<sup>2</sup> Elasmobranch fishes, pp. 79 and 80.

<sup>3</sup> Balfour, *op. cit.* Marshall, *loc. cit.*

<sup>4</sup> Stannius, 'Das peripherische Nervensystem der Fische,' Rostock, 1849.

<sup>5</sup> Gegenbaur, "Die Kopfnerven von Hexanchus," 'Jenaische Zeitschrift,' Bd. vi.

<sup>6</sup> *Esp.* Jackson and Clarke, "The Cranial Nerves of *Echinorhinus spinosus*," 'Journal of Anatomy,' vol. x.

Owing to defective supply of materials, our observations on the stages earlier than  $\kappa$  are too fragmentary to be relied on; this we greatly regret, inasmuch as many features in the early stages are of extreme importance, and would well repay thorough investigation.

In the present paper we propose to confine ourselves to the consideration of the preauditory nerves, reserving the postauditory, which present many features of peculiar interest, for a future occasion.

Our investigations have been conducted almost exclusively by means of sections of hardened embryos of *Scyllium*, and, as on former occasions, we have found a mixture of chromic and osmic acids superior to any other hardening agent. For the specimens from which our best preparations have been made we are indebted to the courtesy of the managers of the Southport Aquarium; our thanks are also due to Mr. A. J. Moss, of Owens College, for his gift of a fine specimen of *Mustelus*, as well as for valuable assistance in connection with the literature of our subject.

THE THIRD (OCULOMOTOR) NERVE.—We do not propose to deal in the present paper with either the olfactory or optic nerves, inasmuch as the former has been already fully described,<sup>1</sup> while concerning the latter we have nothing new to communicate; we therefore commence with the third or oculomotor nerve.

Concerning the development of this nerve we have very little to add to the account given in the paper already referred to.<sup>2</sup> At stage L it arises from the base of the mid-brain, not far from the mid-ventral line, by a large posterior ganglionic root and by several smaller anterior ones clearly distinguished from the former by possessing no ganglion cells. The nerve itself runs backwards as a long slender stem to the interval between the first and second head cavities (fig. 10, 1—2), where it expands into a ganglionic swelling (fig. 10, *c.g.*) wedged in between the tops of the two cavities. From this ganglion the two main branches of the third arise; of these the upper one (fig. 15, III *b*), at a rather later stage, supplies the *rectus superior* and *rectus internus* muscles, whilst the lower one (fig. 15, III *c*), runs down behind the *rectus inferior*, and ends in the *obliquus inferior* muscle (fig. 15, *o.i.*). At stage  $\kappa$ , at which our observations commence, the third nerve has the same point of origin and the same relation to the head cavities;

<sup>1</sup> Marshall, "Morphology of Vertebrate Olfactory Organ," 'Quart. Journ. Micr. Sc.,' July, 1879, pp. 300 *seq.*

<sup>2</sup> Marshall, *loc. cit.*, pp. 78 *seq.*

it differs from the condition described above chiefly in not possessing anterior non-ganglionic roots, and in not having its terminal branches fully developed. At stages later than  $\mathfrak{N}$  the nerve seems to arise from the base of the mid brain by a single large ganglionic root, no smaller non-ganglionic ones being visible (fig. 14, III), though at stage  $\mathfrak{N}$  itself they are very prominent (fig. 13, III).

Besides the branches of the third nerve, mentioned above, there are two others in direct connection with the ganglion *c.g.*: of these the first, at stage  $\mathfrak{L}$  (fig. 10, *N.c.*), is a short nerve, which lies along the top of the second head cavity and serves to connect directly the ganglion *c.g.* of the third nerve with the Gasserian ganglion at the root of the fifth.

The second (figs. 10, 11, and 12, *N*) passes straight forward from the ganglion *c.g.* running through the walls of the first head cavity under the *rectus superior* and *rectus internus*, and through the substance of the sclerotic on the inner surface of the eyeball. Passing out from the orbit, immediately above the *obliquus inferior*, it still pursues its course straight forward, but becomes more superficial. In the earlier stages it crosses the root of the olfactory nerve, with which it lies in very close contact: in the later stages (figs. 12 and 15,  $\mathfrak{N}$ ) it is rather more dorsally situated, and crosses the ophthalmic branches of the fifth and seventh nerves at a considerable angle (fig. 12,  $\mathfrak{N}$ ), ending in the skin at the extreme fore part of the head. After careful and repeated examination we have failed to detect any branch given off from the nerve at any point of its length.

At stage  $\mathfrak{K}$  all the above-mentioned branches of the third nerve are developed, except the upper branch, to the *rectus superior* and *rectus internus*, which we have failed to detect: the ganglion *c.g.* is very conspicuous, and the nerves *N.c.* and *N.* have the same structure and connections as at stage  $\mathfrak{N}$ , the latter of the two stretching forward to the extreme anterior part of the head, in the skin at which it ends.

In the later stages the modifications which the third nerve undergoes are merely ones of detail, all the principal branches of the nerve being already established, and maintaining their relations practically unaltered in the adult. The most important changes concern the ganglion *c.g.*; this, which at stages  $\mathfrak{K}$  and  $\mathfrak{L}$  is a large prominent swelling (fig. 10, *c.g.*), in the later stages becomes far less conspicuous, and the ganglion cells, instead of being concentrated at one spot, occur in small scattered patches at different parts of the nerve. This

change is seen commencing at stage N (fig. 11, *c.g.*), where the ganglion has divided into two main portions, one part retaining its original position, whilst the other becomes connected with the nerve *N.* at some little distance from the third nerve; at stage O-P (figs. 14 and 15) ganglion cells appear to be constantly present at two well-marked points in the course of the third, (1) where the nerve *N.* is given off, and (2) immediately above the *rectus superior*.

At stages K and L (fig. 10) the angle between the nerves *N.c.* and *N.* is very considerable, and this increases in the later stages so much that at stage N (fig. 11) the two nerves are almost at right angles to one another; at stages later than this the nerve *N.* is much more difficult to define, whilst owing to the close proximity of the third to the fifth nerve it is only with extreme difficulty that the nerve *N.c.* can be distinguished at all.

We find, therefore, that the main stem and the branches III *b* and III *c* of stage N become directly the nerves which have the same course and relations in the adult. The ganglion *c.g.* becomes the ciliary ganglion of the adult<sup>1</sup>. The nerves *N.* and *N.c.* become directly continuous with one another, and together form the nerve known as the *Ramus ophthalmicus profundus*. The discussion of the morphological import of these two very remarkable nerves we postpone till after the description of the fifth and seventh nerves.

THE FOURTH (PATHETIC) NERVE.—Concerning the development of the fourth nerve no description has yet appeared, and though our observations do not enable us to give a complete account, still, so far as they go, they are of so definite a character that we think it well to record them here rather than wait for the possibility of completing them at some future time.

The condition of the fourth nerve at stage N is well shown in figures 11 and 13, of which the former shows the greater part of its course, and the latter its root of origin. The nerve arises (fig. 13, *iv*) from the dorsal surface of the extreme hinder border of the mid-brain, so far back indeed that very careful examination is necessary to determine that its origin is really from mid and not from hind-brain. The roots of the two nerves are in close contact on the dorsal surface of the brain. From its root each nerve runs at

<sup>1</sup> Marshall, loc. cit., p. 87, and Schwalbe, "Das Ganglion Oculomotorii," 'Jen. Zeit.,' Bd. xiii,

first almost directly outwards, following the curvature of the brain, until it comes to lie a very short distance below the surface; it then runs backwards and downwards as a long, straight, and very slender nerve (fig. 11, iv), which very commonly branches early in its course, and terminates in the *obliquus superior* muscle. Just before reaching the muscle (fig. 11) the fourth nerve crosses the ophthalmic branches of the fifth and seventh nerves at right angles, lying at a slightly deeper level than these nerves. It also divides into a number of branches, of which one or more appear to become connected with similar branches from the ophthalmic branch of the fifth, the remainder entering the *obliquus superior*, whilst in the later stages (fig. 16) the main nerve is seen to come into very close contact with the two ophthalmic branches themselves of the fifth and seventh nerves.

It will be seen from the above description that the fourth nerve has by stage *n* acquired its adult relations and distribution, and at stages o-p (fig. 15) and q (fig. 16), where it is shown in transverse section, it is still the same. In the adult its course, like that of the other nerves, becomes altered owing to the varying rates of growth of the brain, the skull, and the face; the rapid growth of the skull relatively to that of the brain causing the fourth nerve to run some distance forward within the brain case before passing out, when, as before stated, it runs immediately beneath and in very close contact with the ophthalmic branches of the fifth and seventh nerves.

In attempting to trace the fourth nerve in stages earlier than *n* we have met with considerable difficulties, and have hitherto obtained only a moderate amount of success. At *m* the relations are the same as at *n*, the sole difference being that *the nerve is more slender than at the later stage*. At stage *l*, though we have examined a very considerable number of specimens in excellent histological preservation, we have as yet recognised the fourth nerve in one specimen only, and even in that one not with absolute certainty. In the specimen in question the nerve has the same position and relations as at *n*, but is very much more slender, so thin, in fact, as to be almost unrecognisable. Earlier than *l* we have failed, after the most careful search, to find any trace of the nerve.

Though our observations are imperfect they yet seem to point to certain conclusions of importance touching the morphology of this important nerve. In the first place the fourth nerve is the only one in the body which, in the adult, arises from the dorsal surface of the

brain; it is, therefore, of great importance to notice that from the very earliest stage at which we have seen it the point of origin is that of the adult nerve.<sup>1</sup> Inasmuch as the majority of the cranial nerves, as well as the dorsal roots of the spinal nerves, arise *at first* from the dorsal surface of the brain or spinal cord, it seems natural to suggest that the fourth nerve differs from all the rest, not in its mode of origin, but in the fact that, whilst all the other nerves shift their attachment to a greater or less extent, it alone preserves the primitive position of its roots of origin. This shifting of the roots is, in part,<sup>2</sup> due to the rapid growth of the dorsal part of the brain forcing the roots of the two sides from each other; and it becomes of interest to notice that the fourth nerve arises from a portion of the brain where this rapid growth of the roof does not occur, and where, consequently, one cause of the change in the other nerves is absent.

The fact that the direction of the fourth nerve is at first at right angles, or nearly so, to the axis of the part of the head from which it arises is of importance, as showing that the fourth nerve comes under the category of segmental nerves;<sup>3</sup> and inasmuch as there is no room as regards visceral arches and clefts for a segmental nerve between the third and fifth the fourth would probably be rightly viewed as a separated branch of the third—the only other nerve arising from the mid-brain.

On the other hand, certain other facts in connection with the fourth nerve cannot be explained so easily. Thus, segmental nerves not only arise from the neural crest; they also arise early, are from the first large, and have ganglia at or near their roots. Now, so far as our observations go, the fourth nerve, though it may possibly arise from the neural crest, would not appear to arise early, and certainly is not at first a large nerve, being of much greater size at stage O-P (fig. 15) than at stage N (fig. 11); whilst at stage L, in the single specimen in which it has been detected, it is a nerve of extreme slenderness; moreover, at no part of its length have ganglion cells been found—an important point of difference from segmental nerves.

Another curious feature concerning the fourth nerve is that, in our embryos, it appears almost constantly to divide close to its root into two or even more branches, as is well shown in fig. 11, IV. These

<sup>1</sup> Balfour, 'Elasmobranch Fishes,' pp. 156 and 191.

<sup>2</sup> We say "in part," because it will be shown further on in this paper that another process contributes greatly to this shifting.

<sup>3</sup> Marshall, "Morphology of Olf. Organ," p. 318, 'Quart. Journ. Micr. So.,' July, 1879,

again subdivide near their terminations (figs. 11 and 15), but *all the branches, whether primary or secondary, are distributed to the superior oblique muscle*, with the possible exception of a few of the smaller ones, which appear to join the ophthalmic branch of the fifth. We would suggest that this branching may possibly be an indication of the fourth nerve having previously had a more extended distribution than its present very limited one.

On the whole, our observations lead us to believe that the fourth nerve is to be regarded as a separated part of that segmental nerve of which the third nerve forms the main portion. A further suggestion concerning the fourth nerve will be made after the seventh nerve has been considered.

**THE FIFTH (TRIGEMINAL) NERVE.**—We propose to consider separately the roots and the branches.

**A. *The roots of origin.***—The earliest stage in the development of the fifth nerve that we propose to treat of in the present paper is that represented in fig. 1, taken from a transverse section through the hind-brain of an embryo at a stage intermediate between  $\iota$  and  $\kappa$ , the plane of section passing on the left side through the roots of the trigeminal ( $\nu$ ).

As shown in the figure, the roof of the hind-brain is very thin, and passes rather abruptly into the much thickened sides. To the top of the thickened portion the fifth nerve is attached by a long slender root (fig. 1,  $\nu \alpha$ ). This root passes down alongside the brain, but not in actual connection with it, widening considerably as it does so. Its inner border follows the curvature of the brain until reaching a point about half way down the sides of the neural canal; it then turns suddenly outwards, leaving the brain altogether, and forming, as it does so, a very conspicuous blunt projection (fig. 1,  $\nu \beta$ ), which is in rather closer proximity to the brain than is the part of the nerve immediately above it. The trunk of the nerve then passes outwards and downwards, lying just beneath the superficial epiblast, between it and the outer wall of the second or mandibular head cavity (fig. 1,  $\mathcal{Q}$ ). The whole of the nerve, including its root, consist of closely-packed spherical or polygonal cells, which, like all nerve cells in the early stages, stain very deeply with osmic acid, and differ materially in appearance from the much less closely arranged mesoblast cells.

By stage  $\kappa$  the root of the fifth nerve has undergone very remarkable

changes; as shown in fig. 4, the dorsal attachment (fig. 1,  $v\alpha$ ) to the top of the thickened side of the brain has disappeared completely, and the nerve is now attached to the brain at a point about half way down the side (fig. 4,  $v\beta$ ), *i.e.* at a point exactly corresponding to the conspicuous projection (fig. 1,  $v\beta$ ) already described at the earlier stage. Immediately beyond the root of origin the nerve enlarges suddenly, and presents a distinct dorsal projection at the base of the secondary root of attachment. Although hitherto we have not succeeded in following all the intermediate stages, and have not yet obtained satisfactory preparations of the stages earlier than that drawn in fig. 1, yet we feel justified in putting forward the following explanation of the appearances we have just described, relying for our justification partly upon the description given by Balfour, and still more on our own observations on the development of the roots of the seventh nerve, which will be described immediately.

Balfour has described and figured the fifth nerve as arising at "stage  $\epsilon$ , near the anterior end of the hind-brain, as an outgrowth from the extreme dorsal summit of the brain, in identically the same way as the dorsal root of a spinal nerve."<sup>1</sup>

He has further described<sup>2</sup> how, by the growth of the roof of the brain, the nerves of the two sides, which at first are in contact dorsally, shift their position and become widely separate. His descriptions and our own somewhat fragmentary observations on these stages, when considered in connection with our much more complete series of observations on the seventh nerve, leave no room for doubt that the root of origin ( $v\alpha$ ) shown in fig. 1 is the primary root, the one which at stage  $\epsilon$  was situated at the top of the brain, and which has acquired its present position merely in consequence of the rapid growth of the roof of the brain pushing its two lateral halves apart, and so separating the roots of the nerves.

Concerning the root of attachment ( $V\beta$ ) shown at stage  $\kappa$  in fig. 4 there is more room for dispute. Balfour appears to hold<sup>3</sup> that this further change in position is due to the same cause as the former one, *i.e.* to growth of the roof of the brain; but this explanation, while it would fully account for the first change, would in no way explain such a shifting of the root down the thickened side of the brain, as is

<sup>1</sup> Op. cit., p. 191, and Pl. XIV., fig. 3.

<sup>2</sup> Op. cit., p. 196.

<sup>3</sup> Op. cit., p. 196.



clearly seen to have occurred on comparing fig. 4 with fig. 1. We believe that what really happens is that about the commencement of stage  $\kappa$  the nerve acquires a new and secondary connection with the brain at the point ( $v \beta$ ) opposite the projection already noticed; that the primary attachment ( $v a$ ) is lost; and that the part of the nerve left above the secondary root rapidly diminishes and ultimately disappears altogether, the slight dorsal projection already noticed in fig. 4 being the last rudiment of it. As our arguments in support of this view depend almost entirely on our own observations on the development of the roots of the seventh nerve, we postpone further consideration of the point till a later portion of this paper.

At the commencement of stage  $\kappa$  then, the fifth nerve arises about half way up the sides of the hind-brain by a single large root in which ganglion cells are abundant, and opposite to which there is a well-marked external bulging of the walls of the brain;<sup>1</sup> beyond this root the nerve expands suddenly into a very large ganglionic swelling, the future Gasserian ganglion.

Before the close of stage  $\kappa$  additional roots appear; a long, slender process runs forward from the antero-superior angle of the Gasserian ganglion, and becomes connected with the brain some distance in front of the main root; in addition to which one, two, or more roots of a similar kind appear in intermediate situations.

These anterior roots of the fifth nerve are well shown at the next stage ( $\iota$ ) in fig 10,  $v \gamma$ , which shows that the fifth nerve at this time arises from the brain by three distinct roots, of which the posterior one is much the largest, and is the ganglionic root ( $v \beta$ ) of fig. 4, while the two anterior slender non-ganglionic roots are the new ones. We have studied these roots very carefully, but have been unable to determine with certainty whether they are outgrowths from the brain to meet the ganglion, or from the ganglion towards the brain; our observations, however, though inconclusive, tend very strongly towards the latter of the two alternatives. It has also occurred to us that these new roots may possibly be the original primary root of origin ( $v a$ , fig. 1), which, after losing its original attachment, has acquired a new one lower down; however, though the dates of the disappearance of  $v a$  and appearance of  $v \gamma$  agree fairly well with this hypothesis, we have yet no actual observations in its favour, and do not wish to lay stress upon it.

<sup>1</sup> Marshall, *loc. cit.*, p. 84.

These anterior roots which, during stage L, may be three or more in number, appear in the later stages to be very constantly reduced to two, one of which is the most anterior of the original roots while the other appears to be formed by the fusion of the intermediate ones.

This condition at stage N is well shown in fig. 11, v  $\gamma$ . At a stage between o and p (fig. 14, v  $\gamma$ ) they are rather less conspicuous owing to the interval between them and the secondary root (v  $\beta$ ) being filled up by dense tissue. They are clearly recognisable in the adult, and form, as will be noticed more fully further on, the first or anterior root of the fifth nerve of zootomists.

B. *The branches of the fifth nerve.*—The Gasserian ganglion is, from its first appearance, wedged in between the dorsal ends of the second and third head cavities in the same manner as is the ciliary ganglion between the first and second (fig. 10). From the Gasserian ganglion, at stage  $\kappa$ , two nerves arise; of these, one, which runs straight down between the second and third head cavities, and then along the anterior border of the mandibular arch in front of the ventral portion of the cavity in the latter, is the mandibular branch; and from this, a considerable distance below the ganglion, a small anterior branch—the maxillary nerve—is given off. The second branch from the Gasserian ganglion arises from its anterior inferior angle, runs along the top of the first head cavity and joins the ciliary ganglion; it is the communicating branch between the fifth and third nerves already mentioned, and forms the proximal part of the *ramus ophthalmicus profundus* of zootomists.

At stage L a slender branch arises from the anterior superior angle of the Gasserian ganglion; this is the ophthalmic branch of the fifth (fig. 10, v *a*) which runs forward through the orbit dorsal to all the eye muscles, giving off branches to the neighbouring parts in its course and terminating in the skin of the fore part of the head. One other nerve in connection with the fifth remains to be noticed; this is the connecting branch (*N.c.*, fig. 10) between the fifth and seventh nerves: this is present at  $\kappa$ , at which stage as well as at L it forms a very stout though short nerve, running forwards and downwards from the seventh nerve, over the top of the third head cavity, to join the lower part of the Gasserian ganglion.

The branches of the fifth nerve at stage N are well seen in figs. 11 and 12, which have been constructed so that each of them may show the whole course and distribution of certain nerves: the outlines of

the figures were drawn, with the camera, from individual sections and the branches of the several nerves carefully filled in, again by the aid of the camera, from other sections of the same series. In this way such a view of the nerve is obtained as might be got from a transparent embryo in which the nerves alone stood out as opaque objects. To prevent confusion, from the overlapping of different nerves, two figures have been given, of which the first (fig. 11) shows the roots of the fifth and seventh, the connections of these with one another and with the third nerve, the branches of the latter and of the fifth, the fourth nerve, and some of the branches of the seventh; in the second (fig. 12) the remaining branches of the seventh, with certain branches of the glossopharyngeal are shown, and in addition to these, the whole course of the ophthalmic branches of both fifth and seventh nerves.

The branches of the fifth nerve are seen to be the following:

1. The ophthalmic branch (*v a*), which arises by a slightly ganglionic root, runs forward over the *obliquus superior* (*o.s.*), crossing, as it does so, the fourth nerve at right angles and giving off branches, some of which appear to be connected with the fourth.

2. The communicating branch (*N.c.*) between the Gasserian and ciliary ganglia, the position and relations of which nerve are sufficiently well shown in the figure. We reserve the discussion of this branch, merely noticing here that, though we describe it with the fifth nerve, it appears to belong to the third quite as much as to the latter.

3. The main stem of the fifth, running down behind the first head cavity (1) and the *rectus externus*, receiving the communicating branch (*N.c.*) from the seventh, and after passing downwards and forwards for some distance, dividing into two branches, (*a*) an anterior or maxillary nerve (*v b*) which again gives off numerous branches to the skin of the upper jaw; and (*b*) a posterior or mandibular (*v c*) which runs backwards and downwards, lying in close contact with the outer wall of the lower part of the mandibular head cavity (2) and supplying it with branches. The distribution of ganglion cells is sufficiently shown in the figure; there is a small ganglion at the base of the ophthalmic nerve, and the ganglion cells of the main stem extend some little distance beyond the point of junction with the communicating branch from the seventh.

In figs. 14 and 15 some of these branches (*v a*, *v b*, *v c*) are seen at a stage between o and P: except that the roots of v and VII are much more closely approximated, there is no difference of importance between

this stage and the earlier one which we have more fully described. We have traced all these nerves up to what is practically the adult condition, and have identified them with the branches bearing the same names in the adult. Our observations show that in the fifth, as in the third nerve, all the main branches of the adult nerve are fully established by stage L, and that the after changes are comparatively unimportant.

#### THE SEVENTH, OR FACIAL NERVE.

A. *The roots of origin.*—Fig. 2 represents a transverse section through the roots of origin of the seventh nerve of an embryo between stages I and κ, the same, in fact, of which fig. 1 represents the roots of the fifth nerve. The nerves (VII) are seen to arise from the extreme dorsal summit of the hind-brain, the roots of origin of the two (VII a) being continuous with one another across the top of the brain. It will also be noticed, as contrasted with figure 1, that the two sides of the hind-brain are close together, both above and below, and that the cavity of the hind-brain is a mere vertical slit; that, in fact, the growth of the roof of the brain, which we have seen is the first cause of the separation of the roots of the fifth, has not yet commenced in the part of the brain from which the seventh nerves arise. The section further shows that the nerve on either side extends down as a somewhat club-shaped mass of compactly arranged polygonal cells lying between the external epiblast and the neural canal, but distinct from both, its ventral end having a tendency to pass to the outer side of the third head cavity (fig. 2, 3), just as the fifth nerve passed to the outer side of the second cavity (fig. 1, 2).

The next stage is represented in fig. 3, a section through the hind brain and roots of the seventh nerve of an embryo of stage κ, of the same age though not from the same specimen as fig. 4. The figure shows that very important changes have occurred; the roof of the hind-brain has grown rapidly and considerably, so as to separate widely the two primary roots of the seventh nerves (VII a). On the right side this dorsal primary root is alone seen, but on the left side a considerable portion of the nerve is shown, and it is seen *that, in addition to the primary root (VII a), which is still present, the nerve has acquired a new or secondary root (VII β) about half way down the side of the brain.* Both roots of attachment are perfectly clear and unmistakable, while between them the nerve and brain are quite distinct from one another, and separated by an appreciable interval.

If fig. 3, showing the condition of the root of the seventh nerve at stage  $\kappa$ , be compared with fig. 1, showing the root of the fifth nerve at a rather earlier stage, it will be seen at once that there is a very close resemblance between the two; the sole point of difference being that in fig. 1, though the nerve still retains its primary attachment, the secondary has not yet been actually acquired. Balfour's figures and description, already referred to, show that at a still earlier stage the fifth nerve has exactly the same appearance and relations which the seventh has in fig. 2; and it is mainly on this fact, coupled with the close similarity between such specimens as those represented in figs. 1 and 3, that we rely in support of the explanation we have given above of the root of the fifth nerve.

Inasmuch as figs. 1 and 2 are taken from the same embryo, it would seem that the fifth nerve appears before the seventh, and is, during the earlier phases of its formation, just one stage ahead of it in development. At a time (fig. 1) when the primary roots of the fifth have already become widely separated by growth of the brain-roof, and the secondary attachment ( $v \beta$ ) is on the point of being acquired, the two seventh nerves (fig. 2) are still in contact with one another across the top of the unexpanded brain-roof; and at stage  $\kappa$  the seventh nerve (fig. 3) is in exactly the same condition as the fifth is at the end of stage  $\iota$  (fig. 1).

Our observations appear, therefore, to prove conclusively that as concerns the seventh nerve, while the change of position of the dorsal or primary root ( $vii \alpha$ ) is due *solely* to rapid growth of the roof of the brain, the lower or ventral root ( $vii \beta$ ) is a new and purely secondary attachment.

Whilst these results concerning the roots of the seventh are, we believe, new as applied to Elasmobranchs, they are in perfect accordance with the account previously given by one of us of the development of the seventh nerve in the chick, in which the very same series of changes—the separation of the primary roots by growth of the brain-roof, and the acquiring of new or secondary roots—are shown to occur in a precisely similar manner.<sup>1</sup> The close correspondence between these two very different types of vertebrates is of much interest, partly

<sup>1</sup> Marshall, "Develop. of Cranial Nerves in Chick," 'Quart. Journ. Micr. Sc.,' Jan. 1878, pp. 34 and 35.

The prediction there made, that the secondary attachment of the nerves in Elasmobranchs would prove on further investigation to be acquired in exactly the same manner as in the chick, is now completely verified.

as tending to confirm the correctness of the account, and partly as showing that this curious shifting of the nerve roots, though clearly a change of a secondary nature, must yet have been acquired very early by Vertebrates.

The later stages of development of the roots of the seventh also present points of great interest. Fig. 6 represents a transverse section through the roots of the seventh nerve in the same embryo, at stage  $\kappa$ , of which fig. 5 shows the roots of the fifth nerve. The seventh nerve is seen to arise on either side by two roots, one ( $\text{VII } \alpha$ ) from the top of the side of the brain at the junction of the thickened part with the thin roof, while the other ( $\text{VII } \beta$ ) arises about half way down the side of the brain. Between the two roots the nerve is in contact with the brain, but apparently *not connected with it*. We have traced the intermediate steps between figs. 3 and 6, and find that the upper root ( $\text{VII } \alpha$ ) of fig. 6 is the original dorsal or primary root, and the lower one ( $\text{VII } \beta$ ) the secondary root of fig. 3. In other words, there is an important difference between the fifth and the seventh nerves, *inasmuch as in the former the primary root is lost and the secondary alone retained, whilst in the latter both primary and secondary roots are retained up to stage  $\kappa$ , and indeed, as we shall see immediately, throughout life*. The difference between the roots of the fifth and seventh nerves just noticed does not occur in the case of the chick, in which the primary root of the seventh is lost as completely as is that of the fifth in Elasmobranchs.<sup>1</sup>

This shifting of the roots of origin and acquiring of a secondary connection with the sides of the brain is not confined to the cranial nerves. It has already been shown to occur in the posterior roots of the spinal nerves of the chick,<sup>2</sup> and occurs also in the posterior roots of the spinal nerves of Elasmobranchs. It is a point of much interest to note that the seventh nerve, in the retention of its primary as well as its secondary root, *is not only more primitive than the fifth, but more primitive even than the spinal nerves*.

The condition of the roots of the seventh at stage  $\sigma$  is shown in the left-hand side of fig. 9, representing half of a transverse section through the hind-brain and roots of origin of this nerve. The two roots, the primary ( $\text{VII } \alpha$ ) and the secondary ( $\text{VII } \beta$ ), are even more distinct than at the earlier stages. The primary root ( $\text{VII } \alpha$ ) arises as before from

<sup>1</sup> Marshall, 'Quart. Journ. Micr. Sc.,' Jan., 1878, pp. 24 and 25.

<sup>2</sup> Marshall, "On the Early Stages of Development of the Nerves in Birds," 'Journal of Anatomy,' vol. xi., 1877."

the top of the thickened side of the hind-brain just before its junction with the thin roof; from this origin the root runs downward, alongside of and closely applied to the brain, but unconnected with it, to join the secondary root ( $\text{VII } \beta$ ). This latter is now situated still nearer to the ventral surface than at its first appearance, the distance between the two roots being considerably greater than at the earlier stages, as is evident from a comparison of fig. 9 with fig. 6. The two roots also differ histologically, the dorsal or primary root consisting almost entirely of elongated fusiform cells, whilst the ventral root ( $\text{VII } \beta$ ) is mainly composed of spherical ganglion cells.

This ventral root, at stage o, has, as shown in the figure, two distinct attachments to the brain, one just below the other. It is shown in longitudinal and vertical section at stage  $\text{x}$  in fig. 11, where the brain presents opposite to its point of origin an external bulging precisely similar to that opposite to the secondary root of the fifth ( $\text{V } \beta$ ). The dorsal or primary root ( $\text{VII } a$ ) is shown at the same stage in fig. 12.

In fig. 14 the two roots of the seventh are seen in longitudinal and vertical section, at an age intermediate between stages o and p. The dorsal root ( $\text{VII } a$ ) arises very far up the side of the brain, in fact, as in the earlier stages, from the junction of side and roof; it is of considerable length, is widely separated from the secondary root, and still consists mainly of fusiform cells; the secondary or ventral root, which is overlaid and almost concealed by the primary root, is only seen in part, its most anterior portion alone being visible.

The dorsal or primary root is also well shown at the same stage in fig. 15.

*B. Comparison of the embryonic roots of the fifth and seventh nerves with those of the adult.*—It will be convenient here to briefly summarise our results concerning the roots of origin of the fifth and seventh nerves, and to trace their changes up to the adult form.

About the close of stage  $\text{I}$  the fifth nerve (fig. 1) still retains its primary attachment ( $\text{V } a$ ) to the brain, and is on the point of acquiring its secondary one ( $\text{V } \beta$ ); owing to the growth of the roof of the brain the two primary roots, which were at first continuous across the top of the brain, are now widely separate. The seventh nerve (fig. 2) arises by its primary root from the dorsal summit of the brain, whose roof at this point has not yet commenced its rapid growth, so that the

nerves of the two sides are still directly continuous with one another ; there is as yet no trace of the secondary root of the seventh.

At stage  $\kappa$  the fifth nerve (fig. 4) has completely lost its primary root and is now attached to the brain by the secondary root alone ( $A \beta$ ) ; a slight trace of the former is still present as a small dorsal projection on the nerve just beyond the root of attachment. Towards the close of stage  $\kappa$ , the tertiary or anterior roots have appeared, arising almost certainly as outgrowths of the ganglion towards the brain ; but whether these are altogether new developments or merely new attachments of the primary root is uncertain. The seventh nerve (fig. 3) is in the same condition as the fifth at the preceding stage ; it is now attached by both primary and secondary roots, the former, owing to the growth of the brain-roof, being widely separate from one another.

At stage  $\nu$  the condition of the roots is much the same as at the end of stage  $\kappa$ . The fifth nerve (figs. 5 and 11) is attached by its secondary and tertiary roots, the latter being very constantly two in number, of which the anterior is the larger and attached to the brain some distance in front of the secondary root ( $\nu \beta$ ). The seventh nerve (figs 6, 11 and 12) is attached by both primary and secondary roots, the nerve between the two being in contact, but not in connection, with the brain ; the secondary root (fig. 11) is divided into an anterior or facial, and a posterior or auditory division.

At stage  $\omicron$  (figs 8 and 9) the chief differences are—firstly, that, owing to increased growth of the brain, the distance between the primary and secondary roots of the seventh nerve is much greater than before ; secondly, that the roots of the fifth and seventh nerves, which from the first have been quite independent of one another, are now situated much closer together than they were at the earlier stages.

At stage  $\omicron$ - $P$  (fig. 14) the two roots of the seventh ( $\nu \alpha$  and  $\nu \beta$ ) are still further apart from one another, but are now very close to those of the fifth. The connection between the two nerves which we have already seen is fully established at stage  $\kappa$ , and which is shown at stage  $L$  in fig. 10 ( $N.c'$ ) and at stage  $\nu$  in fig. 11 ( $N.c'$ ) is, by stage  $\omicron$ - $P$ , very much more extensive and intimate than previously. The roots of the nerves are still quite distinct from one another (fig. 14), but immediately beyond these roots the two nerves become so closely and extensively united together that it is impossible to draw a line of separation between them. The connection is rendered still more



intimate by the crossing of one of the branches of the seventh, as will be described fully later on, over the main stem of the fifth, so as to lie in front of the branches of this nerve.

The condition of the roots, as of the branches, of the fifth and seventh nerves at stage o-p differs but little from that of the adult, the sole change of importance as concerns the roots being that the ventral roots ( $v\beta$  and  $vii\beta$ ) approach still closer together, and come into actual contact.

The primitive distinctness, gradual approximation, and ultimate more or less complete fusion of the roots of the fifth and seventh are of great interest, as proving that the fusion of these two nerves, so characteristic of Pisces and Amphibia, is a purely secondary feature; and that the two are at first as independent of one another in these forms as they are throughout life in the higher Vertebrates.

In adult Elasmobranchs the combined roots of the fifth and seventh nerves are usually described together, and the descriptions of different observers, though not quite in harmony with one another in certain details, yet agree fairly well on the main points. Stannius, whose descriptions are the most precise, describes the combined fifth and seventh nerves as arising in Plagiostomes by three roots,<sup>1</sup> of which one is seen on closer examination to be double, giving four roots in all; of these the first, or most anterior one, arises from the ventral surface of the medulla by two short non-ganglionic roots, which unite together shortly after leaving the brain. This root is in *Raja*, according to Stannius, mainly motor, supplying the muscles by which the respiratory movements of the anterior wall of the spiracle are effected, and also certain others in connection with the jaws. The second root of Stannius is large, lies posterior to the first, and is in close proximity behind with the auditory nerve; it may be distinguished into an anterior part which belongs to the trigeminal, and a posterior, more ventrally situated portion, which belongs to the facial. The third root is very large and much more dorsally situated than the others; it is connected by its deeper fibres with the second root, whilst from its superficial fibres are derived, according to Stannius, the *ramus ophthalmicus superficialis* of the fifth, and also, in part, the maxillary and buccal nerves.

Gegenbaur,<sup>2</sup> in his account of the cranial nerves of *Hexanchus*, dis-

<sup>1</sup> Stannius, "Das peripherische Nervensystem der Fische." Rostock, 1849, pp. 29 and 30.

<sup>2</sup> "Ueber die Kopfnerven von *Hexanchus*," 'Jenaische Zeitschrift,' Bd. vi, 1871, pp. 501, 502, and 513, 514.

tinguishes between the roots of the fifth and the seventh. He describes the fifth as arising by the union of two trunks of about equal size, an anterior and a posterior; of these the anterior (*a*) arises from the ventral surface of the medulla by two roots situated very close together; the posterior (*b*) has also two distinct roots, a dorsal one (*a*) arising from the side of the medulla by a large swelling projecting into the fourth ventricle, and a ventral one (*β*) situated immediately above the root of the facial, and in front of, and above that of the auditory.

The seventh nerve in *Hexanchus* is described as arising by two roots, a larger one immediately in front of the auditory, and a smaller one passing to it from the fifth.

Jackson and Clarke<sup>1</sup> describe the combined fifth and seventh nerves in *Echinorhinus* as arising by three roots; an anterior inferior root (*v a*), itself with two well-marked rootlets, a second root (*v β*) arising by a well-marked superior rootlet from the lobus trigeminus and by a smaller inferior one, and a third root (*v γ* and VII) closely connected with the second one,

Balfour<sup>2</sup> describes the fifth nerve in *Scyllium stellare* as arising by three roots:—(1) an anterior more or less ventral root; (2) a root rather behind this arising by two strands, a dorsal and a ventral, and closely connected behind with the root of the seventh; and (3) a quite distinct dorsal and posterior root situated slightly behind the dorsal strand of the second root. The seventh nerve is described as arising by a single root close to, and behind, the second root of the fifth.

On comparing these descriptions of adult Elasmobranchs with our own observations on embryos and adults we are led to the following conclusions.

The fifth nerve in the adult arises by two roots:

*a.* An anterior one arising from the ventral surface of the medulla by two non-ganglionic rootlets, whose distinctness varies much in different adult Elasmobranchs. These rootlets are the tertiary or anterior roots of our embryos (figs. 10, 11, and 14, *v γ*). This root corresponds to the first root of Stannius; the anterior root (*a*) of Gegenbaur; the anterior root (*v a*) of Jackson and Clarke; and the anterior root (1) of Balfour.

*b.* A posterior larger ganglionic root, the ventral or secondary root of our embryos (figs. 10, 11, and 14, *v β*). This is at first quite

<sup>1</sup> "The Brain and Cranial Nerves of *Echinorhinus spinosus*." 'Journal of Anat. and Phys.,' vol. x, p. 81.

<sup>2</sup> Op. cit., pp. 194 and 195.

distinct from the root of the seventh, but during the later stages of development gradually approaches this latter, and in the adult cannot be clearly distinguished from it.

This root is the anterior part of the second root of Stannius; the ventral division ( $\beta$ ) of the posterior root ( $b$ ) of the fifth of Gegenbaur; apparently the inferior rootlet of the second root ( $v \beta$ ) and possibly part of the third root ( $v \gamma$  and VII) as well, of Jackson and Clarke; and the second root (2) of the fifth of Balfour.

The seventh nerve in the adult arises by two roots:

*a.* A dorsal root arising far up the side of the medulla, at the junction of the thickened side and thin roof of the fourth ventricle. This root is the primary or dorsal root of the seventh nerve of our embryos (figs. 2, 3, 6, 9, 12, 14, and 15, VII *a*). It has by previous observers been almost invariably described as a root of the fifth, and never as a true root of the seventh; *our description and figures here given leave no room for doubt that it belongs to the seventh.* As already noticed, the retention of this root marks the seventh as being more primitive than the spinal, and possibly more so than any of the other cranial nerves, all the other nerves apparently retaining their secondary roots only.

This root is the third or dorsal root of Stannius; the dorsal rootlet (*a*) of the posterior trunk (*b*) of the fifth of Gegenbaur; the superior rootlet of the second root ( $v \beta$ ) of Jackson and Clarke; and the dorsal and posterior root (3) of the fifth of Balfour.

*b.* A ventral root arising from the side of the medulla at a rather lower level than the posterior root of the fifth. This is the secondary or ventral root of the seventh of our embryos (figs. 3, 6, 9, 10, 11, and 15, VII  $\beta$ ). The auditory nerve is at first derived from this root, but in the adult appears to be more distinct from the facial than is the case in the embryo. This root is, at first, some little distance behind the secondary one ( $v \beta$ ) of the fifth nerve (figs. 10 and 11), from which it is perfectly distinct; in the later stages the two roots gradually approach one another (fig. 14), and in the adult are usually in close contact.

This root is the posterior part of the second root of Stannius; the root of the seventh of Gegenbaur; part or possibly the whole of the third root ( $v \gamma$  and VII) of Jackson and Clarke; and the single root of the seventh of Balfour.

It would appear, therefore, that the fifth nerve loses its primary

root, retains its secondary, and acquires tertiary roots, while the seventh retains both primary and secondary. Concerning the fourth nerve we would suggest the possibility that it may prove to be the primary root of a nerve of which the third nerve is the secondary root, which has, in this case, acquired complete independence.

*c. The branches of the seventh nerve.*—At stage  $\kappa$  the seventh nerve, which, as we have already seen, has acquired its secondary as well as its primary roots of origin (fig. 3), expands below the secondary root into a large ganglionic swelling lying immediately behind the third head cavity. From this enlargement three branches arise: (1) from the anterior and superior angle of the ganglion a large nerve with a ganglionic base arises, and runs forward along the dorsal surface of the head, lying just beneath the superficial epiblast; this is the ophthalmic branch of the seventh, and is referred to in our figures as VII *a*.

(2) The second branch, which is also large, and has a ganglionic base, arises from the front part of the ganglion immediately below the root of the ophthalmic; its deeper portion runs forwards and slightly downwards over the top of the third head cavity and becomes connected with the main stem of the fifth; it is referred to in the figures as *N.c'*. The more superficial portion passes on further forward in the same direction, crosses the mandibular arch, and enters the maxillary process, lying immediately superficial to the maxillary nerve and just beneath the external epiblast; it is referred to in the figures as VII *d*.

(3) The third branch is the direct continuation of the main stem of the facial nerve; it runs downwards and backwards along the anterior border of the hyoidean arch, and is the rudiment of the posterior or hyoidean branch of the seventh in the adult; it is referred to in the figures as VII *c*.

Of these branches the first, or ophthalmic, is from its earliest appearance connected with the dorsal or primary root of the seventh rather than with the ventral root. The second branch is the most remarkable of the three; its deeper portion forms, as we have seen, a direct connection between the fifth and seventh nerves, a communication which appears to be very early established, inasmuch as by stage  $\kappa$  the connecting branch is already a nerve of considerable size; the superficial portion of this branch (VII *d*) is noteworthy, mainly on

account of its very close relation with the maxillary division of the fifth nerve.

At stage L the only changes of importance are, (1) that the several branches have increased in size, and, excepting the branch VII *d*, which has a very straight course and ends abruptly in the skin, have divided into secondary branches near their terminations; and (2), that a small anterior branch has arisen from the hyoidean nerve (VII *c*), some distance from the brain, which runs forward over the top of the spiracular or hyomandibular cleft, and then down in the anterior wall of the spiracle, *i.e.* in the posterior portion of the mandibular arch; this branch will be referred to as VII *b*.

The several branches of the seventh nerve at stage N are well shown in the diagrammatic figures 11 and 12. The ophthalmic branch (VII *a*) is seen in fig. 12 arising from the base of the primary or dorsal root (VII *a*) as a stout nerve, which expands very shortly after its origin into a large somewhat fusiform ganglion, beyond which the nerve runs forward as a stout trunk to the extreme anterior part of the head. Throughout its course it lies just beneath the external epiblast, and immediately dorsal to the ophthalmic branch of the fifth (V *a*), with which it is in very close relation; like this latter nerve it gives off branches along its whole course to the integument of the neighbouring parts, the branches being few in number at the proximal end, and much more numerous distally. A short distance before its termination this nerve, like the ophthalmic branch of the fifth, is crossed at a considerable angle by the nerve N (fig. 12).

The connecting branch (N.c.) between the seventh and fifth nerves is well seen in figs. 11 and 12; it is now shorter and wider than at stage L (fig. 10), and contains very numerous ganglion cells along its whole length.

The superficial portion of this nerve (VII *d*) is not shown in fig. 11, but is represented along its whole length in fig. 12; it is a stout nerve with a remarkably straight course; it gives off no branches at all along the greater part of its length, but near its distal termination divides rather suddenly into a number of branches, which end in the integument of the maxillary process, the most anterior of them extending forwards almost as far as the hinder border of the olfactory pit. The relations of this nerve to the maxillary branch of the fifth are very curious; the two nerves are very close together, the branch of the seventh lying, as at the earlier stage, immediately superficial

to that of the fifth. These relations are well seen in the transverse section drawn in fig. 6. This shows, as already noticed, the primary and secondary roots of the seventh, and also the proximal portion of the nerve VII *d*. This nerve is seen to be a direct continuation of the primary root; its inner, or deeper, portion is seen on both sides to become continuous with the maxillary branch of the fifth (*v b*), the junction of the two forming the connection between the fifth and seventh nerves already noticed. Beyond this point of union the nerve VII *d* is continued downwards, lying immediately superficial to the maxillary nerve (*v b*). The two nerves preserve this relation up to their terminal distribution, two of the ultimate branches being represented in figure 5 (*v b* and VII *d*). This very remarkable branch of the seventh puzzled us greatly for a long time, and it was only after working out the whole history of its development up to stage *q* that we succeeded in determining its import. The nerve has already been described and figured by one of us,<sup>1</sup> and named tentatively the palatine. This determination now proves to be erroneous; the palatine is a deeply placed nerve, whereas the nerve VII *d* retains its superficial position in the adult.

This nerve (VII *d*) we have now identified as the buccal nerve, the proof of this determination, consisting in our having traced the nerve directly up to the adult. The buccal nerve has always hitherto been regarded as a branch of the fifth, and is described as such by Stannius,<sup>2</sup> Gegenbaur,<sup>3</sup> Jackson and Clarke,<sup>4</sup> Balfour,<sup>5</sup> and others. Stannius<sup>6</sup> points out that the buccal nerve in fishes is very variable; that it may either arise from the main stem of the fifth, from the maxillary or the mandibular, or from both these nerves, or, finally, may be absent altogether.

Up to stage *n*, as shown in figures 6, 11, and 12, the buccal nerve is clearly a branch of the seventh, and could not possibly be taken for a branch of the fifth. After stage *n*, however, the connection between the roots of the fifth and seventh nerves becomes, as we have seen, very much more intimate; and at the stage between *o* and *p*, represented in fig. 14, the buccal nerve (VII *d*), which is now situated completely in front of the maxillary (*v b*), might very easily be taken

<sup>1</sup> Marshall, *loc. cit.*, pp. 86, 87; and Pls. V, fig. 15, and VI, figs. 28 and 29.

<sup>2</sup> 'Handbuch der Zootomie,' p. 158.

<sup>3</sup> *Loc. cit.*, p. 509.

<sup>4</sup> *Loc. cit.*, p. 86.

<sup>5</sup> *Op. cit.*, p. 195.

<sup>6</sup> 'Das Peripherische Nervensystem,' pp. 41 and 42.

for a branch of the fifth rather than of the seventh; careful examination shows, however, that the buccal, which is, as before, the most superficial of all the ventral branches, can be traced up to the dorsal root of the seventh from which it arises, as in the earlier stages.

This origin of the buccal nerve from the root VII *a* has already been noticed by Stannius,<sup>1</sup> who, however, as we have seen, did not refer the root in question to the seventh. Stannius' figure of the nerves in *Chimæra*<sup>2</sup> shows clearly the very superficial position of the buccal nerve and its independence of both maxillary and mandibular nerves.

Of the remaining branches of the seventh the anterior one (VII *b*) is shown in fig. 11 at its origin, arising from a large ganglionic swelling on the main or hyoidean branch of the seventh, and running forward in close contact with the top of the spiracle (*sp.*), in front of which it divides almost at once into two branches, the distribution of which is shown in fig. 12; of these the anterior one (VII *pa*) runs downwards, forwards, and inwards, giving off numerous branches to the roof of the mouth. In fig. 12 the anterior branches of this nerve appear to cross the posterior branches of the buccal, but it must be borne in mind that at this point the two nerves are at very different levels, the buccal being very superficial and the nerve (VII *pa*) lying very deep. This latter is seen in transverse section in fig 7, which shows, on the right side, its origin from the ganglion, and, on the left, its distribution to the mucous membrane of the mouth. By comparing this figure with fig. 6, the difference of levels between the two nerves will be at once apparent. This anterior branch (VII *pa*) is the palatine nerve; it has already acquired by stage *n* its characteristic distribution, and undergoes no further change of importance from this period up to the adult stage.

The second or posterior division (fig. 12, VII *sp*) of the nerve (VII *b*) runs downwards and slightly backwards along the anterior border of the spiracular cleft; it gives off branches along the whole of its length, the great majority of which run backwards to the mucous membrane of the border of the cleft and to the spiracular branchia. This nerve, which at this stage is of about equal size with the palatine, is the spiracular or præ-spiracular nerve of zootomists.

The only branch of the seventh still left for description is the main trunk or hyoidean branch (fig. 11, VII *c*), which forms the direct

<sup>1</sup> Loc. cit., p. 30.

<sup>2</sup> Loc. cit., Taf. 1, fig.

continuation of the main stem of the nerve. This, as is seen from fig. 11, arises from the ventral or secondary root of the seventh, and is at its origin closely connected with the auditory nerve (VIII). Immediately after the auditory nerve leaves it, the facial forms a ganglionic swelling from which the communicating branch (*N.c.*) to the fifth nerve is given off; beyond this point it is continued for a short distance as a stout nerve with comparatively few ganglion cells; this very speedily dilates into the large ganglionic swelling on the top of the spiracular cleft, from which the anterior branch (VII *b*) is given off. The main stem of the seventh (VII *c*) continues its course downwards, running along the anterior border of the hyoid arch and very close to the posterior border of the spiracular cleft; during this part of its course it contains few or no ganglion cells, but gives off a number of branches, of which the first is the largest, from its posterior border, which supply the muscles derived from the wall of the third head cavity (3). A short distance below the lower edge of the spiracular cleft the nerve divides into two branches, of which the anterior (VII *c'*) runs forward along the lower border of the mandibular arch, sending numerous branches to the integument of this part and extending forward so as to come into very close relation with the posterior branches of the maxillary division of the fifth (V *b*). The posterior of the two branches (VII *c*, 2) into which the seventh divides continues the direction of the main stem, and runs down in the hyoid arch just in front of the third head cavity, in the terminal dilatation of which it ends. Of these two terminal branches of the seventh, the anterior, sensory, and superficial one is the *ramus mandibularis externus* of Stannius<sup>1</sup> and Gegenbaur,<sup>2</sup> while the posterior, muscular, and deep branch is the *ramus mandibularis internus* v. *profundus* of the same authors. Both these branches have already acquired, by stage N, not only the characteristic distribution of the adult nerves, but nearly all the minor branches as well.

To recapitulate; we find that by stage N the seventh nerve has acquired all the important branches of the adult nerve, the main trunks and many of the branches being fully developed at a much earlier period—stage L. The seventh nerve at stage N has two roots, a dorsal or primary, and a ventral or secondary. From the dorsal root (VII *a*) arise two branches: (1) the ophthalmic (VII *a*) and (2) the

<sup>1</sup> Loc. cit., p. 65.

<sup>2</sup> Loc. cit., p. 514.



buccal (VII *d*), both of which appear to be purely sensory nerves. The connecting branch (*N.c'*) to the fifth nerve, though it appears in longitudinal section (fig. 11) to be a distinct branch, in transverse sections (fig. 6) seems to be only the deeper portion of the buccal nerve. From the ventral root arises the main or hyoidean branch (VII *c*), from which the branch VII *b* runs forward over the top of the spiracle, dividing, almost immediately, into the palatine (*pa*) and spiracular (*sp*) nerves, whilst the hyoidean itself divides distally into the sensory *ramus mandibularis externus* (*v c*, 1), and the motor *ramus mandibularis internus* (*v c*, 2).

THE SIXTH (ABDUCENS) NERVE.—Concerning the development of the sixth nerve our observations simply confirm the account already given by one of us.<sup>1</sup>

The whole length of the nerve is shown in longitudinal and vertical section in fig. 13 (VI), where it is seen arising from the base of the brain by a number of small non-ganglionic roots which unite to form a slender nerve; this nerve runs forwards a short distance, then turns downwards, pierces the investing mass (*i.v.*), and ends in the posterior extremity of the *rectus externus* muscle (*r.e.*).

Fig. 7 shows the sixth nerve in transverse section at the same stage (*N*): on the left side of the figure the termination of the nerve in the *rectus externus* is seen; while on the right side, which is taken from a more posterior section, one of the roots of origin is shown. This figure shows that the roots of the sixth are considerably nearer the mid-ventral line than are the secondary roots of the seventh nerves, and also that the sixth and seventh nerves are quite independent of one another. At stage *N* the sixth nerve appears to be altogether behind the seventh, but in stage *o* it is situated rather further forwards, so that the same section may pass through the roots of both nerves.

We have not yet detected the sixth nerve in embryos younger than stage *L*: concerning the morphological value of this nerve we adhere to the opinion already expressed that it is to be viewed as bearing the same relation to the seventh that the anterior root of a spinal nerve does to its posterior root.

THE EIGHTH (AUDITORY) NERVE.—This nerve also we can dispose of briefly: at stage *K* it appears as a large ganglionic posterior branch of

<sup>1</sup> Marshall, *loc. cit.*, pp. 89–93

the seventh nerve, given off immediately beyond the root of origin. It is from the first connected with the ventral or secondary root (VII  $\beta$ ). The condition at stage L is shown in fig. 10 (VIII). At stage N (fig. 11) its root, though still intimately connected with that of the facial, shows a very evident line of separation from it; the ganglionic character of the auditory nerve placing it in marked contrast with the non-ganglionic root of the facial. This distinction between the two roots becomes more marked in the later stages.

GENERAL CONSIDERATIONS.—Several questions of a more general character arise out of the facts we have recorded above, and we propose to conclude the present paper with a brief notice of the more important of these. The problems in connection with the roots of origin of the nerves have been already sufficiently discussed, so that we at once turn to the consideration of their branches, concerning which the most important points are the determinations of the equivalence of the branches of the different nerves to one another.

We commence with the ophthalmic branches of the fifth and seventh nerves, the branches named *v a* and *vii a* in our figures. These two nerves, whose course and relations are well shown in figs. 11 and 12, accompany one another very closely along their whole length; they appear to be both sensory nerves, their branches being distributed exclusively to the skin of the top and front of the head, and more especially to the mucous canals of these parts. Of the two nerves the branch of the fifth nerve (*v a*) is the smaller and the more ventrally placed of the two: though the smaller, its branches are, especially in the earlier part of its course, more numerous than those of the seventh. The two nerves in their course through the orbit *lie dorsal to all the other contents of the orbit*. They are at first quite distinct from one another (figs. 11 and 12) and lie close beneath the external epiblast (fig. 5, *vii a*); the branch of the seventh being the more superficial of the two. In the later stages of development, as in the adult, the two nerves lie in very close contact with one another (fig. 16, *v a* and *vii a*), the branch of the seventh lying immediately dorsal to the branch of the fifth; they also, as shown in fig. 16, lie at a deeper level than at the earlier stages.

The fourth nerve bears, as already noticed, a very close relation to these branches. As shown in figs. 11 (IV) and 16 (IV) it crosses the ophthalmic branches at right angles, lying at a slightly deeper level

but turning outwards immediately beneath them, to end in the superior oblique muscle (*o.s.*). At the point of crossing the branches of the two nerves are in very close relation with one another, and we are inclined to believe that a communication exists between the fourth nerve and the ophthalmic branch of the fifth at this point, though we have failed to determine this with certainty.

In determining the morphological value of these ophthalmic branches of the fifth and seventh nerves, very valuable evidence, by which we have been much influenced, is afforded by the condition of the glossopharyngeal nerve. This nerve, at stage L, gives off, just beyond its root of origin, a slender dorsal branch (fig. 10, IX *a*), which, at first passing upwards and backwards, soon curves round the hinder end of the auditory vesicle (*aud.*), and reaching the dorsal surface of the head, runs forward a short distance, lying immediately beneath the superficial epiblast. It gives off branches along its whole course, which are distributed, as shown in the figure, to the integument of the top of the head. At stage N this nerve (fig. 12, IX *a*) has the same course and appearance, the only differences being that it extends rather further forwards than at the earlier stage, so as to reach some distance in front of the middle of the auditory vesicle; and, secondly, that its branches are now seen to be in connection with the commencing mucous canals of this region. This branch of the ninth nerve is clearly the *ramus dorsalis*, and an examination of the figures 11 and 12 will, we think, leave no doubt that the nerves VII *a* and V *a*, which have a similarly superficial course and a like distribution to mucous canals, must be viewed as the equivalent branches of the seventh and fifth nerves. We are, therefore, led to adopt the view put forward by Balfour, that the ophthalmic branches V *a* and VII *a* of the fifth and seventh nerves are the *rami dorsales* of these nerves.<sup>1</sup> Stannius and Gegenbaur speak of the ophthalmics as *rami dorsales*, but refer them entirely to the fifth.

What the causes are which have led to the very marked extension forwards of the *rami dorsales* of these nerves is not very evident; we

<sup>1</sup> In my paper on the head cavities of Elasmobranchs I abandoned the view previously put forward ('Quart. Journ. Micr. Sc.', Jan., 1878, p. 30), that the ophthalmics were persistent remains of the commissure connecting together the roots of the nerves at their first appearance, but did not expressly adopt the view that they were *rami dorsales*. I have been led to adopt this view mainly because it now appears that, instead of being perfectly exceptional in their course, as I had previously supposed them to be, the ophthalmics merely express an exaggerated condition of a state of things shown in a less extreme form by the *ramus dorsalis* of the glossopharyngeal.—A. M. M.

would suggest that it is due mainly to an extension forwards, and accumulation at the anterior end of the head, of the special tegumentary sense organs—the mucous canals—, this extension forwards involving a corresponding extension of the nerves supplying these organs; in connection with this suggestion it is of interest to note that no one of the nerves in front of the fifth sends any branches to these organs. Whether there is any trace of a *ramus dorsalis* to the third is very doubtful; at any rate the fourth nerve cannot be the *ramus dorsalis* of the third, as its course is at right angles (fig. 11) to the *rami dorsales* of the fifth and seventh nerves; and, secondly, it is a motor and not a sensory nerve.

We now come to a far more intricate problem, viz., the import of the connecting branches between the third, fifth, and seventh nerves, with which it will be convenient to consider the nerve *N.* (figs. 10, 11, 12, and 15).

These three nerves, *N.c.*, *N.c.*', and *N.* all appear very early; we have failed to determine the date of their first origin, but by stage κ they are fully established. The posterior one (*N.c.*'), connecting the fifth and seventh nerves together, is the most difficult to investigate, owing to its appearing from the first as merely the deeper portion of the buccal nerve (fig. 6); in longitudinal sections, however, it appears very distinct (fig. 10). It is from the first much shorter than either of the other two nerves we are considering, and in the later stages (fig. 14) and the adult condition, owing to the close approximation of the fifth and seventh nerves, ceases to be visible as a distinct trunk.

The second of the three nerves (*N.c.*, figs. 10 and 11) forms, as already noticed, a direct connection between the Gasserian ganglion of the fifth and the ciliary ganglion (*c.g.*) of the third nerve, and is much more slender than *N.c.*'. Concerning the nerve in question, it is of the utmost importance to notice that not only is it fully established at the stage at which our observations commence, but that it is from the very first a connecting nerve, and that there is no reason whatever in the early stages for considering it as belonging to the fifth rather than to the third nerve. We have, therefore, in this paper given it a perfectly neutral name.

The last of these nerves, *N.*, is still more remarkable; like the others it is present at κ. Starting at this stage from the ciliary ganglion it runs in an almost perfectly straight course to the anterior end of the head,

ending abruptly in the external epiblast, and giving off no branches whatever. At stage L it is in very close relation with the olfactory nerve, and in some specimens seems to be connected with it, though we cannot speak with certainty on this point.

As soon as the eye muscles are established they have very definite relations to this nerve; the *rectus superior* and *internus*, and the *obliquus superior* lying above it, and the remaining three muscles below it (fig. 11). It also passes through the substance of the sclerotic, as noticed in a former portion of this paper.

Concerning the morphological significance of these nerves, bearing in mind their very early appearance and the total absence of evidence for regarding them as branches of either of the nerves they serve to connect, we are disposed to view the nerves *N.c.* and *N.c'* as persistent portions of the neural ridge between the outgrowths to form the third, fifth, and seventh nerves, and as being, therefore, homologous with the primitive commissure connecting the posterior roots of the spinal nerves together.<sup>1</sup> As to the nerve *N.* we are in much more doubt; its apparent connection with the olfactory nerve at L, if confirmed, would tell in favour of its being regarded as a similar commissure between the third and olfactory nerves, and would greatly support views previously advanced by one of us concerning the morphological value of the olfactory nerve.<sup>2</sup> On the other hand, the extension forwards of the nerve *N.* beyond the olfactory nerve to the extreme anterior end of the head must, for the present, be regarded as almost conclusive against its commissural nature. *In this case it can only be a branch of the third nerve*, for the only other nerve with which it is in direct, or indirect, connection is the connecting nerve (*N.c.*) between the third and fifth, which, if it does not belong to the third, there is at any rate no reason for referring to the fifth.

In the adult *Scyllium* this nerve retains the relations to other nerves which it has clearly acquired by stage N; it is described in the adult by Schwalbe<sup>3</sup> as "dieser scheinbare Zweig des Oculomotorius." In *Mustelus* Schwalbe describes it as a branch of the fifth. We much regret that we have had no opportunity of studying the development of

<sup>1</sup> For these commissures in Elasmobranchs, *vide* Balfour, *op. cit.*, pp. 158–160, and Pl. XI, fig. 18, and Pl. XIV, fig. 15b. In the chick, Marshall, 'Quart. Journ. Mic. Sc.', Jan., 1878, Pl. III, figs. 27 and 28.

<sup>2</sup> Marshall, 'Quart. Journ. Mic. Sc.', vol. xix, pp. 300 *seq.*

<sup>3</sup> Schwalbe, 'Das Ganglion Oculomotorius,' p. 16.

this nerve in *Mustelus*: should it prove to arise as in *Scyllium*, then it must definitely be regarded as a branch of the third.

As we have already pointed out, the nerves *N.c* and *N.* together make up the *ramus ophthalmicus profundus* of zootomists, a nerve which seems to have escaped Balfour's notice both in the adult and in the embryo. Balfour does, indeed, in his description of the nerves of the adult *Scyllium*, speak of a *ramus ophthalmicus profundus*, but inasmuch as he says concerning it that "this latter nerve arises from the anterior root of the fifth, separately pierces the wall of the orbit, and takes a course slightly ventral to the superior ophthalmic nerve, but does not (as is usual in *Elasmobranchs*) run below the superior rectus and superior oblique muscles of the eye,"<sup>1</sup> it is clear that he is describing the ophthalmic branch of the fifth and not the true *profundus*, whose existence he has overlooked. There appears to be considerable confusion in the use of the terms *ramus ophthalmicus superficialis* and *ramus ophthalmicus profundus* by different writers, a confusion which our observations on *Scyllium* may help to remove. We find, as already stated, three perfectly distinct nerves to which the term *ophthalmic nerve* can be, and is, applied; of these the two dorsal ones (*v a* and *vii a* of our figures) are the *rami dorsales* of the fifth and seventh nerves, and may be spoken of as the ophthalmic branches of the fifth and seventh nerves respectively. Both these nerves are very superficial along their whole course, and both lie dorsal to all the eye muscles and other contents of the orbit. The two nerves are at first perfectly distinct, but in the adult unite more or less closely together, the extent of their union varying much in different *Elasmobranchs*. The two together constitute the *ramus ophthalmicus superficialis*.

The third of the ophthalmic nerves, the *ramus ophthalmicus profundus*, has a very different course, and is of a totally different nature; it is formed in *Scyllium* by the union of the connecting branch between the fifth and third nerves (*N.c.*) with the branch *N* of the third nerve. It is very definitely characterised by its course ventral to the *superior rectus*, *superior oblique*, and *internal rectus muscles*; by its close relation with the inner wall of the eyeball; by the fact that the ciliary ganglion is either in its trunk or is connected with it directly; by its having at first no branches, and by its close connection with the olfactory nerve.

We believe that the *ophthalmicus superficialis* and *ophthalmicus profundus* always maintain these relations; that the *profundus*, which

<sup>1</sup> *Op. cit.*, p. 194: the italics are our own.

is clearly the nasal nerve of *Mammalia*, is a primitive and very constant nerve, and that it never shifts its position so as to lie dorsal to all the eye muscles, as supposed by Balfour.

The two divisions of the *ophthalmicus superficialis*, on the other hand, appear to be very variable indeed in different Vertebrates; they attain their maximum development in the Elasmobranchs, probably on account of the great development and importance of the organs they supply—the mucous canals. In *Mammalia* the ophthalmic branch of the fifth is represented by the frontal and lachrymal nerves, while the seventh nerve has no ophthalmic branch.

It would hardly be profitable to discuss the various descriptions of these nerves by different writers; we will merely point out here that Schwalbe<sup>1</sup> clearly distinguishes the three ophthalmic nerves. He calls, as we have done, the ophthalmic branches of the seventh and fifth nerves together the *ramus ophthalmicus superficialis*, distinguishing the component parts as *portio major* (VII *a*) and *portio minor* (V *a*) respectively. He also employs the term *ramus ophthalmicus profundus* in the same sense as we have done. Balfour, who was the first to clearly recognise the double nature of the *ophthalmicus superficialis*, is in error in calling the lower portion of it (V *a*) the *ophthalmicus profundus*.

Concerning the other branches of the nerves in question, there can be little doubt that the hyoidean branch (VII *c*) of the seventh and the mandibular branch (V *c*) of the fifth are homologous nerves, supplying respectively the anterior walls of the hyoidean and mandibular arches; and there appear to be good reasons for viewing the nerve (III *c*) as the corresponding branch of the third.<sup>2</sup> All the three nerves in question are either mainly or exclusively motor in function.

The seventh, like the hinder cranial nerves, forks over a visceral cleft—the spiracle. As shown in figs. 11 and 12, there are two branches of the seventh which run down in front of the spiracular cleft, viz., the buccal (VII *d*) and the mandibular (VII *b*), which latter divides almost at once into the palatine and spiracular nerves. The history of their development and a comparison of the branches of the seventh with those of the glossopharyngeal (IX, fig. 12), leave no possible room for doubt that of these the mandibular branch (VII *b*) is the homologue of the anterior branch (IX *b*) of the glossopharyngeal.

<sup>1</sup> "Das Ganglion Oculomotorius," 'Jenaische Zeitschrift,' Bd. xiii., pp. 11 seq.

<sup>2</sup> Marshall, loc. cit., p. 88.

This latter nerve (IX *b*, fig. 12) extends very far forwards in the hyoidean arch, being in this respect very closely imitated by the palatine nerve (VII. *pa*), so that we are disposed to regard the whole of the mandibular division (VII *b*) of the seventh, *i.e.* both palatine and spiracular nerves, as together equivalent to the anterior or hyoidean branch (IX *b*) of the glossopharyngeal.

Balfour<sup>1</sup> describes the mandibular branch of the seventh as being large in the embryo, so large, in fact, that he feels difficulty about identifying it with the adult spiracular nerve. His figures, however, show perfectly clearly that what he describes as the mandibular branch of the seventh is really the nerve we have shown to be the buccal.<sup>2</sup>

The maxillary nerve (V *b*) is, from its time and mode of development, almost certainly to be regarded as the true anterior branch of the fifth corresponding to the mandibular branch of the seventh, although in the absence of a visceral cleft in this region this determination cannot be considered absolutely proved. Whether there is any equivalent branch of the third nerve is very doubtful; at any rate no such branch can be pointed out with certainty.

There now remains for consideration the buccal nerve, the determination of which, as a branch of the seventh, is one of the most striking points we have brought to light. Whether this remarkable nerve has any homologue among the branches of the fifth is a point our investigations have not yet enabled us to determine. It is, perhaps, worth while pointing out that there are many points of resemblance between this nerve and the *ramus ophthalmicus profundus*, points of sufficient importance to render a comparison between the two nerves at any rate a possible and suggestive one. In each case the proximal portion of the nerves in question connects together directly the ganglion of one segmental nerve with that of the nerve next in front, while the distal portion passes forward into the segment anterior to that in which the main branches of the nerve are contained. The early origin, the curiously straight course, and the absence of branches until close to their termination, are features common to the two nerves, and ones in which they stand in marked contrast to most other branches. The deep course of the *profundus* as contrasted with the very superficial one of the buccal nerve may perhaps be attributed to the great development of the eye: in front

<sup>1</sup> Op. cit., p. 202.

<sup>2</sup> Op. cit., Pl. XIV, fig. 2 and fig. 15 *a*.



of the orbit the *profundus* is a superficial nerve, and, like the buccal, is purely sensory in its distribution.

On the other hand, it must be noticed that, as already pointed out, the evidence is distinctly in favour of the distal portion of the *profundus* (beyond the ciliary ganglion) being a branch of the third rather than of the fifth nerve. Another point of distinction between them lies in the fact that the former (the *profundus*) is distributed to what is morphologically the dorsal surface, while the buccal supplies the ventral surface.

In the present paper we have purposely refrained from attempting to determine the homologies between the nerves of *Scyllium* and those of other vertebrates, preferring to wait until, by the study of the development of other types, we are enabled to bring forward positive evidence in support of our determinations.

In conclusion we would express the hope that, by working out the development of the roots and branches of the cranial nerves in a very typical vertebrate, and following these roots and branches through their subsequent changes up to their adult condition, we have rendered more practicable than has hitherto been the case comparisons between the descriptions of embryos and of the corresponding adult animals, and have contributed something towards the establishment of comparative neurology upon a firm and satisfactory basis.

## DESCRIPTION OF PLATES V—VI.

All the figures are taken from sections of *Scyllium* embryos. Figures 1, 2, 3, 4, 5, 6, 8, and 13, were drawn from single sections. Figures 7 and 9 each represent two sections, the right hand half of the figure being in both cases taken from a section a short distance posterior to that represented in the left hand half. The remaining figures, viz. 10, 11, 12, 14, 15, 16, and 17, are of a diagrammatic nature, each being constructed by combining a number of sections from the same embryo; in these, as in the other figures, the outlines were drawn by means of a Hartnack camera.

The numbers attached indicate, in diameters, the magnifying power employed.

*Alphabetical List of References.*

*aud.*, auditory vesicle; *Br.* 1, first branchial arch; *c.g.*, ciliary ganglion; *cr.*, cerebellum; *h.b.*, hind-brain; *Hy.*, hyoid arch; *inf.*, infundibulum; *l.*, lens; *m.*, muscle; *m.b.*, mid-brain; *m.c.*, mucous canal; *Mn.*, mandibular arch; *n.*, notochord; *N.*, nerve from ciliary ganglion to fore part of head, forming the distal portion of the *ramus ophthalmicus profundus*; *N.c.*, communicating branch between third and fifth nerves, forming the proximal part of the *ramus ophthalmicus profundus*; *N.c'*, communicating branch between fifth and seventh nerves; *o.c.*, optic cup; *o.i.*, *obliquus inferior*; *olf.*, olfactory pit; *o.s.*, *obliquus superior*; *pit.*, pituitary involution from mouth; *r.e.*, *rectus externus*; *r.i.*, *rectus inferior*; *r.int.*, *rectus internus*; *r.s.*, *rectus superior*; *spr.*, spiracle, or hyomandibular cleft; 1, first head cavity; 2, second head cavity; 3, third head cavity; I, olfactory nerve; II, optic nerve; III, third, or oculomotor nerve; III *b*, branch of third nerve to *rectus superior*; III *c*, branch of third nerve to *obliquus inferior*; III  $\beta$ , ? secondary root of III; III  $\gamma$ , anterior roots of III; IV, fourth nerve, or *patheticus*; V, fifth, or trigeminal nerve; V *a*, ophthalmic branch of fifth nerve; V *b*, maxillary branch of fifth; V *c*, mandibular branch of fifth; V  $\alpha$ , primary dorsal root of fifth; V  $\beta$ , secondary root of fifth; V  $\gamma$ , anterior, or tertiary roots of fifth; VI, sixth, or *abducens* nerve; VII, seventh, or facial nerve; VII *a*, ophthalmic branch of seventh nerve; VII *b*, mandibular branch of seventh; VII *pa*, palatine division of mandibular branch; VII *sp*, spiracular

division of mandibular branch; VII *c*, hyoidean branch of seventh; VII *c* 1, *ramus mandibularis externus*, or branch of hyoidean to mandibular arch; VII *c* 2, *ramus mandibularis internus*, or terminal branch of hyoidean to walls of third head cavity; VII *d*, buccal branch of seventh; VII *a*, primary, or dorsal root of seventh; VII  $\beta$ , secondary root of seventh; VIII, eighth, or auditory nerve; IX, ninth, or glosso-pharyngeal nerve; IX *a*, *ramus dorsalis* of ninth nerve; IX *b*, hyoidean branch of ninth; IX *c*, branch of ninth to first branchial arch; X, tenth, or pneumogastric nerve; X *b*, anterior branch of tenth nerve in first branchial arch.

Figs. 1 & 2. Transverse sections through the hind-brain of a *Scyllium* embryo, intermediate between stages I and K.  $\times 90$ .

Fig. 1, showing dorsal (primary) and ventral (secondary) roots of fifth nerve; also the second or mandibular head cavity.

Fig. 2, showing origin of dorsal (primary) roots of seventh nerve from neural crest; also the third or hyoidean head cavity.

Figs. 3 & 4. Transverse sections through the hind-brain of an embryo of stage K.  $\times 90$ .

Fig. 4, showing the fifth nerve, arising by its ventral or secondary root alone.

Fig. 3, showing the dorsal and ventral roots of the seventh nerve.

Figs. 5 to 7. Transverse sections through the hind-brain of an embryo of stage N.  $\times 20$ .

Fig. 5, showing the ventral roots of the fifth nerves, the ophthalmic branches of the seventh nerves, and the terminations of the maxillary branch of the fifth and the buccal branch of the seventh nerve; also the first head cavity.

Fig. 6, showing the dorsal and ventral roots of the seventh nerve, the buccal branch of the seventh and maxillary branch of the fifth, and the connection between these two nerves; also the second head cavity.

Fig. 7 shows on the left side the ventral root of the seventh nerve, the termination of the sixth nerve in the *rectus externus*, the mandibular branch of the fifth nerve, the

second head cavity, and the termination of the palatine branch of the seventh nerve. On the right side, which represents a more posterior section, are shown the origin of the sixth nerve, the auditory nerve and vesicle, the palatine branch of the seventh nerve, and the second head cavity.

Figs. 8 & 9. Transverse sections through the hind-brain of an embryo of stage o.  $\times 17$ .

Fig. 8, showing the ventral root of the fifth nerve; also the optic nerves and infundibulum.

Fig. 9, on the left side shows the dorsal and ventral roots of the seventh nerve, and the terminal branches of the buccal and maxillary nerves; on the right side the auditory vesicle, the seventh nerve with its hyoidean branch, and the termination of the palatine nerve.

Fig. 10. Longitudinal and vertical section through the head of an embryo of stage L. The outline is from one section, while the details have been filled in from several consecutive sections in order to show the roots of the fifth and seventh nerves, the relations of the third, fifth, and seventh nerves to the head cavities, and the connections of these three nerves with one another. The figure also shows the glossopharyngeal nerve with its *ramus dorsalis*, and its hyoidean branch.  $\times 25$ .

Figs. 11 & 12. Diagrammatic longitudinal and vertical sections through the head of an embryo of stage N; as in the preceding figure the outline was drawn from one section, and the details from other sections of the same embryo.

The two figures together show the roots and all the branches, with their terminal distribution, of the fifth and seventh nerves, the connections of these nerves with one another and with the third nerve, the principal branches of the third nerve, and the course and distribution of the fourth and ninth nerves, at stage N. No single section could show all or even the greater number of the parts represented in either of these figures, as they lie at very different levels.  $\times 25$ .

Fig. 11, drawn to show the whole course and relations of the fifth nerve and its connections with the third and seventh nerves. The figure also shows the fourth nerve, and the terminal distribution of the hyoidean branch of the seventh.

Fig. 12 shows the whole course of the seventh nerve and its branches, with the exception of the hyoidean branch (shown in the preceding figure); also the course and distribution of the ninth nerve, and certain branches of the fifth and tenth nerves.

Fig. 13. Longitudinal and vertical section through the head of an embryo of stage N; the figure, which is taken from two consecutive sections, shows the origin and main trunk of the third nerve, the root of the fourth, and the roots, course, and distribution to the *rectus externus* of the sixth nerve.  $\times 20$ .

Figs. 14 & 15. Diagrammatic longitudinal and vertical sections through the head of an embryo of an age intermediate between stages O and P.  $\times 15$ .

Fig. 14 shows the roots of origin of the third, fifth, and seventh nerves together with the course and distribution of certain of their branches and the mutual connections between these three nerves.

Fig. 15 shows the dorsal root of the seventh nerve, the ophthalmic branches of the fifth and seventh nerves, the *ramus ophthalmicus profundus*, the fourth nerve, the maxillo-mandibular branch of the fifth, and the buccal and palatine branches of the seventh.

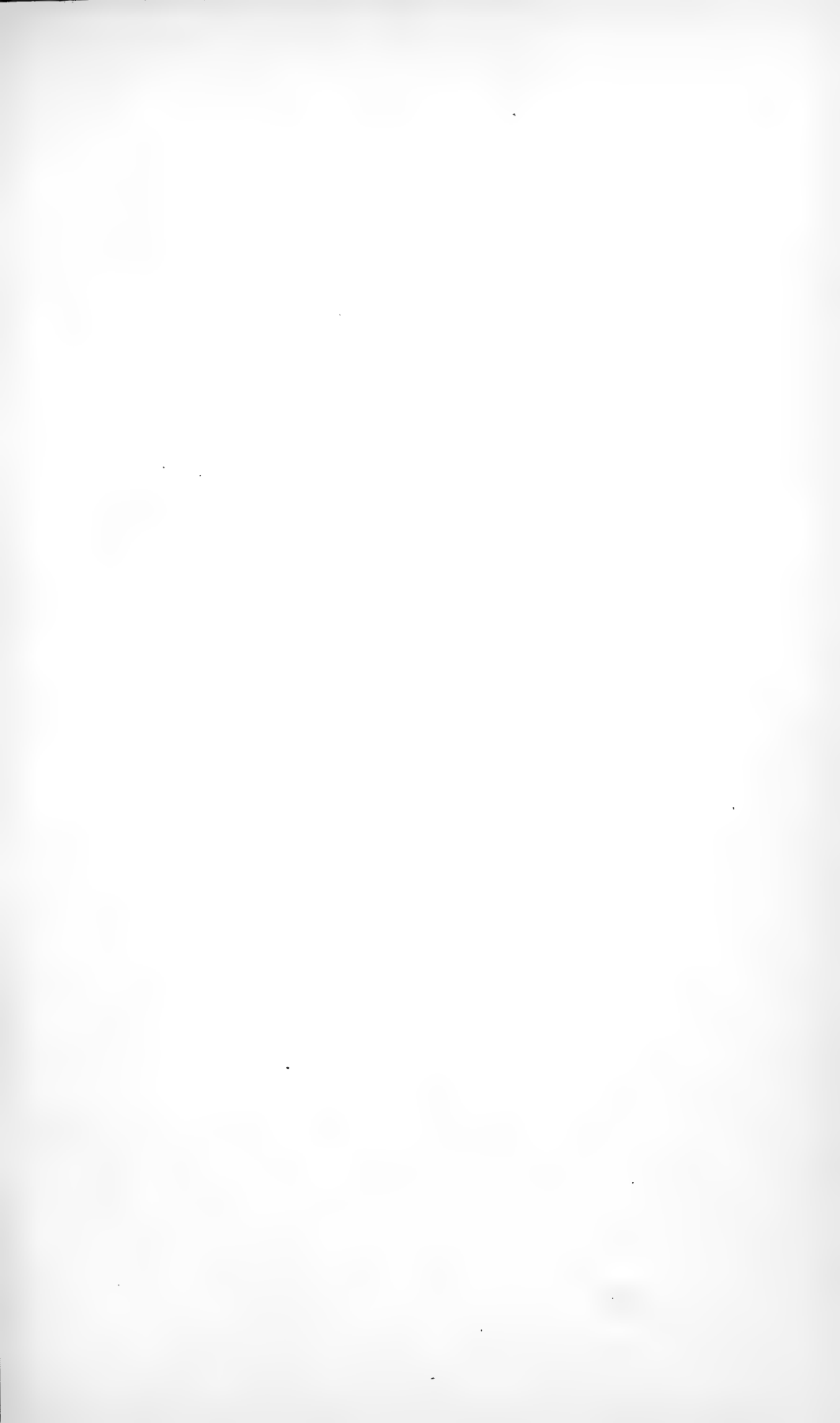
Fig. 16. Transverse section through the head of an embryo of stage Q, shortly before the period of hatching. Shows the origin, course, and distribution of the fourth nerve, and its close proximity to the ophthalmic branches of the fifth and seventh nerves.  $\times 10$ .

Fig. 17. Diagrammatic longitudinal and vertical section through the head of an embryo of stage N. Shows the position and arrangement of the segmental cranial nerves.  $\times 20$ .

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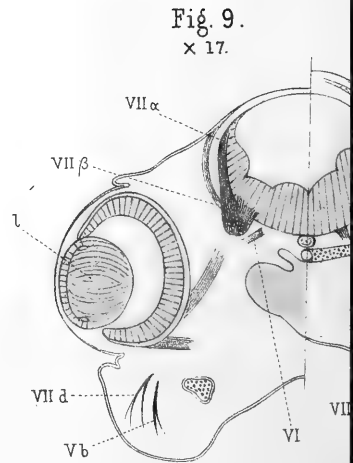
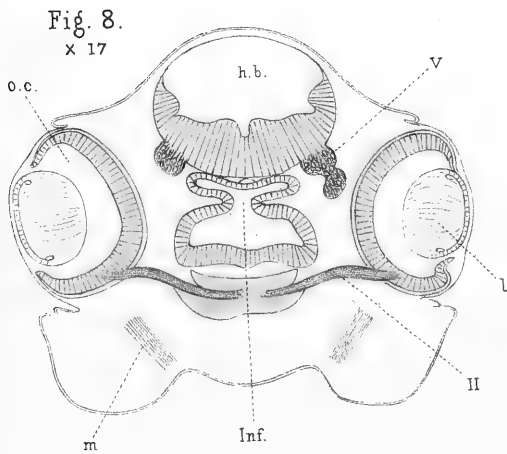
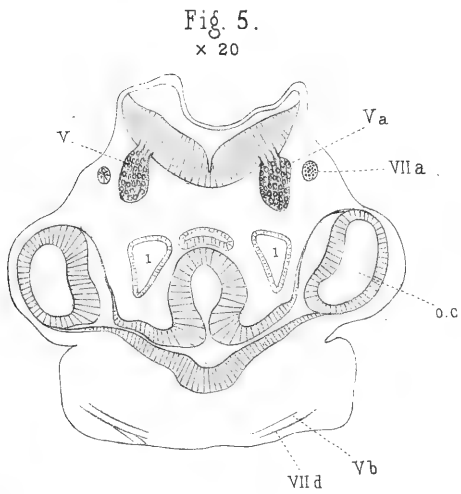
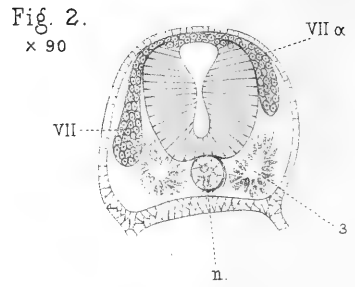
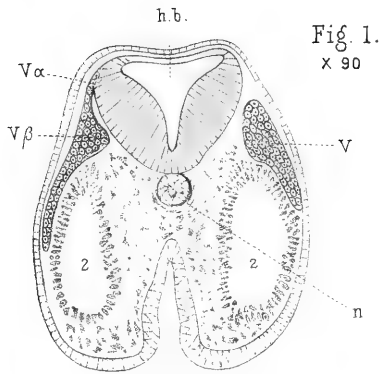




Fig. 3.  
x 90.

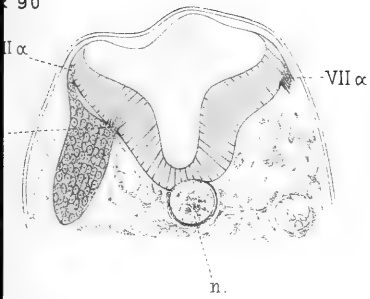
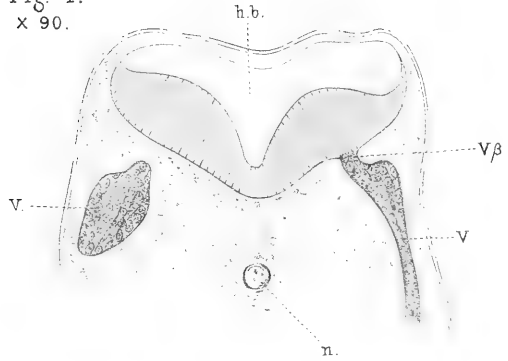


Fig. 4.  
x 90.



6.  
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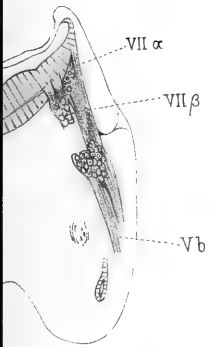


Fig. 7.  
x 20

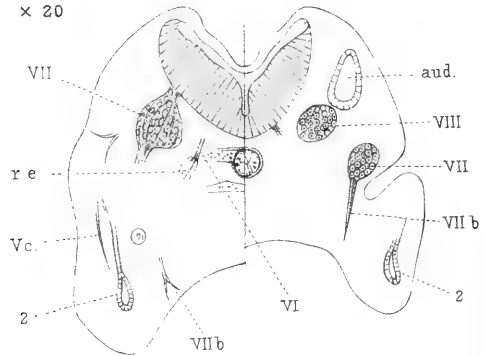
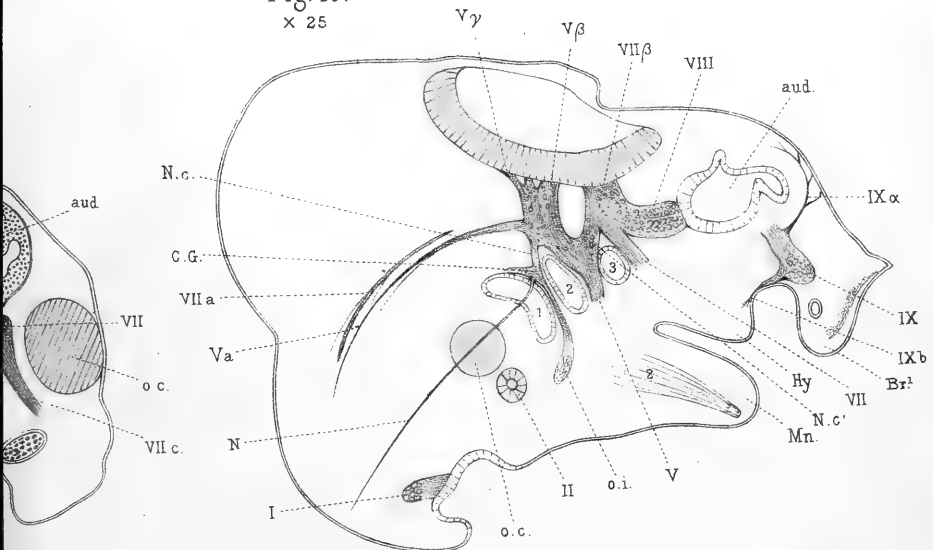
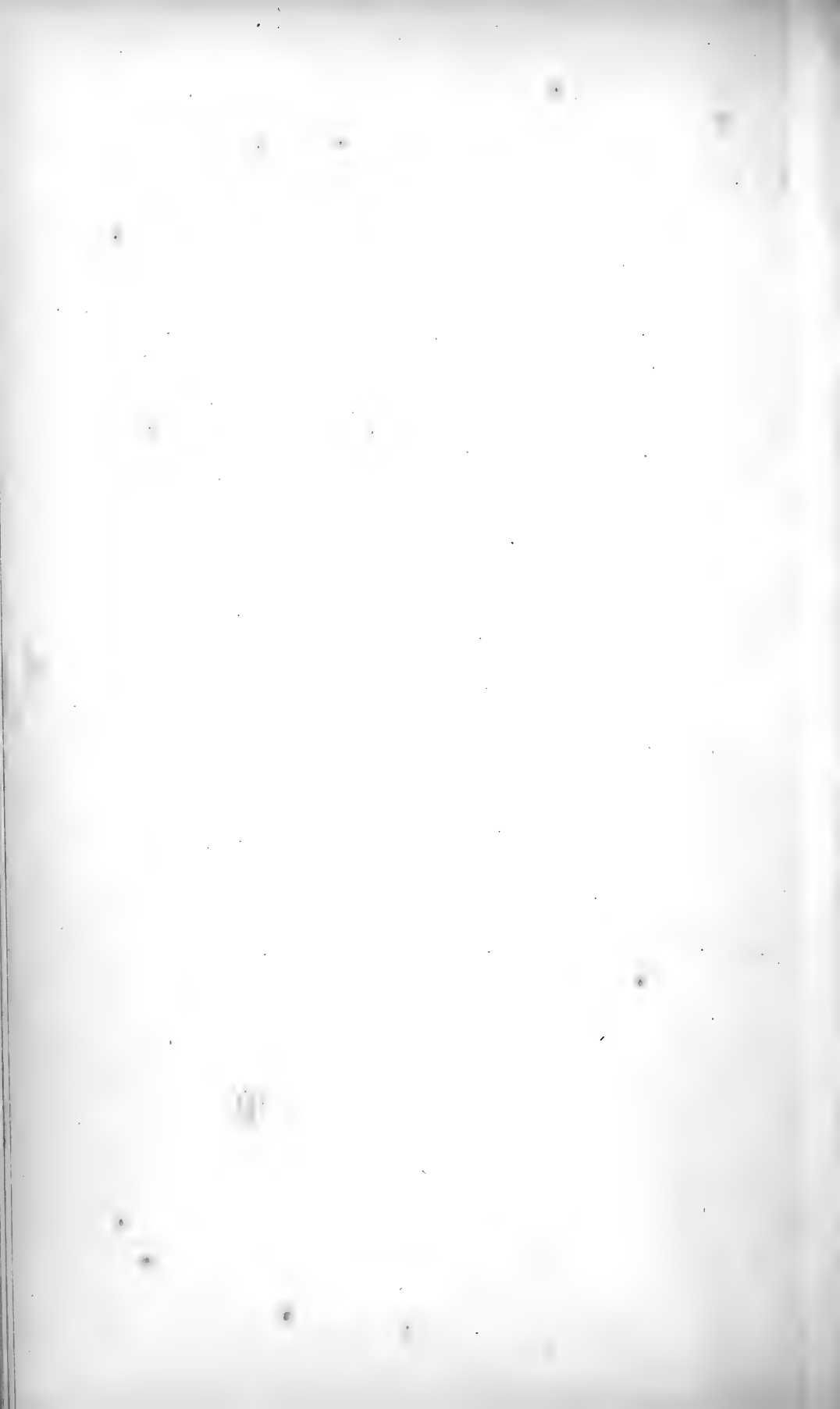


Fig. 10.  
x 25







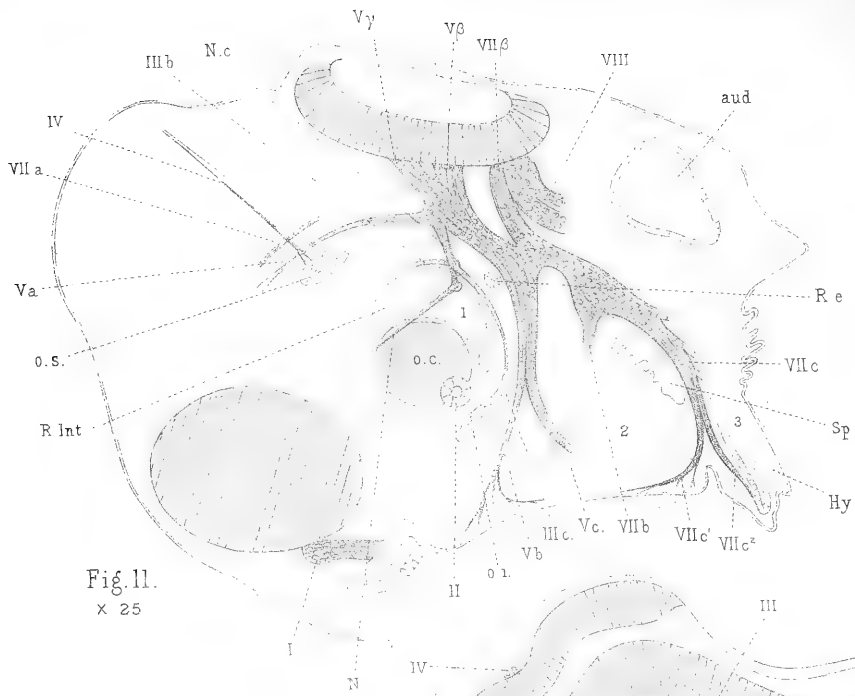


Fig. 13. x 20

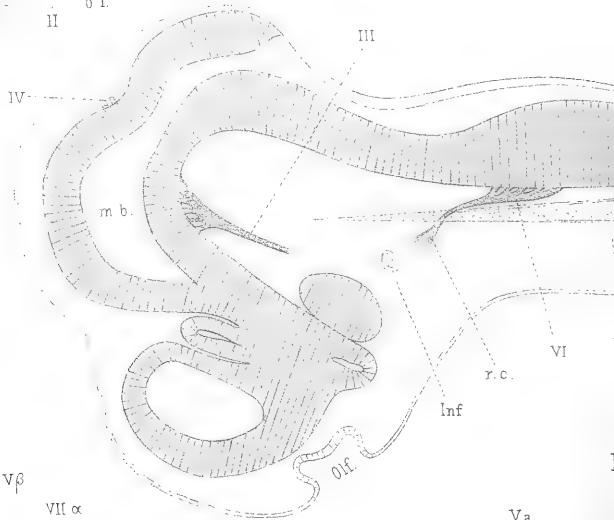


Fig. 14. x 15

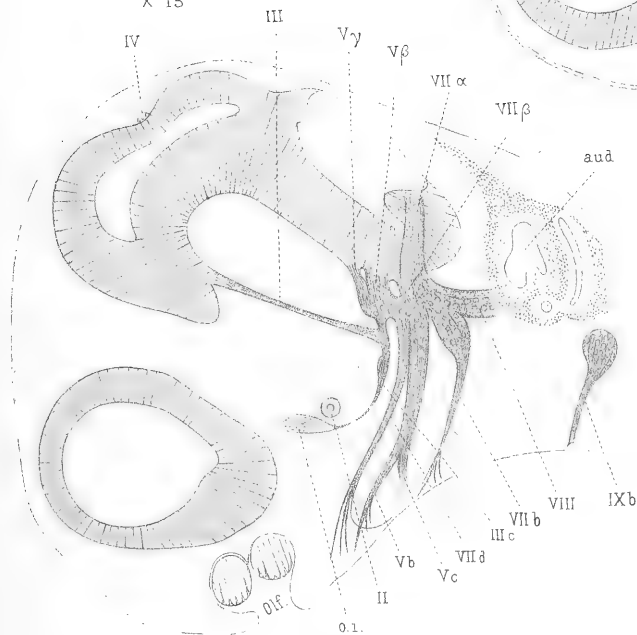


Fig. 12.  
x 25

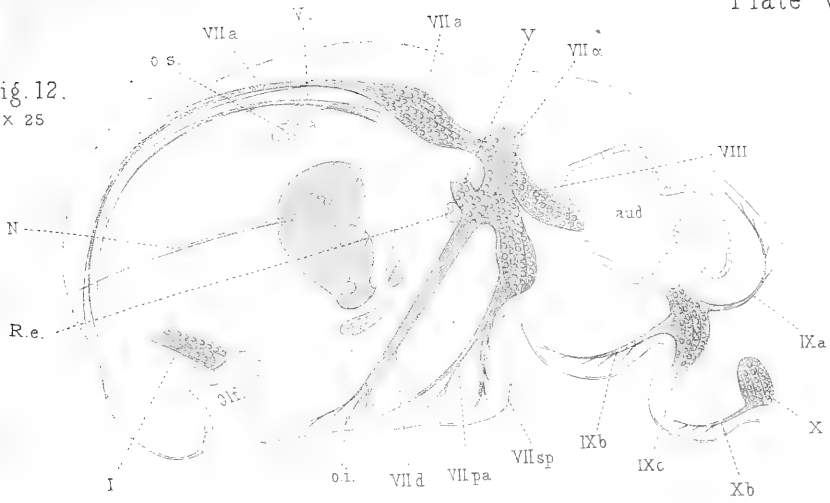


Fig. 17.  
x 20

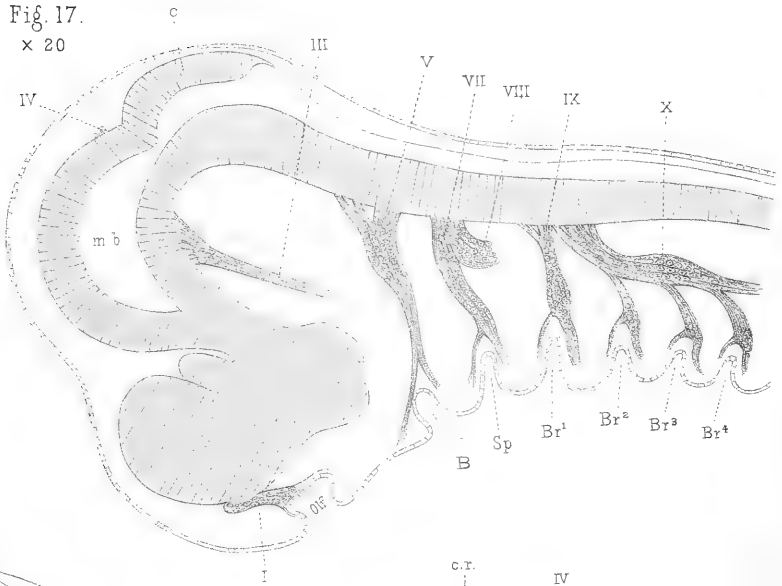
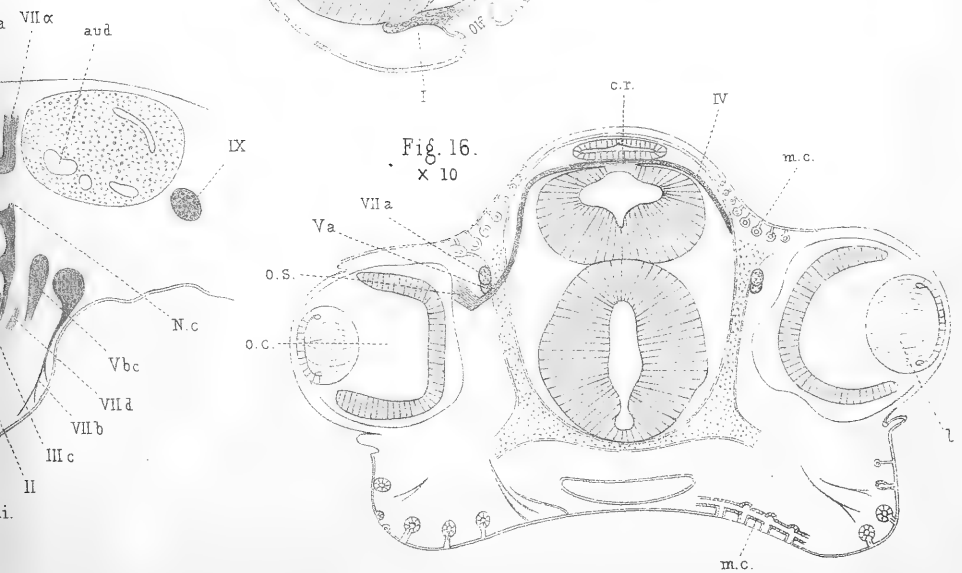


Fig. 16.  
x 10





## THE SEGMENTAL VALUE OF THE CRANIAL NERVES.

*By A. MILNES MARSHALL, M.A., D.Sc., Fellow of St. John's College,  
Cambridge; Beyer Professor of Zoology in Owens College.*

[The references are to Plates V & VI of the preceding paper.]

Whether the nerves arising from the brain are directly comparable to those taking their origin from the spinal cord, and, if so, to how many pairs of the more symmetrically arranged spinal nerves the cranial ones are equivalent, are questions which have attracted the attention and exercised the ingenuity of many of the greatest anatomists, and which have been answered in the most varied senses by the different writers who have attempted to grapple with their difficulties. So long as the problems were attacked from the morphological side alone, as was the case with all the earlier attempts to solve them, the answers obtained were vague, inconclusive, and mutually contradictory; but since the clear light of embryology has been directed upon them, the clouds of uncertainty have been to a very considerable extent dispersed, and there is now, especially amongst those who have most recently dealt with these questions, a very considerable and satisfactory agreement as to the main outlines of the answers to be given, although in many points of detail there is still much discrepancy between the several accounts.

The present paper is an attempt to set forth the actual position of these problems, and the leading phases through which they have passed in their gradual maturation. In preparing it I have made

use of the investigations of others, so far as known to me, as well as of my own published in this *Journal* and elsewhere.<sup>1</sup>

HISTORICAL SKETCH.—The older writers relied exclusively on anatomical evidence in dealing with the problems before us, and their determinations were rather of the nature of guesses than logical endeavours to grapple seriously with the difficulties encountered. Moreover, in the great majority of cases their judgment was influenced in a very prejudicial manner by preconceived ideas on the morphological constitution of the skull.

Inasmuch as these older theories are all based on the same arguments, and differ from one another only in points of minor importance, it will be sufficient to take one of them and examine it critically. For this purpose I select the theory advanced by Stieda, the most recent, indeed the only recent, advocate of the views in question.

Stieda,<sup>2</sup> in attempting to solve the problem of the segmental value of the cranial nerves, commences by stating that as he accepts Oken's theory that the skull consists of three vertebræ, the number of pairs of segmental cranial nerves must necessarily be two; viz., a pair leaving the skull between the first and second skull-vertebræ on either side, and a pair emerging between the second and third skull-vertebræ, the nerves passing out between the skull and the first cervical vertebra being universally considered, when present, the first pair of *spinal* nerves.

Having in this very summary manner determined the number of segmental cranial nerves, Stieda proceeds to divide the nerves actually present into two groups in accordance with this determination. He first rejects the nerves of special sensation, *i.e.* the olfactory, optic, and auditory, on the ground that *embryology shows them to be really parts of the brain, and therefore not directly comparable with the other nerves.*

Concerning the remaining nine pairs of nerves still left for consideration, he holds that the most reliable evidence is afforded by the fact that *in certain groups of animals some of these nerves do not arise independently from the brain, but are represented by branches of other nerves.*

<sup>1</sup> A list of the works consulted is given at the end of this paper.

<sup>2</sup> Stieda, *Studien über das centrale Nervensystem der Wirbelthiere*, Leipzig, 1870. *Separat-Abdruck aus der Zeitschrift für wissenschaftliche Zoologie*, Bd. xx., p. 166, *seq.*



Reasoning from these data, Stieda comes to the conclusion that the component factors of his first cranial segmental nerve are the third or oculomotor, the fourth or trochlear, the fifth or trigeminal, the sixth or abducent, and the seventh or facial nerves; and that of these the third, fourth, sixth, and seventh nerves, and the motor root of the fifth together represent the anterior or motor root, while the sensory portion of the fifth nerve is the representative of the posterior or sensory root. In support of these conclusions he adduces the following arguments:—

1. That the three eye-muscle nerves and the facial nerve may sometimes be replaced by branches of the trigeminal,<sup>1</sup> and therefore may be considered as belonging primarily to that nerve.

2. That the three eye-muscle nerves, the facial nerve, and the *portio minor* of the trigeminal behave with reference to their origin from the brain like the anterior roots of the spinal nerves; the *portio major* of the trigeminal, on the contrary, like a posterior root: meaning by this, the relations of the nerves in question to the nuclei of origin within the substance of the brain.

The second or posterior cranial segmental nerve he considers to be made up of the ninth or glossopharyngeal, the tenth or vagus, the anterior roots of the eleventh or spinal accessory, and the twelfth or hypoglossal nerves; the ninth, tenth, and anterior roots of the eleventh making up the posterior root, and the twelfth nerve representing the anterior or motor root, the main grounds of determination being the same as those relied on in the case of the supposed first nerve.

I have quoted Stieda at some length mainly in order to direct attention to the nature of the evidence on which he attempts to solve the question. The main points on which he relies are contained in the passages I have italicised above, viz., (1) *that the nerves of special sense are contrasted with the other cranial nerves as being, properly speaking, parts of the brain and not nerves in the strict sense of the word*; and (2) *that in certain groups of animals one or more of the cranial nerves may lose their more usual independent character and appear as, or be replaced by, branches of some other nerve; and further, that this is to be taken as indicating that the nerves in question were originally branches of this other nerve, and that their independent origin from the brain, when it does occur, is a secondarily acquired feature.*

<sup>1</sup> All the cases in which this replacement is alleged to occur will be discussed later on in this paper.

Now these two points are of primary importance, forming, as is at once seen, the whole basis of Stieda's argument; and in relying on them he is very far from standing alone. Indeed, until some five or six years ago, their correctness has been assumed, either tacitly or explicitly, by the great majority of those who have dealt with the question, including some of the most eminent anatomists of the time, such as J. Müller,<sup>1</sup> Arnold,<sup>2</sup> Langer,<sup>3</sup> Gegenbaur,<sup>4</sup> and, though in a somewhat less positive manner, Huxley.<sup>5</sup> I direct attention to this at once, because we shall find further on that there are very strong reasons for holding that neither of the points in question is really correct. I have taken Stieda as the most recent representative of a school to which C. V. Carus, Arnold, Buchner, J. Müller, Langer,<sup>6</sup> and many other prominent anatomists belonged, a school which attacked the problem of the segmental value of the cranial nerves by first determining perfectly independently the number of segments or skull-vertebræ in the head, a determination made as a rule on very insufficient and often purely fanciful grounds, and having thus decided the *number* of segments, and therefore of segmental nerves, proceeding to apportion the several nerves to these segments, usually in a very arbitrary manner. The writers named above differ, indeed, in the number of head-segments they respectively adopt, but agree in the principle on which they work, viz., *determining the number of segmental nerves from that of the supposed segments or vertebræ composing the skull.*

Stannius was the first to deal with the question in a more philosophical spirit, and to attempt to determine the number of segmental nerves by a direct study of the nerves themselves. The results of his investigations<sup>7</sup> are contained in his invaluable treatise on the Peripheral Nervous System of Fishes published in 1849. He leaves the three nerves of special sense out of consideration for the same reason as Stieda and the other anatomists we have mentioned, *i.e.* that they are rather parts of the brain than true nerves. He also omits the

<sup>1</sup> Joh. Müller, *Handbuch der Physiologie des Menschen*, 1844, p. 631.

<sup>2</sup> Arnold, *Handbuch der Anatomie des Menschen*, 1851, Bd. ii. pp. 830-834.

<sup>3</sup> Langer, *Lehrbuch der Anatomie des Menschen*, 1865, p. 429.

<sup>4</sup> Gegenbaur, "Über die Kopfnerven von Hexanchus," *Jenaische Zeitschrift*, Bd. vi. 1871, pp. 548-551.

<sup>5</sup> Huxley, *The Anatomy of Vertebrated Animals*, 1871, pp. 71-74.

<sup>6</sup> An excellent summary of the views of these and other writers on the segmental value of the cranial nerves will be found in Stieda's paper already quoted. They all agree in principle with the account given above, the differences being merely in points of detail.

<sup>7</sup> Stannius, *Das periphere Nervensystem der Fische*, Rostock, 1849, pp. 125-131.

eye-muscle nerves, remarking that any attempts to homologise them with spinal nerves "encounter insuperable difficulties on account of their peculiar origin, their absence of ganglia, and their exclusive distribution to the muscles of a sensory apparatus, which are in no way comparable with the muscles of the vertebræ." The remaining nerves, however, Stannius deals with in a very complete and masterly manner. He considers that the fifth, seventh, ninth, and tenth nerves are each equivalent to a spinal nerve, and compares in detail both the roots of origin and the branches of these nerves with those of the spinal nerves.

Stannius was also the first to point out the very important relations of the ventral branches of these segmental cranial nerves to the visceral arches. In the essay quoted above he shows how each visceral arch is supplied by two branches belonging to different nerves, one running along its anterior border, and one along the posterior. He points out how the first branchial arch is supplied along its anterior border by the glossopharyngeal nerve, and along its posterior by the vagus; how the remaining branchial arches are supplied by the vagus, each arch by branches from separate stems; how the hyoid arch is supplied in front by the hyoidean branch of the facial nerve, and behind by the anterior branch of the glossopharyngeal; how the mandibular arch has the mandibular branch of the trigeminal nerve along its anterior border, and along its posterior the anterior branch of the facial, which he identifies as the *chorda tympani* of *Aves* and *Mammalia*; and finally, how the upper jaw is supplied by the ophthalmic and maxillary divisions of the fifth nerve.

He concludes this portion of his treatise with the following very suggestive sentence:—"Hence it follows that the number of the ventral branches of each cranial nerve, and the number of the spinal-like (segmental) cranial nerves is not determined so much by the number of the skull-vertebræ as by that of the visceral arches."<sup>1</sup>

In thus stating that the number of segmental cranial nerves was no longer to be determined by preconceived ideas concerning the composition of the skull, but by direct examination of the nerves themselves, Stannius rendered an invaluable service to morphology. Had he, indeed, gone one step further; had he been able to completely disabuse his mind of this notion of skull-vertebræ which was exercising so pernicious an influence on the zoologists of the day, he would have

<sup>1</sup> Stannius, *op. cit.* p. 131

anticipated by more than twenty years Gegenbaur's announcement<sup>1</sup> of that theory of the vertebrate skull which has since, with some slight modifications, been accepted almost universally.

While the school of morphologists we first dealt with determined the number of the segmental nerves by that of the skull-segments, Stannius showed conclusively that there was no relation whatever between the two, but that there was a very definite and remarkable one between the segmental nerves and the visceral arches. Gegenbaur went a step further, and, starting with the segmental nerves and visceral arches, determined from them the number of head-segments, thus completely reversing the order of proceeding of the older school.

Gegenbaur is sometimes credited with being the first to establish the relations of the cranial nerves to the visceral arches, a determination which, as we have seen, had been already made by Stannius. The often quoted table of the cranial nerves given by Gegenbaur,<sup>2</sup> contains, in fact, nothing that had not been already pointed out by Stannius, except an attempt to rank the labial cartilages as visceral arches, an attempt which has not met with general acceptance. Gegenbaur's real merit consisted in pointing out that the ideal number of skull-vertebræ, as determined by Oken and other "transcendental anatomists," was to be left out of consideration altogether; that the evidence offered by the cranial nerves and visceral arches was to be accepted in full, and was to be taken as the basis for determining the number of segments in the head; and that the vagus nerve was, from the fact of its supplying more than one visceral cleft, to be considered as equivalent to more than one segmental nerve, and to be regarded as formed by the fusion of a certain number of primitively distinct nerves.

Thus it has come to pass that the cranial nerves, while formerly considered of very subordinate importance, are now recognised as affording a very valuable and reliable clue to the solution of that favourite morphological problem—the segmentation of the vertebrate head; and Gegenbaur's paper, which was undoubtedly the chief means by which the cranial nerves were rescued from their former dependent position, must be viewed as marking a most important era in its history.

Attention being thus pointedly directed to the cranial nerves, their comparative anatomy and embryology quickly engaged the attention

<sup>1</sup> Gegenbaur, "Ueber die Kopfnerven von Hexanchus," *Jenaische Zeitschrift*, 1871.

<sup>2</sup> Gegenbaur, *loc. cit.* p. 552.

of zoologists; and during the last five or six years our knowledge on these points has received very material additions, additions which have, on the whole, tended strongly to confirm Gegenbaur's views, while causing modification of them in many secondary points.

The most important of these more recent contributions is undoubtedly the series of facts brought to light by Balfour concerning the early stages of development of the spinal and cranial nerves in Elasmobranch fishes. Balfour showed that,<sup>1</sup> contrary to the generally accepted theory, the nerves are outgrowths from the central nervous system, and therefore of epiblastic origin, instead of being, as formerly supposed, structures arising independently in the mesoblast and only acquiring a secondary connection with the brain and cord.

In the case of the spinal nerves, he showed that the two roots, anterior and posterior, arise separately and independently; that the posterior roots are local outgrowths of a continuous longitudinal band—the neural crest—which grows out along the mid-dorsal line of the spinal cord. By lateral growth of the dorsal summit of the cord the nerve roots of the two sides, which are at first directly continuous with one another across the top of the cord, become separated to a certain extent. The nerve root on either side grows downwards, closely applied to the side of the cord, it then acquires a new or secondary attachment<sup>2</sup> to the side of the cord, some little distance below the primary one. A little later the primary attachment disappears, and the secondary alone remains as the permanent attachment of the posterior root to the cord.

The anterior roots arise later than the posterior, each as an independent conical outgrowth from the latero-ventral angle of the cord. The roots grow rapidly, and soon form elongated bands of fusiform cells, which retain their original points of origin from the cord. Each is at first, and for some time, quite distinct from the posterior root, with which, however, it subsequently unites to form the adult spinal nerve.

Further differences between the anterior and posterior roots are afforded by the fact that the posterior develops at a very early period a large ganglionic swelling—the future spinal ganglion—

<sup>1</sup> Balfour, "On the Development of the Spinal Nerves in Elasmobranch Fishes," *Phil. Trans.* vol. clxvi. pt. 1, 1875; and *A Monograph of the Development of Elasmobranch Fishes*, 1878, pp. 156-161 and 191-205.

<sup>2</sup> The account of this shifting is based on my own observations. Balfour expresses himself as "inclined to adopt this view" (*Comparative Embryology*, vol. ii. p. 372), but does not definitely do so.

while the anterior root is devoid of ganglion cells. The roots of origin of the anterior root are also very generally multiple, while those of the posterior roots, whether primary or secondary, are apparently invariably single.

Balfour's observations were soon extended to birds and mammals, and the description given above is now recognised as that of the general and typical mode of development of the vertebrate spinal nerves. It was further found that the neural crest is not confined to the spinal cord, but extends forwards along the top of the brain, and that certain of the cranial nerves are developed from it in the same way as the posterior roots of the spinal nerves. By this discovery a new and very reliable clue to the segmental value of the cranial nerves is obtained, for it is clear that if certain of the cranial nerves do, and others do not, conform to the mode of development of the typically segmental spinal nerves, there is strong reason for regarding the former as being of segmental value, and the latter as not.

Embryology has furnished us with one further test of the segmental value of cranial nerves, for which again we are indebted to Mr. Balfour, who has shown that in Elasmobranchs (and the observation has since been extended to other groups) the two halves of the cœlom or body-cavity at an early period extend forward on either side of the neck into the head, and that on the appearance of the visceral clefts each of the halves becomes cut up in a series of isolated compartments, one in each visceral arch.<sup>1</sup> If the visceral clefts and arches are segmental, it is clear that these "head-cavities," as they are called, must be also, and that they will therefore afford an additional clue to determining the segmental value of the nerves associated with them.

SUMMARY OF EVIDENCE OF SEGMENTAL VALUE OF CRANIAL NERVES.—From what has been said above it will be evident that we have now several independent tests of the metameric or segmental value of the cranial nerves,—tests with *all* of which a nerve ought to comply to entitle it to rank as segmental. For convenience of reference, these tests, the majority of which have already been discussed, may be enumerated here :<sup>2</sup>—

<sup>1</sup> Balfour, *Elasmobranch Fishes*, pp. 206-209; also Marshall, "Head-Cavities and Associated Nerves of Elasmobranchs," *Quart. Journ. of Micros. Science*, January 1881.

<sup>2</sup> Cf. Marshall, "The Morphology of the Vertebrate Olfactory Organ," *Quart. Journ. of Micros. Science*, July 1879, p. 317.

1. Segmental nerves develop at a very early stage as outgrowths from the neural ridge on the dorsal surface of the brain.

2. At an early period they shift downwards, and acquire new or secondary roots of attachment to the sides of the brain.

3. The general course of the main stem of a segmental nerve is at right angles, or nearly so, to the axis of the head at the point of origin of the nerve. This feature, which is explained more fully in the paper quoted above, is evident from an inspection of fig. 17, in which the directions of the segmental nerves are shown, and from the consideration that the course of segmental nerves must be approximately parallel to the boundaries of the segments to which they belong: a segmental nerve could not run transversely across a number of segments.

4. Segmental nerves have the characteristic relations to the visceral clefts and arches, and, therefore, also to the head cavities in these arches, first pointed out by Stannius as noticed above, each nerve supplying the borders of one cleft, and therefore of two arches. Concerning this test, it may be noted that, although from the constancy of the relations of visceral clefts to other structures in all vertebrates above *Amphioxus*, there can be no doubt that Gegenbaur, Huxley, Semper, and others are correct in maintaining the segmental value of these clefts, yet that the total absence of any correspondence between the visceral clefts and the body segments in *Amphioxus*, and still more in the Ascidians, makes it very doubtful whether this segmental character is a primitive one.

5. Segmental nerves very constantly present ganglionic enlargements, either at or near their points of division into their two main ventral branches.

Having thus cleared the ground, and explained what we mean by a segmental nerve, and why it is of importance to determine which of the cranial nerves are of segmental value, and which are not, I propose to consider these nerves and discuss their claims in order, beginning with the most anterior ones, and taking them in the sequence usually adopted by anatomists.

I. THE FIRST OR OLFACTORY NERVE.—This nerve was until recently supposed, by reason of its development, to stand quite apart from the rest of the cranial nerves, and to be, properly speaking, a part of the

brain rather than a nerve in the strict sense of the word.<sup>1</sup> Instead of developing like the other nerves, the olfactory was stated to arise as a hollow outgrowth from the anterior part of the cerebral hemisphere—the so-called olfactory lobe or vesicle: it was also stated to arise considerably later than the posterior cranial nerves.

It is now known that these supposed distinctions between the olfactory and the other nerves do not really obtain,<sup>2</sup> but, on the contrary, that the olfactory nerves develop in precisely the same way as the other cranial nerves; that they arise at first from the upper part of the fore-brain and gradually shift downwards, acquiring by so doing a secondary connection with the cerebral hemispheres, of which they are at first completely independent; and, finally, that the olfactory lobe or vesicle so far from being the earliest part to be developed is actually the last, no vestige of it appearing in the chick until the seventh day of incubation, in the salmon till long after hatching, or in dogfish until stage O of Balfour's nomenclature.

If, then, the olfactory nerve agrees in all important features of its development with the other cranial, and the spinal nerves, the further question at once suggests itself,—has it segmental value? An examination of the evidence at our disposal, which is unfortunately far from complete, shows that there is much to be said in favour of such a view; thus, applying to the olfactory nerve the several tests of the metameric value of cranial nerves in the order given above, we obtain the following results:—

1. The olfactory nerve develops very early: the actual date of its first appearance is very difficult to determine, and has not yet been ascertained with certainty in any case, but in both the chick and the dogfish it appears at a very early stage of development, and in the chick, indeed, an attempt has been made to show that the olfactory nerve is “one of the first nerves in the body to appear,”<sup>4</sup> arising before any of the spinal nerves. There is also evidence, though as yet inconclusive, in favour of the origin of the olfactory nerve in the chick from the neural crest.

<sup>1</sup> Vide, e.g., Huxley, *Anatomy of Vertebrated Animals*, p. 71; and Gegenbaur, *Elements of Comparative Anatomy*, English Translation, p. 515.

<sup>2</sup> Marshall, “Morphology of Vertebrate Olfactory Organ,” *Quart. Journ. of Micros. Science*,” July 1879; and Balfour, *Comparative Embryology*, vol. ii, 1881, pp. 336 and 382.

<sup>3</sup> I have dealt with this question at some length in a former paper on “The Morphology of the Vertebrate Olfactory Organ,” *Quart. Journ. of Micros. Science*, July 1879, to which I would beg to refer the reader who may desire further details than I can give here.

<sup>4</sup> Marshall, “The Development of the Cranial Nerves in the Chick,” *Quart. Journ. of Micros. Science*, Jan. 1878, p. 23.



2. The olfactory nerve resembles the segmental nerves in undergoing during the earlier stages of its development a very considerable displacement of its root of attachment to the brain, and as this feature is one of the most remarkable characters of these segmental nerves, and is, so far as we know, confined to them, its occurrence in the olfactory nerve must be admitted to be of much weight.

In both the dogfish and chick the olfactory nerves are clearly recognisable before the cerebral hemispheres have commenced to develop, the nerves at this stage arising from the dorsal part of the sides of the original fore-brain or anterior cerebral vesicle. The hemispheres in the chick are lateral outgrowths of the fore-brain, and are from the first situated above, *i.e.* on the dorsal side of, the roots of the olfactory nerves; they grow forwards and upwards with great rapidity, driving the olfactory nerves down to the base of the brain, and so causing these nerves to appear to arise from their under and anterior part. Whether the root of the olfactory nerve undergoes any change comparable to the secondary attachment described above as occurring in the spinal nerves, has, however, not yet been ascertained.

3. The general course of the olfactory nerve in the early stages of development is, like the segmental nerves, at right angles to the axis of the head at the point of origin of the nerve, although, owing to cranial flexure, it is very far from being parallel to the hinder segmental nerves. This feature is shown in fig. 17, I. In the later stages of development, owing to the forward growth of the nasal region this relation becomes completely lost.

4. Concerning the relations of the olfactory nerve to visceral arches and clefts, I must beg to refer the reader to the paper quoted above, in which I have drawn attention to "the very close resemblance as to form, structure, general relations, time of appearance, &c., existing between the olfactory organ and the gill clefts," and have adduced other arguments on which I have attempted to establish the following conclusions:—"That the olfactory organ is the most anterior visceral cleft; that the olfactory nerve is the segmental nerve supplying the two sides of that cleft in a manner precisely similar to that in which the hinder clefts are supplied by their respective nerves; and that the Schneiderian folds are homologues of gills."<sup>1</sup>

<sup>1</sup> "Morphology of Vertebrate Olfactory Organ," *Quart. Journ. of Micros. Science*, July 1879, p. 330.

5. The olfactory nerve is distinctly ganglionic near its root of origin from the brain in Elasmobranchs and in the chick.

It would thus appear that although the evidence is at present far from conclusive, and although further information is needed on many points, notably concerning the earliest stages of development of the olfactory nerve, yet that the nerve answers fairly well to the tests of segmental value as defined above; and it is important to note that the points in which it responds incompletely are precisely those on which our knowledge of the nerve is avowedly imperfect, and that in no case is a test directly contradicted. I am therefore disposed, while fully admitting the need for further investigation, to rank the olfactory nerve as the most anterior of the cranial segmental nerves, the nerve belonging to the first head-segment.

The segmental value of the olfactory nerve has recently been advocated by Wiedersheim, who draws attention to the fact that in *Epicrion*, and probably in other *Gymnophiona* as well, there are on either side two olfactory nerves, one dorsal and one ventral, the roots of the two being perfectly independent, and some little distance apart.<sup>1</sup> Wiedersheim considers that these two roots are homologues of the dorsal and ventral roots of a spinal nerve, and that by their discovery the segmental rank of the olfactory nerve may be considered to be established.

A similar condition of the olfactory nerve in *Pipa dorsigera* has been figured, though not described, by Fischer.<sup>2</sup>

These two cases, in both of which the additional root is the dorsal one, tend strongly to confirm the view taken above of the primitive connection of the olfactory nerve with the dorsal surface of the brain, and therefore presumably with the neural crest; but in the absence of any observations on either the development or the physiological properties of the two roots in question, I do not think that much weight can be attached to Wiedersheim's suggestion of their homology with the roots of a spinal nerve.

Balfour<sup>3</sup> argues against the segmental value of the olfactory nerve, on the ground that it is incompatible with the views which he holds concerning the primitive vertebrate mouth, and concerning the relations between the nervous systems of vertebrates and invertebrates. His

<sup>1</sup> Wiedersheim, *Die Anatomie der Gymnophionen*, Jena, 1879, pp. 59, 60, and pl. iv. fig. 35, pl. vi. fig. 62.

<sup>2</sup> Fischer, *Amphibiorum nudorum neurologiæ specimen primum*, 1843, Tab. ii. fig. 1.

<sup>3</sup> Balfour; *Comparative Embryology*, vol. ii. 1881, pp. 260-265 and 383.

views on these points are of very great interest and importance ; but inasmuch as they involve the descent of Chætopods and Vertebrates, not from a common segmented ancestral type, but from a common unsegmented type, and also the existence of a group of segmented animals, which “appears now to have perished” without leaving any trace behind, it would clearly be impossible to discuss them here in full. His theory that the vertebrate fore-brain is the homologue of the supra-oesophageal ganglia of Arthropods and Chætopods is, however, to my mind open to very serious objections, some of the more weighty of which he has himself mentioned, viz., (1) that there is no actual anatomical or embryological break between the fore-brain and the hinder portion of the central nervous system, such as one might reasonably expect to find on his hypothesis ; (2) that the lowest known vertebrate, *Amphioxus*, instead of lending any support to this view, distinctly contradicts it, the fore-brain being less differentiated from the hinder portion than in any other vertebrate, while “the termination of the notochord immediately behind the fore-brain”—almost the only direct evidence he adduces in favour of the “morphological distinctness” of the fore-brain—again fails completely, the notochord in *Amphioxus*, as is well known, extending to the extreme anterior end of the head, some distance beyond the front end of the brain.

II. THE SECOND OR OPTIC NERVE.—Although, as we have just seen, the statement that the olfactory nerve is rather a part of the brain than a nerve in the strict sense of the word, is found on examination not to hold good, yet, as regards the optic nerve, it is certainly correct ; the mode of development of the optic nerve, which is too well known to require a detailed description here, placing it in this respect in marked contrast to every other nerve in the body.

From the fore-brain or anterior cerebral vesicle two hollow lateral outgrowths arise—the optic vesicles. These become constricted at their origin from the brain, the constricted portions or optic stalks becoming ultimately the optic nerves. By a process of unequal growth of the different parts, coupled with a direct pushing in of the outer wall by the formation of the lens, each vesicle becomes doubled up on itself, the outer wall being pushed back into the inner, and so giving rise to the double-walled “optic cup” or secondary optic vesicle.

! This mode of development, which, with secondary modifications,

applies to all vertebrates except *Amphioxus*, and must therefore be considered as primitive so far as vertebrates are concerned, differs so fundamentally from the development of the hinder cranial or spinal nerves that no comparison whatever is possible between them. The optic nerve must therefore be regarded as one *sui generis*, and which can accordingly have no claim to be considered of segmental value.

The existence of this clearly non-segmental nerve between the olfactory and the hinder nerves is undoubtedly an objection to the view advocated above concerning the segmental value of the olfactory nerve; but until we obtain a clearer light than we are at present able to throw on the phylogenetic history of the vertebrate eye, and indeed of the vertebrate race altogether, it is difficult to gauge properly the weight of the objection.

**THE EYE-MUSCLE NERVES.**—Concerning the morphological value of these three nerves—the third, fourth, and sixth pairs—opinions have perhaps differed more than in the case of any of the other cranial nerves.

The nerves in question are small, with a singularly limited and constant distribution to the muscles moving the eyeball, and to certain other parts in connection with the eye, the third nerve supplying the *rectus superior*, *rectus internus*, *rectus inferior*, and *obliquus inferior* muscles of the eye ball, also the *levator palpebræ superioris* and the circular muscle of the iris; the fourth nerve supplying the *obliquus superior* muscle, and in many vertebrates sending sensory branches to the conjunctiva and the skin of the upper eyelid; and the sixth nerve supplying the *rectus externus* muscle, and in many cases the suspensory muscle of the bulb of the eye and the muscles of the nictitating membrane. In dealing with these it will be convenient to consider them at first collectively, inasmuch as many points of importance concern them all alike, and afterwards to consider briefly the several points of individual interest which they present respectively.

Until very recently it was the almost universal custom amongst anatomists, when discussing the segmental value of the cranial nerves, to exclude the eye-muscle nerves altogether from consideration, on the ground that *they were not constant in their distribution, but that one or more of the muscles normally supplied by them might under special circumstances be supplied by branches of the fifth nerve*, the further

inference being drawn from these special cases that the eye-muscle nerves were primitively branches of the fifth nerve, which have in the majority of existing vertebrates attained independence and acquired the appearance of distinct nerves, a title to which they have really no claim.<sup>1</sup>

This view has very recently been advocated by Wiedersheim, whom I quote in order to illustrate my statements. In dealing with the fourth nerve in the frog he notices that it usually forms anastomotic communications with the ophthalmic branch of the fifth nerve as it crosses it, and that the number of these communicating branches is very variable. He then says:—"Dies eben beschriebene Verhalten sowie auch dasjenige des *Abducens* und des später abzuhandelnden *Oculomotorius* liefert eine hübsche Illustration zu der in höheren Thiergruppen in immer stärkerer Weise hervortretenden Tendenz der Augenmuskelnerven, sich von ihrem Stammboden, der Trigemini-gruppe, zu emancipiren, um endlich eine gut individualisirte Selbstständigkeit zu erlangen."<sup>2</sup>

As this view, so definitely expressed by Wiedersheim in the above passage, appears to have met with very general acceptance, and as it very seriously affects and concerns the subject of the present paper, I have taken some trouble to collect all the recorded cases in which the distribution of the eye-muscle nerves or the supply of the eye-muscles in vertebrates is said to present any constant deviation from the normal arrangement as noticed above; and I propose now to examine critically these alleged exceptions to the general rule.

1. *Amphioxus*.<sup>3</sup>—The azygos character of the eye and its extreme simplicity of structure render any comparison with the eyes of higher and more typical vertebrates perfectly futile.

## 2. *Marsipobranchii*.

(a) *Hyperotreti*.—Among the myxinoid fishes, according to Stannius<sup>4</sup>, J. Müller,<sup>5</sup> Huxley,<sup>6</sup> Gegenbaur,<sup>7</sup> and others, the eye-muscle nerves are completely absent. Here again we are dealing with animals

<sup>1</sup> *Vide, e.g.*, Gegenbaur, "Ueber die Kopfnerven von *Hexanchus*," *Jenaische Zeitschrift*, 1871, pp. 548, 549; Huxley, *Anatomy of Vertebrated Animals*, 1871, p. 73; also Stieda and the various authors quoted by him in his "Studien ueber das centrale Nervensystem der Wirbelthiere," *Zeitschrift für wissenschaftliche Zoologie*, Bd xx. 1870.

<sup>2</sup> Wiedersheim, in Ecker's *Anatomie des Frosches*, Zweite Abtheilung, 1881, p. 24, note 1.

<sup>3</sup> Stannius, in his *Handbuch der Anatomie der Wirbelthiere*, Zweite Auflage, 1854, p. 161, notices the absence of the eye-muscle nerves in *Amphioxus*.

<sup>4</sup> Stannius, *op. cit.*, p. 161.

<sup>5</sup> J. Müller, *Vergleichende Neurologie der Myxinoiden*, p. 49.

<sup>6</sup> Huxley, *Vertebrates*, p. 73.

<sup>7</sup> *Hexanchus*, p. 549.

in which the eyes are in a very rudimentary condition, and the eye-muscles either absent or extremely imperfectly developed; so that, as pointed out by Schwalbe,<sup>1</sup> no importance can be attached to them in determining the question of the primitive independence of the eye-muscle nerves, and this consideration is much strengthened by the strong evidence we possess of the Myxinoids being degenerate or degraded forms.<sup>2</sup>

(b) *Hyperoartii*.—Attention has been directed to the condition of the eye-muscle nerves in the lampreys by a number of writers. According to Schlemm and d'Alton,<sup>3</sup> the lampreys have independent eye-muscle nerves, but their number is diminished, and some of the muscles are supplied by the fifth nerve. The fourth nerve is described as having its usual origin behind the optic lobes and entering the orbit in company with the third, which has an independent origin in front of that of the fifth. The combined nerve, formed by the union of the third and fourth, divides into two main branches, an *upper one* supplying the *rectus superior*, and a *lower one* supplying the *rectus internus* and *obliquus superior*. The three other muscles, viz., *rectus inferior*, *rectus externus*, and *obliquus inferior*, are said to receive their nerves from the trunk of the fifth nerve.

Fischer<sup>4</sup> and Stieda<sup>5</sup> also refer to the peculiar distribution of the eye-muscle nerves in the lampreys, but avowedly draw their information from Schlemm and d'Alton's paper, from which it would appear that Huxley,<sup>6</sup> and probably Owen<sup>7</sup> and Günther<sup>8</sup> also, derive their accounts.

Gegenbaur<sup>9</sup> gives a slightly different account. He says that in *Petromyzon* there is an independent fourth nerve, but that the sixth is a branch of the fifth, which supplies the *rectus inferior* as well as the *rectus externus*, while the third nerve is limited in its distribution, supplying the *rectus superior*, *rectus internus*, and *obliquus inferior*. He gives no reference in support of his statement, and must therefore be supposed to make it on his own authority, especially as it differs

<sup>1</sup> Schwalbe, "Das Ganglion Oculomotorii, *Jenaische Zeitschrift*, Bd. xiii., p. 71.

<sup>2</sup> Cf. Balfour, *Comparative Embryology*, vol. ii., 1881, p. 263, note 2.

<sup>3</sup> Schlemm u. D'Alton, "Ueber das Nervensystem der Petromyzon," *Müller's Archiv*, 1838

<sup>4</sup> Fischer, *Amphibiorum nudorum Neurologice Specimen Primum*, 1843, p. 47.

<sup>5</sup> Stieda, *loc. cit.*, p. 174.

<sup>6</sup> Huxley, *Anatomy of Vertebrated Animals*, p. 73.

<sup>7</sup> Owen, *Anatomy of Vertebrates*, 1866, vol. i., p. 300.

<sup>8</sup> Günther, *Introduction to the Study of Fishes*, 1850, p. 105.

<sup>9</sup> Gegenbaur, *Hexanchus*, p. 549, note 1.

notably from the accounts of all other writers whom I have been able to consult.

Concerning the above accounts, it appears that they can be reduced to two sources—(1) the description given in 1838 by Schlemm and d'Alton, which I have assumed to be the source from which Owen, Huxley, and Günther obtain the accounts given in their text-books quoted above, because their descriptions, which are very brief, agree exactly with that of Schlemm and d'Alton, and add nothing to it; and (2) Gegenbaur's description in 1871, which must be independent, inasmuch as it does not quite agree with Schlemm and d'Alton's. According to Gegenbaur, the only peculiarity is that the sixth nerve is not independent but a branch of the fifth, which supplies the *rectus inferior* as well as the *rectus externus*; while, according to Schlemm and d'Alton, three of the muscles—the *rectus inferior*, *rectus externus*, and *obliquus inferior*—are supplied by the fifth nerve; and, in addition to this, the third and fourth nerves unite together, a point which Gegenbaur does not notice.

The dissection is a difficult one, on account of the small size of the nerves concerned; and additional evidence from direct observation is necessary before we can decide whether either of the above descriptions is perfectly correct.

There are, however, certain points of considerable importance which concern not only *Petromyzon*, but many other forms as well, and may be conveniently dealt with here.

Both the third and fourth nerves are distinctly stated to have independent roots of origin, and to arise from the normal situations in the brain; and this being the case, I wish to point out that the anatomical arrangement of the nerves would probably be more correctly described by saying that the third nerve, though having a separate root of origin, becomes connected with the fifth, so that in the adult some of its branches appear to be derived from the fifth; than by saying, with Huxley, Stieda, and Günther, that the muscles in question are supplied by branches of the fifth.

Fischer long ago adopted this view. He describes, on Schlemm and d'Alton's authority, the condition of the nerves in *Petromyzon* in these words:—“Genus *Petromyzon* duas Oculomotorii ostendit partes, alteram liberam, parisque quarti quoque continentem fibras, alteram cum Trigemino conjunctam;”<sup>1</sup> and Stannius gives still clearer ex-

<sup>1</sup> Fischer, *op. cit.*, p. 47, note 1.

pression to it ; for after referring to Schlemm and d'Alton's observations, he says :—" Offenbar ist hier ein Theil der Wurzelemente des N. oculorum motorius, so wie auch die Wurzel des N. abducens, in die Bahn des N. trigeminus, übergetreten,"<sup>1</sup> and remarks that it is quite possible that a very careful examination of the nerve-roots would show that the *abducens* has really an independent root of origin.

The point at issue is an important one, and must be clearly stated. When we find two nerves—the third and fifth, which in the great majority of vertebrates are independent of one another both in origin and distribution—in certain forms, as the lampreys, arising from the brain independently and normally, but becoming united together at some point or other of their course, so that it is no longer possible from mere anatomical observation to say with certainty to which of the two a given branch belongs, are we to infer, as is done tacitly or explicitly by many writers,<sup>2</sup> that the condition shown by the lamprey is the more primitive one, and represents an intermediate stage in the process by which the eye-muscle nerves gradually emancipated themselves from their parent nerve—the fifth—and attained ultimately the complete independence they show in the great majority of existing vertebrates? Or, on the other hand, are we to infer that the independent origin of the third nerve is primitive, and that its connection with the fifth, when, as in the lamprey, it does occur, is a secondarily acquired one? To my mind there can be no doubt whatever that the latter is the correct explanation ; and the chief reasons that lead me to think so are the following :—

(a) Though we know of instances—notably in the case of the vagus—of nerves originally distinct and independent gradually becoming fused, and then this fusion getting thrown back to a very early developmental stage ; yet we know of no established case of a branch attaining independence, and acquiring the character of a distinct nerve.

(b) Supposing it were possible for such a process to occur, it would certainly be very surprising if, as in the supposed case of the third nerve, the process of differentiation should commence at the proximal end, and that there should be a stage in which the roots were independent and the two nerves still fused distally.

(c) There are very strong reasons, which we shall discuss later on,

<sup>1</sup> Stannius, *Das peripherische Nervensystem der Fische*, p. 18.

<sup>2</sup> Cf. the authors mentioned above, and especially the passage quoted from Wiedersheim on p. 139 above.



for viewing both the third and fifth nerves as segmental, and therefore primitively independent of one another.

(d) If Wiedersheim's view were correct, we should certainly expect the third nerve of higher vertebrates in its early stages of development to show some indication of its supposed primitive connection with the fifth. So far, however, is this from being the case, that in all cases where the development of the third nerve has yet been traced, it is a perfectly independent nerve from the start.<sup>1</sup>

(e) A crucial test is afforded by the fact that other nerves—*e.g.* the fifth and seventh—though, as a rule, separate from one another throughout the vertebrate series, may in some forms become more or less closely united together, so that it is impossible by mere anatomical evidence to distinguish branches of the one from those of the other; the forms in which this fusion of the fifth and seventh nerves occurs being, as we shall see more fully later on, in many cases the same as those in which the fifth and the eye-muscle nerves tend to fuse. In the case of the dog-fish, in which this fusion of the fifth and seventh nerves is a marked feature of the adult state, all the stages of development are now known,<sup>2</sup> and it is found that, so far from the state of fusion being a primitive one, the two nerves are in their early stages quite independent and some distance apart, as in other vertebrates, and that their subsequent gradual approximation and fusion are purely secondary characters.

The above arguments appear to me to establish the proposition that the third nerve is primitively an independent one,<sup>3</sup> and that its partial fusion with the fifth, when it occurs, is a purely secondary and not a primary character.

If they prove the case for the third nerve, so also for the fourth and sixth nerves. The presence of independent roots of origin from the brain must be held to establish that, however close may be the connection of their trunks with the fifth nerve, they are really independent nerves, and are *not* to be described as being "replaced by branches of the fifth nerve."

<sup>1</sup> Marshall, "On the Development of the Cranial Nerves in the Chick," *Quart. Journ. of Micros. Science*, Jan. 1878, pp. 23—27; and "On the Head Cavities and Associated Nerves of Elasmobranchs," *Quart. Journ. of Micros. Science*, Jan. 1881, pp. 78—88.

<sup>2</sup> Marshall and Spencer, "Observations on the Cranial Nerves of Scyllium," *Quart. Journ. of Micros. Science*, July 1881, pp. 482—486.

<sup>3</sup> The independence of the third nerve has recently been upheld on anatomical grounds by Schwalbe—*Das Ganglion Oculomotorii*; and by Balfour, on embryological ones—*Comparative Embryology*, vol. ii.

In the case of the lampreys, then, I hold that we have no reliable evidence of the third or fourth nerves being in any way abnormal in their distribution to the eye-muscles; while, as regards the sixth nerve, although no distinct root of origin has yet been seen, I hold, with Stannius, that a much more careful and searching investigation must be made for it before any statement as to its absence can be accepted.

3. *Ganoidei*.—In the majority of Ganoids the nerves of the eye-muscles have the normal arrangement, and are completely independent of the fifth, except where the third unites with the ophthalmic branch of the fifth at the ciliary ganglion. Only one exception is known.

In *Lepidosteus* according to J. Müller,<sup>1</sup> the arrangement is abnormal, the third and fourth nerves entering the orbit closely united with the ophthalmic division of the fifth, of which they appear as branches. The sixth nerve is described and figured as accompanying the main trunk of the fifth, but distinct from it.

Stieda, in his essay<sup>2</sup> before referred to, quotes Müller's account, but does so incorrectly, making Müller say that there is a distinct fourth nerve, but that the third and sixth are replaced by the branches of the fifth; whereas Müller really says that the sixth is a distinct nerve, and that the third and fourth are, not "replaced by branches of the fifth," but contained in the ophthalmic nerve.

Stannius,<sup>3</sup> referring to Müller's account, observes that it is probably merely another instance of juxtaposition of originally distinct nerves.

Concerning this alleged exception, we notice in the first case that it rests on a solitary description, which has not yet been confirmed, and that confirmation is needed is evident from the figures referred to. Müller gives two figures of the cranial nerves of *Lepidosteus*, which do not agree in all points; indeed, the points of difference are so marked that the two figures are by no means easy to reconcile with one another. Müller's figure 3 appears to me to present nothing exceptional, except that the third and fourth nerves enter the orbit as one trunk, and that the fourth nerve at the point where it crosses the

<sup>1</sup> J. Müller, *Ueber den Bau und die Grenzen der Ganoiden*, 1846, p. 97, and plate iv. figs. 2 and 3.

<sup>2</sup> Stieda, *loc. cit.*, p. 174.

<sup>3</sup> Stannius, *Das peripherische Nervensystem der Fische*, p. 19.

*portio minor* of the *ramus ophthalmicus superficialis*<sup>1</sup> is rather more extensively connected with this nerve than is usually the case. The nerve which Müller marks  $\gamma$ , and calls the "ophthalmic branch of the fifth," but which he does not seem to have followed to the brain, I see no reason for considering as other than what one would naturally suppose it to be from its distribution to all the eye-muscles except the *rectus externus*, i.e. the combined third and fourth nerves. In Müller's figure 2 there is a remarkable point of difference, inasmuch as the nerve which I have considered in the former figure to be the proximal part of the *portio minor*, or trigeminal portion of the *ramus ophthalmicus superficialis*, is *entirely omitted*. No mention is made either in the text or in the description of the figures of this very important difference. I would further notice that, although the two figures in question are drawn of the same size and to the same scale, yet that the relative proportions of the several nerves, and more especially the extent to which they are fused with one another, are so very different in the two cases that one is driven to suppose either that the figures are taken from different specimens, in which case there must be considerable individual variability in the very points alleged to be exceptional, or else that one or others of the figures is taken from an incomplete dissection.

The above considerations lead to the conclusion that, in the absence of direct confirmation, Müller's account of the eye-muscle nerves in *Lepidosteus* does not prove that they are in any way exceptional, except in the fact of the third and fourth nerves entering the orbit as one trunk.

Very important information concerning these nerves in *Lepidosteus* has recently been afforded by Schwalbe, who finds, from a careful examination of the nerves and brain, that *both the third and fourth nerves have independent origins from the brain*;<sup>2</sup> a fact which, as in the case of *Petromyzon*, must be held to conclusively prove that such connection as may actually occur between the fifth nerve on the one hand, and the third and fourth on the other, beyond their roots of origin, is of a purely secondary character, and that it does not in the very slightest degree militate against the claims of the third and fourth to rank as independent cranial nerves.

<sup>1</sup> For the nomenclature of these ophthalmic nerves, *vide* Marshall and Spencer, "Observations on the Cranial Nerves of Scyllium," part i., *Quart. Journ. of Micros. Science*, July 1881.

<sup>2</sup> Schwalbe, *Das Ganglion Oculomotorii*, pp. 23, 72, and 73.

4. *Teleostei*.—The only recorded instances that I can find of deviation from the normal arrangement of the eye-muscle nerves among osseous fish are :—

(a) *Amblyopsis*,<sup>1</sup> the blind fish of the Mammoth cave of Kentucky, in which the eyes are rudimentary and functionless, and the eye-muscle nerves, as might be expected, absent.

(b) *Silurus glanis*, in which, according to Stannius,<sup>2</sup> the eyes are small, the eye-muscles very slender, and the eye-muscle nerves *outside the skull* closely united with the ophthalmic branch of the fifth. Stannius points out, however, that careful examination shows that all three nerves arise independently from the brain at the normal situations, and expressly notices that, but for the discovery of these extremely slender roots, the eye-muscle nerves of *Silurus* would have been beyond all doubt described as branches of the fifth nerve. It is of course probable that in the other species of blind fish, whether living in caves, as *Typhlichthys*, *Stygicola*, *Gronias*, *Ailia*, &c., or living at great Ocean depths, as the *Scopelidae*, the eye-muscle nerves are, as in *Amblyopsis spelæus*, rudimentary or absent; but it will be sufficiently evident, from what has been already said, that neither these blind fish nor such cases as *Silurus* tell in any way against the independent rank of the eye-muscle nerves.

5. *Dipnoi*.—In his account of the African *Lepidosiren* (*Protopterus*) *annectens*, Professor Owen<sup>3</sup> notices that the optic nerves “are remarkably small, in correspondence with the feebly-developed organs of vision;” also that the eye-ball “has no special muscles, whence the absence of the third, fourth and sixth cerebral nerves.”

According to Hyrtl<sup>4</sup> in the South American form, *Lepidosiren paradoxa*, in which also the eyes are very small, the four *recti* muscles are present, but the two *obliqui* not represented. The eye-muscle nerves were not found, but were believed to be replaced by two fine branches of the ophthalmic division of the sixth nerve, which branches, however, were not traced into the *recti* muscles.

Professor Humphry's<sup>5</sup> description of *Lepidosiren* (*Protopterus*) *annec-*

<sup>1</sup> Noticed by Stannius, *Das peripherische Nervensystem*, p. 18; and Schwalbe, *loc. cit.*, p. 71.

<sup>2</sup> Stannius, *op. cit.*, pp. 18, 19.

<sup>3</sup> Owen, “Description of the *Lepidosiren annectens*,” *Trans. Linnæan Soc.*, vol. xviii., 1839, p. 340.

<sup>4</sup> Hyrtl, “*Lepidosiren paradoxa*,” Prag. 1845, p. 44.

<sup>5</sup> Humphry, *Observations in Myology*, 1872; *The Muscles of Lepidosiren annectens with the Cranial Nerves*, pp. 77 and 79.

*tens* very closely agrees with Hyrtl's of *L. paradoxa* in the points with which we are now concerned. He finds, contrary to Owen, that the four *recti* muscles "may clearly be distinguished," though there are no *obliqui*. "Special nerves to these muscles (the third, fourth, and sixth) were not found;" but the ophthalmic division of the fifth is described as giving off in the orbit "ciliary and oculo-motor nerves," which, however, do not appear to have been traced to their distribution.

Gegenbaur<sup>1</sup> simply quotes Hyrtl to the effect that all three eye-muscle nerves are represented by branches of the fifth; which, however, is a wider and more positive statement than Hyrtl really made.

Stannius<sup>2</sup> also states, on Hyrtl's authority, that the eye-muscle nerves have no independent roots; and Huxley<sup>3</sup> notes that in *Lepidosiren* "the three motor nerves of the eyeball are completely fused with the ophthalmic division of the fifth," a condition which he is disposed to view as the most primitive arrangement met with among vertebrates.

In considering what importance is to be attached to this often-quoted exception to the general rule, we have first to notice that we are dealing with animals in which the eyes are "very small" and "feebly developed;" secondly, that the eye-muscles are so small that their very existence was not only overlooked, but expressly denied, by so competent an anatomist as Professor Owen; thirdly that the two anatomists, Hyrtl and Humphry, who have described these muscles, agree in saying that the *recti* muscles are alone present, a condition clearly not fully realised by those who state, on Hyrtl's authority, that the fourth nerve is, like the third and sixth, represented by a branch of the fifth; fourthly, that in neither of the cases mentioned were the nerves actually traced into the muscles in question.

To these points we must add one, urged with great force by Schwalbe,<sup>4</sup> and which acquires much weight from the cases of *Petromyzon* and *Lepidosteus* already considered, viz. that a sufficiently careful examination of the brain has not been made to render us certain as to the alleged absence of independent roots of origin for such of the eye-muscle nerves as may be present.

The importance of Schwalbe's warning is strikingly exemplified by

<sup>1</sup> Gegenbaur, *Hexanchus*, p. 549.

<sup>2</sup> Stannius, *Das peripherische Nervensystem*, p. 18.

<sup>3</sup> Huxley, *Anatomy of Vertebrated Animals*, p. 73, note.

<sup>4</sup> Schwalbe, *Das Ganglion Oculomotorii*, p. 72.

the recent observations of Wiedersheim<sup>1</sup> on the nervous system of *Lepidosiren (Protopterus) annectens*. Wiedersheim describes a moderately long but exceedingly slender nerve which leaves the skull through a special foramen in front of that of the fifth, and loses itself in the eye-muscles in a manner which he was unable to determine with certainty. In spite, however, of taking "all conceivable pains," he was unable to ascertain whether this hitherto overlooked eye-muscle nerve arises independently from the brain, or is a mere branch of the fifth, though he is inclined himself to regard it as an independently arising third nerve.

Under these circumstances, and especially when we consider Wiedersheim's discovery of a distinct eye-muscle nerve, and his statement of the extreme difficulty he experienced in tracing this nerve even to the limited extent which he succeeded in doing, we must, I think, conclude that, whatever subsequent investigation may tell us, *Lepidosiren* at present offers no definite or reliable evidence against the statement that the eye-muscle nerves are independently arising nerves in all vertebrates in which the eye-muscles themselves are present.

6. *Amphibia*.—Statements of exceptional innervation of one or more of the eye-muscles among *Amphibia* are by no means uncommon; and though I have devoted some time to making my list as complete as possible, I am far from certain that I have succeeded in collecting all the alleged cases. The following list includes all I have been able to refer to, and certainly all that are mentioned in the standard works and papers on the subject:—

A. *Apoda (Gymnophiona)*.—Wiedersheim, in his monograph on this group,<sup>2</sup> mentions that in *Cecilia* the eye-muscles are present, but of exceedingly small size, so small indeed that he could not make out either their number or arrangement; neither was he able to ascertain anything concerning their innervation; indeed, he makes no mention whatever of the eye-muscle nerves. Fischer<sup>3</sup> also failed, from his dissection of a single specimen, to make out anything definite concerning the eye-muscle nerves. Inasmuch as the eyes of *Cecilia* are very small, it would seem probable that we have here another instance of rudimentary eyes, accompanied very possibly by a reduction in the number of eye-muscles; and we have already seen that the evidence

<sup>1</sup> Wiedersheim, *Morphologische Studien*, Heft 1; III. *Das Skelet und Nervensystem von Lepidosiren annectens*, 1880.

<sup>2</sup> Wiedersheim, *Die Anatomie der Gymnophionen*. Jena, 1879, pp. 55, 56, and 61.

<sup>3</sup> Fischer, *op. cit.*, p. 47.

yielded by such cases cannot be accepted as in any way affecting the question of the primitive independence of the eye-muscle nerves.

B. *Caudata* (*Urodela*).

(a) *Proteus*.—The specimen of *Proteus* dissected by Fischer<sup>1</sup> was, like that of *Cocilia*, too imperfectly preserved to permit him to make any positive statement concerning the eye-muscle nerves; indeed, he calls attention to and expressly regrets his inability to determine whether these nerves are present or absent. The eyes of this cave-dwelling amphibian are situated beneath the skin, and are of very rudimentary structure, being arrested at what is in other vertebrates a very early embryonic condition.<sup>2</sup> As has been pointed out by Schwalbe,<sup>3</sup> Fischer does not in any way deny the existence of eye-muscle nerves, but merely records his inability to find them in a very imperfectly preserved specimen.

(b) *Salamandra* and *Triton*.—I take these two genera somewhat out of their proper zoological order, because they afford perhaps the most widely-known and frequently-quoted examples of abnormal innervation of the eye-muscles—instances which must accordingly be carefully considered.

Fischer, who was the first to draw attention to the point,<sup>4</sup> states that in *Salamandra* and *Triton* the third nerve, though rising independently from the brain, only supplies three of the eye-muscles—the *rectus internus*, *rectus inferior*, and *obliquus inferior*—the *rectus superior* receiving a special branch from the “nasal division” of the fifth, which branch is absent in *Anura* in which the innervation is normal. In discussing the importance of this, he says:—“*Quid igitur veri possit esse similius, quam quod partium duarum, in quas penes Salamandrina divisum sit oculomotorius, altera eandem, quam in Ecaudatis retinuerit formam, altera cum Trigemino se conjunxerit?*” The fourth nerve in the same two genera, according to Fischer, “seems to have coalesced with the fifth pair;” at any rate, he was unable to discover any independent nerve, and the *obliquus superior* muscle is supplied by the “nasal branch” of the fifth. The sixth nerve is perfectly normal both in its origin and distribution; it passes very close to the Gasserian ganglion, but is really distinct from it, and leaves the skull by an aperture distinct from that of the fifth.

<sup>1</sup> Fischer, *op. cit.*, pp. 35 and 47.

<sup>2</sup> For a description and figure of the eye of *Proteus*, *vide Semper, Animal Life, International Science Series*, pp. 73, 79.

<sup>3</sup> Schwalbe, *Das Ganglion Oculomotorii*, p. 72.

<sup>4</sup> Fischer, *op. cit.*, pp. 24, 25, 32, and 47.

Fischer's careful descriptions, which have the great advantage of being illustrated by as careful figures,<sup>1</sup> have been referred to by many writers—Stannius,<sup>2</sup> Gegenbaur,<sup>3</sup> Hoffmann,<sup>4</sup> Stieda,<sup>5</sup> &c.—who, however, have added nothing to our knowledge on the subject from direct observations of their own.

Schwalbe,<sup>6</sup> who appears to be the only anatomist since Fischer's time who has directly investigated this interesting point, has furnished additional information of great value concerning it. He finds, in confirmation of Fischer's statement, that the nerve to the *rectus superior* muscle is derived, not from the third nerve, but from the "nasal branch" of the fifth; but points out that before this nerve is given off the third and nasal nerves cross and lie in very close contact with one another. He considers it probable that at this point there is direct connection between the two, although he was unable to prove it; and he accordingly supports the view, held also by Fischer and Stannius, that the supply of the *rectus superior* by the fifth is only apparent and due to the close connection and partial fusion of the third and fifth nerves at this point of crossing.

Concerning the fourth nerve, Schwalbe's results are more positive, and of great importance. He finds that although in the majority of specimens of *Salamandra maculosa* he dissected, the arrangement described by Fischer obtained, the nerve to the *obliquus superior* appearing as a branch of the nasal nerve, yet that in some cases, one of which he figures,<sup>7</sup> the fourth may be a completely independent nerve, arising from the brain in the normal position.

Reviewing, then, these much-quoted cases of *Salamandra* and *Triton*, we find that Fischer's account of the anatomical arrangement of the nerve is confirmed by Schwalbe. We find that the sixth nerve is perfectly independent both at its root and along its whole course—is, in fact, in every way normal. That the fourth nerve is, as a rule, an apparent branch of the "nasal branch" of the fifth, but, at least in *Salamandra*, may be not uncommonly an independent nerve, normal in every respect. That the third nerve always arises independently from the brain; that it crosses the "nasal branch" of the fifth, lying

<sup>1</sup> Fischer, *op. cit.*, tab. ii., fig. 2 (*Salamandra*), and fig. 3 (*Triton*).

<sup>2</sup> Stannius, *Das peripherische Nervensystem*, p. 19.

<sup>3</sup> Gegenbaur, *Hexanchus*, p. 549, note 1.

<sup>4</sup> Hoffmann, *Bronn's Thierreich*, Bd. vi., Heft ii., *Amphibia*, p. 204.

<sup>5</sup> Stieda, *loc. cit.*, p. 174.

<sup>6</sup> Schwalbe, *Das Ganglion Oculomotorii*, pp. 25—27.

<sup>7</sup> Schwalbe, *Das Ganglion Oculomotorii*, Tab. xiii., fig. 13.



in close contact with it as it does so; and that it supplies only three muscles—the *rectus internus*, *rectus inferior*, and *obliquus inferior*—the *rectus superior* receiving its branch from the “nasal nerve,” and this branch coming off beyond the point of crossing of the third and nasal nerves; and that this condition of things is interpreted by both the writers who have investigated it directly—Fischer and Schwalbe—as merely implying that the third nerve has become partially fused with the fifth.

Concerning this “nasal nerve,” from which, in the two genera in question, the branch to the *rectus superior* always, and that to the *obliquus superior* usually, arises, there is a further point of importance. Schwalbe<sup>1</sup> has attempted to prove that this “nasal nerve” really corresponds, in part at least, to the *ramus ophthalmicus profundus* of Selachians. The point could only be decided by a study of the development of this nerve in *Urodela*, of which at present we know nothing; but should Schwalbe prove to be correct, the very slight amount of deviation from the normal condition which we have found to be all that really occurs in *Salamandra* and *Triton* would be still further reduced; for embryology teaches us that the *ramus ophthalmicus profundus* of Selachians is really a connecting branch between the third and fifth nerves, which cannot be said to belong distinctly to either the one or the other, and that the portion of this nerve beyond the point at which it crosses the third nerve, from which portion we have seen that the branch to the *rectus superior* arises, has nothing whatever to do with the fifth, but belongs really to the third nerve.<sup>2</sup>

From what has been said above, I think that no other conclusion can be drawn than that the cases of *Salamandra* and *Triton* do not afford any reason for regarding the eye-muscle nerves as other than independent and constant nerves.

(c) *Menobranchus*.—Gegenbaur<sup>3</sup> states, on Fischer's authority, that in *Menobranchus*, as in *Salamandra* and *Triton*, the fourth nerve is replaced by a branch of the fifth. I have been unable to refer to Fischer's account, so that any discussion of the case would be unprofitable. It is, however, very possible that the condition is really what Schwalbe has shown to occur in *Salamandra*.

<sup>1</sup> Schwalbe, *Das Ganglion Oculomotorii*, p. 26.

<sup>2</sup> Marshall, “Head Cavities and Associated Nerves of Elasmobranchs,” *Quart. Journ. of Micros. Science*, January 1881, p. 89; and Marshall and Spencer, “Cranial Nerves of Scylium,” *Quart. Journ. Micros. Science*, July 1881, pp. 494 seq.

<sup>3</sup> Gegenbaur, *Hexanchus*, p. 549, note 1.

(d) *Siredon*.—Fischer<sup>1</sup> has established that the third and fourth nerves are normal in origin and distribution, but was unable to make out anything definite concerning the sixth nerve.

(e) *Cryptobranchus*.—Schmidt, Goddard, and V. d. Hoeven are quoted by Hoffmann<sup>2</sup> as stating that in the *Cryptobranch* the third and fourth are independent nerves, but that the sixth is a branch of the nasal division of the fifth.

Professor Humphry<sup>3</sup> remarks that the dissection of the cranial nerves is difficult, on account of the "tough areolar tissue of the animal and the numerous accompanying veins." He was unable to "discover the third, fourth or sixth nerves in the orbit." The third and fourth were, however, found in the cranial cavity, but not the sixth.

Here, again, our information is too imperfect to allow definite conclusions to be drawn. If the sixth nerve really appears as a branch of the fifth, it is of importance to note that, as is evident from Professor Humphry's figure, the fifth and seventh nerves are quite distinct from one another—a point to which we shall refer when considering the *Anura*.

C. *Anura*.—The condition of the eye-muscle nerves in *Anura* has been carefully investigated by a number of anatomists, notably by Fischer<sup>4</sup> and Schwalbe.<sup>5</sup> The results of these investigations are as follows:—In all *Anura* that have been examined, the third and fourth are distinct and independent nerves, with normal origin and distribution. In *Pelobates* and *Bombinator* the third leaves the skull by the same foramen as the fifth, with which it is in very close contact, though the two nerves are really distinct.

The sixth nerve in all cases has an independent origin from the brain in the normal position. In *Bufo*,<sup>6</sup> the sixth nerve preserves its independence along its whole course, and is in all respects perfectly normal. In the other *Anura* examined—viz. *Pipa*, *Rana*, *Pelobates*, *Bombinator*, and *Hyla*—the sixth nerve, though arising independently, unites with the Gasserian ganglion, and the branch to the *rectus externus* is derived from the "nasal branch" of the fifth.<sup>7</sup>

<sup>1</sup> Fischer, *Anatomische Abhandlungen über die Perennibranchiaten und Derotremen*, Hamburg, 1864, p. 127.

<sup>2</sup> *Bronn's Thierreich*, Bd. vi.

<sup>3</sup> Humphry, *Observations in Myology*, p. 45, and pl. iv. fig. 22.

<sup>4</sup> Fischer, *Amphibiorum nudorum Neurologiæ specimen primum*, pp. 3—22 and 45—48.

<sup>5</sup> Schwalbe, *Das Ganglion Oculomotorii*, pp. 28—31.

<sup>6</sup> Fischer, *op. cit.*, p. 5, and tab. ii fig. 1.

<sup>7</sup> Cf. Fischer, *op. cit.*, pp. 3—22, and tab. i. fig. 2 (*Hyla*), fig. 3 (*Bombinator*), fig. 4 (*Pelobates*); and tab. ii. fig. 1 (*Pipa*), fig. 4 (*Rana*); also Wyman, *Anatomy of the Nervous System of Rana pipiens*, New York, 1853, pp. 26—28; also Wiedersheim in Ecker's *Anatomie des Froeschens*, Zweite Abtheilung, 1881, pp. 20—21.

But little criticism is called for by the above account. As was urged in the case of *Lepidosiren*, the presence of a distinct root of origin in the normal position must be held to prove that the sixth nerve is in the cases quoted above really an independent nerve, in spite of its apparent fusion with the fifth at the Gasserian ganglion. The fact that the sixth in an allied genus (*Bufo*) retains its independence, is an additional argument in favour of the fusion being secondarily acquired; and this view must be considered to be established by the statement made by Stannius,<sup>3</sup> on Fischer's authority, that the sixth nerve is independent of the fifth in the larval stages of those forms which, when adult, have the two nerves fused.

This concludes the list of recorded instances of exceptional innervation of the eye-muscles. Leaving out, as we are fairly entitled to, the cases of *Amphioxus* and of those forms in which, as in *Amblyopsis*, the eyes are rudimentary and functionless; the results of an examination of the remaining instances may be stated thus:—

1. That in no single instance has it been established that any one of the eye-muscle nerves is replaced by a branch of the fifth, or of any other nerve—the cases in which this is alleged to occur being far more naturally explained by supposing partial fusion between the nerves concerned to have occurred.

2. That in the alleged cases of replacement of one or more of the eye-muscle nerves by a branch of the fifth nerve, the "branch of the fifth" in question is very probably the *ramus ophthalmicus profundus*, which is really a communicating nerve between the third and fifth, belonging as much to one as to the other in its posterior portion, and in its anterior part belonging exclusively to the third.

3. That the instances in which the absence of one or other of the eye-muscle nerves has been alleged are either, as in *Petromyzon*, *Lepidosteus*, *Pipa*, *Hyla*, &c., cases in which the nerves in question arise from the brain in a perfectly normal manner, and after running a certain distance within the skull become connected more or less intimately with the fifth nerve; or else cases in which, as in *Lepidosiren*, the eyes are small, the eye-muscles imperfectly developed, and the descriptions of their anatomy incomplete and unsatisfactory.

4. That such cases do not in any way invalidate the proposition that the third, fourth and sixth are independent nerves throughout the vertebrate sub-kingdom.

<sup>3</sup> Stannius, *Hanäbuch der Zoologie*, Zweites Buch, *Die Amphibien*, 1856, p. 150, note 3.

I propose now to consider briefly the leading features exhibited by the eye-muscle nerves individually.

III. THE THIRD, OR OCULOMOTOR NERVE.—Since the third nerve is found to be an independent nerve throughout the vertebrate series, it becomes of interest to inquire whether or not it possessés segmental value.

Observations by different investigators during the last few years have tended very strongly to support, if, indeed, they may not be said to have established, the claim of the third nerve to rank among segmental nerves. Inasmuch as this point has been very fully discussed recently<sup>1</sup> I do not propose to go over the whole of the evidence here, but shall merely apply, in a somewhat summary manner, the several tests of segmental value in the order given on a previous page.<sup>2</sup>

1. Though the earliest stages of development of the third nerve have not yet been ascertained with precision in any case, yet there is very strong reason for thinking that in the chick, at any rate, the third nerve develops, like the hinder cranial nerves and the posterior roots of the spinal nerves, as an outgrowth from the neural crest on the top of the mid-brain.<sup>3</sup>

2. Inasmuch as, at a rather later, though still early period—about the sixtieth hour in the chick, and stage K of Balfour's nomenclature in the dog-fish—the third nerve arises from the base of the mid-brain, very near the mid-ventral line, it is clear that, if the observations on the earlier stages are correct, the roots must shift downwards at an early period, and to an extent unequalled by any other nerve.

Kölliker has described the later stages of this shifting, as seen in rabbit embryos, as follows:<sup>4</sup>—In an embryo 12 days 5 hours old, and 7 mm. long, the third nerve arose from the mid-brain, not from its ventral surface, but about half-way up its side; later on it shifts ventralwards, "like the ganglionated cranial nerves and the sensory spinal roots," being found on the ventral surface of the mid-brain in an embryo of the 14th day, and 15 mm. long.

<sup>1</sup> Marshall, "Development of Cranial Nerves in Chick," *Quart. Journ. Micros. Science*, January 1873, pp. 23—27; and "Head Cavities and Associated Nerves of Elasmobranchs," *Quart. Journ. Micros. Science*, January 1881, pp. 78—83; also Schwalbe, *Das Ganglion Oculomotorii*.

<sup>2</sup> *Supra*, p. 11.

<sup>3</sup> Cf. Balfour, *Comparative Embryology*, vol. ii., p. 379.

<sup>4</sup> Kölliker, *Entwicklungsgeschichte des Menschen und der höheren Thiere, Zweite Auflage*, 1879, p. 613.

3. The course of the main stem of the nerve is (fig. 17, IV) at right angles to the axis of the head at the point of origin of the nerve.

4. Morphologists are very far from agreeing as to the existence of a visceral cleft in front of the mouth, so that it would be premature to discuss the relations of the third nerve to this "lacrimal cleft," for whose existence there is, however, much to be said. Concerning the head cavities, however, the evidence yielded by the third nerve is of a perfectly definite and convincing character. The nerve in Elasmobranchs passes downwards and backwards from its root of origin to the interval between the dorsal ends of the first and second head-cavities, where it expands into a ganglionic swelling—the ciliary ganglion. Beyond this point the main trunk of the nerve passes down between the two cavities, *the relations of the third nerve to the first and second cavities being precisely the same as those of the fifth nerve to the second and third cavities.*<sup>1</sup>

5. As just noticed, there is a very evident ganglionic swelling at the point of division of the third nerve into its two main branches.

These considerations are, I think, when taken in conjunction with its previously established constancy throughout the vertebrate series, sufficient to establish the proposition that the third nerve is of segmental nature. The further question, whether the third represents an entire segmental nerve, or only a portion of one, will be best answered by considering the fourth nerve.

IV. THE FOURTH, OR TROCHLEAR NERVE.—Having established the constancy of this nerve, we have now to consider its morphological import. Concerning its development we know very little, but that little is of importance. In the dog-fish it has been shown<sup>2</sup> that the fourth nerve, at the earliest period at which it has been recognised, arises from the brain at the same spot as in the adult, *i.e.* the dorsal surface of the hinder end of the mid-brain; further, that its course is from the first that of a segmental nerve.

Now, if the visceral clefts and arches, and the head-cavities give us, as they most certainly do, reliable clues as to the segmentation of the head, then it is seen at once *that there is no room for a segmental nerve*

<sup>1</sup> Marshall, "Head Cavities and Associated Nerves of Elasmobranchs," *Quart. Journ. of Micros. Science*, Jan. 1881, pp. 78 *seq.*

<sup>2</sup> Marshall and Spencer, "Cranial Nerves of Scyllium," *Quart. Journ. of Micros. Science*, July 1881, pp. 672—674.

between the third and fifth nerves; and therefore, if the fourth is of segmental nature, it must belong to one or other of these nerves.

The following considerations seem to point very strongly to the third and fourth nerves being connected together, and favour the view that they are together equivalent to a segmental nerve.

1. The two nerves in question, the third and fourth, both arise from the mid-brain or middle cerebral vesicle. Furthermore, they are the only nerves that arise from this division of the brain, either in the embryo or the adult. There are independent reasons for thinking that these brain-vesicles have segmental value;<sup>1</sup> and though these reasons may not be considered conclusive on the point, they nevertheless lend some support to the view that the two nerves arising from one of these vesicles belong to the same segment.

2. The third and fourth nerves, though arising separately from the brain, may be connected together more or less intimately beyond their roots of origin. This, for instance, is a marked feature both in *Petromyzon* and *Lepidosteus*, also in *Salamandra* and *Triton*, if Schwalbe is correct in identifying the "nasal branch" of the fifth with which both the third and fourth nerves are connected as the *ramus ophthalmicus profundus*.

3. According to Meynert,<sup>2</sup> the third and fourth nerves arise in the adult from a common nucleus. This has, however, been denied by Forel,<sup>3</sup> though supported by other investigators, and probably requires confirmation.

4. The fourth, though chiefly known as a motor nerve, is really in many animals a nerve of mixed function, giving off in *Selachians* and *Amphibians*<sup>4</sup> sensory branches to the conjunctiva and skin of the upper eyelid. This point is of importance, because if the third and fourth are together equivalent to a segmental nerve, it would be only reasonable to expect that certain of its fibres should be sensory; and analogy would certainly lead us to look for sensory branches in the portion with the more dorsally situated root, *i.e.* the fourth nerve, which, as we have just seen, does actually present such sensory fibres.

<sup>1</sup> Vide Foster and Balfour, *Elements of Embryology*, part i., p. 138; and Marshall, *Development of Nerves in Birds*, *Journ. Anat. Phys.*, vol. xi., p. 510.

<sup>2</sup> Meynert, "The Brain of Mammals," *Stricker's Histology*, New Sydenham Society's Translation, vol. ii., pp. 444, 445.

<sup>3</sup> Forel, *Haubenregionen*.

<sup>4</sup> Schwalbe, *Das Ganglion Oculomotorii*, p. 14; Wiedersheim, *Morphologische Studien*, p. 21; and in Ecker's *Anatomie des Frosches*, p. 24; also Hoffmann, *Bronn's Thierreich*, Bd. vi., p. 203.

5. That the fourth nerve is itself not an entire segmental nerve is rendered probable by the fact, noticed by Schwalbe, that it has no ganglion, and is strongly supported by the further fact that the third nerve almost certainly arises at first from the dorsal surface of the brain, and beyond all doubt is, during its early stages, attached much higher up the side of the brain than it is at a later stage, *i.e.* that the third nerve behaves like a posterior spinal root.

Since, as we have seen, there is no room for a separate segmental nerve between the third and the fifth, I am inclined to view the third and fourth nerves as together equivalent to a segmental nerve, which has divided into two portions, whereof one—the fourth—has remained in its primitive position on the top of the brain, while the other—the third—has, like the other cranial nerves and the posterior spinal roots, shifted downwards, the extent of the shifting being greater than that of any of the other nerves, but the several steps of the process probably the same as in these. This view will be found to be very closely in accordance with that advocated by Schwalbe.<sup>1</sup>

V. THE FIFTH, OR TRIGEMINAL NERVE.—It will be convenient to continue the consideration of the cranial nerves in the usual sequence, and to take the remaining eye-muscle nerve—the sixth—after the trigeminal.

The fifth nerve completely fulfils all the conditions of a true segmental nerve.<sup>2</sup> It appears very early as an outgrowth from the neural crest. The root of origin from the brain shifts down at an early period, acquires a secondary attachment to the side of the brain, and loses its primary attachment completely. The direction of the stem is at right angles to the axis of the head at the point of origin of the nerve. The maxillary and mandibular branches are related to the maxillo-mandibular or buccal cleft in the manner characteristic of the posterior segmental nerves, as was first pointed out by Stannius. The relations of the fifth nerve to the second and third head cavities are of a perfectly typical nature; and finally a ganglionic enlargement—the Gasserian ganglion—is present in the nerve a short distance above its division into the two main branches.

<sup>1</sup> Schwalbe, *Das Ganglion Oculomotorii*, pp. 77, 78.

<sup>2</sup> For the development of the fifth nerve in Elasmobranchs, *vide* Balfour, *Elasmobranch Fishes*, 1878, pp. 196—198; also Marshall and Spencer, "Observations on the Cranial Nerves of Scyllium," *Quart. Journ. of Micros. Science*, July 1881, pp. 474—479; in the Chick, *vide* Marshall, *Quart. Journ. of Micros. Science*, Jan. 1878, pp. 28—32; and in the Rabbit, Kölliker, *Entwickelungsgeschichte*, 1879, pp. 610—712.

The only possible doubt as to the independent segmental value of the fifth nerve hinges on the fact that in the two lower classes of vertebrates—*Pisces* and *Amphibia*—the fifth is very generally fused more or less completely with the seventh in the adult condition; the fusion sometimes, as in most fishes, involving the roots to a greater or less extent, sometimes, as usually in *Amphibians*, occurring a short distance beyond the roots and close to the Gasserian ganglion.

This approximation or fusion of the fifth and seventh nerves has, as mentioned above, been employed by J. Müller, Stieda, and others, as an argument against the two nerves being of independent segmental value.

A crucial test of the force of this argument is afforded by a study of the development of the roots of the two nerves in Elasmobranchs, in which the fusion of the roots in the adult is so complete that what is really one of the roots of the seventh has hitherto been almost invariably described by anatomists as a root of the fifth.<sup>1</sup> In the dog-fish it has been shown that the two nerves, though so intimately connected in the adult, are in the early embryonic stages perfectly distinct from one another, and some distance apart, as far from one another, indeed, as they are in corresponding stages of such forms as the chick or lizard in which they remain completely separate throughout life; and that the gradual approximation and fusion of the two nerves, which occur during the later developmental stages, and all the steps of which have been traced, must, like the partial fusion which we have seen may occur in some forms between the third and fifth nerves, be viewed as purely secondary features.

In early stages of both *Teleosteans* and *Amphibians*, I have also noticed that the roots of the fifth and seventh nerves are perfectly distinct from one another, and some distance apart, and that their subsequent approximation must accordingly be, as in Elasmobranchs, of a purely secondary nature.

The claim of the fifth nerve to rank as an independent segmental nerve must, I think, from what has been said above, be considered as definitely established.

VI. THE SIXTH, OR ABDUCENT NERVE.—The proper morphological position of this nerve is by no means easy to determine with any

<sup>1</sup> A full account of the development of the roots of the fifth and seventh nerves in the dog-fish, and of the relation of the embryonic to the adult roots, will be found in the paper by Mr. Spencer and myself quoted above, *Quart. Journ. of Micros. Science*, July 1881, pp. 482—486.



degree of certainty; and the views of different writers on the point are far from being in harmony with one another.

In a former section of this paper we have established the fact that the sixth is an independent nerve throughout the vertebrate sub-kingdom. It always supplies the *rectus externus* muscle of the eyeball, and may supply other parts as well; thus, in reptiles it supplies the retractor muscle of the bulb of the eye, and in *Batrachia* the suspensor muscle of the bulb and the muscles of the nictitating membrane.<sup>1</sup> *In all cases it is a purely motor nerve.* Indeed, if we omit the eleventh and twelfth pairs, which are not constant cranial nerves, the sixth is not only the most purely motor cranial nerve, but the only exclusively motor one throughout the vertebrate series.

Its point of origin from the brain in adult vertebrates is also a remarkable and constant one. It arises from the under surface of the medulla, very close to the mid-ventral line, and vertically below, or more usually slightly posterior to the common root of origin of the seventh and eighth nerves. In some cases the root may be in front of that of the seventh nerve. The root is always slender, and devoid of ganglion cells.

Concerning the development of the sixth nerve, we unfortunately know but little. At the fifth day in the chick,<sup>2</sup> and at a corresponding stage in the dog-fish<sup>3</sup>, it has been detected and described, its appearance and relations being practically identical in the two cases. It is a slender nerve, with no ganglion cells at any point in its length, arising from the ventral surface of the hind-brain, below the seventh nerve, by a number of small slender roots, and running forward to the *rectus externus* muscle, in which it ends. The roots are, from the earliest period at which the nerve can be recognised, close to the median ventral line (fig. 7, VI), and some distance below the root of the seventh (fig. 7, VII), from which they are from the start perfectly distinct. So far as can be inferred from negative evidence, the sixth nerve appears to develop later than the seventh and other segmental nerves.

From the above account it is clear that the sixth has no claim whatever to segmental rank, inasmuch as it distinctly fails to answer any one of the tests of such rank laid down on page 133. It does

<sup>1</sup> Stannius, *Handbuch der Zoologie*, Zweite Auflage, Zoologie der Amphibien, 1856, p. 150.

<sup>2</sup> Marshall, "Development of Cranial Nerves of Chick," *Quart. Journ. of Micros. Science*, Jan. 1878, pp. 23—25.

<sup>3</sup> Marshall, "Head Cavities and Associated Nerves of Elasmobranchs," *Quart. Journ. of Micros. Science*, Jan. 1881, pp. 89—93.

*not* develop from the neural crest. The roots of origin do *not* shift downwards, but are from their first appearance in the adult position. The course of the nerve is nearly parallel to, and certainly *not* perpendicular to the axis of the head. It has *not* the definite relations to the visceral clefts and arches, and to the head cavities, characteristic of a segmental nerve. And it has *no* ganglion cells at any point in its length.

As the nerve is not an independent segmental nerve, it must either belong to one of the segmental nerves or else be a nerve of altogether exceptional nature. The latter supposition should, I think, only be adopted as a last resource if all the other attempts at explanation fail, and I therefore propose now to consider the relations of the sixth to the segmental nerves, or rather to the fifth and seventh nerves, which are clearly the only ones which could claim it.

By the majority of writers who have discussed this point, the sixth is referred to the fifth. Thus, Gegenbaur considers the sixth to be an independently arising motor root of the fifth, a view which Schwalbe<sup>1</sup> also adopts. Wiedersheim speaks of the fifth and sixth nerves as together making up a segmental nerve; while Huxley<sup>2</sup> is disposed to view the sixth as primarily part of the fifth.

Notwithstanding the weight of authority against me, I think that the sixth nerve should be grouped with the seventh, and not with the fifth, for the following reasons:—

1. In the early stages of both chick and dog-fish the roots of the sixth are completely behind those of the fifth nerve. Indeed, the majority of the roots are even behind the roots of the seventh; and although a transverse section may, as in fig. 7, pass through the roots of both sixth and seventh nerves, yet the root of the sixth in such a section is the most anterior of the series, the other roots being further back, and completely behind the seventh root.

2. In adult vertebrates, also the sixth nerve usually arises beneath or slightly behind the seventh, very rarely in front of it.

3. Though the sixth nerve may, beyond its root, be closely connected with the fifth, yet it is important to notice that *all the cases—Petromyzon, Lepidosiren, Pipa, Rana, Anura—in which it is described as fusing with the fifth, are also cases in which the seventh and fifth nerves are very closely connected together*, so that the connection between

<sup>1</sup> Schwalbe, *Das Ganglion Oculomotorii*, p. 74.

<sup>2</sup> Wiedersheim, *Morphologische Studien*, p. 23.

<sup>3</sup> Huxley, *Anatomy of Vertebrated Animals*, p. 73, note.

the sixth and fifth in these instances by no means proves that the sixth belongs to the fifth, but is more probably due to the same cause—whatever it may be—that determines the approximation or fusion of the seventh and fifth nerves.

Concerning the actual value of the sixth nerve, I see no reason to alter the opinion I have previously expressed, that the sixth nerve may be regarded as having the same relation to the seventh that the anterior root of a spinal nerve has to its posterior root. I shall return to this point when considering the seventh nerve.

VII. THE SEVENTH, OR FACIAL NERVE.—As to the segmental value of the seventh nerve there can be no doubt whatever; for, like the fifth, it completely and indisputably fulfils all the conditions of a segmental nerve laid down on page 133.

It develops<sup>1</sup> very early as an outgrowth from the neural crest on the dorsal surface of the hind-brain (fig. 2). At an early stage the nerve acquires a new or secondary attachment to the side of the brain (fig. 3); but, unlike all the other nerves, cranial or spinal, *the original or primary root is retained as well as the secondary root*, whereas in all the other nerves the primary attachment appears to be lost. The general course of the nerve is at right angles to the axis of the head at its point of origin. The relations of its branches to the hyo-mandibular cleft, first pointed out by Stannius, and afterwards insisted on by Gegenbaur, are those of a typical segmental nerve, as are also its relations to the head-cavities; whilst, finally, it is ganglionic at its division into the two main ventral branches.

As to the independent rank of the seventh nerve, I have already discussed fully the theory that the seventh and fifth nerves are connected together primarily, and have stated the arguments leading to the conclusion, that although in many vertebrates—fish and amphibians—the two nerves are more or less closely fused together, yet that embryology shows that this fusion is a secondarily acquired character.

The relation between the sixth and seventh nerves is of still greater importance, from its bearing on the disputed question of whether there

<sup>1</sup> For an account of the development of the seventh nerve in Elasmobranchs, *vide* Balfour, *Elasmobranch Fishes*, 1878, pp. 198–202; and Marshall and Spencer, *Quart. Journ. Micros. Science*, July 1881, pp. 679–691; in the Chick, *vide* Marshall, *Quart. Journ. Micros. Science*, Jan. 1878, pp. 34–36; and in Mammals, Kölliker, *Entwicklungsgeschichte*.

are to be found in any of the cranial nerves roots strictly comparable to the anterior roots of the spinal nerves.

In dealing with this question, it is first necessary to establish certain general conclusions concerning the cranio-spinal nerves. As was first pointed out by Balfour, the posterior roots of the spinal nerves must be regarded as of a more primitive nature than the anterior roots, the grounds on which this conclusion is based being the following:—

1. The actual mode of development of the two kinds of roots in the spinal nerves. As noticed in a previous page, the posterior roots appear before the anterior ones, and are also in their mode of development of a more primitive character than these latter, the posterior roots consisting at first entirely of undifferentiated spherical or polygonal cells, while the anterior roots are almost from their first appearance fibrillar.

2. The condition of the nervous system in *Amphioxus*, in which, according to Balfour, all the nerves arise by single roots, which roots correspond to the dorsal or posterior roots of other vertebrates, and must clearly in *Amphioxus* be of mixed motor and sensory function.

From these facts the further conclusion is drawn "that primitively the cranio-spinal nerves of vertebrates were nerves of mixed function with one root only, and that root a dorsal one; and that the present anterior or ventral root is a secondary acquisition."<sup>1</sup>

Concerning the several steps by which these anterior roots have been acquired, the evidence at our disposal is of an imperfect, and in great part merely conjectural character. Still I think that, although we may not be able to solve the problem completely, we can at any rate define its limits fairly accurately, and perhaps indicate the path along which the solution will ultimately be found.

The problem is how, from animals resembling *Amphioxus* in possessing only dorsal roots to the nerves, and these dorsal roots consequently of mixed function, has the type of spinal nerve met with among existing vertebrates, with two distinct roots, dorsal or sensory and ventral or motor, been derived?

It appears to me that there are two ways in which we can conceive this change as having come about:—

<sup>1</sup> Balfour, *Elasmobranch Fishes*, p. 193.

Firstly, we might suppose that in some way, and for some reason, the sensory and motor portions of the originally single root became completely separated from one another, and that while the sensory portion of the nerve retained the primitive mode of development and position of attachment of the root, the motor portion acquired a new mode of development and a new position of attachment, and then united farther on with the posterior root to form a spinal nerve. On this view the motor and sensory roots of a spinal nerve correspond to the motor and sensory portions of the single root of *Amphioxus*.

Or, secondly, we might imagine the anterior root to be, not the motor portion of the original root, but an altogether new development, an independent outgrowth from the spinal cord to supply the more complicated system of muscles that would necessarily accompany the gradual perfection and complication of the internal skeleton; that this new root was at first completely independent of the original or dorsal root, and for a time coexisted with a dorsal root of mixed function; that in the case of the spinal nerves the whole motor function gradually got transferred to, or usurped by, the new root; while the two roots, originally separate along their whole length, became united to form the mixed trunk of the spinal nerve.

Now, although there are very considerable and obvious difficulties in the way of accepting either of these alternatives, yet it appears to me that the second is far more in accordance with the actual facts than the first, and that it offers a ready explanation of many points unintelligible on the first hypothesis. Thus, the second view explains why in actual development the anterior spinal roots appear later than the posterior, and why they are for some time quite distinct from these latter; it also explains such cases as *Petromyzon*, in which the anterior and posterior roots of the spinal nerves are said to remain distinct from one another throughout life.

By far the most important argument, however, in favour of the second hypothesis is afforded by the explanation it yields of the condition of the cranial nerves as compared with the spinal; and in connection with this point I would direct special attention to the statements already made concerning the sixth and seventh nerves.

It has been shown above that the seventh nerve in Elasmobranchs develops in a manner precisely similar to the posterior roots of the spinal nerves; that it arises as an outgrowth from the neural crest (fig. 2, VII), the nerves of the two sides being at first directly and

widely continuous with one another across the top of the brain; that by growth of the mid-dorsal roof of the brain the two nerves get separated from one another; that the root acquires a secondary attachment to the side of the brain (fig. 3, VII), *but that, unlike the other cranial or spinal nerves, it retains the primary as well as the secondary root throughout life. In this respect the seventh is, with the possible exception of the fourth, the most primitive nerve in the body*, inasmuch as it exists throughout life in a condition which is only a transitory one in all the other nerves. However unexpected this point may be, I cannot but think that it is one of the greatest importance in the determination of any question concerning the morphology of the cranial and spinal nerves.

The seventh being a very primitive nerve, there is strong *a priori* reason for thinking that the sixth nerve, which we have seen reason for grouping with the seventh, is also of a primitive nature, and it is clear that on the second hypothesis such is the case, the complete independence of the sixth nerve being merely the retention of a primitive character, while its limited and special distribution to muscles not present in *Amphioxus* affords a very possible explanation of its appearance in higher vertebrates. On the first hypothesis, on the other hand, the sixth nerve would be, not a root which *had retained its primitive independence of the seventh*, but a root which had as a perfectly exceptional occurrence *acquired independence*, a view directly contradicted by the primitive condition of the seventh itself.

It must surely be regarded as a very significant fact that a transverse section through the hind-brain of *either an embryo or adult Elasmobranch* passing through the roots of the sixth and seventh nerves (fig. 7) agrees absolutely in all essential points with a section *at an early embryonic stage* through the roots of a spinal nerve in the same animal, *i.e.* that a condition which is transitory in the case of the spinal nerves is permanently retained in the case of the sixth and seventh nerves. This fact, which is the strongest possible argument in favour of the second hypothesis, clearly directly contradicts the first.

If the doctrine that the cranial nerves are more primitive than the spinal appear at first sight paradoxical,<sup>1</sup> I would point out that there

<sup>1</sup> I have myself on a former occasion both felt and urged this objection ("Head Cavities of Elasmobranchs," *Quart. Journ. of Microsc. Science*, Jan. 1881, p. 91). Further investigation has convinced me that I was then wrong, and that Balfour was right in considering (*Elasmobranch Fishes*, p. 198) the cranial nerves as more primitive than the spinal, though do not agree with his conclusion that the cranial nerves have no anterior roots.

is independent evidence in favour of the head retaining a more primitive condition than the body. Thus the skull, though subjected to very extensive secondary modifications, is really in a more primitive state than the vertebral column, for the skull represents the permanent retention of a condition, that of a continuous unsegmented cartilaginous tube, which is only transitory in the case of the vertebral column except in the lowest vertebrates; the division of the cartilaginous tube into segments or vertebræ never occurring, and in all probability never having occurred in the skull, though so constantly present in the vertebral column. The fact that it is in the lowest vertebrates alone that this unsegmented condition is retained in the trunk as well as in the head is a strong argument in favour of the view that the head is really in a more primitive condition than the trunk as regards skeletal elements.<sup>1</sup>

On the second hypothesis, the mixed—motor and sensory—nature of the seventh nerve is explained as due, like the persistence of the primary root and the independence of the sixth nerve, to retention of the primitive condition, and the extreme variability presented by the relative importance of the sensory and motor functions of the seventh nerve in different vertebrates may help to render intelligible how the posterior spinal roots, which were originally of mixed function, have become converted into purely sensory roots.

If the hypothesis advanced above should prove correct, it would be only reasonable to expect that the posterior roots of the spinal nerves should in some exceptional cases be found to retain in part their primitive mixed character, and to co-exist as mixed posterior roots with exclusively motor anterior roots. I am not aware of any such cases, or of the existence of any residual physiological phenomena that would support such a view, but would suggest that a direct investigation of the functions of the spinal roots in the lampreys, where the two roots are stated to remain distinct from one another throughout life, might conceivably lead to interesting results.

The application of the hypothesis to the remaining cranial nerves is sufficiently obvious from the accounts given of these. The main point of difficulty concerns the determination of the presence or ab-

<sup>1</sup> The following quotation from Balfour, which I only became acquainted with after the above passage was written, strongly confirms this view:—"This development (of the skull) probably indicates that the basilar plate contains in itself the same elements as those from which the neural arches and the centra of the vertebral column are formed, but that it never passes beyond the unsegmented stage at first characteristic of the vertebral column."—*Comparative Embryology*, vol. ii., p. 467.

sence of anterior motor roots to these nerves; and on this point I have no additional evidence beyond what I have already stated elsewhere.<sup>1</sup>

VIII. THE EIGHTH, OR AUDITORY NERVE.—In all the forms in which the development of the auditory nerve has been ascertained, it arises as part of the seventh nerve. Neither its development nor its anatomical relations afford the slightest ground for thinking it to be of segmental rank.<sup>2</sup>

IX. THE NINTH, OR GLOSSOPHARYNGEAL NERVE.—Like the auditory, the ninth nerve can be disposed of very briefly, but for a directly opposite reason. Since Gegenbaur confirmed Stannius' account of its relations to the first branchial cleft, the claim of the glossopharyngeal to rank as an independent segmental nerve has been very generally admitted; and as the history of its development<sup>3</sup> shows that it conforms in all respects to the characters of a segmental nerve as defined on page 133, it would be superfluous to discuss in detail its now universally recognised claims to segmental value.

X. THE TENTH, OR VAGUS NERVE.—The tenth nerve stands in much the same position as the ninth, with the exception that while the glossopharyngeal is a single segmental nerve, the vagus, from its relations to a number of visceral clefts, must be considered as equivalent to an equal number of segmental nerves fused together. This was first pointed out by Stannius, and subsequently developed in much more detail by Gegenbaur; and since the publication by the latter of his memorable essay on the cranial nerves of *Hexanchus*, has been accepted almost universally as the true theory of the morphological value of the tenth nerve. It is only necessary to add here that the study of its development shows that it completely fulfils all the conditions required of segmental nerves.

Concerning the number of primitively separate segmental nerves fused together to form the vagus, we cannot speak positively. The

<sup>1</sup> Marshall, "Head Cavities and Associated Nerves of Elasmobranchs," *Quart. Journ. of Micros. Science*, Jan. 1881, pp. 91—93.

<sup>2</sup> For the development of this nerve in Elasmobranchs, *vide* Balfour, *Elasmobranch Fishes*, p. 198; in the Chick, Marshall, "Development of Cranial Nerves in the Chick," *Quart. Journ. of Micros. Science*, Jan. 1873, pp. 34—36.

<sup>3</sup> For the development of the glossopharyngeal and vagus nerves in Elasmobranchs, *vide* Balfour, *Elasmobranch Fishes*, pp. 202, *seq.*; in the Chick, Marshall, *loc. cit.*, pp. 36—39.



greatest number of clefts supplied by it in vertebrates above *Amphioxus* is met with among the *Marsipobranchii* and in *Notidanus*, where it supplies the six posterior branchial clefts, and must therefore be equivalent to *at least* six segmental nerves. Whether this is the full number, however, is a point not yet decided.

XI. AND XII. THE ELEVENTH, OR SPINAL ACCESSORY, AND TWELFTH, OR HYPOGLOSSAL NERVES.—Neither of these nerves is constant as a cranial nerve throughout the vertebrate series, a fact which renders it very doubtful whether the claim of either of them to segmental value could be entertained. For this reason, and partly because I am at present engaged in investigating their development, about which we know as yet very little, I do not propose to deal further with them in the present paper. Forming, as they do, the connecting links between cranial and spinal nerves, they may be expected to yield valuable evidence concerning the validity of the hypothesis propounded above concerning the relations between these two groups of nerves.

SUMMARY.—The conclusions arrived at concerning the segmental value of the cranial nerves may be expressed in a tabular form thus (*cf.*, fig. 17):—

Segment.	Nerve.	Visceral Cleft.	Visceral Arch.
1. Præoral.	I. Olfactory.	Olfactory.	
2. Do. }	III. Oculomotor, } IV. Trochlear. }	Lachrymal.	
3. Oral.	V. Trigeminal.	Buccal.	Maxillary.
4. Postoral. {	VII. Facial, } VI. Abducent. }	Spiracular or } hyomandibular. }	Mandibular.
5. Do.	IX. Glossopharyngeal.	1st Branchial.	Hyoid.
6. Do.	X. Vagus, 1st branch.	2nd "	1st Branchial.
7. Do.	" 2nd "	3rd "	2nd "
8. Do.	" 3rd "	4th "	3rd "
9. Do.	" 4th "	5th "	4th "
10. Do.	" 5th "	6th "	5th "
11. Do.	" 6th "	7th "	6th "

*List of chief Works and Papers referred to, arranged according to date of publication.*

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THE SYSTEM OF BRANCHIAL SENSE ORGANS AND  
THEIR ASSOCIATED GANGLIA IN ICHTHYOPSIDA. A  
CONTRIBUTION TO THE ANCESTRAL HISTORY OF  
VERTEBRATES.

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[PLATES VII, VIII, & IX.]

INTRODUCTION.

Among the many weighty questions which have arisen with the rise and progress of comparative embryology, that of the origin and ancestral history of Vertebrates has occupied, and still occupies, an important place.

That the question, if capable of solution at all, would be solved by the discoveries of embryology, is now, and has been for the last ten years, a general opinion among zoologists. So much for a general agreement. But as to the particular line of descent one might recall half a dozen different theories supported by different schools of workers.

The impulse to these speculations was first given by the discovery of the tadpole-like larva of Ascidians, and the opinion that Vertebrates were derived from Ascidians we owe to Kowalewski and Kupffer. This view has had its day, and is now only a reminiscence.

Another important theory, important because clothed with the authority attached to the name of Balfour, is the theory that Verte-

brates arose from unsegmented worms, in which two lateral nerve-cords were supposed to have coalesced dorsally instead of ventrally, as in Annelida.

Following this, one is reminded of Hubrecht's theory, which allies Vertebrates with Nemertines, and sees the Vertebrate notochord reflected in the Nemetine proboscis sheath.

By no means least important is the celebrated Annelidan theory of the origin of Vertebrates first originated by Dohrn<sup>1</sup> and Semper.<sup>2</sup> A theory which, in spite of all attacks, still survives, and at present seems to be more probable than any other.

Finally, the alliance of *Balanoglossus* with Ascidians, Amphioxus, and Vertebrates, recently advocated by Bateson,<sup>3</sup> must be mentioned. Interesting though this is, it cannot yet be considered as sufficiently established to be accepted without reserve; but if more evidence for it be forthcoming, it is a moot point whether our existing notions of the relations of Vertebrates and Annelida will not have to be modified, for we know of no existing Annelid which has relationships with *Balanoglossus*. And here I would point out that my own researches on the cranial nervous system and sense organs of Vertebrates, instead of supporting the alliance of *Balanoglossus* with Vertebrates as high as fishes, present rather a hindrance in the way of such alliance, whilst they are still more opposed to the alliance of Vertebrates with existing Annelida.

That Vertebrates have their nearest allies, except *Balanoglossus*, in the group of Annelida, is becoming more and more obvious from recent researches, especially from those of Dohrn; but the links of such an alliance seem to have been rather in long extinct Annelida than in any at present existing.

In the following pages an account will be given of the morphology and development of the branchial sense organs and associated ganglia in Amphibians and Fishes, chiefly in Elasmobranchs. The branchial sense organs are those sense organs which have usually been called organs of the lateral line, and were formerly called "segmental sense organs" by me. The name "organs of the lateral line" is bad, because it chiefly refers to those sense organs along the lateral line of

<sup>1</sup> Dohrn, 'Ursprung der Wirbelthiere,' 1875.

<sup>2</sup> Semper, "Verwandschaftsbeziehungen der gegliederten Thiere," 'Arbeiten a. d. Zool. Institut zu Würzburg,' 1875.

<sup>3</sup> Bateson, W., "Development of *Balanoglossus*," 'Quart. Journ. Micro. Sc.,' Supplement, July 1885.

the trunk, which morphologically form only a small portion of the sense organs. I have myself seen reason to reject the name segmental sense organs, because although originally they are segmental, and in later life may occur one in each segment of the trunk, still *at first they are confined to one region only of the body, the gill-bearing region*, and only extend into the trunk much later. Originally they are seated one above each gill-cleft or over the site of each cleft, and may, therefore, be called branchial sense organs.<sup>1</sup>

The so-called ganglia of the posterior roots of the cranial nerves arise in connection with them, and must be regarded as originally special ganglia of these sense organs.<sup>2</sup>

One general conclusion may be referred to here, and that is, that *at present we are acquainted with no invertebrate nervous system which is built upon the same plan as that of Vertebrates.*

The matter will be discussed later on, and I only refer to it here in order that from the outset the branchial sense organs may be raised from their present position of neglect and obscurity, and may be given that important morphological (and physiological) place which their relationships to the gill-clefts on the one hand, and to the ganglia of the posterior roots of cranial nerves on the other, most certainly entitle them to.

Unlike many previous observers, I have found that it is absolutely impossible to study the branchial sense organs of fishes without at the same time dealing with the posterior roots of the cranial nerves, which are morphologically as well as physiologically inseparably connected with the former.

It would take up too much time and space to give here a history of all the researches on these two sets of organs, which have hitherto been usually treated apart from each other as if they had no connection.

The work has been mainly carried out on embryos of *Torpedo ocellata*, for which I have to thank the Zoological Station at Naples. But I have also studied Teleostei and Amphibians, and have had a few embryos of *Mustelus* and *Pristiurus*. However, in the descriptions in the following pages, unless otherwise stated, the condition of affairs in *Torpedo* will be understood to be under discussion.

<sup>1</sup> Beard, "Cranial Ganglia and Segmental Sense Organs," 'Zool. Anzeig.', 192, 1885; also Froriep, "Ueber Anlagen von Sinnesorganen am Facialis, &c.," 'Archiv für Anat. and Physiol.', 1885.

<sup>2</sup> Beard, op. cit.; Froriep, op. cit.; and Spencer, "Notes on the Early Development of *Rana temporaria*," 'Quart. Journ. Micro. Sc.', Supplement, July 1885.

In the first place, I think it will be of great advantage and will tend to simplify matters very much if the general schema of the development of a cranial nerve (dorsal root) of an Elasmobranch, such as *Torpedo*, be given.

Then those cranial nerves, which I regard as segmental, will be discussed: olfactory, nerve of ciliary segment, trigeminal, facial, auditory, glossopharyngeal, and vagus.

The optic nerve is left entirely out of consideration. Firstly, because I have made no investigations, and hence have no new facts about it to record; and secondly, as is well known, its whole development is different from that of the other cranial nerves; and I can only agree with those zoologists who class the optic nerve entirely apart from the other cranial nerves.

Not so, however, with the olfactory and auditory nerves and organs. Partly following Marshall, I feel bound to place these nerves in the category of cranial segmental nerves, and to class the olfactory and auditory organs<sup>1</sup> as specialised branchial sense organs.

Finally, after the account of the various nerves, the bearing of the facts described on the morphology and ancestral history of Vertebrates will be discussed.

#### GENERAL SCHEMA OF THE DEVELOPMENT OF A DORSAL ROOT OF A CRANIAL NERVE.

According to the existing views of the development of a dorsal root of a cranial nerve in Elasmobranchii, based mainly on the researches of Balfour,<sup>2</sup> Marshall,<sup>3</sup> and Van Wijhe,<sup>4</sup> the nerve, soon after its development from the neural ridge, divides into two main branches, a dorsal one and a ventral one. The dorsal branch is sensory, and supplies the so-called organs of the lateral line. The ventral one is mainly motor; it soon divides again into two branches, which, as Stannius<sup>5</sup> first showed, pass one on each side of a visceral cleft. The posterior branch is mainly concerned with the innervation of the

<sup>1</sup> Beard, "On the Segmental Sense Organs, &c.," *Zool. Anzeiger*, 161, 162, 1884; "On Cranial Ganglia, &c.," *Zool. Anzeiger*, 192, 1885.

<sup>2</sup> Balfour, "Elasmobranch Fishes."

<sup>3</sup> Marshall, "The Development of the Cranial Nerves in the Chick," *Quart. Journ. Micr. Sc.*, 1878; Marshall, "On the Head Cavities, &c.," *Quart. Journ. Micr. Sc.*, 1880; Marshall, "On the Segmental Value of the Cranial Nerves, &c.," *Journ. of Anat. and Physiol.*, 1882; also separate.

<sup>4</sup> Van Wijhe, 'Ueber die Mesodermsegmente und die Entwicklung der Nerven des Selachierkopfes,' Amsterdam, 1882.

<sup>5</sup> Stannius, 'Das Peripherische Nervensystem der Fische,' 1849.

gill muscles. According to Van Wijhe, the dorsal branch becomes intimately connected with the skin, and is there in connection with the rudiments of the so-called sense organs of the lateral line. He further holds that the sensory epithelium takes part in the formation of the nerve. In this respect the dorsal branch differs from the ventral one, which does not, according to any writer, arise either wholly or partially from the skin, but is a direct outgrowth of the neural crest (Marshall). The branch in front of the cleft is developed later than the other branches, but how is still uncertain. At any rate, both Professor Froriep and I have failed to gather from Van Wijhe, who alone has studied the development of this branch, how this branch and the *Ramus pharyngeus* are developed. In Amphibians, Götte<sup>1</sup> long ago held that the so-called dorsal branches were split off from the skin.

These various branches have all received general names, some of which require alteration in view of the researches contained in this paper. The branch posterior to the cleft is called the main or posterior branch (Balfour), and post-trematic by Van Wijhe; in this paper it will be spoken of as the post-branchial nerve. The branch in front of the cleft, viz. the præ-trematic of Van Wijhe, I shall call the præ-branchial nerve.

The *Ramus pharyngeus* of Van Wijhe will retain the same name when spoken of here. But now for the so-called dorsal branches; of all the general names this is by far the worst. It is true that the name has been employed by many distinguished zoologists, Stannius, Gegenbaur, Balfour, Marshall, and Van Wijhe, and that therefore to propose a change, except for very weighty reasons, would be a very high-handed and arbitrary proceeding. However, it must be done, and on grounds to be afterwards stated.

Though some of these various so-called dorsal nerves may come to occupy a dorsal position, still, as was first mentioned to me by Professor Dohrn, it is morphologically wrong to regard them as dorsal. Of the truth of this I have fully convinced myself, and hope soon to convince the reader also. I have, however, no means of knowing whether my reasons for rejecting the name are the same as Professor Dohrn's. These branches will be described by the general name of *supra-branchial*.

So much for a general view of the adult condition. A schema of the development in Elasmobranchii would be as follows. (This

<sup>1</sup> A. Götte, 'Entwicklungsgesch. d. Unke,' 1875.



account is in accordance with my own researches, and contains some additions to the accounts given by my predecessors.)

The nerve grows outwards and downwards from the neural ridge towards the lateral surface of the head. In its course it lies directly under, but unconnected with, the epiblast. In the case of those nerves which are connected with gill-clefts, and are therefore typical, the nerve lies just over the cleft (fig. 50). All this is well known, and has been described by Balfour, Marshall, Van Wijhe, &c.

The subsequent events are as follows :<sup>1</sup>

1. When the nerve reaches the level of the notochord, or a little below that level, it fuses with the epiblast (fig. 34).

2. Part of the nerve, however, passes on to the lateral muscle-plates of the segment (figs. 34, 50).

3. At the point of fusion mentioned in 1. a local thickening of epiblast has previously taken place (fig. 14).

4. After the fusion has taken place a proliferation of some of the cells composing the thickening ensues. The proliferated cells form a mass of actively dividing elements still connected with the skin and fused with the dorsal root (fig. 16).

5. This mass of cells is *the rudiment of the ganglion of the dorsal root*, and externally to it is situated the *rudiment of the primitive branchial sense organ of that root* (figs. 12 and 13).

6. For some time cells continue to be given off from the thickened epiblast, and of those already given off many show nuclear figures (fig. 8) indicating rapid division.

7. While the ganglion is still fused with the epiblastic thickening the latter begins to grow in length, and to push its way either forwards or backwards, as the case may be, between the general epiblast cells (figs. 40 and 41).

8. The general epiblast cells thus pushed away are probably lost (figs. 40 and 41, *i.e.*).

9. Concomitantly with this growth of the sensory thickening, the ganglion begins to separate from the skin, and so comes to lie deeper in the mesoblast (fig. 35). As it separates there arises a nerve from the sensory thickening (figs. 11, 13, &c.). This nerve grows centrifugally from the ganglion, arising from the elements of the thickening, and being in fact split off from the latter along its whole length. It

<sup>1</sup> Beard, "On the Cranial Ganglia and Segmental Sense Organs," 'Zool. Anzeig.,' 192, 1885; also, on some points, Spencer, "Notes on the Development of *Rana temporaria*," 'Quart. Journ. Micr. Sc.,' Supplement, July 1885.

is the so-called dorsal branch, and, as previously stated, will be here called the *supra-branchial branch*.

10. The sensory thickening of a segment, which gives rise to the branchial sense organs of that segment, may remain very small or may increase to a very considerable length, but in any case the nerve connecting the whole length of the thickening with its ganglion is split off from the thickening, and split off simultaneously with the growth of the latter.

11. The præ-branchial nerve is also formed as the ganglion separates from the skin, and is probably in all cases also split off from the epiblast in front of each cleft.

12. Of the development of the *R. pharyngeus* nothing can be here recorded; but I think, from the nature of the case, that this nerve also probably arises from the cells on the upper wall of the cleft.

Thus, as the general result of these observations, the existing views of the development of the dorsal root of a cranial nerve will have to undergo some modification. That in Elasmobranchs the main root of the nerve is a direct outgrowth from the neural ridge, as stated by Balfour and Marshall, is certainly true. The shifting and acquisition of a secondary point of attachment described by Marshall also seem to take place. The post-branchial branch also appears to arise from the direct outgrowth from the neural ridge, but in the formation of the rest the epiblast probably plays a part. In the case of the supra-branchial branches this is certain, and it is highly probable in the case of the ganglion. That the other branches, viz. the præ-branchial and *R. pharyngeus* of Van Wijhe, are derived from the skin is probable, and in one case it can be proved, viz. the præ-branchial nerve of the hyoid.

Having now a general view of the development of a typical cranial nerve, the various nerves may be considered. In the above schema we have the key to all the cranial nerves. Some, such as the ninth, or glossopharyngeal, we shall find to fit in pretty exactly with the schema. But in others the story that ontogeny often omits or distorts ancestral history is also repeated.

Some of the branches may be absent even in the ontogeny, while others may be abnormally developed. Others, again, may be partially fused with neighbouring nerves, as has been abundantly demonstrated by previous writers. But whatever the adult condition of any of the dorsal roots of the cranial nerves, whatever the actual condition of

olfactory nerve, nerve of the ciliary ganglion, fifth, seventh, eighth, ninth, and vagus complex, all can, by the consideration of their actual development, and of the condition of the various organs which are, or would be if present, related to them, be reduced to the general schema.

The divergencies between the various nerves are, as might be suspected, naturally dependent on the presence or absence of gill-clefts in connection with the segment to which the nerve belongs.

For this reason I shall consider the nerves out of their natural order, taking those of the true gill-clefts first. Their order of treatment will thus be as follows :

Nerve.	Cleft.	Segment. <sup>1</sup>
Seventh.	Spiracle, and one absent.	Fourth and fifth.
Ninth.	First branchial.	Seventh.
Vagus.	Second, third, fourth, and fifth branchial.	Eighth, ninth, tenth, and eleventh.
Fifth.	Mouth.	Third.
Ciliary.	Hypophysis. (?)	Second.
Olfactory.	Absent.	First.
Auditory.	Absent.	Sixth.

In the above list it will be noticed that the cleft of the fifth nerve is described as the mouth. This view, which we owe to Prof. Dohrn, seems to me to receive very considerable support from my researches. I shall refer to the matter subsequently.

For the ciliary, olfactory, and auditory nerves I have hesitated to assign clefts, because the evidence for their existence is uncertain, and the nature of the three nerves is more easily explicable if we regard the clefts as absent or metamorphosed. Here it will suffice to say that clefts have been assigned to these nerves by various zoologists, with what justification we shall see later on.

#### DORSAL ROOT OF THE FOURTH AND FIFTH SEGMENTS, SEVENTH NERVE OR FACIALIS.

As already described by Balfour<sup>2</sup> and Marshall,<sup>3</sup> the seventh nerve arises from the neural crest in the region of the hind-brain and just in front of the auditory capsule.

<sup>1</sup> The numbering of the segments is in accordance with those conclusions from my researches which appear to me to be fairly certain. Probably the facial nerve is a complex of two segmental nerves, apart from the auditory segmental nerve. If this be the case, then there are eleven segments at least from the olfactory nerve to the fourth root of the vagus inclusive.

<sup>2</sup> Balfour, 'Comp. Embryol.,' vol. ii, p. 377.

<sup>3</sup> Milnes Marshall, "Head Cavities and Associated Nerves in Elasmobranchii," 'Quart. Journ. Micr. Sc.,' 1880; also, "Nervous System of Chick," 'Quart. Journ. Micr. Sc.,' 1878.

These authors further agree in assigning a common root of origin to the seventh and auditory nerves. Marshall has, however, in one of his early works, drawn attention to a line of division between the ganglia of the auditory and facial nerves in the chick. Now, although the rudiments of the facial and auditory nerves lie very closely together, I consider that at first the two are really distinct. The facial grows downwards and outwards from the neural crest, and just under the epiblast. When it reaches the level of the notochord part of it fuses with the sensory thickening above the hyoid arch, and just above the future hyoid cleft. The rest passes on (fig. 20) to the lateral muscle plates of the hyoid arch. At the point of fusion with the sensory thickening the ganglion is formed. Of this, one stage is figured in fig. 20. In this condition the nerve is to be regarded as passing through an ancestral stage. Its condition is then figured in the diagram of a typical dorsal root (fig. 50), which passes from the brain to the primitive branchial sense organ and its associated ganglion above a gill-cleft, and from which ganglion a nerve passes along the posterior side of the cleft to the muscles of the gill.

In later stages the ganglion is still partly fused with the skin, but it soon separates, leaving behind it the rudiments of several branches.

These branches are the supra-branchial, the præ-branchial, and the pharyngeal. The development of the pharyngeal branch has not yet been traced. The other branches are split off from the epiblast. The supra-branchial (figs. 21 and 22) is formed at the expense of the deeper portion of the sensory thickening, which has begun to grow forwards over the face.

Very soon *this nerve divides into two branches*; that is, the sensory thickening grows forwards as two divergent thickenings, from each of which nerve-fibres are split off, and thus two branches are formed (fig. 51, *p.b.n.*). This development from the dichotomously dividing rudiment has been described by Van Wijhe.<sup>1</sup> These two branches have been described by Marshall and Spencer.<sup>2</sup> The upper one is the portio facialis of the oph. superficialis (Marshall), the lower one the ramus buccalis (Marshall and Spencer). The upper one Balfour, Marshall, and Spencer classed as a ramus dorsalis of the seventh. As stated by Van Wijhe,<sup>3</sup> they are concerned in the innervation of the supra- and

<sup>1</sup> Van Wijhe, *op. cit.*, pp. 26, 27.

<sup>2</sup> Marshall and Spencer, "On the Cranial Nerves of Scyllium," *Quart. Journ. of Micr. Sc.*, 1881.

<sup>3</sup> *Op. cit.*, p. 27.

infra-orbital sense organs respectively (branchial sense organs). These branchial sense organs, it is hardly necessary to state, are developed from the dichotomously divided sensory thickening mentioned above.

The portio facialis of the ophth. superficialis (fig. 51, *p.f.*), is obviously enough, as pointed out by Marshall, Balfour, and Van Wijhe, a so-called dorsal branch; that is, what we have here called a supra-branchial. Van Wijhe has, and I fully agree with him, classed the r. buccalis (fig. 51, *r.b.*) as a "dorsal branch," and gives these reasons: (1) Its origin from the same rudiment as the former nerve; (2) its simultaneous appearance with that nerve; (3) its similar development and distribution to (branchial) sense organs. Van Wijhe, indeed, regards the two as branches of one nerve, and as therefore equivalent to one so-called dorsal branch. Dohrn<sup>1</sup> has advanced very weighty reasons for the existence of a hyomandibular segment in front of the hyoid and behind the mouth, but has not adduced the cranial nerves in support of his view. I would here venture to suggest that an additional ground for his view is to be seen in the existence of two supra-branchial nerves in the facial. It would indeed be remarkable if Van Wijhe were correct in regarding these two nerves as merely branches of one nerve, for in no other single and simple cranial nerve do we meet with more than one supra-branchial nerve. To my mind the best explanation of the presence of these two branches is that the facial is composed of the fusion of two cranial segmental nerves, and this apart from its fusion with the auditory. The reader may compare Dohrn's views on the nature of the hyomandibular with this explanation. Except for this the facial seems to be a fairly typical cranial nerve, and agrees well with the general schema. It should be noticed that the supra-branchial branches grow *forwards*, for this point will be referred to in discussing the vagus. Though I agree fully with Van Wijhe's<sup>2</sup> view that there are two segments in the hyoid arch, and this apart from the hyomandibular portion, I cannot treat the auditory nerve here. The special modifications it has undergone will be best considered after some of the other nerves have been discussed. In their earliest appearance I believe the auditory and facial nerves are not fused, and even in the later stages (figs. 21, 42), as already noticed by Marshall in the chick, the ganglia of the two nerves are partially separated, and the line of division is easily recognisable. For the

<sup>1</sup> Dohrn, "Studien zur Urgeschichte des Wirbelthier-Körpers," No. vii, 'Mittheil. a. d. Zool. Stat. zu Neapel,' vol. vi, part i.

<sup>2</sup> Op. cit., pp. 9 and 28.

later stages of the facial the reader is referred to Marshall's works and to the paper by Marshall and Spencer.

#### NERVE OF THE SEVENTH SEGMENT—GLOSSOPHARYNGEAL.

This nerve arises from the neural ridge (Balfour) immediately behind the auditory organ, and grows down the lateral wall of the body to just above the point of origin of the first true branchial cleft. Its fusion with the skin is represented in fig. 32, and the origin of its ganglion from the skin and in connection with the branchial sense organ of this segment in fig. 42. The main portion of the nerve grows downwards behind the cleft, and proceeds to the lateral muscle plates of the first branchial arch.

Later, as the ganglion separates from the skin, the supra-branchial nerve is developed. Like other supra-branchial nerves it splits off from the skin in connection with a sensory thickening which gives rise to the supra-temporal sense organs.

Marshall described the course but not the development of this branch in the embryo.

The direction of growth of this nerve is somewhat different from that of the corresponding branches of the seventh. It grows dorsally and forwards (fig. 51, *s.t.g.*)

In late stages præ-branchial and pharyngeal nerves are developed, but I have no observations as to their mode of origin to record.

It is obvious that the glossopharyngeal agrees exactly with the general schema. The sole peculiarity to be noticed is the direction of growth of its supra-branchial branch. As in the cases of other nerves, the shifting and secondary attachment described by Marshall probably occur; I have, however, not studied them.

#### NERVES OF THE EIGHTH, NINTH, TENTH, AND ELEVENTH SEGMENTS— VAGUS COMPLEX.<sup>1</sup>

The actual development of this complex has been fairly accurately described by Van Wijhe. However, as in the cases of other nerves, he omitted to record some steps in the process of development, and referred the actual connection of the complex with the skin to a later stage than that in which it first arises.

He further, though describing the connection of the supra-branchial

<sup>1</sup> For the vagus the condition in *Torpedo* is taken, in which there are at least four nerves concerned; in *Hexanchus* the vagus has five elements, in *Heptanchus* six (*Gegenbaur*).

branches with the skin, and though figuring the actual fusion of the vagus ganglia with the sensory thickening, does not ascribe to the skin any part in the formation of the ganglia.

Like Van Wijhe, I cannot find in the vagus outgrowth itself any real segmentation in its earliest stages. The first outgrowth from the neural crest (fig. 33) is a broad uninterrupted band stretching from just behind the glossopharyngeal, which it almost joins, to a considerable distance backwards.

Like other posterior roots, this outgrowth grows outwards and downwards towards the portion of epiblast just above the second, third, fourth, and fifth branchial clefts, which are now just forming (fig. 33). Here the epiblast forms a longish sensory thickening, with which the vagus fuses.

Portions of the vagus pass on (fig. 34) behind the rudiments of each of the above-mentioned clefts, and form, as in other cases, the post-branchial nerves.

At the point of fusion with the skin, cells are proliferated from the epiblast to form the ganglia.

Soon, as pointed out by Van Wijhe, we get the ganglion of the first vagus cleft separated from the rest of the mass and fused with an isolated thickening above the second true branchial cleft.

For the rest of the vagus there is usually only one ganglionic mass, which, however, ventrally, and by its post-branchial branches, shows a division into three portions. This mass lies over the last three clefts, and is to be regarded as made up of the fused ganglia of the three branchial sense organs of these clefts, with the addition, however, of rudiments of nerve elements of a certain number of clefts which have disappeared, and even in the ontogeny hardly present traces of their former existence. In *Torpedo*, however, as first noticed by Wyman,<sup>1</sup> there is a rudiment of one cleft which never breaks through to the surface, and which is therefore never functional.<sup>2</sup> The rudiment of this cleft is very obvious in horizontal longitudinal sections of certain stages, and is represented in fig. 47. Here there is a considerable hypoblastic depression (*cl. vi*) of the pharynx just behind the last or fifth branchial cleft.

Corresponding to it is a shallower but still marked epiblastic

<sup>1</sup> Wyman, "Observations on the Development of *Raja batis*," 'Mem. Amer. Acad. of Arts and Sciences,' vol. ix, 1864.

<sup>2</sup> This paper of Wyman's was not accessible, and the statement in the text is given from Balfour's 'Embryology,' vol. ii.

involution. Along the posterior side of this hypoblastic depression the intestinal branch of the vagus runs. Gegenbaur has regarded this branch of the vagus as containing rudiments of post-branchial branches of aborted clefts; and I think that in the relationship of this intestinal branch in *Torpedo* to rudiments of a sixth cleft we have a new support for this view.

The ramus intestinalis is, as Van Wijhe states, mainly made up of the post-branchial branch of the last true visceral arch; but, as just stated, it must also contain portions of the post-branchial branches of one or more aborted clefts. Certainly this is the case in *Torpedo*.

In the question of the homology of this nerve I can only agree with Van Wijhe in rejecting Balfour's view that the ramus intestinalis is a commissure.

The statement just made concerning aborted clefts is also in accordance with Van Bemmelen's researches on the thymus. His discovery of thymus elements behind the vagus is mentioned by Dohrn<sup>1</sup> in his last great work, as supporting his view that Vertebrates formerly possessed many more gill-clefts than they do at present. The question will be returned to later on.

It is thus seen that in *Torpedo* at any rate the vagus contains the elements of at least four segmental nerves and the rudimentary portion of a fifth.

The first one of the lot is, shortly after its first development, slightly separated from the fused mass which contains the sense organs and ganglionic portions of the rest.

Hence vagus I. can be treated alone. As mentioned before, its post-branchial branch passes along the posterior wall of the second branchial cleft to the musculature of the cleft. The skin takes no part in its formation. Above the cleft the main nerve fuses with the skin, and there, as in other cases, the ganglion and primitive branchial sense organ are formed. In this case too—and fig. 34 shows it fairly well—the sensory thickening must be considered as taking part in the formation of the ganglion.

Later, the ganglion separates from the skin, and, along with this separation, the sensory thickening grows forwards and takes also a dorsal direction, a supra-branchial nerve splits off, and the sense organs formed are part of the supra-temporal branchial sense organs (fig 51,

<sup>1</sup> Dohrn, "Studien zur Urgeschichte, &c., No. vii, 'Mittheil. a. d. Zool. Stat. zu Neapel,' Bd. vi, Heft 1.



*st.v.*). Here, as in the glossopharyngeal, the supra-branchial branch has a dorso-anterior direction.

Vagus I. also fits into the schema very well. It is formed just in the way described in the schema, has the same relation to a cleft, develops a primitive branchial sense organ and associated ganglion, &c. In fact, its development might have been taken in giving the schema.

For the rest of the vagus there is only one ganglionic mass, and one long, broadish thickening with which the ganglionic mass is associated.

When the common nerve rudiment grows from the neural ridge and fuses with the epiblast, at the point of fusion the ganglionic mass is proliferated, probably entirely from the skin. From the ganglionic mass branches are sent off along the posterior sides of each of the three last clefts to the musculature of the clefts. They are the post-branchial branches, and are not developed from the skin. The last of the three is the so-called intestinal branch of the vagus. Along with the separation of the ganglion from the skin, the sensory thickening begins to grow backwards along the lateral surface of the trunk (fig. 39). This thickening is the rudiment of the so-called lateral line. The description of its development to be given here is in the main identical with that given by Van Wijhe.<sup>1</sup> It agrees with Götte's<sup>2</sup> and Semper's<sup>3</sup> researches in so far as it describes the origin from the skin of the so-called lateral nerve, and in this point it differs from Balfour's account.<sup>4</sup> It is, as Semper stated, very easy in Elasmobranchs, though by no means so in Teleostei, to follow the whole development of the lateral line and nerve.

In horizontal longitudinal sections the whole process is obvious enough, and I can fully endorse Van Wijhe in the opinion that Balfour would have had no doubt about the matter had he studied the point with horizontal sections instead of with transverse ones. The question of the direction of sections is here a vital one. In fig. 39, (*vg.gl.*) the compound vagus ganglion is represented as fused with the skin, and the lateral line, *ll.*, has commenced to grow backwards.

It is an interesting and by no means an unimportant point that the lateral line increases in length not by the actual conversion of the epiblast cells behind the growing point of the line into sensory cells

<sup>1</sup> *Op. cit.*, pp. 34, 35.

<sup>2</sup> Götte, 'Entwicklungsgesch. d. Unke,' p. 672.

<sup>3</sup> *Op. cit.*, p. 256.

<sup>4</sup> Balfour, 'Elasmobranch Fishes,' p. 141.

similar to those already present in the line, *but that there is an actual growth backwards of the lateral line itself* (figs. 40 and 41). That is, the sensory cells which compose the rudiments of the "line," and which anteriorly give rise to the compound vagus ganglion (*vg.* 2, 3, and 4), repeatedly and rapidly divide, and in such a manner that the "line" is increased in length and pushes its way between the indifferent epiblastic cells behind it (fig. 40). These indifferent epiblastic cells (figs. 40 and 41, *i.e.*) are actually thrust aside and *probably lost along the whole course of the "lateral line" and concomitantly with its growth.*

Part of the epiblast which is cast off is figured in figs. 40, 41, *i.e.* It is possibly this temporary epiblast seen in transverse section which led to Balfour's view of a special origin of the canals of the sense organs in the trunk of Elasmobranchs.

As in other cases the nerve of the sense organs, the so-called lateral nerve, is formed from the deeper portion of the sensory thickening. This mode of origin of the lateral nerve was first described by Semper, and afterwards more fully by Van Wijhe in Elasmobranchs.

The point is far easier to determine here than in the case of other supra-branchial nerves; indeed, it attracts the eye with startling distinctness in horizontal longitudinal sections of embryos of the proper age. The nerve is formed as the sensory thickening grows backwards along the body. It is well shown in figs. 40 and 41, *l.n.*, and can be traced from the vagus ganglion (*vg.gl.*) backwards along the thickening, gradually becoming thinner and less differentiated until finally it ceases in the cells of the sensory thickening.

That here there is no actual growth backwards of the nerve is obvious enough, for when the development has taken place for some length, then near the ganglion the nerve is fibrillar and has few nuclei, these latter increasing as the nerve proceeds backwards, and the fibres becoming, *pari passu*, fewer, and ending gradually in the protoplasm of the sensory thickening.

Where the compound vagus ganglion (*vg.gl.* 2, 3, 4) separates from the skin (fig. 36) it is easily seen that above each of the three branchial clefts fibres are given off from the separating ganglion to the sensory thickening. In fact, each of the elementary nerves making up the vagus compound, *viz.* *vg.* 2 and 3, and the intestinal branch, *vg.* 4 and 5, takes part in the formation of the so-called "lateral line." In other words, the lateral line is made up of supra-branchial branches

of at least four segmental nerves, probably of more than four, viz. vagus 2, 3, 4, 5. The fifth root is the rudiment of the nerve of the rudimentary cleft mentioned before.

We have seen that the facial, which is probably a compound nerve, has a large forked supra-branchial branch, and we shall find that the fifth and ciliary also, as already well known, have each a very long supra-branchial nerve, extending over the snout (fig. 51, *op.s.* and *oph. pro.*), and hence we need not be much surprised that a supra-branchial nerve, which is made up of the elements of at least four supra-branchial branches, should grow right away to the tail, and supply a very long series of branchial sense organs.

In a former note<sup>1</sup> I put forward certain hypotheses concerning the posterior roots of spinal nerves to account for the apparently abnormal innervation by the vagus, that is by a cranial nerve complex, of a region extending right to the tail. These hypotheses I now see reason to reject, and after a study of the actual facts of development in Elasmobranchs, as now recorded, I can only conclude that the so-called lateral line only differs in length and direction of growth from the other branchial sense organs. Its length is sufficiently accounted for by its containing the elements of at least four supra-branchial nerves, and its direction offers in itself nothing really remarkable, for the direction of growth of the other supra-branchial branches is not always the same. Those of the fifth, seventh, and ciliary grow forwards; those of the glossopharyngeal and vagus I. grow dorso-anteriorly, and that of the rest of the vagus grows backwards (figs. 46 and 51).

In fact, the direction of growth of sense organs and nerves would seem to be determined by the usefulness or need of having branchial sense organs in regions of the body other than the region just above the gill-clefts where they primitively occur.

Judging by the great variations one meets with in the arrangement of these branchial sense organs in Ichthyopsida it would seem as though different families of fishes and Amphibians had independently solved the matter for themselves. *The great morphological point to be noticed, and I shall lay great stress on it later, is that at first there is the rudiment of one branchial sense organ with its associated ganglion over each gill-cleft or over the site of a potential gill-cleft.*

With reference to the hypotheses about spinal nerves mentioned above, I may here state that I see no reason now for assuming that

<sup>1</sup> Beard, "On Segmental Sense Organs, &c." 'Zool. Anz.', 161, 162, 1884.

true spinal nerves were ever connected with branchial sense organs. So far as my researches go there is a wide difference both in morphology and development between the cranial and spinal nerves.

The mode of development of the lateral nerve here described is, as previously mentioned, in the main the same as that ascribed to it by Van Wijhe. The only author who has assigned to it a different origin in Elasmobranchs is Balfour, who was inclined to the view that the nerve really grows backwards from the vagus ganglion.

My own researches on Teleostei<sup>1</sup> led me to accept Balfour's view, but since I have had the opportunity of investigating the matter in Elasmobranchii I conclude that my interpretation of the matter in Teleostei was erroneous.

No doubt the account given by Hoffmann<sup>2</sup> of the development in Teleostei is correct. It accords well with the facts as recorded for Elasmobranchs here and by Van Wijhe.

But none the less it may not be superfluous to point out that the existing accounts of the development of what I have called supra-branchial nerves in Teleostei, Elasmobranchii, and Amphibians—that is, the accounts given by Semper, Götte, Hoffmann, and Van Wijhe—contain in them one element of uncertainty. That is, as to how the nerve thus developed from the skin acquires its connection with the appropriate ganglion.

Most of the accounts are quite silent on this point. Götte, it is true, recognised the importance of the matter, and stated that the nerve in any particular case separates from the skin along part of its length and grows to its ganglion. This view, however, is not in accordance with the facts, and I have reason to believe that Prof. Götte has now himself ceased to hold it.

The apparent absence of connection between the nervous structure of the brain and the branchial sense organs of the head was to Balfour a great objection to Götte's and Semper's view. He said, and to a certain extent he was right, that at first there is no nerve in connection with the developing sensory thickening.

This is right so far as its growing point is concerned, for there the nerve has not developed.

But, as Van Wijhe has pointed out, it is not really the case so far as relates to entire absence of nerve in connection with the sensory

<sup>1</sup> *Op. cit.*

<sup>2</sup> Hoffmann, "Zur Ontogenie der Knochenfische," 'Archiv für Micr. Anat.,' Bd. xxiii, p. 45.

thickening, and further, the connection between sense-thickening and nerve is best made out in early stages, and is afterwards not so easy to trace.

Van Wijhe himself, though he has given a true, accurate, but somewhat incomplete account of the development of these supra-branchial branches to the sense organs, cannot be said to have solved the difficulty under discussion. He has rather ignored it, and though possessing the material for its solution, has not mentioned the matter.

It is very curious that, although he has figured the fusion of various ganglia with the skin, he has apparently not noticed that the supra-branchial branches grow in the various cases out of the various ganglia so fused, *and therefore are in connection with their appropriate ganglia from the first.*

In fact the whole *rationale* of the formation of supra-branchial nerves is to be seen in the deploying of the branchial sense organs, and in the connection of these organs with the ganglionic centre by longer or shorter conducting fibres—the supra-branchial nerves. Originally the sense organs were restricted *to one over each gill-cleft, with an associated ganglion.*<sup>1</sup> This increased, and gave rise to two by division, and so on. This is the more certain when we remember that even in late stages, according to Malbranc,<sup>2</sup> the sense organs of Amphibia increase by division. I have myself noticed and recorded this mode of increase in embryonic Teleostei.<sup>3</sup>

It is hardly necessary to repeat that Gegenbaur's view of the composition of the vagus out of a number of typical posterior roots is quite true. We have seen that it really contains rudiments of at least five such elements in Torpedo.

It follows from this that the vagus agrees with the schema given in the preceding pages. It is equivalent to and shows the development of, at least four such schematic nerves. True, there is only one supra-branchial branch,<sup>4</sup> the lateral nerve, for all the elements of the vagus except the first. But this is probably secondary, and due to the fusion

<sup>1</sup> Beard, "Segmental Sense Organs and Associated Ganglia," 'Zool. Anz.,' 192, 1885; also Froriep, "Ueber Anlagen von Sinnesorganen am Facialis, &c.," 'Archiv für Anat. und Physiol.," 1885.

<sup>2</sup> Malbranc, "Von der Seitenlinie u. ihren Sinnesorganen bei Amphibien," 'Zeit. f. wiss. Zool.' vol. xxvi, 1876.

<sup>3</sup> Beard, "Segmental Sense Organs of Lateral Line," 'Zool. Anzeiger,' Nos. 161, 162, 1884.

<sup>4</sup> In Torpedo and many other forms. In other cases the "lateral line" is more complicated; especially is this the case in Amphibia, *vide* Malbranc, *op. cit.*

of the posterior elements of the vagus, and, as stated before, *vg.* 2, 3, and 4, all give fibres to the lateral line.

It is worth mentioning here, because these researches confirm one of Balfour's views, that the "lateral line" was originally, as he believed, restricted to the anterior part of the body. The whole development of all these branchial sense organs shows the truth of this. But it is, at the same time, a very curious fact that these sense organs along the trunk of Teleostei are segmental (fig. 44, *br.o.*). This is well known, and is figured in the above figure, which is part of a horizontal section of a salmon hatched about six weeks.

At one time I believed, with Eisig and others, that great morphological importance could be attached to these facts; but I feel now compelled to adopt Balfour's view, and in discussing the morphology of these sense organs I shall strongly urge that in face of the facts of development here recorded, the morphological connection between these branchial sense organs of Vertebrates and the "Seitenorgane" of Capitellidæ, first suggested by Eisig,<sup>1</sup> becomes of a very doubtful nature. And here again I may be permitted to remind the reader that Balfour<sup>2</sup> long ago rejected the existence of any homology between these two sets of organs.

#### VAGUS IN AMPHIBIA.

Mr. Spencer has recorded in this Journal<sup>3</sup> certain observations on the nerves of Amphibians. He has found that not merely the ganglia of the dorsal roots of cranial nerves of Amphibians, but that the whole of the nerves themselves are split off from the skin. I have figured the origin of the vagus nerve and ganglion in the frog in fig. 27. I have investigated the facts in Amphibians, and can fully confirm Mr. Spencer in most points. The development as seen in Amphibians is interesting, as in some respects showing a very primitive condition of the nervous system, *viz.* a nerve sheath or part of one; in other respects it is impossible in them to get as good a view of the primitive nerve composition of the head as in Elasmobranchs.

In Amphibians a considerable amount of fusion of once separate nerves has taken place, not only behind the auditory organ, but also in front of it. As an instance, it may be mentioned that the ciliary

<sup>1</sup> Eisig, "Die Seitenorgane der Capitelliden," 'Mittheil. a. d. Zool. Stat. z. Neapel,' vol. i., 142.

<sup>2</sup> Balfour, 'Comp. Embryol.,' vol. ii., p. 142.

<sup>3</sup> 'Quart. Journ. Micr. Sc.,' Supplement, July 1885.

ganglion, which in Elasmobranchs, and even in birds, is quite distinct in its development, is in the Amphibians fused with the Gasserian, and the two arise together as one fused mass.

Vagus 1, 2, 3, and 4 are also all fused into one mass in Amphibia; the figure (27) is a transverse section through this mass. In it the nerve has not separated from the skin, and the ganglionic portion is readily recognisable as a mass of yolk-filled cells on the level of the lateral line. Later, both ganglion and nerve leave the skin as in Elasmobranchs.

NERVE OF THE THIRD SEGMENT—TRIGEMINAL LESS OPHTHALMICUS  
PROFUNDUS.

The fifth nerve is well suited for studying the development of the ganglion of a dorsal root.

It is well known, from Balfour's and Marshall's researches (*opercit.*), that it arises from the third of the brain vesicles. In fact, from their researches and those of Van Wijhe, the development of the fifth is fairly well known with the exception of three stages. These are the fusion with the skin, the formation of the Gasserian ganglion, and the mode of development of the supra-branchial nerve (portio minor of the ophthalmicus superficialis, Schwalbe).

To explain these stages it will be necessary to repeat some facts which are already known.

The outgrowth from the neural ridge, which forms the rudiment of the fifth, is broad and extends backwards almost to the region of the seventh. Anteriorly it stretches forwards almost to the roots of the ciliary, to be hereafter mentioned.

But the region between the two ganglia is well defined in the earliest stages by the indifferent epithelium between them, and by the position of the second head cavity which lies between them (fig. 11, *h.c.* 2).

The nerve rudiment grows down to the level of the notochord (fig. 14) and fuses with an epiblastic thickening, just as the other nerves do. Here cells can be seen leaving the thickening to form the ganglion (fig. 15).

In this case and in that of the ciliary there can be little doubt as to the actual mode of formation of the ganglion. The thickening which gives rise to the ganglion is situated just dorsal to the mouth, and in fact has just the position of a branchial sense organ.

The ganglion is figured in fig. 17, still connected with the skin, and

possessing then what we may regard as its primitive branchial sense organ.

Later, the sensory thickening grows in an anterior direction, and as it does so the ganglion separates from the skin, leaving behind it, as in other cases, a nerve which is split off from the sensory thickening, and which is the supra-branchial branch of the fifth (fig. 51, *op.s.*). Its course, &c., have been described by Marshall and Spencer, and it is usually called the *portio minor* of the *ophthal. superfic.* It was first classed as the *r. dorsalis* of the fifth by Balfour, and Marshall and Spencer afterwards expressed their agreement with this view. Where the main nerve fuses with the skin its course is continued along the mandibular arch by a number of cells of the nerve. These form the post-branchial branch, and innervate the musculature of the mandibular arch. Later, a præ-branchial nerve is developed (Van Wijhe and others), which hooks over the angle of the mouth in the way that other præ-branchial branches hook over gill-clefts.

Another apparent branch of the fifth is the nerve which Marshall has called a communicating nerve between the ciliary and Gasserian ganglia (fig. 51, *c.b.*). Its true nature has been worked out by Van Wijhe, who has shown that it really belongs to the ciliary ganglion. As I accept this statement I shall describe the nerve, as Van Wijhe has done, as part of the nerve of the second segment.

The *ophthalmicus profundus* (fig. 51, *oph. pro.*) is also a part of the nerve of the second segment. This has been recognised by Marshall and Spencer, and also by Van Wijhe.

The later fusions which occur between the fifth and seventh and the fifth and ciliary are in the early stages absent. In fact, in its development the *fifth has the typical characters of the posterior root of a gill-bearing segment.* It fulfils in every way, as Marshall found, the requirements of a segmental nerve as laid down by him, and it accords with our schema. It possesses a primitive branchial sense organ and an associated ganglion *just above a cleft, the mouth.* It has the homologues of post-branchial and præ-branchial branches, and it develops a supra-branchial nerve in connection with the branchial sense organs over the snout (fig. 51, *op.s.*).

The new additional light thrown on the nature of the mouth will be referred to in discussing the general morphological considerations arising out of these researches. Suffice it here to say that the facts



given above seem to me to confirm Dohrn's<sup>1</sup> conclusion that the mouth arose from a pair of coalesced gill-clefts.

SECOND SEGMENTAL NERVE—OPHTHALMICUS PROFUNDUS, CILIARY  
GANGLION, AND RADIX LONGA.

A good deal of confusion exists as to the actual nerve components of this segment.

Marshall<sup>2</sup> regards the motor oculi as the main stem of the ciliary ganglion, and attributes to it the character of an anterior and posterior root. In Marshall and Spencer's<sup>3</sup> paper the ophthalmicus profundus is also classed as part of this segment. Schwalbe<sup>4</sup> had previously shown that the ciliary ganglion was really the ganglion of the posterior root of this segment, a demonstration which Marshall confirmed embryologically. Following on and extending these discoveries, Van Wijhe recognised the most important component of this segment in the ophthalmicus profundus, which he classed as the posterior root of the segment. While accepting to a certain extent Van Wijhe's view, I feel bound to admit that from Van Wijhe's researches alone, the matter does not stand in a very clear light.

Here, as in other cases, Van Wijhe's preconceived notions as to the correspondence of the roots of cranial nerves to those of the spinal nerves, interfered with the proper interpretation. Marshall<sup>5</sup> first gave an account of the development of the ciliary ganglion; this account Van Wijhe added to, but it is still by no means complete. And although the development of no cranial ganglion is easier to follow, and no fusion of the epiblast more obvious than the development and fusion of the ciliary ganglion, this fusion has never before been figured, and Van Wijhe's earliest stage figured (fig. 31, *g.l.c.*, *op. cit.*) is a stage at which the ganglion is in great part separated from the skin, and in which the ophthalmicus profundus which runs from the ganglion along the snout and forms the supra-branchial branch, has just begun to develop.

A glance at the diagrams (figs. 45 and 46) of the cranial nerves, according to my views, will simplify matters and pave the way for the account shortly to be given.

<sup>1</sup> Dohrn, "Studien, &c.," No. 1, 'Mittheil. a. d. Zool. Station zu Neapel,' Bd. iii., p. 252.

<sup>2</sup> Marshall, "Segmental Value of Cranial Nerves," 'Journ. of Anat. and Physiol.,' 1882.

<sup>3</sup> *Op. cit.*, p. 29.

<sup>4</sup> Schwalbe, 'Das Ganglion Oculomotorii.'

<sup>5</sup> Marshall, "Head Cavities and Associated Nerves, &c.," 'Quart. Journ. Micr. Sc.,' 1880.

Taking the ninth nerve, or glossopharyngeal, as a type of a cranial nerve to a true gill-cleft, we see that there is a main stem (*p.r.*), a ganglion with associated sense organ, and then three other branches. These are a post-branchial (*p.n.*), a præ-branchial (*p.b.n.*), and a supra-branchial (*s.b.n.*). As their names imply, the post-branchial and præ-branchial run behind and in front of the cleft respectively. The supra-branchial nerve is the nerve connected with the later developed additional branchial sense organs.

Now we may turn to the nerve of the second segment. The first thing noticeable is *that the cleft is absent*,<sup>1</sup> or at any rate the gill muscles are not present even in the ontogeny.

As a natural corollary to the absence or metamorphosis of the cleft, and absence of its muscles, *the post-branchial and præ-branchial nerves are also aborted.*

In the diagram this abortion is represented by dotted lines (fig. 46). Hence all that we can expect to find of the posterior root of this segment is a supra-branchial branch to the branchial sense organs, the ganglion of the branchial sense organs, and the main stem connecting the ganglion with the brain. The ganglion is the ciliary, the main stem is the radix longa, connecting the ciliary and Gasserian ganglia, and the supra-branchial branch is the ophthalmicus profundus.

This identification is very similar to that given by Van Wijhe, but the matter is approached from an entirely different point of view.

The actual development is as follows: From the neural crest of the mid-brain, just before the closure of the neural folds, cells grow outwards and downwards to a thickened patch of epiblast just above and behind the eye (fig. 7).

This outgrowth has been seen and described by Marshall and Van Wijhe. But Marshall recognised in it the first rudiment of the motor-oculi, and Van Wijhe that of the ophthalmicus profundus. Neither observer saw the skin fusion or the development of the ganglion. When the outgrowth reaches the thickened patch of epiblast it fuses with it (fig. 6). Cells are then proliferated off from the skin to form the ganglion, and the outer portion of the thickening begins to form the *primitive branchial sense organ* (figs. 8 and 9). From the thicken-

<sup>1</sup> Or metamorphosed. Dohrn has recognised what he believes to be a cleft behind the nose and in front of the mouth in the *hypophysis*. He does not say that it is the cleft of the ciliary ganglion, but this would seem to follow if Dohrn's view were accepted. As at present, though possible, no relationship of this supposed cleft to the ciliary ganglion has yet been demonstrated, Dohrn's view must be accepted with reserve.

ing, cells are given off for some time, until a large ganglionic mass is formed, which still for some time remains fused with the skin.

In fact, in the case of the ciliary ganglion the mode of development is well marked and very easy to study. The sensory thickening soon begins to grow forwards over the snout, and as it does so the ganglion begins to leave the skin. As this takes place a nerve is developed from the thickening, and connects the ganglion with its branchial sense organs.

From its course, relations, &c., this nerve is seen to be the ophthalmicus profundus.<sup>1</sup> It is *morphologically the supra-branchial nerve of the second segment.*

The distance between the ciliary and Gasserian ganglia, even in early stages, is very short. The outgrowth from the neural ridge which forms the main stem of the ciliary ganglion is practically continuous with the outgrowth which forms the main stem of the fifth. Van Wijhe has also drawn attention to this.

Hence it can hardly be wondered at that the connection of the two ganglia with the brain soon becomes a common one, which distally divides into two portions, one of which is continued to the Gasserian ganglion, while the other goes somewhat obliquely to the ciliary, and forms its so-called *radix longa* (fig. 51, *c.b.*).

Although I have no observations to record as to the development of the third or motor-oculi nerve, still Marshall's opinions on the nature of the nerve must be discussed, and as his views are inconsistent with the other facts as recorded in this paper, I shall state what seem to be urgent reasons for modifying them.

Marshall has advanced the suggestion that the third and fourth nerves together make up a segmental nerve. He says:<sup>2</sup> "There is very strong reason for thinking that, in the chick at any rate, the third nerve develops, like the hinder cranial nerves and the posterior roots of spinal nerves, as an outgrowth from the neural crest on the top of the mid-brain." Since the third nerve later on arises from the base of the mid-brain, "very near the mid-ventral line," he infers that the nerve must shift downwards, and to an extent unequalled by any other nerve.

Now, leaving aside the fact that the shifting in the case of the third

<sup>1</sup> Apparently also Van Wijhe's identification, but not very obvious from his description.

<sup>2</sup> Marshall, "Segmental Value of Cranial Nerves," 'Journ. of Anat. and Physiol.,' p. 35  
1882.

nerve, if it does take place, occurs, by Marshall's admission, to a greater extent than in the case of the other cranial nerves, a point which is surely of some importance, there are other objections which cannot, I think, be ignored. Marshall's views have also been contested by Van Wijhe, for whose reasons the reader is referred to his oft-quoted work on the nerves of the Elasmobranchii.

In any discussion as to the nature of the third nerve the morphology of the head cavities is bound to have an important place. The second or mandibular head cavity undoubtedly gives rise to the superior oblique muscle (fig. 12, *h.c.*<sub>2</sub>). On this point I can fully confirm Van Wijhe.

This fact alone ought to dispose of the fourth nerve, which Marshall considers as part of the nerve of the second segment—that is, as part of the third nerve. The mandibular head cavity arises from the meso-blast plate of the mandibular arch, according to Balfour, Marshall, and Van Wijhe. It gives rise to the superior oblique muscle, therefore the nerve of this muscle, the fourth nerve, must also belong to the mandibular segment, as Van Wijhe insists.

Further, if the first head cavity is morphologically of the same nature as the second and third head cavities, then the third nerve, which innervates the muscles derived from the third head cavity, is, *a priori*, of the same nature as the fourth and sixth nerves.

Marshall himself regards the sixth nerve as a ventral root of the seventh nerve,<sup>1</sup> and says: "Concerning the actual value of the sixth nerve, I see no reason to alter the opinion I previously expressed, that the sixth nerve may be regarded as having the same relation to the seventh that the anterior root of a spinal nerve has to its posterior root."

We have also seen reason to believe that the fourth is a ventral root of the trigeminal nerve. And from all these facts we might fairly regard the third as also a ventral root.

But further, the dorsal root of no other cranial nerve, if we except the third, innervates the structures arising out of a head cavity. The dorsal roots, so far as they are motor, only innervate those structures derived from the lateral muscle plates (Van Wijhe).

According to Van Wijhe, the third nerve develops after the ciliary ganglion, and hence could not be its dorsal root. The third, at any rate, is an exceedingly fine nerve, and is much thinner than the ophthal-

<sup>1</sup> 'Segmental Value, &c.,' pp. 42-44.

micus profundus; hence, if the third nerve be the dorsal root of the second segment, then the proximal stem of the nerve is thinner than one of its distal branches. Hence there seems to be no avoiding the conclusion, in which I agree with Krause and Van Wijhe, that the third is not the dorsal root of the ciliary ganglion, but is the ventral root of the second segment.

Returning to the general schema of the development of the dorsal root of a cranial nerve, it is found that, so far as its development goes, the nerve of the second segment agrees with the schema. In this instance allowance has to be made for the absence of a gill-cleft, and more especially, of a gill-musculature. In this the absence even in the ontogeny of post-branchial and præ-branchial branches is accounted for. Otherwise the development is normal. There is a main stem with primitive branchial sense organs and an associated ganglion, the ciliary. There are no other branches except the later developing supra-branchial nerve (*ophth. profund.*). This nerve, as elsewhere, is developed in connection with the extension forwards of the branchial sense organs (fig. 51, *oph. pro.*). The reduction which has probably taken place in the nerve of the second segment prepares the way for the recognition and interpretation of the still greater specialisation which the two remaining cranial segmental nerves have undergone; and affords a better insight into the true nature of the olfactory and auditory nerves.

#### FIRST SEGMENTAL NERVE—OLFACTORY NERVE.

The olfactory nerve has usually been classed with the auditory and optic nerves apart from the true segmental cranial nerves.<sup>1</sup> Dohrn, in his essay on "Die Ursprung der Wirbelthiere," first suggested that the nose was a gill-cleft, and Marshall<sup>2</sup> very strongly advocated this view as the result of his researches on the chick and in Elasmobranchii. He insisted, and as I believe with justice, on the segmental nature of the olfactory nerve. His reasons for this view were based on the actual development of the olfactory nerve; and he states—and so far as my researches go they only confirm his statement—that "the olfactory nerves develop in precisely the same way

<sup>1</sup> Huxley, 'Anat. of Vertebrates,' p. 71; Gegenbaur, 'Elements of Comp. Anat.,' English trans., p. 515; Götze, *Entwicklungsgesch. d. Unke, &c.*

<sup>2</sup> Marshall, A.M., "The Development of the Cranial Nerves in the Chick," 'Quart. Journ. Micr. Sc., 1873, p. 23; and also, 'Morphology of the Vertebrate Olfactory Organ,' 'Quart. Journ. Micr. Sc.,' 1879.

as the other cranial (segmental) nerves: they arise at first from the upper part of the fore-brain and gradually shift downwards, acquiring by so doing a secondary connection with the cerebral hemispheres, of which they are at first completely independent; and finally the olfactory lobe or vesicle, so far from being the earliest part to be developed, is actually the last, no vestige of it appearing in the chick until the seventh day of incubation, in the salmon till long after hatching, or in the dogfish until stage O. of Balfour's nomenclature."<sup>1</sup>

For the rest it is hardly necessary to repeat here the evidence advanced by Marshall of the segmental nature of the olfactory nerve. Though in my opinion not quite conclusive, it is of value so far as it goes, and it will be summarised later on after additional evidence has been adduced in favour of the segmental nature of the olfactory nerve.

But Marshall recognises in the olfactory organ the rudiment of a gill-cleft, and, as I am led to a somewhat different view, it may be of advantage to give a summary of Marshall's reasons for this opinion.

For the detailed account the reader is referred to the paper on "The Morphology of the Vertebrate Olfactory Organ." The following abstract is taken from Wiedersheim's 'Lehrbuch der Vergleichenden Anatomie,' p. 375. The epitome there given is so concise and clear that I do not feel it necessary to offer any excuse for reproducing it here.

Starting from the fact that the olfactory nerve agrees in its development with the other cranial nerves—that is, that it represents a spinal-like nerve which springs from the neural ridge—Marshall regards the olfactory groove as a primitive gill-cleft, which in exactly an analogous position to that in which the true gill-clefts are supplied by branches of the glossopharyngeal and vagus, has an anterior (upper) and a posterior (lower) branch of the olfactory nerve, these branches being respectively in front of and behind the supposed olfactory cleft. The Schneiderian folds of the nasal mucous membrane are comparable to the gill-filaments of fishes. As a consequence of the above view a communication between the nasal and oral cavities must once have existed in all Vertebrates, including fishes. Leaving aside the fact that such a condition is still present in Myxinoids, traces of it are to be seen in the naso-oral groove of Selachians, and also in the development of other fishes. Thus Marshall found in salmon embryos obvious diverticula of

<sup>1</sup> Marshall, 'Segmental Value of Cranial Nerves,' p. 13.

the oral mucous membrane, which stretched towards the nasal groove, but which later in the development disappeared. Smelling, argued Marshall, is only a modified breathing, and thus no violent physiological change is necessary to convert a gill into a smelling organ.

Wiedersheim<sup>1</sup> himself formerly supported Marshall's view, and pointed out that in *Epicrion*, and probably in other *Gymnophiona* as well, there are on either side two olfactory nerves, one dorsal and one ventral, the roots of the two being perfectly independent and some little distance apart. He considered these roots to be homologous with the dorsal and ventral roots of a spinal nerve, and that by their discovery the segmental rank of the olfactory nerve was established. But, as Prof. Wiedersheim has kindly informed me by letter, he has, since the appearance of Blaue's paper ("Ueber Bau der Nasenschleimhaut bei Fischen und Amphibien," 'Archiv für Anat.,' 1884), seen reason to change his views on this subject.

The contents of this really important paper will be referred to shortly, and here I need only express my conviction that the results of Blaue's work, taken in conjunction with the light which I hope to throw on the development of the nose and its relationship to the other branchial sense organs, settle in a very definite and satisfactory manner the true homology of the nose.

What has now to be demonstrated is that the *nose is really a branchial sense organ, that is, the sense organ of a non-existent gill-cleft, and not a gill-cleft itself.*

It ought here to be mentioned that Hoffmann has already expressed a very similar view of the nature of the nose.<sup>2</sup> That is, he compares its whole development to that of the ear and of the so-called organs of the lateral line, and rejects Marshall's view entirely.

Although I have very little that is new to add concerning the development of the olfactory nerve, still the novel way in which its development will be regarded is not without importance.

It was seen in discussing the nerve of the second segment—the root of the ciliary ganglion—that the whole nature of the nerve of this segment was obvious enough when it was noticed *that the musculature of the lateral plates, that is, the gill-musculature, was absent, even in the ontogeny.*

<sup>1</sup> Wiedersheim, 'Anatomie der Gymnophionen,' 1879, pp. 59, 60.

<sup>2</sup> Hoffmann, "Zur Ontogenie der Knochenfische," 'Archiv f. Micros. Anat.,' Bd. xxiii., p. 88.

As a consequence, *post-branchial and præ-branchial nerves were absent*, and the whole segmental nerve was reduced to a ganglion and a supra-branchial sensory nerve, this nerve, as its name implies, being connected with the innervation of the still-existing branchial sense organs. Of course the main stem of the nerve connecting ganglion and brain was also present.

A very similar condition of things exists in the nose. The early development has its exact parallel in the development of the nerve of the second segment. The sole difference is that the sense organs of the nose have not, as in the case of those of the second segment, undergone further development in a linear direction (fig. 46), but have confined that development to a somewhat circular area; that is, they have developed in many directions, but to a limited extent in each. A change of function has also probably occurred. In higher forms this, of course, is certain.

A glance at the diagram (fig. 46) will illustrate the meaning of the above remarks. The supra-branchial nerve of the second segment (*s.b.n.*) is represented by a line. In the nose (*olf.o.*) a supra-branchial nerve can hardly be said to be present. The sense organs have developed within an enclosed figure.

For the rest, the development of the nerve of the first segment is practically that of a typical segmental nerve in which post- and præ-branchial branches are aborted.

The nerve grows down from the brain to a thickening of epiblast, it fuses with this thickening (fig. 1), and a ganglion is formed at the point of fusion (figs. 2, 3, and 4). Even with the limited amount of material at my disposal, it can fairly well be shown that the ganglion is formed from the skin.

When the nerve first fuses with the skin, just as in other cases, no ganglion is present (fig. 1).

The ganglion first develops after the fusion, and from the inspection of figs. 2, 3, and 4, which are camera drawings of actual sections, it will be plain that there are strong reasons for believing that, as in other cases, the ganglion is proliferated from the sensory thickening. At any rate, in a later stage, which has also been figured by Marshall (fig. 5), it is seen that the state of affairs exactly resembles that in the ciliary ganglion and thickening (fig. 8), Gasserian ganglion and thickening (fig. 17), &c. The only difference between the olfactory ganglion and thickening and the complete segmental



nerve, ganglion, and thickening of a gill-bearing segment, is the absence in the olfactory segment of any præ- or post-branchial nerves.

Fig. 2 shows us a ganglion fused with an epiblastic sensory thickening and connected with the brain by a short nerve stalk. In fact, it is the picture of a branchial sense organ and its associated ganglion.

The facts of development here given, which accord so marvellously with the development of the other cranial segmental nerves, certainly render necessary a modification of Marshall's view as to the nature of the olfactory organ, and in fact a modification in the sense of the above passage, in which the nose is regarded not as a gill-cleft, but as the sense organ of a gill-cleft.

Marshall based his views firstly on the correspondence in anatomical and histological structure between the nose and other gill-clefts; secondly, on the frequent occurrence of two branches of the olfactory nerve, one on each side of the supposed cleft; and he further compared the Schneiderian folds of the nasal mucous membrane, as Stannius<sup>1</sup> had previously done, to the folds of a gill.

The facts of development, as stated by Marshall, have been here admitted, but at the same time slightly extended, and in such a wise that the development of the olfactory nerve and organ is shown to agree very closely with that of the nerve, ganglion, and branchial sense organs of any other cranial segmental nerve.

But now as to the relationships of the branches of the olfactory nerve to the supposed cleft, and as to the nature of the branches themselves.

In its earliest development the olfactory nerve shows nothing that can really be homologised with the post-branchial branch of a cranial nerve. Such a resemblance, when present at all, is only existent in much later stages.

But the post-branchial branch of a cranial nerve, whenever developed, is, *par excellence*, concerned with the innervation of the gill musculature, and if it contains sensory fibres its main portion is motor. There is nothing like a gill-musculature, even in early stages, connected with the olfactory organ.

No one has yet described an arterial arch, gill-cartilage, or musculature in connection with the supposed nasal visceral arch. The Schneiderian folds have indeed, in Elasmobranchii and other forms, a certain resemblance to gill-folds, but this alone would not be sufficient

<sup>1</sup> Stannius, 'Lehrbuch der Vergleichenden Anatomie,' ii. Theil

to homologise the two structures, and the folding could be more easily explained as brought about by the mere physiological need of increased surface. But surely it is a great change from a respiratory structure and function to a sensory structure and function ; a change which, in spite of the basis of truth in Dohrn's law of change of function, has not, so far as I am aware, been shown to have occurred in any other case. True, Dohrn<sup>1</sup> has recognised a gill-cleft in the hypophysis, but he has declined to ascribe a sensory function to that structure.

Froriep,<sup>2</sup> also, in discussing my views as to the nature of the Vertebrate auditory organ, has suggested that the ear is really a modified gill-cleft. But, as I shall presently show, this suggestion cannot be accepted, or even be held with any amount of reserve, for it is based on erroneous ideas of the primitive nature of the dorsal roots of cranial nerves.

If my discoveries stood alone, I should conceive it as highly probable, if not certain, that the nose is really a branchial sense organ. But this view of its nature is confirmed in a most striking manner, and rendered as certain as anything can possibly be by the researches of Blaue.<sup>3</sup>

These researches have been carried out on a considerable series of fish and Amphibians, and have led to the conclusion that in the lowest form of adult nose met with, viz. the nose of some fishes and Amphibians (*Belone*, the herring, and *Proteus*), the structure of the nasal membrane is essentially made up of a series of "smell buds" (*Riechknospen*), and between these an indifferent stratified epithelium. These smell buds are identical in structure with the so-called taste-buds of the *papilla foliata* of the tongue, say of a rabbit, and are also identical with the structures in the skin of fishes, which are here called branchial sense organs, and which are usually known as sense organs of the lateral line.

In the common *Triton* those structures described by Blaue are readily found in transverse sections passing through the nasal cavities. One such section is figured in outline in fig. 48, and a part of the section, showing two sense bulbs of the nose, or smell buds, is figured under high magnifying power in fig. 49.

<sup>1</sup> Dohrn, "Studien, &c.," No. 2, 'Mittheil. a. d. Zool. Stat. zu Neapel,' Bd. iii.

<sup>2</sup> Froriep, "Ueber Anlagen von Sinnesorganen am Facialis," 'Archiv für Anat. und Physiol.,' 1885.

<sup>3</sup> Blaue, "Ueber Bau der Nasenschleimhaut bei Fischen und Amphibien," 'Archiv für Anat. und Physiol.,' 1884.

In Triton I have fully convinced myself by actual investigation that Blaue's results are true and accurate. And I have also somewhat examined the state of things in a few fishes. There can really be no doubt as to the accuracy of Blaue's results; and here it only remains to give a very short *résumé* of the paper, referring the reader who desires further detail to the original, which is illustrated by a number of very beautiful drawings.

In many Amphibians and fishes the nasal membrane has the structure mentioned above, but in others the indifferent epithelium becomes reduced, so that the bulbs come to lie nearer together. This reduction of the indifferent epithelium begins around the bases of the buds. The basal epithelium is pushed away, and in such a fashion that the bulbs are in contact basally, but are separated distally by indifferent epithelium (*Exocoetus*).

In *Trigla* typical smell buds are found along with others that have increased in width and pushed the indifferent epithelium away.

In *Cottus* the smell buds are almost completely fused together, but there is still a little indifferent epithelium, and a few buds still remain isolated.

Lastly, in *Fierasfer* and others the indifferent epithelium has disappeared entirely from the folds of the nasal membrane, and a continuous sensory epithelium is present.

Thus Blaue has furnished very valuable evidence, from which, in conjunction with our knowledge of the development in *Elasmobranchii* the nature of the nose can be decided with greater probability than hitherto.

In *Elasmobranchii* separate bulbs are not present even in the embryo. The indifferent epithelium has disappeared even in the ontogeny; but from Blaue's researches on the structure of the nasal membrane in adult fishes generally, and from the mode of development of the nose, its ganglion and nerve, there can really be no hesitation about classing the nose with the branchial sense organs, and hence we are justified in calling it the modified sense organ of a gill-cleft.<sup>1</sup> F. E. Schultze<sup>2</sup> had previously stated his conviction that the "*Geschmackorgane*" of taste buds were the last remains of the skin sense bulbs of fishes, and Blaue now homologises the smell buds and the sense bulbs of the skin of fishes.

<sup>1</sup> Beard, "Cranial Ganglia and Segmental Sense Organs," 'Zool. Anz.,' 192, 1885.

<sup>2</sup> F. E. Schultze, "Ueber die becherförmigen Organe der Fische," 'Zeit. f. wiss. Zool.,' Bd. xii, 1863.

But though he is convinced of this homology, he nowhere hints that the nose is to be regarded as a specialised portion of the so-called organs of the lateral line, and in fact accepts and supports Marshall's gill theory of the nature of the nose, and derives his smell buds from skin sense bulbs which, originally present on the nasal visceral arch, as in other cases, have wandered into the nasal-cleft.

Now, although sense bulbs are present on and along the visceral arches of many fishes, they are not primitively there, their primitive position being *above* the cleft, not along it. Their presence along the arch is a later development. This fact and the facts of development as given before are entirely opposed to Blaue's supposition.

It is a curious commentary on the influence of the same set of facts on the views of different zoologists, that while Blaue, as the result of his researches, advocates the gill nature of the nose, Prof. Wiedersheim, as he has kindly informed me by letter, since reading Blaue's paper, considers it necessary, as most morphologists would, to give up entirely the notion that the nose is a gill-cleft.

My own opinion does not rest on the researches of Blaue alone. Apart from those discoveries, I should believe myself justified in holding, as against the views of Prof. Dohrn and of my own teacher, Prof. Marshall, that the nose is the modified sense organ of a gill-cleft rather than a gill-cleft itself.

But though maintaining that Blaue's results are not necessary to support this view, yet, blending together those results and the facts recorded in this paper as to the development, &c., of the supra-branchial sense organs and of the nose itself, I believe that my view of the nature of the nose has so solid a foundation in facts that even the most sceptical zoologist can have little hesitation in accepting it.

Shortly stated, the olfactory organ is a branchial sense organ, and the olfactory nerve is a segmental nerve, the post-branchial and præ-branchial branches of which, in consequence of the absence of a nasal-cleft, are not developed. In fact, the olfactory nerve is the sensory remnant of the most anterior segmental nerve.

#### DEVELOPMENT OF THE NOSE IN AMPHIBIA AND TELEOSTEI.

Hoffmann has described the development in *Salmo*, but has not ascribed an epiblastic origin to the nerve; this, however, is the case in both Teleostei and Amphibians. In Amphibia, Götte held that the olfactory nerve was developed in mesoblast. In fig. 4. the develop-

ing olfactory nerve and organ of a Teleostean, *Rhodeus amarus*, is figured, and in fig. 3 a similar stage in *Rana temporaria*. In both cases there is an epiblastic thickening, with which is united the rudiment of a ganglion, and there is also the rudiment of a nerve, the future olfactory nerve (*olf.n*), just splitting off from the skin. The development here is precisely similar to the development of the fifth nerve in the frog as described by Spencer, or to that of the vagus in the same animal as described in the preceding pages.

It is hardly necessary to say that these facts confirm what has been said of the nature of the nose in Elasmobranchii.

#### NERVE OF THE SIXTH SEGMENT—AUDITORY NERVE.

In a former paper<sup>1</sup> I suggested the homology of the auditory organ with the so-called organs of the lateral line or branchial sense organs. Subsequent investigation has only confirmed this suggestion.

Gegenbaur originally ranked the auditory nerve as a dorsal branch of the seventh. On embryological grounds Marshall and Balfour had also been led to the conclusion that the auditory nerve was not in itself entitled to segmental rank, but was in its development only a dorsal sensory branch of the seventh. Marshall, indeed, held that there was not room for another segmental nerve between the seventh and ninth.

Recent researches have led different zoologists to the opinion that the hyoid arch is composed of two originally distinct arches.

Van Wijhe considers that the obliterated cleft was behind the facial nerve, while Dohrn holds that it was in front of the hyoid cleft. The possibility that both are right appears to me not unlikely. Dohrn sees remains of a former cleft in the hyo-mandibular and in the thyroid body. The only evidence afforded by the nerves in support of this appears to be the existence of two supra-branchial nerves for the seventh. Alone it is not convincing evidence, but taken in connection with Dohrn's facts<sup>2</sup> it is, I think, of importance.

That a cleft formerly existed behind the hyoid cleft and in front of the first branchial is not admitted by Dohrn, and he has declined to

<sup>1</sup> Beard, "On the Segmental Sense Organs, &c.," 'Zool. Anzeig.,' Nos. 161, 162, 1884.

<sup>2</sup> Dohrn even goes further, and postulates a separate spiracular visceral arch just behind the mandibular arch. Thus, according to Dohrn, there are four arches included between the fifth nerve and the seventh nerve, viz. mandibular, spiracular, hyomandibular, and hyoid. So far as my researches extend, I have found nothing in the nerves that would suggest a spiracular arch. However, bearing in mind what has taken place in the case of the vagus, I should hesitate to cast even a doubt on the truth of his view.

attach any weight to the reasons which Van Wijhe urged for this opinion, which was based on the presence of two head cavities in the hyoid arch. Van Wijhe does not appear to have attached much importance to the evidence offered by the nerves, for he did not regard the auditory nerve as in itself of segmental value, and he never suggested the homology of the auditory organ with the branchial sense organs.

#### DEVELOPMENT OF THE AUDITORY NERVE.

In Elasmobranchii the facts of development for this segment are exactly comparable to those described for the olfactory segment. The arrangement is here the same. There is no gill-cleft, and of course, as a consequence of the absence of that, we cannot expect to find a post-branchial nerve.

The following line of argument may, as in the case of the olfactory, be used for the auditory segment. The sense organs and ganglion connected with the ciliary segment are without doubt homologous with the sense organs and ganglion of a cleft-bearing segment such as the glossopharyngeal. The ciliary has no præ- or post-branchial nerves because there is no gill-musculature or cleft. The auditory segment has no præ- or post-branchial branch just as the ciliary, but its sense organs, ganglion, and nerve are exactly like, and have the same structure as the sense organ, ganglion, and nerve of the ciliary segment. Therefore the auditory nerve, organ, and ganglion are homologous with the nerve, sense organ, and ganglion of the ciliary segment, and therefore are also the homologues of the nerve, sense organ, and ganglion of the glossopharyngeal segment. But the sense organ and ganglion of the latter are a branchial sense organ and its ganglion, therefore the auditory organ is also a branchial sense organ, and the auditory nerve the remnant of a segmental nerve.

Immediately behind and somewhat overlapping the sensory thickening which gives rise to the facial branchial sense organ is a long and broad auditory thickening (fig. 23). Behind the outgrowth of the neural crest which forms the facial nerve there is at a certain stage a small short outgrowth. This is the rudiment of the auditory nerve (fig. 23). It soon reaches the auditory thickening, fuses with it (figs. 24 and 25), and the ganglion begins to be formed at the point of fusion, and probably from the thickening itself as a proliferation, just as in other cases.

Before the auditory involution has proceeded very far there is a considerable ganglion formed, and fused with the auditory thickening (fig. 29). At this stage the whole nerve, sense organ, and ganglion correspond exactly with the nerve, sense organ, and ganglion of the ciliary segment (fig. 8).

Soon the involution is carried to such an extent that the auditory organ forms a sac, but it still opens on to the surface, and in Elasmobranchs does so throughout life. Even after the formation of the sac, cells continue to be given off from the thickening to form the ganglion (fig. 31). The later-formed semicircular canals, &c., are obviously secondary complications, which have as their motive the extension and perfection of the sensory surface, and which resemble somewhat the formation of a supra-branchial nerve and its sense organs.

The resemblance in structure between the sensory cells of the ear and those of the branchial sense organs is obvious enough, and need not be dilated upon here.

In Amphibia (*Rana temporaria*) the auditory organ, nerve, &c., are formed just like the sense organ, nerve, &c., of the trigeminus of the same animal. The nerve is split off from the epiblast, the auditory thickening is developed from the deeper layer of the epiblast opposite the notochord, and, as in the stage figured (fig. 28), there is no auditory ganglion, it is fair to assume that it is formed just as in other cranial posterior nerves in Amphibia in connection with the auditory thickening.

In Elasmobranchii, &c., the auditory ganglion and nerve become so fused with the facial that the nerve has usually been described as a branch of the facial. We have seen that it develops separately from the facial, and even when partially fused (fig. 21), the line dividing the two nerves is readily seen (*cf.* Marshall).

#### GENERAL CONSIDERATIONS.

*Morphology of the branchial sense organs.*—It is pretty clear from the facts recorded in the preceding pages that the so-called organs of the lateral line have some physiological relationship with the gill-clefts. They arise at the same time as the latter, are originally seated one over each gill-cleft, and have each a ganglion of a dorsal root of a cranial nerve arising with and attached to them. From the ganglion nerve-fibres pass to the gill-musculature on the one hand, and to the

brain on the other. In fact, these sense organs may very well be regarded as *special sense organs of the gill-clefts or as branchial sense organs*. This conclusion Prof. Froriep and I have independently arrived at.

From the above considerations, and from the facts of development recorded here, it also follows that the ganglia of the posterior roots are primitively ganglia of these branchial sense organs. Originally connected directly with this branchial sense organ, the ganglion of the posterior root has now left its primitive position and has come to lie in the mesoblast, being only connected with its sense organ by nerve-fibres. In this conclusion as to the nature of the ganglion I am again independently in agreement with Froriep and Spencer.

In describing the schematic development of a dorsal root I have, I think, sufficiently emphasised its true nature. Primitively, a dorsal root of a cranial nerve is the nerve of a gill-cleft, and is apparently only connected with the innervation of its cleft. It sends fibres from the brain to the sense organ and ganglion above the cleft, thence other fibres pass to the musculature and walls of the cleft (fig. 50).

It is not without importance to notice that any division of the dorsal root of a cranial nerve into so-called dorsal and ventral branches is *primitively absent* (fig 50). Such divisions only occur in the later development in consequence of the separation of the ganglion from the skin, and of the formation of a greater number of branchial sense organs. Of course the ventral branch is there from the start, but in itself it is mainly motor and gives rise to no ganglion, and probably never has sense organs in connection with it. It certainly is not directly concerned in the innervation of a primitive branchial sense organ. Through a misunderstanding of this point Prof. Froriep has been led into rather serious errors as to the nature of the dorsal roots. He concluded from Van Wijhe's researches, and, I must admit, not without reason, for the matter is there very vaguely stated, that the branchial sense organ and ganglion could occur on the ventral branch of a cranial nerve as well as on a dorsal. This conclusion led him to the opinion that the auditory nerve is a ventral branch. The blame of the matter lies very much at the door of Van Wijhe, for he described a cranial nerve (dorsal root) as typically possessing two branches, a dorsal and a ventral one, both of which could possess a ganglion. Now, we have seen in the development that the so-called dorsal branch (supra-branchial nerve) forms late in the development,



and arises simply from the necessity of extension and increase of the branchial sense organs, with which it is solely concerned, the ventral branch as such being probably solely concerned with the innervation of the gill-clefts.

A few words may be devoted to the researches of Bodenstein<sup>1</sup> and Solger,<sup>2</sup> which have led to the conclusion that in the sense organs of the lateral line in Teleostei nerve strands connecting the various sense organs together are present. From the account of the development given here such a connection might be expected to occur, for I have shown that the "lateral line" has arisen solely by the extension and multiplication of the primitive branchial sense organs of the vagus: these are, as we have seen, connected in development, being formed from one continuous sensory rudiment, and as they form one physiological whole, we could expect a connection in the adult. Although I have not attempted here to give an account of the development of the "lateral line" in Teleostei, I may perhaps be allowed a few words on it, as it seems to confirm the researches under discussion.

In this case in the growth backwards of the sensory rudiment there are found thicker portions, which are segmental, and thinner portions connecting them. The nerve is split off along the whole length, just as in Elasmobranchs. The thicker portions give rise to the sense organs, the thinner portions only to nerve structures, and probably to those connecting strands described by Bodenstein and Solger.

#### REMAINS OF BRANCHIAL SENSE ORGANS IN HIGHER VERTEBRATES.

Prof. Froriep's paper, leaving aside the small error just mentioned, is a very interesting and very important addition to our knowledge of the ancestry of Mammalia. It is mainly concerned with the description of rudiments of these branchial sense organs of the facial, glossopharyngeal, and vagus in Mammalia, viz. cow and sheep embryos. These rudiments are only found in certain stages, and disappear later. When they still exist the corresponding ganglia of these cranial nerves, viz. the ganglia of facial, glossopharyngeal, and vagus, are fused with the skin; indeed, the conditions seem to be much the same as in Elasmobranchii. That the ganglia are wholly or partly derived from the skin in Mammalia, Prof. Froriep hesitates to decide. It is some-

<sup>1</sup> Bodenstein, E., "Der Seitencanal von Cottus Gobio," 'Zeit. f. wiss. Zool.,' Bd. xxxvii, Heft 1.

<sup>2</sup> Solger, "Ueber die Seitenorganen Ketten der Fische," 'Zool. Anzeig.,' 1882, No. 127, p. 660

what remarkable that Prof. Froriep should have failed to find rudiments of such sense organs in connection with the Gasserian and ciliary ganglia, and I cannot help expressing a firm conviction that such rudiments exist at some stage or other in Mammalian development. This conviction rests on a twofold basis—an *a priori* one, that in Elasmobranchii the sense organs of the ciliary and Gasserian ganglia are very well developed; and, secondly, on the discovery, of which I hope soon to give a full account, that *such rudiments occur, and are very obvious in embryo chicks*. They are in the chick especially obvious in the cases of the ciliary and trigeminal segments, but they also occur in the segments of the facial, glossopharyngeal, and vagus.

Of course here, as in Mammalia, they disappear after the fish stage has been passed through, but when they attain the maximum of their development one could almost fancy, in studying them, that it was an Elasmobranch embryo which was under examination, the state of affairs in both cases being so alike that one can only marvel that these rudiments have hitherto escaped notice in the chick. So much for the present.

#### THE NOSE AND EAR AS BRANCHIAL SENSE ORGANS.

In the preceding pages abundant evidence has, I think, been adduced to show that the nose and ear are specialised branchial sense organs. Whether they ever had gill-clefts in connection with them is a point which, from the evidence at present at our disposal, we cannot decide, and can only suspect that such was once the case from the relationship of the other branchial sense organs to gill-clefts, and from the known facts that certainly Vertebrates once possessed more clefts than at present. At any rate, at present the thymus or thyroid of the nose and ear, or their equivalents, have still to be found.

The only zoologists who have suggested a different view of their nature are Froriep and Blaue, who have suggested that the ear is a gill-cleft. Apart from the evidence given in the preceding pages, which is inconsistent with this view, one may reasonably ask that the supporters of such a view shall give us more evidence than that afforded by an epiblastic depression that an organ is a gill-cleft.

In this matter the nose and ear stand on equal terms, and until we have a few more of the structures which compose a gill-cleft and visceral arch, such as arterial arch, cartilage, &c., assigned to them, we can reasonably regard the matter with a certain amount of reserve.

It is interesting to notice that if my views be correct the nose and ear are the only remains of the branchial sense organs<sup>1</sup> in the adults of higher Vertebrates. They have survived with a possible change of function, while the other branchial sense organs have disappeared except in the first stages of the embryo, and are then only transitory structures.

#### THE MORPHOLOGY OF THE SUPRA-BRANCHIAL NERVES.

This point has, I think, been sufficiently demonstrated in the general part of this work. The supra-branchial nerves are merely concerned in extensions of the branchial sense organs to a distance from the ganglia. They are erroneously called dorsal, for this condition when acquired is purely secondary.

Any commissural nature of some of these branches, as suggested by Marshall and Spencer, is out of question. None of them are remains of the neural ridge. Still less can I accept Spencer's recent suggestion,<sup>2</sup> that "the two curious branches which unite respectively the fifth and seventh and fifth and third cranial nerves . . . . may be regarded as persistent parts of the lateral nerve which united the ganglia of the sense organs along the lateral line in the head, and which, separating from the skin, have come in the course of development to occupy a much deeper position, together with the ganglia, with which they preserve their primitive connection."

These "curious branches" are portions of fused supra-branchial nerves, as a glance at the diagrams (figs. 46 and 51) will show.

#### THE RELATIONS OF THE HEAD AND TRUNK IN VERTEBRATES.

Many attempts have been made to homologise the components of the segments of the head and trunk, and naturally such attempts have extended to the nerves. The spinal nerves, it is hardly necessary to say, present anterior and posterior roots, the latter of which are ganglionated. Such a state of affairs has been sought for also in the head, but in face of the facts previously recorded it is at least doubtful, even if the existence of cranial anterior and posterior roots be granted, whether these can be homologised with those of the spinal nerves. The posterior roots of cranial and spinal nerves develop differently, for

<sup>1</sup> Professor F. E. Schultze notwithstanding, the possibility that the taste buds of the tongue of higher Vertebrates are also to be referred to those sense organs must be borne in mind. Their innervation by the glossopharyngeal is, in this connection, very suggestive.

<sup>2</sup> Spencer, 'Notes on the Early Development of *Rana temporaria*,' p. 12.

the spinal have no connection with the skin in early stages ; that is, the ganglion is never fused with the skin, and their roots are never connected with gill-clefts or with special sense organs.

One of the most striking results of these researches is the great distinction of the body of Vertebrates into a gill-bearing region and a non-gill-bearing region ; and at present, with the sharply-defined differences which obtain in the development of the organs of these two regions, attempts to homologise organs in the two different regions would seem to meet with indifferent success. That Balfour was right in regarding the cranial nerves as more primitive than the spinal is probable enough, but at the same time it is very questionable whether the spinal nerves ever had the *same* primitive characters as the cranial.

Dohrn's idea that the anus arose from a pair of coalesced gill-clefts may be rejected without more ado, for there seems to be no evidence for it. Not so, however, his mode of regarding the mouth as a pair of coalesced gill-clefts ; that is probably true. In dealing with the relations of head and trunk, the vexed question of anterior roots of cranial nerves crops up, and with it the nature of the head cavities. I have no observations to record on the so-called anterior roots of cranial nerves except on the hypoglossus, which has certainly nothing to do with the cranial nerves, as Dohrn has pointed out. Van Wijhe regarded the hypoglossus as made up in Elasmobranchs of three anterior roots of the vagus. In this point my researches agree with those of Dohrn and Froriep. The hypoglossus has nothing to do with the vagus.

Froriep's<sup>1</sup> account of the development of the former in Mammalia seems to hold good also for Elasmobranchs. As in Mammalia, the hypoglossus of Elasmobranchs is derived from the anterior roots of the first three spinal nerves. The posterior roots are developed in the embryo, but afterwards abort. I have not figured them, because the spinal nerves really lay beyond the scope of this work.

As to the head cavities themselves, their persistence in the anterior part of the head may, as other observers have stated, be due to their functional connection with the eyes. That they once occurred in all the segments of the head is probable enough, though with what organs they were originally connected is not so plain. Possibly from their muscular nature, and the apparent absence of sensory elements, even in development, in their nerves, they may have been the muscles of

<sup>1</sup> Op. cit., pp. 5 and 48.

neural parapodia. That they had nothing to do with the gill-clefts themselves is pretty certain.

#### NATURE OF THE MOUTH.

A few words may be here said on the bearing of these researches on the nature of the mouth.

Dohrn<sup>1</sup> first suggested that the mouth was primitively a pair of gill-clefts, which have coalesced and come to open mesially. He afterwards showed<sup>2</sup> that it arises in Teleostei as two lateral depressions just like gill-clefts. In the preceding pages I have shown that in Elasmobranchs there is a primitive branchial sense organ over the angle of the mouth, and with this sense organ an associated ganglion, the Gasserian; and also that, just as in the nerves of other gill-clefts, a supra-branchial nerve is afterwards developed from this ganglion in connection with the extension of the branchial sense organs of the mouth cleft. I need hardly say that I see in these facts a strong additional support for Dohrn's view.

#### SEGMENTATION OF THE HEAD.

Admittedly this is one of the most difficult problems in Vertebrate morphology, and I cannot flatter myself that I am nearer a solution of it than other zoologists. But it may be remarked that the tendency of recent researches has been to increase the number of segments recognisable in the Vertebrate head. In ordinary sharks with five true gill-clefts, Marshall and Van Wijhe recognised nine segments, but Van Wijhe rejected Marshall's olfactory segment, and Marshall did not regard the hyoid as composed of two segments. I should increase the number to at least eleven in sharks, with four roots to the vagus, and apparently Dohrn would agree with this number, but his segments might not be quite the same.

Indeed, at present it is impossible to solve the problem with any degree of probability, and it is a question whether it ever will be solved. Hence the following table is only a tentative one, and is only meant to give a general view of the results of the researches recorded here. In passing I may remark that Dohrn's recent criticism of Ahlborn's<sup>3</sup>

<sup>1</sup> Dohrn, 'Ursprung der Wirbelthiere.'

<sup>2</sup> Dohrn, "Studien, &c," 'Mittheil. a. d. Zool. Station zu Neapel.' Bd. iii I. "Der Mund der Knochenfische."

<sup>3</sup> Ahlborn, "Ueber die Segmentation des Wirbelthier-Körpers," 'Zeit. für wiss. Zool' Bd. xl, p. 309.

Segment.	Dorsal Nerve-roots.	Cleft.	Nature of Sense Organ of Cleft.	Ganglion.	Supra-branchial Nerve.	Head Cavity.	Ventral Nerve-root.
I ...	Olfactory	None	Olfactory org.	Olfactory	None	None	None.
II ...	Radix longa of ciliary ganglion	None, or hypophysis	Branchial	Ciliary	Ophthalmicus profundus	First	Motor oculi.
III ...	Trigemimus	Mouth	Branchial	Gasserian	Ophthalmicus superficialis less portio facialis	Second	Trochlear.
IV ...	} Facialis	} Absent	Branchial	} Facial	{ Portio facialis of ophthalmicus superficialis	Third	Abduccens.
V ...			Branchial			} Auditory org.	
VI ...	Auditory	None	Auditory	None	None		None.
VII ...	Glossopharyngeal	First branchial	Branchial	Glossopharyngeal	Supra-temporal branch	?	None.
VIII ...	Vagus I	Second branchial	Branchial	Vagus I	Supra-temporal branch	None	None.
IX ...	} Vagus II, III, and IV	} Third, fourth, and fifth branchials	} Branchial	} Vagus II, III, and IV	} Lateral nerve	} None	} None.
X ...							
XI ...							

paper on this point seems to me to meet the objectionable points so successfully that further criticism is unnecessary.

In connection with this table the reader would do well to consult the three diagrammatic figures (figs. 43, 46, 51). The same results are there shown. Fig. 43 is a diagrammatic horizontal section through the various sense organs and ganglia, and with fig. 45, which is a side view of the same structures, shows the primitive condition. Fig. 45 shows the primitive position of these sense organs over the gill-clefts: in it, for simplicity, the post-branchial nerves are left out; but in fig. 46 these and the præ-branchial nerves are shown. The closed gill-clefts are also given, with the absorbed branches, in dotted lines. Finally, fig. 51 is meant to show the adult condition of the supra-branchial nerves, which are very diagrammatically given in fig. 46.

#### THE RELATIONS OF THE BRANCHIAL SENSE ORGANS TO THE "SEITEN-ORGANE" OF CAPITELLIDÆ.

Eisig<sup>1</sup> first suggested that these two sets of organs were homologous. Since then no one has added anything to the grounds for this homology furnished by Eisig. Until now it may truly be said that we knew nothing of the morphology of these branchial sense organs of Vertebrates. Now we do know a little, and this appears to me to place the homology of the "Seitenorgane" of Capitellids with the branchial sense organs in a very doubtful light. We have seen that primitively these branchial sense organs are not found in all segments of the body, but are limited to the head, that they have special ganglia, and are special sense organs of the gill-clefts.

In all these points they differ from the Seitenorgane of the Capitellidæ, and, interesting and important as Eisig's researches are, we must at present, I think, hesitate to accept the proposed homology.

#### PHYSIOLOGY OF THE BRANCHIAL SENSE ORGANS.

Of this we really know nothing. Leydig, who has the honour of having first described these sense organs, thought they were organs of a sixth sense. By others they have been regarded as touch organs, and as organs for testing the water breathed. Lastly, Mayser<sup>2</sup> suggested that they were a low form of auditory organ, and Emery<sup>3</sup>

<sup>1</sup> Eisig, "Die Seitenorgane und beckerförmigen Organe der Capitelliden," *Mittheil. a. d. Zool. Station zu Neapel*, Bd. i.

<sup>2</sup> Mayser, "Studien über das Gehirn der Knochenfische," *Zeit. f. wiss. Zool.*, vol. xxxvii, 1881.

<sup>3</sup> Emery, "Fierasfer," p. 48, 'Fauna and Flora of the Bay of Naples.'

instituted a comparison between the auditory labyrinth and branchial sense organs, and concluded that the two sets of organs have an analogous function. That this is the case seems now very possible; that they are concerned in the perception of wave motion is obvious enough from their structure.

I have here shown, and Professor Froriep<sup>1</sup> has also come to the same conclusion, that they are the special sense organs of the gill-clefts. On this view we may assume that they give notice of impending danger to the gill-clefts, and so enable the latter to be closed. Of course they were existent long before an operculum was developed in any fish.

After this demonstration that these sense organs stand in some important relationship to the gill-clefts, it may reasonably be expected that experimental evidence of their real nature will shortly be forthcoming. Here a valuable field of research is open for the physiologist, and a very important one too, for researches in it may lead to a better knowledge of other Vertebrate sense organs, such as the nose and ear, which appear to have been primitively of the same nature as these branchial sense organs.

If the researches recorded here should give any impulse to the physiological study of these organs, they will have done a great deal; for in spite of the many brilliant researches on the structure of these branchial sense organs, which have undoubtedly told us much about their structure and distribution, we cannot till now be said to have gained a clearer insight into their true nature than we possessed after Leydig's researches. This honoured histologist and zoologist showed that they were really sense organs, but there the matter has remained for thirty-five years.

My researches on the lateral line were commenced over two years ago in Professor Semper's laboratory at Würzburg. In consequence of difficulties with the only material I then had, viz. embryos of Teleostei, they led to very little result. Afterwards they were for a time laid aside for other work. Although the results of the work in Würzburg were very barren, being made in what appeared to be a dreary and empty field, still my gratitude is none the less due, and is here expressed, to Professor Semper for his untiring advice and assistance.

<sup>1</sup> This was stated by Professor Froriep and myself independently.



To Professor Milnes Marshall, in whose laboratory the later researches on Elasmobranchs were made, my acknowledgments are due not only for the privilege of the use of his library of zoological works, but also for his valuable assistance, criticism, and advice. I also wish to express my best thanks to Professor Wiedersheim for good counsel, and to my friend Dr. L. Will, who very kindly made a number of useful extracts from Götte's great "Unke" work, a work which was inaccessible to me in Manchester.

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12. STANNIUS, H.—‘Handbuch der Anatomie der Wirbelthiere,’ 1854.
13. WIEDERSHEIM, R.—‘Anatomie d. Gymnophionen,’ 1879.

## DESCRIPTION OF PLATES VII, VIII, AND IX.

In most cases the objective and ocular used for each drawing are denoted by letters, such as Z. D, oc. 2, which signify Zeiss's objective D, ocular No. 2. The figures are mostly camera drawings, and are all reduced to one third of their apparent enlargement.

## ALPHABETICAL LIST OF REFERENCES.

I, III, V, VII, &c., Olfactory, motor oculi, trigeminal, facial, &c., nerves. *al.c.* Alimentary canal. *aud.* and *au.o.* Auditory organ. *au.gl.* Auditory ganglion. *au.n.* Auditory nerve. *br.* Brain. *br.gl.* Branchial ganglion. *br.o.* Branchial sense organ. *c.b.* Nerve connecting ciliary and Gasserian ganglia. *c.gl.* and *cil.gl.* Ciliary ganglion. *cil.* Ciliary. *cl.* Cleft. *cl. vi* Sixth cleft. *ep.* Epiblast. *f.br.* Fore-brain. *f.gl.* Facial ganglion. *fac.* Facial. *Gass.* Gasserian. *G.gl.* Gasserian ganglion. *gl.gl.* Glossopharyngeal ganglion. *gloss.* Glossopharyngeal. *h.br.* Hind-brain. *h.c.* Head-cavity. *h.c.<sub>2</sub>* Second head-cavity. *hy.cl.* Hyoid cleft. *i.e.* Indifferent epiblast. *l.l.* Lateral line. *l.n.* Lateral nerve. *l.m.* Lateral muscle plates. *m.* Mouth. *m.br.* Mid-brain. *me.* Mesoblast. *ms.* Inter-muscular septa. *n.* Notochord. *n.c.gl.* Nerve of ciliary ganglion. *n.s.* Nervous system. *olf.gl.* Olfactory ganglion. *olf.n.* Olfactory nerve. *olf.o.* Olfactory organ. *oph.pro.* Ophthalmicus profundus. *op.s.* Ophthalmicus superficialis of fifth nerve. *p.br.o.* Primitive branchial sense organ. *p.b.n.* Præ-branchial nerve. *p.f.* Portio facialis of ophthalmicus superficialis—one supra-branchial nerve of facial, *p.n.* Post-branchial nerve. *p.r.* Posterior root. *r.b.* Ramus buccalis, the second supra-branchial nerve of the facial. *sb.n.* Supra-branchial nerve. *sp.c.* Spinal cord. *sp.gl.* Spinal ganglion. *sm.b.* Smell-buds. *s.t.v.* Supra-temporal branch of vagus I. *s.t.g.* Supra-temporal branch of glossopharyngeal. *vg.gl.* Vagus ganglion. *vg. I.* Vagus ganglion I.

## PLATE VII.

Fig. 1. Olfactory nerve just fusing with olfactory thickening. *Torpedo ocellata*. Z. D, oc. 2, camera. *olf.n.* Olfactory nerve. *olf.o.* Olfactory thickening.

Fig. 2. Olfactory ganglion (*olf.gl.*) and olfactory thickening (*olf.o.*) fused together. *Torpedo ocellata*. Z. D, oc. 2, cam. luc.

- Fig. 3. Transverse section of olfactory organ (*olf.o.*) and nerve (*olf.n.*) in *Rana temporaria*. Z. c, oc. 2, cam. luc.
- Fig. 4. Transverse section of embryo of *Rhodeus amarus*, showing olfactory nerve and thickening both fused with skin. Letters as before. *f.br.* Brain. Z. F, oc. 2, cam. luc.
- Fig. 5. Transverse section through fore-brain and olfactory organ of an embryo of *T. ocellata*. Combined from several sections. Shows olfactory nerve and ganglion fused with thickening and connected with brain. Letters as before. Z. A, oc. 2, cam. luc.
- Fig. 6. Somewhat horizontal section through mid-brain, showing nerve of ciliary ganglion (*n.c.gl.*) just fusing with skin. *T. ocellata*. Z. F, oc. 2, cam. luc.
- Fig. 7. Low-power view of same section. Z. c, oc. 2, cam. luc.
- Figs. 8 & 9. High- and low-power drawings respectively of a somewhat horizontal section through fore- and hind-brain. Shows ciliary ganglion rudiment (*c.gl.*) and its primitive branchial sense organ (*p.br.o.*). The ganglion is in course of formation from the epiblast. *f.br.* Fore-brain. *h.br.* Hind-brain. *Torpedo ocellata*. Z. D and A, oc. 2, cam. luc.
- Fig. 10. Horizontal section through a young *Torpedo* embryo, showing ciliary ganglion still fused with its sensory thickening. Also shows motor oculi nerve, (III). *c.gl.* Ciliary ganglion. *p.br.o.* Primitive branchial sense-organ. III. Motor oculi. *G.gl.* Gasserian ganglion. *hy.cl.* Hyoid cleft. *m.br.* Mid-brain. *h.c.* Head-cavity. *f.gl.* Facial ganglion. Z. A, oc. 2, cam. luc.
- Fig. 11. Drawing under high power of ciliary ganglion and its primitive sense organ of the preceding section. Late stage, but still intimate fusion with skin. Also origin of supra-branchial nerve of ciliary ganglion (*ophth. profund.*) from skin. Supra-branchial nerve (*s.br.n.*). Z. F, oc. 2, cam. luc.
- Figs. 12 & 13. Similar drawings to figs. 10 and 11 respectively. Letters as before. *gl.gl.* Glossopharyngeal ganglion.
- Fig. 14. Horizontal section through mid-brain and anterior portion of hind-brain. Shows course of fifth nerve, which lies just under skin, but is not yet fused with it. No ganglion yet present. *T. ocellata*. Z. c, oc. 2, cam. luc.

- Fig. 15. Fifth nerve fused with its sensory thickening (*p.br.s.o.*), and proliferation of Gasserian ganglion from the skin. *G.gl.* Gasserian ganglion. *T. ocellata*. Z. c, oc. 2, cam. luc.
- Fig. 16. Similar figure to preceding one. *T. ocellata*. Z. c, oc. 2, cam. luc.
- Fig. 17. Section through hind- and fore-brain. Shows Gasserian ganglion just before its separation from the skin. *T. ocellata*. Z. A, oc. 2, cam. luc.
- Fig. 18. Small piece of a horizontal section of a *Torpedo* embryo. Shows hyoid præ-branchial nerve (*pb.n.*) lying in epiblast and not yet separated from it. *G.gl.* Gasserian ganglion. *f.gl.* Facial ganglion.

## PLATE VIII.

- Fig. 19. Transverse section through hind-brain of a *Torpedo* embryo. Facial nerve (vii) just on point of fusion with its sensory thickening. Gill-cleft (hyoid) just about to form. Z. c, oc. 2, cam. luc.
- Fig. 20. A similar section. Facial nerve just fused with skin, and its post-branchial (*p.n.*) passing on to muscles of cleft. Z. A, oc. 2, cam. luc. A later stage of facial ganglion in fig. 42.
- Fig. 21. Facial ganglion leaving skin, and still connected by two supra-branchial nerves (*s.b.n.* 1, and *s.b.n.* 2). Z. D, oc. 2, cam. luc.
- Fig. 22. Horizontal section of a *Torpedo* embryo. Facial ganglion fused with auditory, but line of demarcation is obvious. Facial has just left the skin, and is leaving a supra-branchial nerve (*s.b.n.*) behind it.
- Fig. 23. Part of a transverse section through the auditory region of a *Torpedo* embryo. Auditory nerve (viii) not yet fused with auditory thickening (*au.o.*). Z. F, oc. 2, cam. luc.
- Figs. 24 & 25. Auditory just fused with auditory thickening, and ganglion proliferating. Letters as before. *T. ocellata*. Z. F, oc. 2, cam. luc.
- Fig. 26. Low-power drawing of a horizontal section, such as the two preceding figures form part of.
- Fig. 27. Transverse section, rather oblique, through hind-brain of a frog embryo. Shows auditory nerve and thickening on one side, and vagus nerve and ganglion on the other. *au.n.*

- Auditory nerve. *vg.* Vagus nerve. *vg.gl.* Vagus ganglion.  
Z. A, oc. 2, cam. luc.
- Fig. 28. High-power drawing of auditory portion of preceding. Shows auditory nerve not yet separated from skin. Z. F, oc. 2, cam. luc.
- Fig. 29. Transverse section through auditory region of an Elasmobranch embryo. Shows auditory ganglion and auditory thickening intimately fused together. Auditory involution as yet only partial.
- Fig. 30. Similar section under low power. Auditory involution complete.
- Fig. 31. Highly-magnified drawing of auditory portion of last section. Shows intimate fusion of ganglion and thickening, and proliferation of cells of thickening into ganglion. Many nuclear figures near proliferating portion.
- Fig. 32. Transverse section through hind-brain of a Torpedo embryo. Shows glossopharyngeal nerve (ix) just fused with its primitive branchial sense organ (*p.br.o.*). Z. c, oc. 2, cam. luc.
- Fig. 33. Similar section to preceding. Vagus nerve (x) just before fusion. Z. c, oc. 2, cam.
- Fig. 34. Similar section. Vagus nerve just fused with its thickening. Post-branchial branch (*p.n.*) passing on to muscles of cleft. Z. D, oc. 2, cam.
- Figs. 35 & 36. Portions of similar sections to preceding. Portions of vagus ganglion (*vg.gl.*) above gill-cleft, and just separating from skin; in separating, leaving a nerve behind. *p.br.o.* Branchial sensory thickening. *phr.* Pharynx. *cl.* Cleft. *T. ocellata.* Z. D., oc. 2, cam. luc.

## PLATE IX.

- Fig. 37. Horizontal section through head of a Torpedo embryo. Shows hyoid præ-branchial nerve (*p.br.n.*) forming in epiblast.
- Fig. 38. High-power view of small piece of preceding section, showing hyoid præ-branchial nerve (*p.br.n.*) in epiblast. Z. F, oc. 2, cam.
- Fig. 39. Horizontal section through Torpedo embryo. Vagus ganglion separating from the skin. Lateral line (*l.l.*) growing backwards and pushing indifferent epiblast (*i.e.*) away. *sp.gl.* Spinal ganglion. Z. A, oc. 2, cam. luc.



- Fig. 40. High-power view of part of preceding section, showing lateral line forming (*l.l.*), indifferent epiblast (*i.e.*) being pushed away, and lateral nerve (*l.n.*) splitting off from thickening. *me.* Mesoblast. *T. ocellata.* Camera luc.
- Fig. 41. Later stage of lateral line. Further back in trunk. High-power, camera lucida. Shows same things as preceding drawing. *T. ocellata.*
- Fig. 42. Drawing combined under camera from several horizontal sections of an Elasmobranch embryo. Shows several cranial ganglia fused with their branchial sense organs. *p.br.o.* Primitive branchial sense organ. *m.br.* Mid-brain. *c.gl.* Ciliary ganglion. *G.gl.* Gasserian ganglion. *f.gl.* Facial ganglion. *au.gl.* Auditory ganglion. *gl.gl.* Glossopharyngeal ganglion. *vg.gl. I.* First vagus ganglion. *vg.gl.c.* Second, third, and fourth vagus ganglion. *l.l.* Lateral line. *l.n.* Lateral nerve. *au.* Ear. *n.* Notochord. *sp.c.* Spinal cord.
- Fig. 43. Diagrammatic horizontal section through the various branchial sense organs and their ganglia. The reader should conclude nothing from the cerebral vesicles figured here: there is probably at least one between the trigeminal and seventh nerves, and it is not figured here.
- Fig. 44. Part of a horizontal section of a six week's old salmon. Shows the position and segmental arrangement of the branchial sense organs (*br.o.*) in the trunk. *in.s.* Intra-muscular septa. *n.* Notochord. *me.* Mesoblast.
- Fig. 45. Diagram of lateral view of an Elasmobranch embryo. Shows the central nervous system as plate not yet involuted, the posterior roots of the cranial nerves (*p.r.*) the branchial sense organs, the dorsal eye (*oc.*), mouth, and gill-clefts. Letters as before.
- Fig. 46. Similar diagram, to show the branches of nerves to gill-clefts. The aborted branches in dotted lines. Also shows formation and direction of various supra-branchial nerves (*s.b.n.*). Vagus represented as supplying in all five clefts. This figure is a more diagrammatic view of fig. 51, which represents nature more or less accurately.
- Fig. 47. Horizontal section of a Torpedo embryo, showing rudiment (*cl. vi*) of a sixth true branchial cleft.

- Fig. 48. Low-power drawing of transverse section through nose of an adult Triton showing Blaue's smell-buds (*sm.b.*).
- Fig. 49. High-power drawing of two such smell-buds. Z. F., oc. 2, cam. luc.
- Fig. 50. Diagrammatic transverse section through the gill-bearing region of an Elasmobranch or other Ichthyopsid. Nervous system not yet closed in. On the left side the gill muscle plate is shown, and on the right the gill-cleft. *h.c.* Head cavity. *n.s.* Nervous system. *p.r.* Posterior root. *n.* Notochord. *p.br.o.* Branchial sense organ. *br.gl.* Branchial ganglion. *l.m.* Lateral muscle plate. *p.n.* Post-branchial nerve. *al.c.* Alimentary canal.
- Fig. 51. Diagram taken partly from my own drawings and partly from Prof. Marshall's. Shows the ganglia and various branches of the cranial nerves. Also mouth (*m.*) and gill-clefts (*cl.<sub>1</sub>*, *cl.<sub>2</sub>*), &c. For lettering, see general list.
-



Fig. 1.

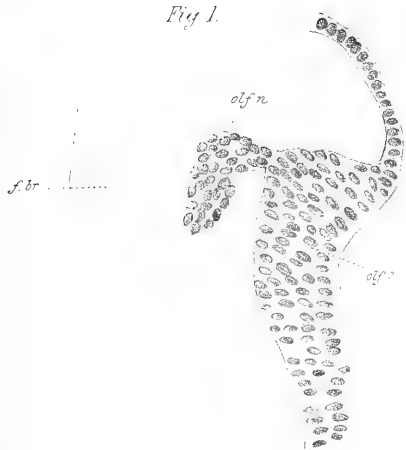


Fig. 2.



Fig. 6.



Fig. 3.



Fig. 4.

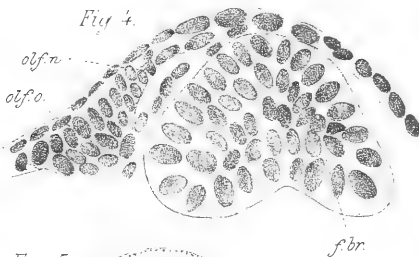


Fig. 8.



Fig. 5.



Fig. 14.

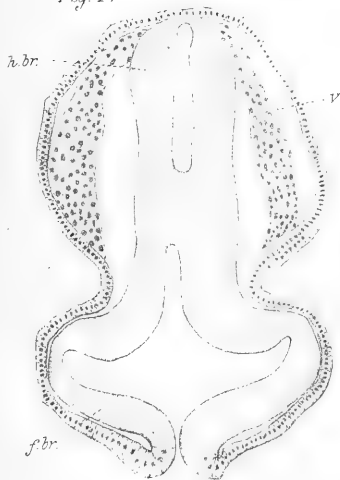


Fig. 15.



Fig. 16.

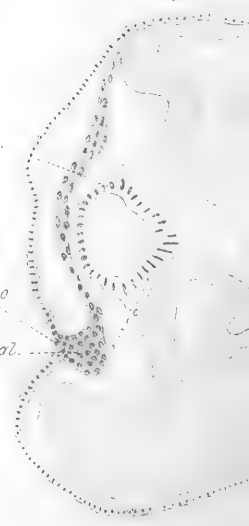


Fig. 7.



Fig 10.



Fig 12.

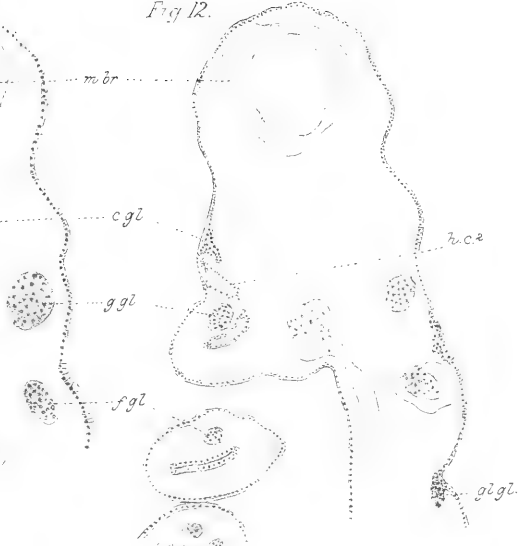


Fig. 9.



Fig. 11.

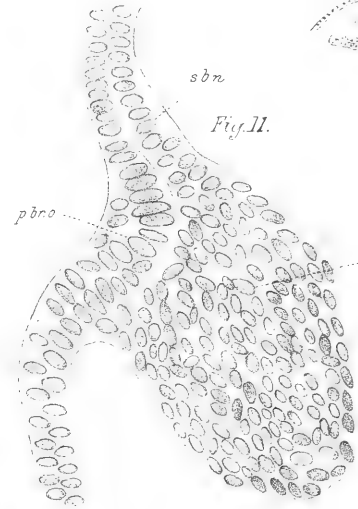


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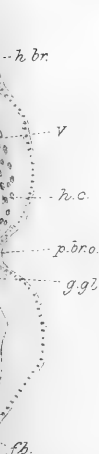
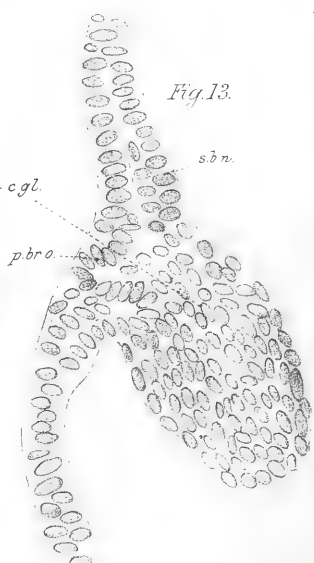


Fig 17.



Fig. 18.

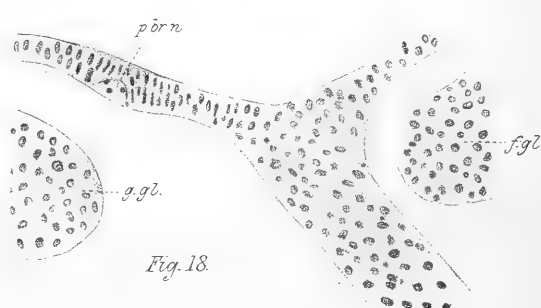






Fig. 19.

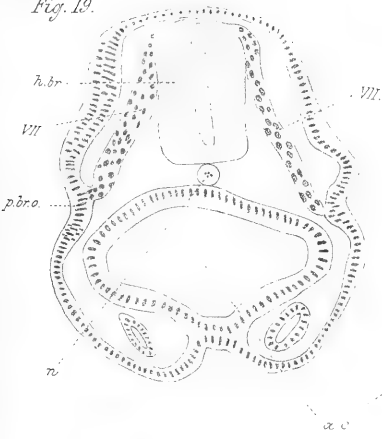


Fig. 20.

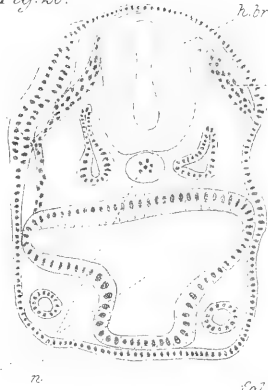


Fig. 21.

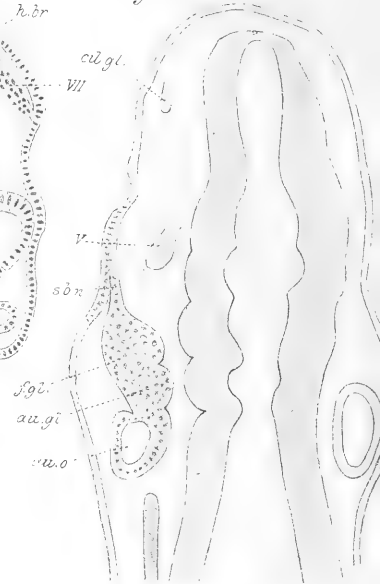


Fig. 22.

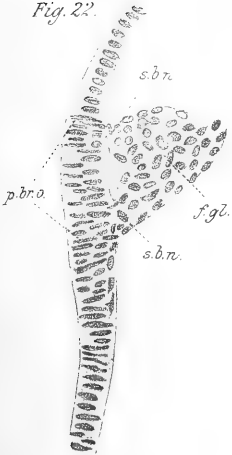


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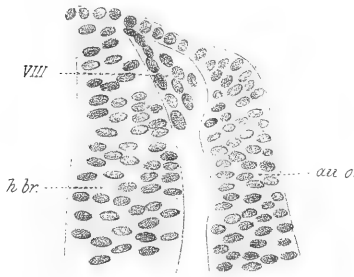


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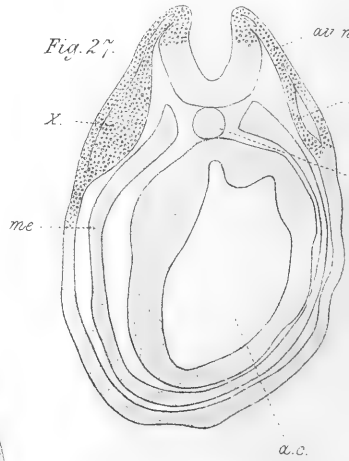


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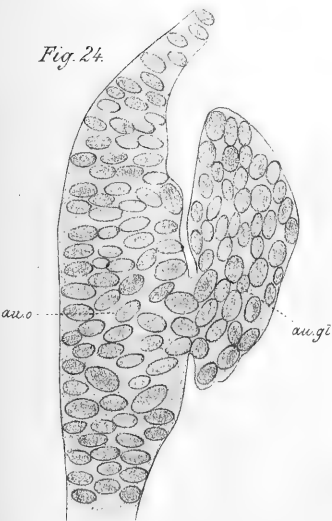


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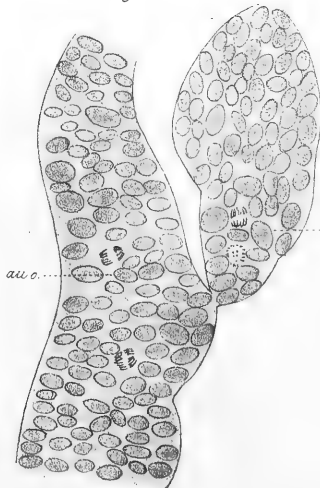


Fig. 26.





Fig. 32.

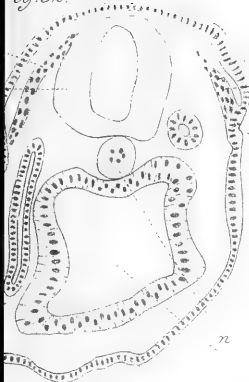


Fig. 33.

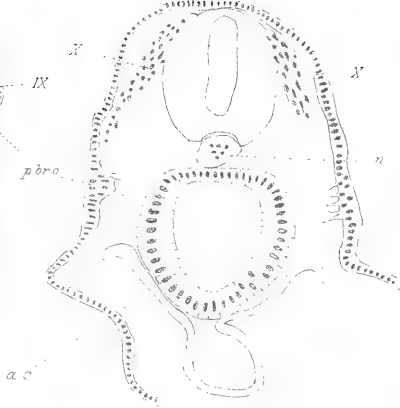


Fig. 34.

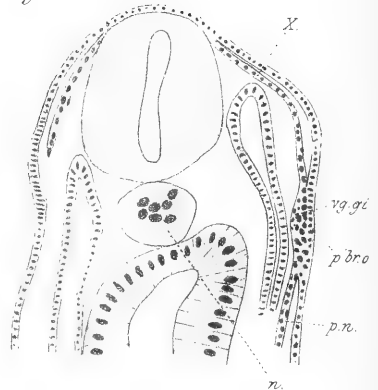


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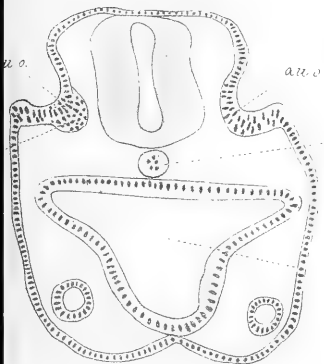


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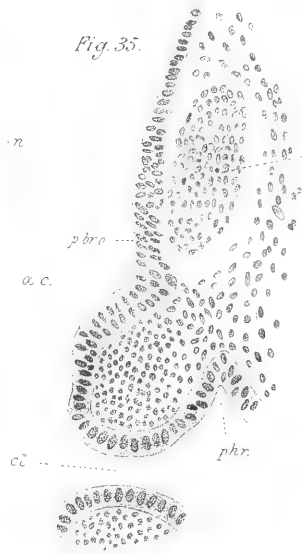


Fig. 36.



Fig. 30.

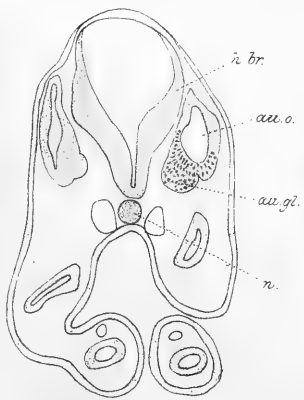


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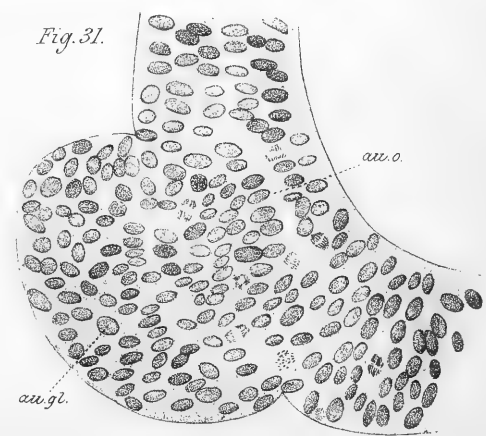






Fig. 37.



Fig. 39.



Fig. 42.



Fig. 38.



Fig. 40.



Fig. 41.

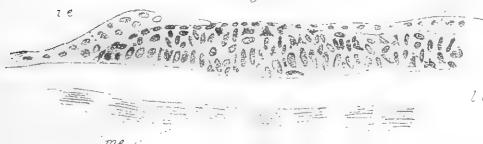


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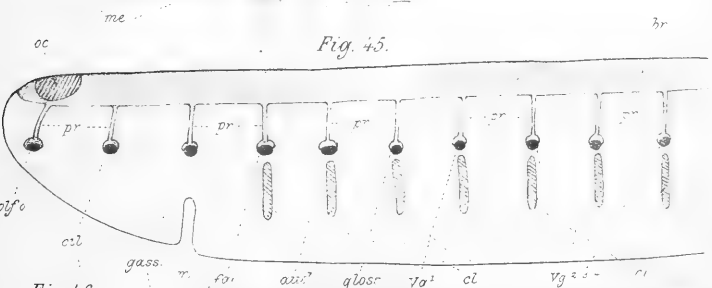


Fig. 46.

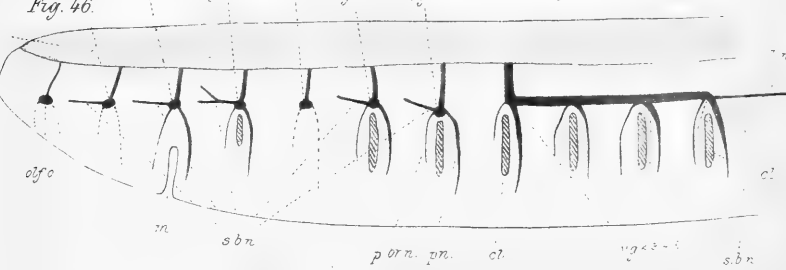


Fig. 47.

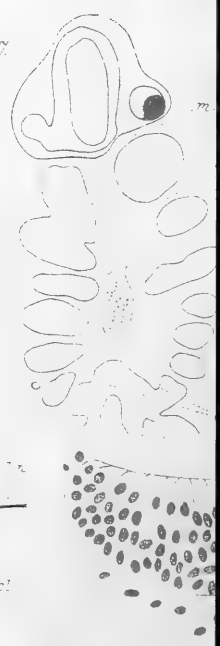


Fig. 43.

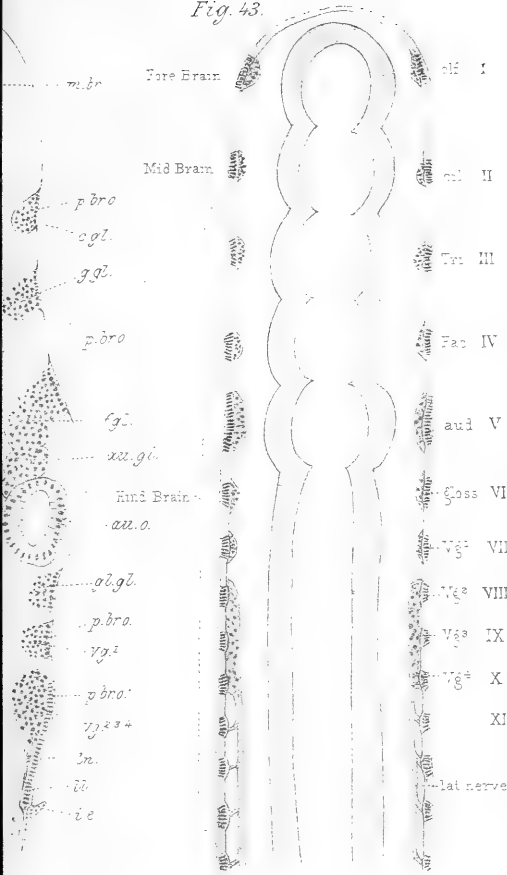


Fig. 44.

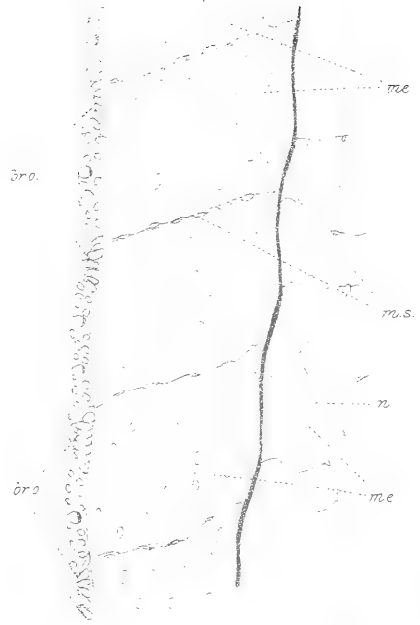


Fig. 50.

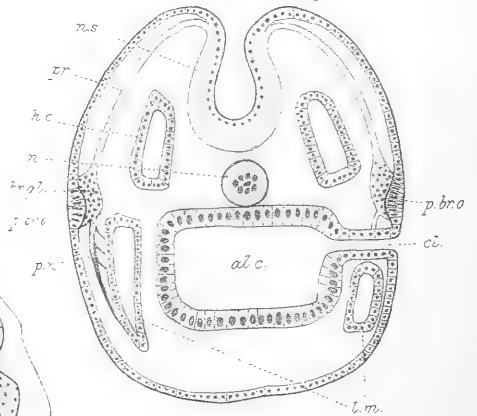


Fig. 48.

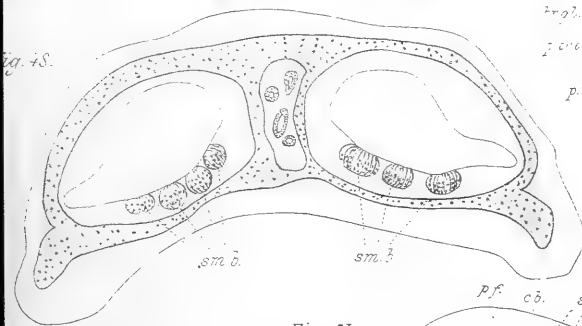


Fig. 51.

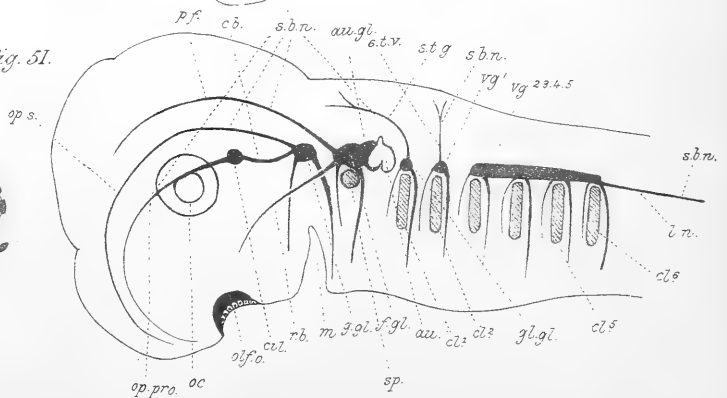
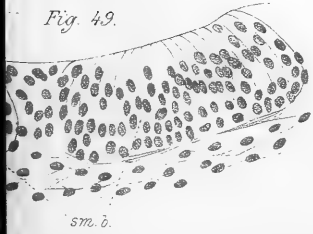


Fig. 49.





## A SIMPLIFIED VIEW OF THE HISTOLOGY OF THE STRIPED MUSCLE FIBRE.

By B. MELLAND, B.Sc., *Platt Physiological Scholar in the  
Owens College.*

[PLATE X.]

### INTRODUCTION.

Everyone who has considered the subject must admit the essential identity from a physiological point of view of all those tissues which possess in a special degree contractility. The contraction of a white blood corpuscle or amœba is essentially the same phenomenon as the contraction of an involuntary fibre cell or a striped muscle fibre.

When we consider these three contractile tissues from a histological point of view we are struck by an apparently essential difference in character between the striped muscle fibre and the elements of the other two contractile tissues, and indeed cells generally. The voluntary muscle fibre is morphologically a cell like a muscle fibre cell and the amœboid corpuscle. Yet it differs from the latter and from all other cells in showing a characteristic transverse striation.

According to Klein,<sup>1</sup> the protoplasm of the simpler contractile tissues, (1) the amœboid cell, (2) the ciliated cell, and (3) the involuntary fibre cell, agrees, inasmuch as it consists of two parts—a matrix and an arrangement of fine fibrils, the intracellular network. The actual arrangement of the fibrils differs somewhat in the three cases.

<sup>1</sup> 'Klein, 'Atlas of Histology,' diagrams 1 and 4, and fig 2, pl. xv.

In the white blood corpuscle they are arranged in a network or meshwork with polygonal meshes. In the ciliated cell they also form a network which seems to be in peculiar relation with the cilia. In the ciliated cell of the Mollusc, according to Engelmann,<sup>1</sup> the fibrils are arranged in a longitudinal manner as fine varicose filaments running the whole length of the cell, and in connection with the bases of the cilia. In the protoplasm of the involuntary fibre cell the fibrils are arranged in a central or axial bundle, anastomosing at the poles of the nucleus with the intra-nuclear network.

Observations on which I have been engaged for some time past, and which have been partly worked out in the Physiological Laboratory of Owens College, lead me to the belief that the striated muscular fibre really agrees fundamentally as regards histological structure with the other contractile tissue elements, in containing an intracellular network, differing from them merely in the greater amount of differentiation, and more regular arrangement, of the network.

I believe, further, that the various conflicting descriptions given by different observers, and those points on which competent histologists differ more materially, can be explained and brought into harmony with one another by this view.

I have observed this network in the fibres of *Dytiscus*, the Bee, Crayfish, Lobster, Frog, and Rat, prepared by a somewhat special method of gold staining, the network being the only part of the fibre stained by the gold.

It may be specially stained also by treating the fibre with acetic acid, and subsequently staining with hæmatoxylin.

It may be demonstrated, though not so completely, in the living fibre, and in acetic and osmic acid preparations. I have submitted my drawings and preparations to the examination of Prof. A. Gamgee and Prof. Milnes Marshall.

#### DEMONSTRATION OF AN INTRACELLULAR NETWORK IN THE STRIPED MUSCLE FIBRE.

##### I. THE MUSCLE FIBRE PREPARED WITH GOLD CHLORIDE.

###### (a) *Dytiscus marginalis*.

*Method of gold staining.*—Decapitate a *Dytiscus*, open the thorax, remove a portion of a leg muscle, and place in 1 per cent acetic acid

<sup>1</sup> Engelmann, 'Pflüger's Archiv,' xxiii, 1880, and 'Quain's Anatomy,' 9th edition, vol. ii, fig. 240.



from five to fifteen seconds, then into gold chloride solution 1 per cent for forty-five minutes, and leave in formic acid 25 per cent for forty-eight hours in the dark. Tease and mount in glycerine.

If now examined with a magnifying power of about 700 diam. the appearance shown in figs. 1, 2, 3, 12, 13, and 14 will be seen in certain of the fibres. The method of preparation has a great tendency to soften the fibre, so that it becomes much expanded on compression by the cover-slip; it also has a great tendency to split the fibre into transverse discs.

Fig. 1 represents a fibre which has retained its natural size and form. Narrow transverse bands of granular substance, deeply stained with the reduced gold, are seen crossing the fibre, separated by wider bands of lighter substance. These deeply-stained granular bands correspond in position to Krause's "membranes." The usual separation into light and dim discs of about equal thickness is lost by this method of preparation. Traversing the wider unstained discs, and giving the fibre the appearance of longitudinal striation, are seen fine longitudinal lines.

In fig. 2 is seen a portion of a fibre which has been more flattened out by pressure. In it the deeply-stained, narrow granular band is seen to consist of a transverse row of dots. The longitudinal lines are seen to represent fine rod-like bodies traversing the position usually occupied by the dim stripe, and being continued into the dots at either end. In some fibres a minute thickening of the rod is apparent midway in the position of the so-called "Hensen's disc" (in the middle of the dim stripe).

This method, as was before stated, has a tendency to split the fibre into transverse discs. These *isolated discs* are found in many parts of the preparation; they present the appearances seen in figs. 4 and 5. They are seen plainly in all cases to consist of two parts—(1) a network of fine highly refracting lines, stained by the gold, and having thickenings at the nodes; and (2) an unstained substance lying in the interstices of the network.

The appearance of this network differs somewhat with the degree of compression of the discs. When much compressed the network appears more open, and the nodal dots less marked. Towards the outside of the fibre the meshes appear more oblong, the network extending mostly in a radial direction. This network evidently corresponds when it is in its transverse position in the fibre with the deeply-stained,

beaded disc occupying the position of "Krause's membrane." This is shown in certain fibres in which the discs are not seen perfectly edgewise but in perspective (fig. 6). The beaded disc at each membrane of Krause is here seen to consist of a transverse or horizontal network, united to the discs above and below by fine thread-like lines. This method of gold staining, then, brings out a network arranged in a manner represented diagrammatically in diagrams 1, 2, 3, and 4.

This network differs chemically from the rest of the fibre, inasmuch as it resists to a larger extent the action of acetic acid, and possesses in a greater degree the power of reducing gold.

It will be shown later, by other methods of preparation, that this network differs again from the matrix in its physical properties. The network is isotropous and highly refractile. The refractive power is somewhat altered by gold staining, but certain optical effects are still produced by the refractive action of the network upon light. These optical effects can be more definitely seen in *isolated portions* of the network than in the whole fibre.

#### *Optical Effects produced by the Network.*

Fig. 12 represents a small piece of the network isolated from the rest of the fibre, consisting of nine or ten rows of dots and the connecting longitudinal bars. There is a single layer only of network and dots. This isolated piece seems to be a portion of sarcolemma stripped off the fibre, along with the portion of network immediately below the sarcolemma, and attached to it by each transverse network.

When exactly focussed (fig. 14,  $\iota$ ) each dot appears as a dark granule surrounded by a bright halo. The blending of these haloes causes a crenated bright transverse band. The effect of alternating light and dim bands is thus obtained, the bright band being crossed transversely by a row of dots, the dim band longitudinally by a series of fine lines.

On altering the focus (raising .0025 millimetre, about), the refractive effects are to a certain extent transposed (fig. 14,  $\upsilon$ ). The dots now appear bright, surrounded by a dark border. By coalescence the appearance of a narrow bright disc is produced, separated from the dim disc at each side by a dark crenated line.

Similar refractive effects and transposition on focussing are seen in the discs isolated by transverse splitting of the fibre.

*Transposition of the Bands.*

The effect known as "transposition" of the bands has been noticed by many observers. On raising the objective what was previously the bright band appears now darker than the dim band.

This so-called transposition is seen in fibres prepared by the gold method, better in fibres prepared with osmic acid; diag. 6,  $\cup$  represents a fibre at the upper focus. The light band in the position of Krause's membrane appears very bright, and is bordered by a dark line at the junction of the light and dim bands. On focussing about  $\cdot 0025$  mm. lower down (with Zeiss D obj.) the appearance seen in L is obtained. The darkest part of the fibre is now in the centre of what was the bright band, that is, in the position of Krause's membrane. Bordering on this dark band, and separating it from the dim band, is a bright zone. The dim band remains much the same as before, though by contrast with the now dark Krause's membrane it may appear lighter.

The bright haloes round the nodal dots of the network may be compared with the similar effects observed whenever any highly refractile particle, such as a micrococcus or minute oil globule, is observed in a medium of lower refracting index.

In the oil globule suspended in water similar and very definite transposition effects are seen on altering the focus. If focussed low it appears as a dark spot surrounded by a bright halo or border (L, diag. 7). On raising the objective (about  $\cdot 0025$  mm., Zeiss D) the oil globule appears bright, surrounded by a dark border.

The effect produced when a row of oil globules are seen side by side is, at the lower focus (L), a bright band (formed by the coalesced haloes), with a series of dark dots traversing it; at the upper focus ( $\cup$ ) a narrower bright band, bordered by dark edges. The beads at the nodes of the transverse network may be looked upon as refracting and reflecting the light, in the same way as an oil globule in water, and as causing the so-called "transposition" of the bands seen on altering the focus.

*Identity of Network with Schäfer's Muscle Rods.*

We cannot but be struck by the resemblance of the appearances brought out by gold staining with those described by Schäfer<sup>1</sup> in the living fibre as muscle rods. The two views differ, however, on two

<sup>1</sup> E. A. Schäfer, "On the Minute Structure of the Leg Muscles of the Water-beetle." 'Phil. Trans.,' xii, 1873.

points: (1) Schäfer describes in a transverse section of the fibre a bright ground substance with a number of minute specks or dots; no appearance of a network. (2) He considers that there is typically a double transverse row of dots in the middle of each bright stripe.

Concerning the appearance on *transverse section* we must not forget that Schäfer's conclusions were drawn from the *living* fibre in *optical* transverse section. Probably he saw all that it is possible to see of the transverse network in the living fibre, namely, the thickenings or dots at the nodal points, the fine network, seen so plainly in a transverse view when stained with gold, not being visible in the fresh fibre examined in this way.

*Is there a single or a double row of dots* in the middle of the bright stripe? In the fresh fibre sometimes a single, sometimes a double, row of dots is seen, the two appearances often alternating with a higher or a lower focus. The same variation is seen in alcohol and some other preparations.

In the gold preparations, when the fine granular disc or transverse network is seen perfectly edgewise and in focus, it appears invariably made up of a *single transverse line of dots*.

When the transverse network is not seen perfectly edgewise, through not lying in a plane quite at right angles to the longitudinal axis, but slightly obliquely or in perspective, it may appear as a double row of dots or as a granular or dotted band crossing the disc transversely.

In a perspective view of the fibre (figs. 3, 6, and 17), not only the dots (nodal points of the network) at the near side of the fibre are seen, but at the same time those deeper down or at the far side. Hence the appearance of two or more rows of dots crossing the fibre. When, by raising the focus, the nearer edge of one of these obliquely-arranged discs is alone focussed it is seen to consist of a single row of dots.

It was noticed a few moments ago, when speaking of transposition of the bands, that at the upper focus (diag. 6,  $\nu$ ) the coalesced bright dots form a bright band bordered at each side by a dark crenated line. Each dark line is not unlike a row of dots. Schäfer<sup>1</sup> seems to have figured muscle at this upper focus, and hence describes two lines of dots traversing the light disc where it borders on the dim disc.

<sup>1</sup> 'Quain's Anatomy,' vol. ii, 9th edition, fig. 119.

*(b) Bee.*

Insect muscle may be very conveniently obtained from the thorax or leg of a bumble bee.

Prepared with acetic acid and gold chloride, by the method already described, it shows a network identical with that described in *Dytiscus*.

In order to obtain muscle in as uncontracted a condition as possible, gold preparations were made from the leg muscles of a bee, rendered insensible and immovable by chloroform vapour, in which presumably there was complete relaxation of the muscle fibre. These preparations, however, could not be distinguished from those prepared without chloroform.

As the fibres are rendered soft by the method of preparation their size and the size of their elements varies with the pressure of the cover-slip; hence measurements are of little or no value.

*(c) Frog.*

The fibres from the gastrocnemius of the frog treated by the same gold method as before yield an unmistakable network. The fibres when examined are seen to be more changed by the process than is the case with insect muscle. They become very much softened, and when pressed upon by the cover-slip expand to many times their natural diameter, and thus often altogether lose their shape. Owing to this disturbance of the fibre the network usually shows no distinct differentiation into horizontal or transverse, and longitudinal portions. Hence there is no transverse striation.

In many places in the preparation isolated portions of fibre show a network with polygonal meshes as in fig. 7. This network is also seen at the ends of certain fibres which, curling up, show a transverse section. The meshes are often, when the fibre is much expanded by compression, large enough to be seen with Zeiss A. obj., at other times much smaller, approximating in size to the meshes of the horizontal networks in insects' muscle. The size of the meshes seems to depend entirely on the degree of compression of the fibre. When the meshes are small, distinct thickenings or dots are seen at the intersections of the fibres composing the network. This network is particularly sharply defined and is plainly seen to be a true network, that is, the lines represent linear fibres only. It is not a honeycomb work. The lines do not represent the edges of plates of interfibrillar material.

*(d) Crustacean.*

An exactly similar network can be brought out in the muscle of the lobster. My friend Mr. C. F. Marshall has made preparations of lobster muscle with acetic acid and gold which show this network in a most beautiful manner. The muscle in this case was left in 15 per cent acetic acid for fifteen minutes (a much longer time than I use), in gold chloride thirty minutes, and in 25 per cent formic acid in a warm chamber for three hours exposed to the light.

This network represents the transversely and longitudinally arranged network described in insects' muscle pulled out of shape. In some of the fibres indeed it is still seen arranged in the rectangular manner. Fig. 8 represents a portion of a fibre in which transverse are crossed by longitudinal lines with dots at the intersections. In this case the ordinary light and dim transverse striation is obtained by refraction round the nodal dots.

At first sight the meshes of the irregular network described in the frog and lobster look too large to correspond in size with the meshes of the horizontal network in *Dytiscus*, that is, with the end view of sarcous elements. But we must not forget the effect of pressure; it expands the fibre to about ten times its normal diameter, and a corresponding increase in the size of the meshes takes place. Fig. 11 represents a transverse section of the fibre of the frog cut fresh with the freezing microtome and stained by the gold method. It has not been much enlarged by pressure, and hence the meshes of the network are small.

Fig. 10 represents a portion of a fibre of the lobster which has split into fibrils; an uncommon effect in gold preparations. When muscle splits into fibrils the fibres of the transverse network rupture midway between the nodal points; the longitudinal threads and dots remain often attached to the fibril of sarcous substance, and cause it to appear transversely striated.

The muscular fibres of the crayfish show exactly the same network, the precise method of gold staining seems to make little difference. Isolated portions of network are seen pulled out of shape, and thus with polyhedral meshes as in fig. 7. At other points the network is seen still arranged in its typical manner as in fig. 8.

*(e) Rat.*

In the Rat most of the fibres show the typical arrangement into transverse and longitudinal portions (fig. 9). The transverse network

is most marked. In certain isolated portions the dots at each nodal point of the network are seen surrounded by bright haloes as already described.

Such then is the effect of gold staining on the muscular fibre. Can this network be demonstrated in any other way? Any method which fixes the fibre in that condition in which it is when living gives rise to appearances closely resembling those described. Acetic and osmic acids seem to act in this way.

## II. ACETIC ACID PREPARATIONS.

Muscular fibres from the leg of the bee were placed in dilute acetic 1 per cent for from five to fifteen seconds, then into glycerine, and mounted.

On examination they are seen to present a transverse row of dots at each membrane of Krause and longitudinal connecting rods. The network, like the sarcolemma, seems to resist the action of acetic acid more than the matrix or sarcous substance. If the fibre be stained in hæmatoxylin after the action of the acetic, the network becomes stained to a greater extent than the matrix, which remains relatively unstained.

The fibre now presents the appearance seen in fig. 15. Thin granular deeply-stained discs are seen crossing the fibre in the position of each Krause's membrane. They are attached to the sarcolemma at the edges, and appear to divide the fibre into compartments. If the near edge of one of these discs be focussed it appears as a transverse row of dots crossing the fibre, and in many fibres fine longitudinal lines may be seen joining the dots of two adjacent discs.

In some fibres the appearance of a double row of dots crossing the fibre in the position of the transverse network is seen. This is represented in fig. 16. It is noticed in the preparations made with acetic acid, that the double rows of dots are met with, as a rule, in those fibres which have undergone least pressure. In fibres expanded by pressure a single row of transverse dots is alone observed.

Fig. 17 represents a fibre treated with acetic acid and afterwards stained in watery solution of logwood. At the upper part of the fibre the thin dotted transverse discs are not seen edgeways but partially from below. Lower down in the fibre the discs are seen more nearly edgeways, and appear in perspective view as narrow granular bands. These granular bands appear crossed longitudinally, and more or less broken up into short parallel longitudinal segments, by fine bright

lines. These bright lines are caused by refraction from the longitudinal rods of the network.

### III. OSMIC ACID PREPARATIONS.

Preparations made by placing living muscles from the bee in osmic acid 1 per cent for ten minutes, and mounting in balsam, give on examination the appearances figured in fig. 18 and diag. 6. Thickenings (Engelmann's "fixed waves of contraction") are seen on many of the fibres.

In diag. 6,  $\perp$  the fibre is seen crossed at intervals by a dark well-marked line, Krause's "membrane," or the horizontal network. On focussing upwards this line appears as a thin bright disc, and the appearance  $\cup$  is obtained.

In certain fibres (fig. 18), by careful examination, it can be seen that this dark line consists of a row of dots, and occasionally fine longitudinal lines may be seen joining them.

A *fixed wave of contraction* is shown in this figure. The contracted part of the fibre is widened out transversely and the distance between the transverse networks diminished. The series of haloes round the rows of dots extends to the whole of the now diminished interval between the successive rows. There is consequently a bright band in the position usually occupied by the dim band. Traversing this bright band longitudinally are seen fine lines joining the dots of adjacent networks. Between this fully contracted and the relaxed part of the fibre is the portion showing the "homogeneous stage" of Engelmann. The transverse marking is here to a large extent lost, and this can be easily understood, when we consider that at the onset of contraction the transverse network would be probably more or less pulled out of shape. The individual dots would no longer lie in the same transverse plane, and hence the haloes would not blend into a continuous bright transverse disc. This agrees with the fact mentioned by Schäfer,<sup>1</sup> that mechanical shifting of the elements of a fibre causes a disappearance of the transverse striations.

Another point often observed in osmic acid preparations is a caving in of the sarcolemma between each transverse network, that is opposite the dim stripe. In other preparations the sarcolemma usually bulges at these points, and appears to be contracted at its attachment to the transverse network or Krause's membrane. This may be explained if it be supposed that in osmic acid preparations there is a certain

<sup>1</sup> Quain's Anatomy, vol. ii, 9th edition, p. 129.



amount of contraction of the matrix or sarcous substance, by exosmosis for instance. The sarcolemma will follow this decrease in bulk but will be prevented from doing so at those points where it is held outwards by the more rigid transverse networks.

#### IV. THE LIVING FIBRE.

The fibres from the leg of *Dytiscus*, or the bee, mounted without the addition of any fluid, and examined whilst fresh or living, give the appearances seen in figs. 19 and 20. Most of the fibres are seen to present the appearance of alternate dim and bright bands, the dim bands being the thicker. Each dim band is traversed by a series of longitudinal lines of a highly refractile substance. Running across the middle of the bright band transversely is seen a single row of dots. The fine dark lines crossing the dim stripe are traceable at either end into the dots of the bright stripe. In this case, just as in the acetic acid preparations, there often appears to be a double row of dots in the centre of the bright stripe. Fibres are seen side by side, one with a single row, another with a double row of dots in this position. When a double row is present, the corresponding dots of the two rows appear to be always joined longitudinally by fine lines across the middle of the bright stripe. This is mentioned by Haycraft<sup>1</sup> but not by Schäfer.

Sometimes again the appearance shown in fig. 20 is observed. A series of short parallel longitudinal lines is seen in the position of the transverse network. These lines appear dotted on careful examination. This appearance is similar to that described in the acetic acid preparation (fig. 17), and may be explained in the same way as a perspective view of the network crossed by longitudinal bright lines, caused by refraction from the longitudinal rods. "Transposition" of the bands may be seen on altering the focus, similar to that already described. The line of dark dots, with its series of bright haloes forming the bright disc, becomes now a line of bright dots bordered by two crenated dark lines. The above observations on the living fibre were made by means of the gas chamber. The chitinous integument of the leg of the bee was slit longitudinally, the muscle scooped out, and quickly teased on a cover-glass and inverted over the moist gas chamber. This method may be used for studying the phenomena of contraction, by blowing air charged with alcohol vapour into the chamber, and thus causing the fibre to contract by chemical stimulus.

<sup>1</sup> 'Quart. Journ. Micr. Sc.,' April, 1881, p. 23.

On contraction the fibre becomes shorter and thicker, the transverse rows of dots approach one another and appear darker, probably by contrast with the now bright "dim" disc. These appearances are similar to those seen in the "fixed waves of contraction," described in the osmic acid preparations.

In a preparation of fresh muscle I have seen a fibre undergo slow rigor mortis, commencing at one end and gradually extending towards the other. It exactly resembled a very slow contraction wave passing over the fibre, and the changes undergone by successive discs, as the contraction affected them, were similar in appearance to those described in fig. 18, and could be observed with more deliberation than usual.

*The Fibre under Polarised Light.*—The effects observed in the living fibre with crossed Nicols were exactly similar to those figured and described by Brücke and Schäfer ('Quain's Anat.,' 9th ed., vol. ii, fig. 125). Brücke's drawing is almost identical with diagram 3.

The fibre is chiefly made up of doubly refractile or anisotropic material, but a band of singly refractile or isotropous material crosses the fibre transversely in the position of each Krause's membrane, and this band is seen with a high power to consist of a row of rhomboidal dots. Fine lines of isotropous material are described running longitudinally across the anisotropic discs and joining the rhomboidal dots. The appearance of the muscle fibre under polarised light leads us to the belief that the network consists of isotropous, the matrix or ground substance of anisotropic or doubly refracting, material.

#### V. ALCOHOL PREPARATIONS.

Alcohol preparations of muscle show, in most cases, a somewhat different character to those prepared by the preceding methods.

*Spirit* has a tendency to split the fibre into fibrils and sarcous elements. After the muscle has been in alcohol it may be stained with some reagent; Kleinenberg's hæmatoxylin, for instance, gives excellent results. Alum carmine may also be used. Mount in Canada balsam.

*Absolute alcohol* has a somewhat different effect from ordinary spirit. It sometimes seems to fix the fibre as it appears during life—that is, there is no differentiation into sarcous elements, but transverse rows of dots, and longitudinal lines are alone seen, as in the living fibre. Fixed waves of contraction may also be found.

Fig. 21 represents a portion of a fibre of *Dytiscus* stained in hæmatoxylin after the action of spirit. It shows an alternation of bright and dim discs, the dim discs stained a deep purple and made up of a series of sarcous elements side by side. Across the middle of the bright discs a granular or transverse line is seen. Fine longitudinal lines, the longitudinal bars of the network, may occasionally be seen crossing the bright discs.

This account agrees for the most part with that given by Klein<sup>1</sup> as to the structure of muscle. He, however, figures a continuous line—the homogeneous Krause's membrane—in the middle of the bright stripe, and no longitudinal fibrillation in the bright disc.

Let us consider the influence of the intracellular network in producing the appearances known as *sarcous elements*, and *Cohnheim's areas*, in the muscle fibre.

The matrix, or substance which lies in the interstices of the network, is of far greater bulk than the network. It is homogeneous throughout; nevertheless, it may be looked upon as being *partially* divided into columns or fibrils by the longitudinal bars of the network, and partially into discs—the contents of muscle compartments—by the transverse networks. By the action of spirit the matrix becomes split into fibrils. The reagent causes this "sarcous substance" to shrink (possibly by abstraction of water), and the homogeneous mass now separates into fibrils along the lines of greatest weakness—that is, along the guide lines formed by the longitudinal bars of the network. These fibrils may again divide transversely at the horizontal networks, producing *sarcous elements* (diag. 8). Thus the appearance of sarcous elements is seen, as described by Klein,<sup>2</sup> to be a *post-mortem* phenomenon. In consequence of shrinking the sarcous substance no longer entirely fills up the skeleton "muscle caskets," and the division into sarcous elements, which was foreshadowed only before by the bars of the network, becomes evident by the development of intervening spaces between adjacent elements. The appearance known as Cohnheim's areas is somewhat differently described by different observers. For the present we may follow Klein's<sup>3</sup> description. The prismatic sarcous elements which lie side by side in the living fibre with no intermediate substance, shrink through coagulation on dying, and become separated from one another by a transparent interstitial fluid substance. In a

<sup>1</sup> 'Atlas of Histology,' p. 77.

<sup>2</sup> Loc. cit., p. 76.

<sup>3</sup> Loc. cit.

transverse view there are thus seen small polygonal areas separated by clear lines, each polygonal area corresponds to a sarcous element.

Cohnheim's areas may be described as the appearances produced by coagulation and splitting of the matrix along the guide lines formed by the transverse network; they represent an end view of sarcous elements, and are *post-mortem* phenomena (diag. 9).

#### PREVIOUS VIEWS.

I think it unnecessary to give a historical account of the different views which have been published with regard to the structure of the striped muscle fibre.

An epitome of the historical results may be found in Schäfer's<sup>1</sup> paper on the leg muscles of the water-beetle; or by the same author in 'Quain's Anatomy,' 9th ed.

Reference has already been made to most of the appearances described by different observers, and the way in which these appearances may be explained as caused by the presence of a highly refracting network.

The relation of this network to Krause's<sup>2</sup> views may be noticed. Krause's "muskel-kästchen" are bounded above and below by Krause's membrane, and laterally by the boundaries of Cohnheim's areas. Brücke<sup>3</sup> regards the isotropous lines which traverse the anisotropous disc as optical sections of the partitions between "muskel-kästchen." These partitions correspond with the longitudinal bars of the network and with Schäfer's rods. The alternation of bright and dim transverse bands has been looked upon by several observers as an optical effect, and not due to any anatomical differentiation here present.

Hepner<sup>4</sup> and Stricker look upon the bright band as the expression of total reflexion, which occurs at the line of demarcation between Krause's membrane and the chief substance of the fibre.

Bowman suggested that the transverse striping shown by the fibrillæ was caused by the moniliform shape. Haycraft<sup>5</sup> has recently developed this view, and extended it to the whole fibre.

Striped muscular fibres are met with in the animal kingdom, from the Cœlenterata upwards; there is no reason to suppose that the

<sup>1</sup> Loc. cit.

<sup>2</sup> "Ueber den Bau der quergestreiften Muskelfaser," 'Zeitschr. f. rat. Med.,' xxiii.

<sup>3</sup> 'Quain's Anatomy,' p. 127; and "Muskelf. im polarisirten Licht," 'Wiener Denkschr.,' xv.

<sup>4</sup> 'Stricker's Handbook' (Syd. Socy.), p. 548, vol. iii.

<sup>5</sup> 'Quart. Journ. Micr. Sc.,' April 1, 1881.

cause of the transverse striation is different here from that in the insect.

I have received the greatest sympathy during this investigation from my friend Mr. C. F. Marshall, with whom I have verified most of my results. The drawings of the network in the fibres of the Rat and Lobster are from gold preparations by him. Mr. Marshall is at present working on the histology of the muscle fibre, from the lowest types of the animal kingdom in which it occurs upwards, and has already obtained interesting results. A study of the comparative development or phylogeny of this network, and at the same time of its embryology, may lead to its undoubted recognition as an ordinary intracellular network.

My thanks are also due to Professor Milnes Marshall, who has kindly examined my drawings and specimens, and suggested alterations in the paper, and to Mr. J. Priestley.

#### BRIEF SUMMARY OF RESULTS.

The chief results at which I have arrived may be summarised as follows :

There is an intracellular network present in the striped muscle fibre of *Dytiscus*, Bee, Lobster, Crayfish, Frog, and Rat, which may be most clearly demonstrated by certain methods of gold staining. The network alone is stained by the reduced gold, and, owing to this differentiation, is plainly visible even with comparatively low powers. This network may be demonstrated, though not so completely, in the living fibre, and in acetic and osmic acid preparations.

Crossing the fibre transversely, united to the sarcolemma, and more or less separating the muscle fibre into compartments, are network partitions—the transverse network.

Running longitudinally down each compartment, and joining the dots at the intersections of the fibres of the transverse network, are a series of fine rods. The arrangement of this network will be made evident by reference to diagrams 1, 2, 3, and 4.

This network consists of an isotropous material, and is more highly refractile than the rest of the muscle substance, which is anisotropous. This network serves to explain the transverse striation and other complicated appearances presented by the muscle fibre, and brings into harmony many of the conflicting statements of histologists on this subject.

## DESCRIPTION OF PLATE X.

Diags. 1, 2, 3, and 4.—Diagrammatic views of the network in striated muscle.

Diag. 1.—Perspective view of the fibre, showing the transverse network, *a*, at each membrane of Krause, and the longitudinal lines.

Diag. 2.—Perspective view of a portion of the network, showing:—*a*. The transverse networks, with polygonal meshes and dots at the nodes. *b*. The longitudinal bars of the network ending in the dots.

Diag. 3.—The fibre seen in longitudinal view. The transverse network, *a*, appears as a row of dots crossing the fibre (in the position of Krause's membrane). *c*. Minute thickenings on the longitudinal bars of the network, midway between the transverse networks.

Diag. 4.—The fibre seen in transverse section.

Diag. 5.—Network as seen in a longitudinal view of the fibre, showing the production of alternating bright and dim bands by refraction around the nodal dots.

Diag. 6.—So-called transposition of the bands, as seen in an osmic acid preparation of muscle of Bee. *u*. Appearance at upper focus. *l*. Appearance at lower focus.

Diag. 7.—Oil globules in water, showing their refractive effect upon light. *u*. At the upper focus, each globule surrounded by a dark border. *l*. At the lower focus, each globule surrounded by a bright halo.

Diag. 8.—Production of sarcous elements by contraction of the matrix and splitting along the guide lines formed by the bars of the network (seen in spirit preparations).

Diag. 9.—Formation of Cohnheim's areas by contraction of the matrix as above. In this transverse view of the fibre the prismatic sarcous elements are seen on end, and appear as polygonal areas separated by bright lines.

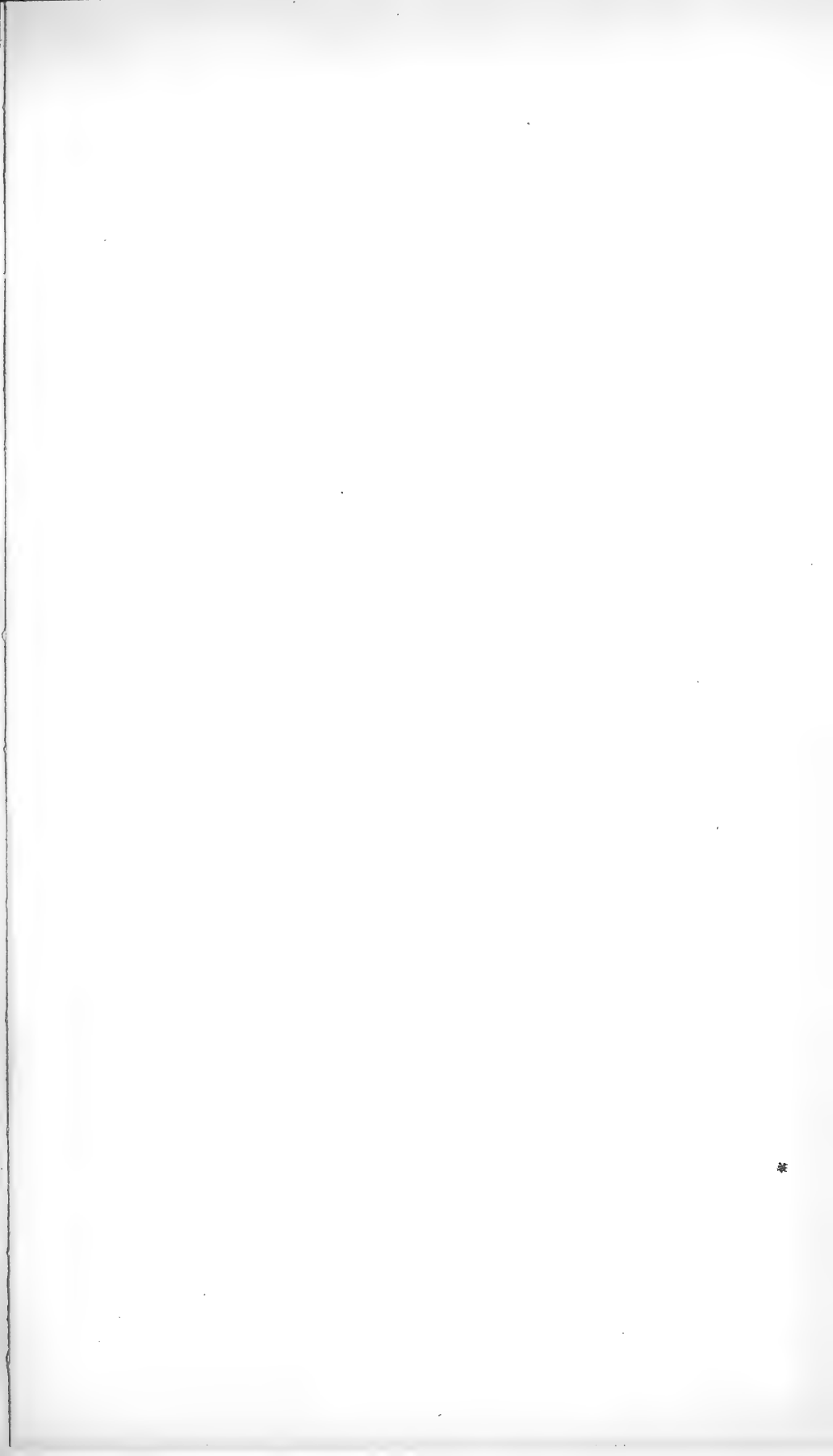
Fig. 1.—Fibre of *Dytiscus*, prepared by the gold method. Zeiss, D obj., No. 5 oc.

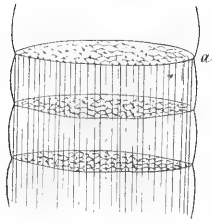
- Fig. 2.—Dytiscus, gold method, portion of a fibre more compressed than in Fig. 1.
- Fig. 3.—Fibre of Bee, prepared by the gold method; transverse networks in perspective.
- Figs. 4 and 5.—Dytiscus, gold method, showing isolated discs consisting of a network.
- Fig. 6.—Fibre of Dytiscus, gold method, splitting into discs.
- Fig. 7.—Lobster fibre, gold chloride; isolated portion of a fibre, network pulled out of shape. Exactly similar networks are seen in the Frog and Crayfish.
- Fig. 8.—Frog, gold method; network arranged typically, and showing transverse striping.
- Fig. 9.—Rat, gold chloride; longitudinal view of a portion of a fibre. (Preparation by C. F. Marshall.)
- Fig. 10.—Lobster, gold chloride, splitting into fibrils.
- Fig. 11.—Frog. Transverse section of the frozen fibre, stained by the gold method.
- Fig. 12.—Dytiscus, gold method; isolated portion of the network.
- Fig. 13.—The same, more highly magnified. (Zeiss, F obj., No. 5 eyepiece.)
- Fig. 14.—The same, showing refracting effect of the network. L. Lower focus. U. Upper focus.
- Figs. 15 and 16.—Fibres of Bee, treated with acetic acid, then Kleinenberg's hæmatoxylin.
- Fig. 17.—Fibre of Bee, treated with acetic acid, then watery solution of logwood. The transverse networks seen more or less obliquely.
- Fig. 18.—Fibre of Bee, prepared with osmic acid, shows a fixed wave of contraction.
- Fig. 19.—Living fibre of Bee, showing longitudinal view of network, ( $\frac{1}{15}$  immersion obj).
- Fig. 20.—Living fibre of Bee, transverse networks seen somewhat obliquely.
- Fig. 21.—Portion of a fibre of Dytiscus, stained in hæmatoxylin after the action of spirit. Shows sarcous elements.

Where not otherwise stated, the drawings were made from Zeiss, D. obj., No. 5 oc.

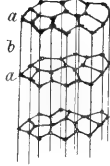




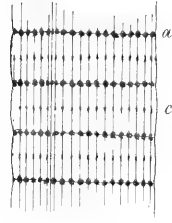




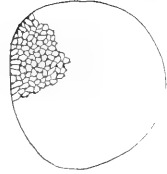
Diag. 1.



Diag. 2.



Diag. 3.



Diag. 4.



Diag. 5.



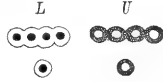
Diag. 8.



Diag. 9.



Diag. 6.



Diag. 7.

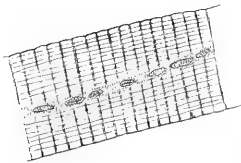


Fig. 1.

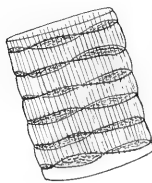


Fig. 3.

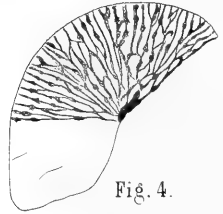


Fig. 4.

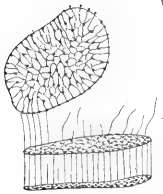


Fig. 6.

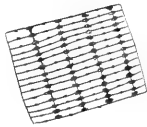
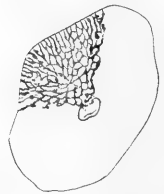


Fig. 2.



Fig. 5.



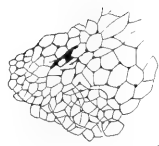


Fig. 7.

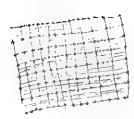


Fig. 8.



Fig. 9.



Fig. 10.

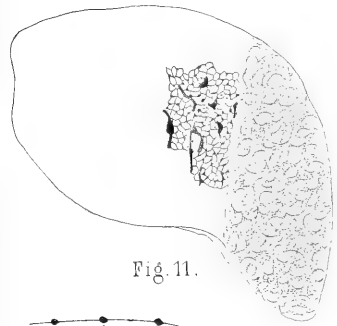


Fig. 11.

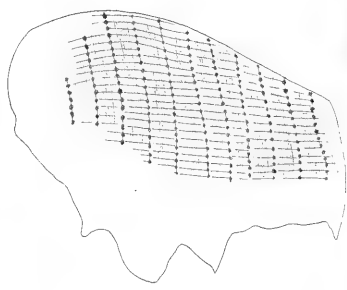


Fig. 12.

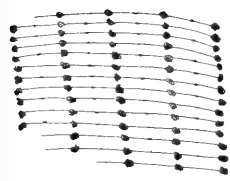


Fig. 13.

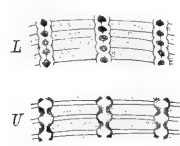


Fig. 14.

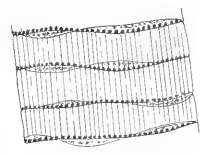


Fig. 15.

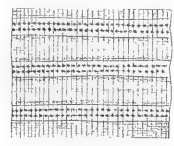


Fig. 16.

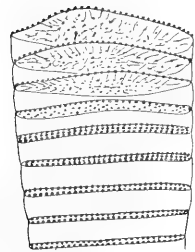


Fig. 17.

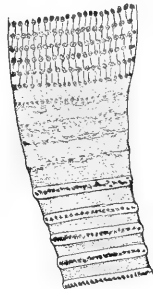


Fig. 18.

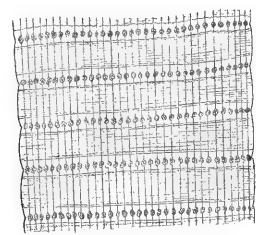


Fig. 19.

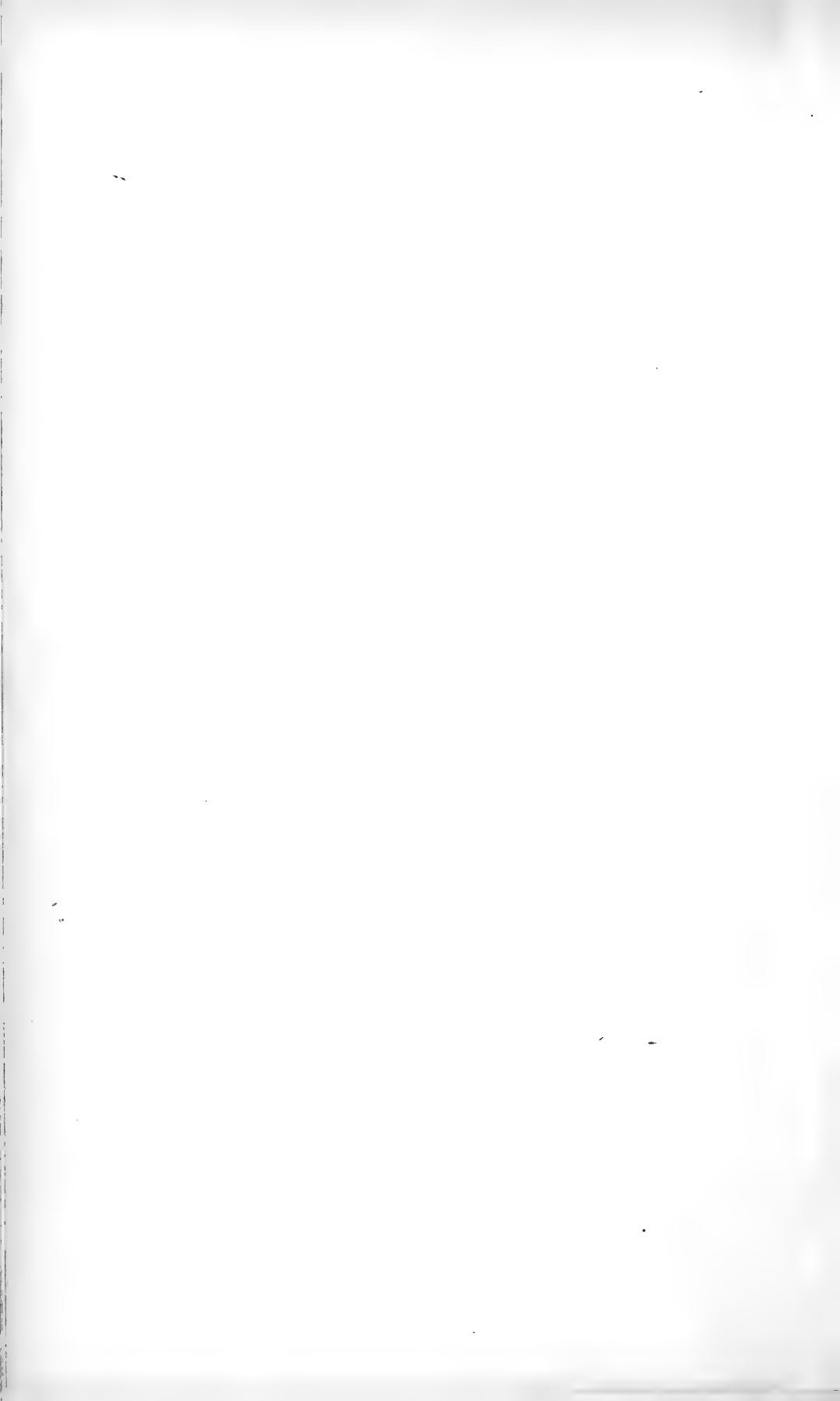


Fig. 20.



Fig. 21.





## THE ANATOMY OF THE MADREPORARIA. I.

By G. HERBERT FOWLER, B.A., *Keble Coll., Oxon; Berkeley Fellow of the Owens College.*

[PLATES XI, XII, & XIII.]

By the kindness of Professor H. N. Moseley I have been enabled to study the anatomy of certain Madreporaria obtained by him during the voyage of H.M.S. "Challenger." As in lecture-courses and text-books but little information is given relative to this ancient and interesting group, and the few papers on the subject are scattered, a short sketch of the more recent researches is prefixed to my own results. Two forms only are described in this paper, *Flabellum patagonicum* and *Rhodopsammia parallela*: others, it is hoped, will follow shortly. The text will be reduced as far as possible throughout.

An acquaintance with the anatomy and development of an ordinary Actinia is presupposed in the reader, this being the type by which comparisons are made; but a list of the more technical names used to describe the anatomical parts of the polyp is given, with the synonyms for the use of those desirous of consulting the literature of the subject.

Mouth-disc = Mundscheibe, Peristome.

Body wall = Leibeswand.

Stomatodæum (oesophagus) = Schlund-, oder Magen-rohr.

Cœlenteron = Darmhöhle, Leibeshöhle, Estomac.

Mesenteries (sarcosepta) = Scheidewände, Parietes, Replis mesenteroïdes.

Mesenterial filament (craspedon) = Mesenterialfaden, Cordon pelotonné.

Septa (sclerosepta) = Sternleisten, Cloisons, Lames.

Theca = Mauerblatt, Muraille.

A "pair" of mesenteries is constituted by two mesenteries whose longitudinal muscle fibres are ranged on their adjacent faces (except in the case of the two "directive pairs," each of which is placed at one end of the longer axis of the mouth oval, and in which the arrangement of the muscles is reversed). For the chambers (Radial-taschen, Loges) into which the coelenteron is periaxially divided by the mesenteries, I am compelled to coin new names. To those chambers which lie between a "pair" of mesenteries the term *entocœle* is applied (fig. 1, B); to those chambers of which one lies between every two pairs of mesenteries the term *exocœle* (fig. 1, A). The septa lying in these two classes of chambers are similarly called *exosepta* and *entosepta*.

The classification adopted will be found at the end of the paper, together with the bibliography.

#### RECENT RESEARCHES INTO THE MORPHOLOGY OF THE GROUP.

In 1873 Lacaze Duthiers (1), studying the development of *Astroïdes calycularis* on the coast of Algiers, found that it agreed in every important point with the development of *Actinia*, his observations on which (2) were corroborated and corrected by the Brothers Hertwig (3). With regard to the developing skeleton, he recorded two facts of importance—firstly, as appears in his pl. xiv, fig. 27, that it was formed *outside the polyp*; and secondly, that the theca arose *independently of the septa*. Owing to various practical difficulties his investigation was incomplete.

The chief worker in this field has been Georg von Koch, who, in the course of several investigations, has arrived at the conclusion that the theca is a secondary structure, derived from fusion of the peripheral ends of the septa. The evidence adduced in support of this theory appears to me to be at present insufficient for complete proof, though from our slight knowledge of the group it is injudicious to absolutely deny its truth.

Von Koch first published this theory in 1879, founding it on the following observations on *Caryophyllia* (4). There is no living tissue on the greater part of the exterior of the corallum; but at the apex the peripheral edge of the mouth-disc overlaps the lip of the calyx in such a way that in the highest sections the septa appeared to stand free in the coelenteron, in sections a little lower to have fused peripherally into a theca. The costæ are, according to him, and

as will be seen by the figures, the outermost ends of the septa (Pl. XI, A, B).

Further, the mesenteries and chambers between them appeared to be continued into this external part of the polyp. These appearances he explained by supposing that, as the peripheral ends of the septa approximated and fused, they surrounded the mesenteries, dividing them ultimately into a central and a peripheral part. As a further proof he adduced the observation that in microscopic sections of the corallum sutures were visible in the theca at the points where he supposed the septa to have fused.

I venture to think with Moseley (5) that this explanation is erroneous; that the appearances in the first section (Pl. XI, A) are due merely to the fact that in this, as in many corals, the secretion of calcium carbonate is most active about the septa, which consequently rise slightly above the level of the theca, as may be seen in any figure of *Caryophyllia*; and further that, in the second section (Pl. XI, B), the apparent continuation of the mesenteries and chambers between them over the lip of the calyx is not due to their having been cut into two portions by fusion of the septa, but to more or less abnormal contraction due to the use of alcohol; in life the polyp, when fully expanded, undoubtedly stretches over the lip, but in these forms, so far as I can ascertain, in *natural* contraction it is completely within the calyx. Further, as appears from his own researches and those of others on different forms, the whole skeleton, instead of being, as he describes, free in the coelenteron, is shut off from it by a layer of endoderm and mesoderm, and as much outside it as the rest of the corallum; these layers he himself figures as clothing another part of the septum, though of this portion no histological details are given. Von Heider, in a paper shortly to be referred to, states that Von Koch has overlooked the fact that the whole of the corallum is covered externally by ectoderm and mesoderm; certainly this form requires more complete investigation. Again, having ground many microscopic sections of corals, I can afford no credence to "sutures;" in the process cracks fly through the coral in all directions. But if evidence of a directly contrary character is needed, the case of *Flabellum* may be adduced, in which (5), according to Moseley, sutures run not between the fused ends of the septa—*i.e.* through the theca—but down the centre of each septum.

Though Von Koch gives no detailed description of the anatomy of Caryophyllia, the following account may be inferred from his figures and text (4) (6). The polyp is built on the Actinian type, consisting of mouth-disc, stomatodæum, mesenteries; the muscles of the latter being arranged as in Actinia. No external body wall, its place being taken by the theca; inner body wall of mesoderm and endoderm, lining the cœlenteron, and clothing the interior of the calyx, both theca and septa. Mouth-disc drawn down in abnormal (?) alcoholic contraction over the lip of the calyx. Entosepta and exosepta both present. No mention made of tentacles.

Of *Madrepora variabilis* he records, in 1880, the following facts (6): Structure Actinian; in the end-polyps of the colony six pairs of mesenteries, six entosepta, and six exosepta; in the side-polyps also six pairs of mesenteries, but six entosepta only.

Von Koch has also studied *Stylophora digitata* in somewhat greater detail (7). The form of the colony resembles that of *Alcyonium digitatum*; the polyps live in small calyces on the surface of the colony, but the living tissues are not continued down into its centre, as in *Alcyonium*; the lower part of the cavity formerly inhabited by the polyp being shut off by a kind of tabula as it grows upwards. Over the surface of the colony lies the cœnosarc, the fleshy rind of the otherwise calcareous colony, which puts the polyps in communication with one another, being permeated by canals which are continuous with their cœlentera, and similarly lined by endoderm. The polyp possesses six pairs of mesenteries, six larger tentacles, six smaller tentacles, and six entosepta. There are two distinct types of nematocyst. Longitudinal muscles occur on the mesenteries, but the smallness of the latter rendered it impossible to detect whether their arrangement agreed with Actinia or not.

In 1881 Dr. von Heider, of Graz, published a description of *Cladocora astræaria* and *Cl. cespitosa* (8). These species are also built on the Actinian type; and Heider describes for them the same continuation of the mesenteries and mesenterial spaces that v. Koch mentions as occurring in *Caryophyllia cyathus*. I have examined macroscopically and by sections *Cl. cespitosa* in a completely retracted state, and can find no trace of such a condition, an observation which confirms my belief that this appearance is due to partial contraction, owing to the use of alcohol. There is no true cœnosarc such as occurs in *Stylophora*, just as there is no cœnenchyme, the calyces being free



outwardly from the rest of the colony. In luxuriant growth and budding, however, according to Heider, both skeletons and soft tissues of adjacent polyps may fuse; an observation interesting as probably indicating the history of the formation of the cœnosarc and cœnenchyme which characterise many other forms. There is one correction to be made in his work which, for the sake of future workers in this field, ought to be mentioned here, namely, that in his Pl. III he frequently figures as endodermal cells small spherical bodies with a well-staining nucleus, which are zooxanthellæ or symbiotic unicellular Algæ, living free in the cœlenteron in such numbers as often to completely obscure the true endoderm, with which they, of course, have no connection. While accepting v. Koch's theory as to the origin of the theca from fusion of the septa, he differs from it in some details, regarding the "sutures" as merely cracks artificially produced in the corallum. Septa and tentacles both entocœlic and exocœlic; mesenteries and their muscles arranged as in *Actinia*. For further details, which are very thoroughly worked out, his paper should be consulted. One point of importance deserves mention: between the corallum and the structureless mesoderm-lamella which overlies it immediately, and was generally understood to secrete it, v. Heider detected certain cells, for the most part scattered, but in some places forming a definite layer. To these he gave the name calyccoblasts, and assigned the function of coral-secretion, with great justice, as later researches proved, though their origin was a matter of doubt till cleared up by v. Koch.

The latter, in a paper on the development of *Astroïdes calycularis*, brought into notice the following facts (9). When first fixed, and before the secretion of the skeleton has commenced, the embryo is plano-convex, and its ectoderm may be divided into two regions, corresponding to its surfaces, the plane disc of attachment, or basal ectoderm, and the convex portion, or lateral ectoderm, the centre of which is invaginated as the stomatodæum. The skeleton first appears as small pellets of calcium carbonate lying *between the basal ectoderm and the foreign body to which the embryo is attached*, and is therefore outside the animal, and consequently *the result of secretion by the ectoderm*. As the corallum is always described in text books as a product of the mesoderm, this observation cannot be too strongly insisted upon. These pellets become first a ring-shaped disc, then a complete disc lying between the basal ectoderm and the foreign body to which the

embryo is attached. Where septa are to be formed the three body layers, endoderm, mesoderm-lamella, and basal ectoderm, rise upwards as a fold into the cœlenteron; and as they rise, coral is deposited beneath them which fuses with the original disc; the septa are thus also deposited outside the basal ectoderm. Then they begin to bifurcate at their distal ends. The originally basal ectoderm to which the secretion of the skeleton is attributable *persists in the adult as the calycoloblasts* of v. Heider.

Von Koch further asserts that the theca results from the fusion of the bifurcating ends of the septa; but, though not venturing to deny this, I would point out that he neither describes the process nor gives figures to illustrate it; whereas, on the other hand, we have the direct evidence of L. Duthiers to the effect that the theca and septa arise independently of each other (“les septa et la muraille ne sont pas unis”), and a figure, which appears to bear out his statement. It must, however, be borne in mind that Lacaze Duthiers may have described as theca what v. Koch terms epitheca, a secretion of the lower portion of the lateral endoderm of the embryo which fuses with the periphery of the original basal disc, and ultimately combines also with what he terms the true theca, formed as above mentioned, to become the outer wall of the corallum. Were this the case, however, the costæ could not be, as he regards them, the peripheral ends of the septa. But the question can only be finally settled by a study of the embryonic development of widely different forms.

Professor Moseley has published a preliminary note on *Seriatopora* and *Pocillopora* (10). These forms were originally classed with the Tabulata, but his account of their anatomy brings them into close connection with the other Madreporaria at present described. The polyps of *Seriatopora* are oval in outline, with twelve short tentacles, which in complete retraction are covered over by the indrawn margins of the disc, a condition common in Actiniaria, but very rare in Madreporaria. There are twelve mesenteries, only two of which, the same two in every polyp, are enormously long and bear mesenterial filaments and generative organs. The elongation of this pair of mesenteries deep into the colony suggests an inevitable comparison with the Alcyonaria; and the similarity is strengthened by the marked orientation of the polyp, for a division into “dorsal” and “ventral” halves is clearly distinguishable in both soft tissues and corallum. Two of the septa are very rudimentary, and

both this fact and the absence of mesenterial filaments on ten of the mesenteries would seem to indicate a *degeneration*, of which I hope to bring forward a second instance in a future paper. Between the polyps runs a similar canal system to that already described by v. Koch in Stylophora. The anatomy of Pocillopora, so far as mentioned, appears to agree in all respects with that of Seriatopora, and the polyps exhibit the same marked orientation.

Moseley has also described the microscopic anatomy of three other Madreporarian polyps (II). His observations on *Flabellum* are mostly incorporated with my own below, and need not, therefore, be recapitulated here; and of *Stephanophyllia* I hope to give a detailed description in a future paper.

Of *Bathyaectis*, which is planoconvex in shape, the plane being the basal surface, he records that on decalcification a lamina of ectoderm and mesoderm separates off from the base. This fact, together with its shape, suggests that the original basal ectoderm of the embryo persists in this species throughout life, in its primitive position, except for such part as grows up with the skeleton (the calyco blasts).

To sum up the undoubted facts elucidated by these observers:—

1. The adult Madreporarian polyp is built distinctly on the *Actinian type*, except for the absence of an external body-wall in some cases (Caryophyllia, Cladocora), which is then replaced physiologically by the imperforate theca.

2. The corallum is a product of the *ectoderm*, and deposited outside the embryo.

This ectoderm persists in the adult as the layer of *calyco blasts*, to which the continual growth of the corallum is attributable; thus the skeleton is *morphologically external* to the polyp throughout life.

4. Between this layer and the cavity of the cœlenteron, and clothing every part of the skeleton, is a layer of mesoderm and endoderm, forming the internal body-wall.

5. Septa, when present, always lie between a pair of mesenteries (*entosepta*), sometimes also in the spaces intermediate between pairs of mesenteries (*exosepta*).

6. Tentacles may be exocoelic as well as entocoelic, but exosepta may be present without corresponding tentacles.

The present classification of the Madreporaria is admittedly unscientific. I have therefore laid stress on what may perhaps seem the trivial point of the relations of septa and tentacles to the mesen-

terial spaces, as it is probable that, since the morphological differences of the whole group of *Zoantharia hexacoralla* are very slight, such structural variations might be useful for a new classification, which, if based upon the relations of polyp to skeleton, will be on a far sounder foundation than the present one, which rests upon the skeleton alone.

FLABELLUM PATAGONICUM (Moseley).

This is an imperforate Madreporarian, belonging to the family Turbinolidae. As Moseley (II) has given a full description of the specific characters of the corallum in his "Challenger" Report (to which reference should be made for figures of the complete calyx), only a few of them will be mentioned here.

i. The *corallum* is solitary and conical, the apex of the cone forming a pedicle by which the polyp is attached when young; in the adult the pedicle becomes obliterated and the coral free (*vide* figs. 2, 3, *Pl.*). The outline of the mouth of the calyx is oval (fig. 1). There are four orders of septa, all of which are entocœlic; six of the first order, which meet in an elementary form of columella; six of the second, which are nearly as long as the primary septa; twelve of the third, and twenty-four of the fourth order. In some specimens the full number is not developed. The corallum is about 2 cm. high in a well-grown specimen; and the longer axis of the calyx mouth about  $2\frac{1}{2}$  cm., the shorter axis 2 cm. in length.

Along the lines which correspond on the exterior surface of the theca with the attachments of the septa on the interior, are shallow but distinct grooves running from lip of calyx to tip of pedicle, each corresponding exactly in position with a septum. These do not agree with v. Koch's views as to the origin of the theca from fusion of the septa, to accord with which costæ should be developed in this position, such as occur in many forms.

The whole of the exterior surface of the theca shows well-marked lines of growth (fig. 4), so arranged as to appear to indicate that the chief centres of activity for the secretion of coral lie in the septa. Hence the lip of the calyx is slightly dentate (figs. 3, 4).

While the upper fourth of the external surface of the theca is, like the whole of the interior of the calyx, glistening, white, and hard, the lower three-fourths are soft in texture and brownish. This latter portion was described by Moseley as a "light-brown epitheca." But on decalcification the brown substance falls off as soft flakes, which, by

means of sections are found to consist of dead tissues and algal (?) parasites. There is really no epitheca present, recognisable as such, in the adult.

The columella (fig. 3, *col.*) is incomplete, the septa not always meeting regularly along their free edges.

In the retracted condition of the polyp there is no tissue external to the corallum (figs. 1, 2), nothing corresponding to the condition described by Heider in *Cladocora* and by v. Koch in *Caryophyllia*. When expanded, however, the soft tissues almost certainly stretch outwards and downwards over the upper fourth of the exterior of the theca, which is thus kept white and hard, as mentioned above. Were the polyp thus completely expanded to be plunged into a killing fluid, the same appearances would ensue as the above-named observers have described.

ii. *Anatomy.*—This agrees in all essential details with the Actinian type, except in the absence of an external body-wall, the whole polyp being enclosed in the corallum (figs. 1, 2). Moseley mentions that in some specimens tissues external to the theca were observed round the lip, and figures them (II), pl. xvi, fig. 10, as consisting of ectoderm and mesoderm, but had not the means of studying them by sections. None of my specimens had any trace of such, and from observations on *Desmophyllum*, a closely-allied form, I imagine that these tissues were simply due to the expansion of the polyp, and contained a continuation of the coelenteron such as was described by v. Heider in *Cladocora*. On decalcification the polyp appears conical, and divided into a series of wedges by the spaces where the septa had been. At the base of the polyp—*i.e.* the apex of the cone—these wedges appear to be connected together by little bridges of tissue. These latter are of no morphological importance, being due apparently merely to the incompleteness of the columella, and their arrangement varies in different specimens. The polyp consists of a mouth-disc bearing tentacles; a stomatodæum, which opens into the coelenteron, the latter being periaxially divided into exocoelæ and entocoelæ by the mesenteries.

The *mouth-disc* (fig. 2, MD) is peripherally fastened to the extreme edge of the lip of the calyx, and is centrally invaginated into the typical Anthozoan stomatodæum.

On the disc are borne the *tentacles*, which are simple hollow evaginations of the entocoelæ—*i.e.* one is placed over each septum.

They are covered by small prominences, each of which is a "battery" of nematocysts. I have not been able to determine whether they possess an opening at the tip or not. They vary in size and position according to the order to which they belong, the primary tentacles being the largest and nearest to the mouth. (*Vide* Moseley (II), pl. xvi, fig. 12.)

The *mouth* is oval in outline, and at each end of its long axis there is in most cases a well-marked gonidial groove.

Through the periphery of the mouth-disc protrude the acontia. I have by a fortunate section been able to satisfy myself that they are ejected through definite openings, not by rupture of the disc; these are therefore directly comparable to the cinclides of Actiniæ.

A *mesentery* of the first order is drawn in fig. 5 to show the general trend of the muscles, though they are much more numerous than there represented. They are best seen by mounting the mesentery whole in glycerine.

In the arrangement of the longitudinal muscles on the inner (entocœlic) faces of the mesentery, Flabellum agrees with Actinia; these are the retractors of the polyp. On the outer (exocœlic) faces are ranged the protractors, oblique in direction; these differ slightly in the species, being confined in *Fl. alabastrum* to the upper third of the mesentery, while the longitudinal fibres extend for its whole length. Both sets of fibres are continued into the tentacles, the oblique muscles of the mesentery becoming their external longitudinal coat, the longitudinal muscles of the mesentery passing into the internal and approximately circular fibres of the tentacle. This apparent change of direction will be understood by fig. 5.

The two pairs of "directive mesenteries" at the ends of the longer axis of the mouth appear to possess the same general direction of the muscle fibres, though bearing them on reverse faces; but the oblique protractor muscles (in this case entocœlic) are, proportionately to the retractors, somewhat more strongly developed, implying, perhaps, that the expansion of the polyp is their especial function.

There are no perforations through the mesenteries, such as are described in Actiniæ, putting the chambers in communication.

Both the primary and secondary orders of mesenteries are attached to the stomatodæum for its whole length; the tertiaries are attached to the mouth-disc, but, as the latter passes imperceptibly into the stomatodæum, no importance is to be attached to this.

What Moseley has termed "the contorted mesenterial filaments," a mass of coils lying on the side of the mesenteries, appear to me, after careful investigation to be, in part, at least, organs corresponding to the *acontia* of Actiniæ, namely, long lamellar offsets of the free edge of the mesentery, with one edge thickened to correspond to the mesenterial filament, and charged with very large nematocysts. They protrude in some instances, as above stated, through definite openings in the mouth-discs. Their exact origin from and relation to the mesenteries I have not been able to detect, owing to the brittle condition of the specimens, which did not allow of their being dissected out.

The ova are developed on all three orders of mesenteries. As their origin and position do not appear to differ from the type described by the brothers Hertwig for Actinia, no figures are given. I have not seen the testes, hence Flabellum may be regarded as dioecious. The filament is present along the whole course of the free edge of the mesentery, including that region in which ova are developed. The latter is mostly below the part which is characterised by great contortion of the free edge and by (?) the giving off of *acontia*.

iii. *Histology*.—The ectoderm of the *mouth-disc* (fig. 6) is characterised by deeply-staining, very numerous nuclei; and has distinctly the appearance of a secreting layer. It probably produces a similar secretion to the slime poured forth in quantities by an irritated Actinia.

This figure (which is a section along the line *a*, fig. 2) is taken from a well-grown polyp, and shows traces of the originally basal ectoderm which secretes the corallum (the *calycoblasts* of v. Heider) (*ch.*, fig. 6). In a younger and actively-growing polyp these are much more definitely marked (*ch.*, fig. 7). The nuclei lie in a gelatinous-looking matrix, which stains slightly with borax carmine, but in which no cell outlines are distinguishable. In the calycoblast layer surrounding the septum, at the same height and in the same polyp, the nuclei are much rarer (*ch.*, fig. 8).

The characters of the ectoderm alter considerably on the *tentacles*; as above mentioned, it is on them raised into a series of knobs, each of which is a "battery" of nematocysts. A transverse section through the wall of a tentacle is shown in fig. 9, and exhibits the structure of a battery; the nematocysts are confined to the peripheral part, and behind them lie a very large number of nuclei, probably instrumental, as was first suggested by v. Heider, in the formation of the cells which

replace the ejected nematocysts. On the peripheral face of the mesoderm-lamella lie longitudinal muscle fibres continuous with the transverse fibres of the mesentery; on the central face, oblique fibres.

The stomatodæal ectoderm is not essentially different from that of the mouth-disc; and though there are well-marked gonidial grooves (food grooves, Mundwinkelfurchen), they show no differentiation of ectoderm comparable to that of Alcyonarians (the "siphonoglyphe" of Hickson).

The whole of the *coelenteron* is lined by endoderm of cubical or columnar cells; generally it is only one cell deep, and in the living animal presumably ciliated throughout. At the point where it passes into the thickening known as the *mesenterial filament* (if that be indeed endodermal in origin) its characters change, and the number of nuclei increases enormously, together with the length of the cells. Its histological appearance entirely bears out what physiological investigation has also shown for the similar filament in Actiniæ, that it is secretory in character, producing a proteolytic fluid (fig. 10).

*Nematocysts* do not occur apparently in the true mesenterial filament, but only on that portion of it which is continued on to the contorted lamellæ, which I regard, in part at least, as equivalent to the acontia of Actiniæ. Those occurring on the tentacles are of a different size and shape from those which characterise the acontial filament, though in the latter both forms are found. The smaller, occurring on the tentacles, is  $\cdot 06\text{mm.} \times \cdot 01\text{ mm.}$ ; the larger, which is only to be found on the acontial filament, is  $\cdot 1\text{ mm.} \times \cdot 025\text{ mm.}$  The thread of the latter form is covered with minute barbs, which give it, when coiled up in the capsule, a granular appearance.

#### RHODOPSAMMIA PARALLELA (Semper).

This form, belonging to the family Eupsammidæ, affords a very good example of a perforate Madreporarian. Budding sparsely, it forms no cœnenchyme, so that the polyp can be studied easily and without the complications incident to cœnenchymatous species.

i. Of the *Corallum* the systematic characters have been already described by Semper (12), but certain corrections are to be made in his account relative to the arrangements of the septa. Beautiful figures of the colony will be found in his paper, which contains much valuable and curious information about the group Madreporaria.

The corallum of a polyp is about 30 cm. in height; the calyx, which



is, as usual, oval in outline, measures about 18 mm. in the longer axis, and 9-13 mm. in the shorter. Fresh polyps may be budded off from the side, or, more rarely, from the calyx.

The *theca* has the porous appearance characteristic of the *Perforata*, and is marked on the external surface by distinct spinous *costæ* or ridges; each of which corresponds externally to the attachment of a septum on the interior surface of the theca (fig. 14).

Both exosepta and entosepta occur in this form. Of true—*i.e.* entocœlic—*septa* there are only three orders, with occasional traces of a fourth; from the sides of each primary and secondary entoseptum grows out an exoseptum (fig. 14), and the relations of these two classes to each other are rather complicated. Such a system as *a-a* in fig. 22 shows, in a transverse section taken high up in the polyp, the arrangement diagrammatised in fig. 19, consisting of five true entosepta (each of which lies between a pair of mesenteries), and four exosepta alternating with them. In a lower section (fig. 20), the two exosepta which grow out from the sides of adjacent primary and secondary entosepta, fuse over and with the intermediate tertiary septum into one. Lower yet (fig. 21) the two compound septa thus produced in each system meet over and with the secondary septum, so that the *columella* is due to the irregular fusion (fig. 15) of twelve primary entosepta, distinct for their whole length, and twelve other septa thus elaborately compounded.

ii. *Anatomy*.—In *Rhodopsammia*, which, like all the other forms as yet described, bears a close resemblance to an *Actinia*, the mouth-disc, unlike the case in *Flabellum*, passes into a distinct *external body wall* of ectoderm, mesoderm, and endoderm (extending in some specimens very much further down than is represented in the diagram, fig. 13). Between this and the theca lies a narrow space in which run, parallel to the long axis of the corallum, lamellæ of tissue, connected on the one hand with this external body-wall, on the other with the tissues clothing the exterior surface of the theca (figs. 13, 14, 17, M'). These lamellæ correspond externally to the attachments of the mesenteries on the interior surface of the theca, and are apparently continuous with them over the lip of the calyx (fig. 13). They thus divide the space between body-wall and theca into a series of long chambers, corresponding to the exocœles and entocœles, in each of which lies a *costa*. Between these chambers and the exocœles and entocœles a system of ramifying canals permeates the theca, placing the two sets

of cavities in communication with one another. The columella is perforated by a similar system of canals, which unites the whole circle of entocœles and exocœles; there is thus free communication throughout the whole of the polyp, despite the comparative preponderance of skeleton over soft tissue. The canals are composed of endoderm and mesoderm, continuous with the same layers that clothe all the rest of skeleton; and in the meshes of the network lies the corallum, theca or columella.

The polyp thus consists of an external body-wall, mouth-disc with tentacles, stomatodæum, and mesenteries; with a cœlenteron divisible into columellar canal system, exocœles, entocœles, thecal canal system, and chambers exterior to the theca, corresponding to and continuous over the lip with the mesenterial chambers.

The body-wall and mouth-disc are composed of simple ectoderm, endoderm, and mesoderm, agreeing with those of other Hexactiniae.

The outline of the stomatodæum is oval, as usual; but I have not observed any trace of gonidial grooves at the ends of the longer axis.

The *tentacles*, which are simple evaginations, appear to be entocœlic only; they are so invaginated into pockets on each side of the septum that it is impossible to make out their exact size and shape. This condition is probably due merely to alcoholic contraction, and does not imply that involution is the normal method of tentacular contraction. A similar invagination had taken place at the bases of the tentacles of Flabellum. They are covered with nematocysts, which are not so sharply defined into batteries as was the case in Flabellum.

At a varying depth below the lip of the calyx (but generally at a lower point than is represented in fig. 13, which is considerably shortened in the longer axis) the external body-wall perishes, owing probably to the various parasites that infest the external surface of most coral thecæ and polyps; notably a sponge, which in some places eats its way right into the theca. The cavity marked *f* in fig. 15 is thus filled with sponge spicules. Below the point at which the body-wall ends there is visible in some places a thin line of tissue indicated in fig. 15, *g*, which may or may not be a part of the polyp. The appearance of the periphery of the theca in such a section suggests very strongly that a secondary line of corallum has been deposited round the circumference to protect the canals from communication with the sea water and against the parasites. At the top of fig. 15 the semi-circular outline of the canals seems to indicate such a formation.

The mesenteries vary in number, and are, like the entosepta, generally of three orders. They are divisible into "pairs," as in the other forms described, and possess the same arrangement of longitudinal retractor muscles on their entocœlic faces, with the usual difference in the two directive pairs. The trend of these muscles is roughly indicated in fig. 13; but their minuteness renders it impossible to recognise the arrangement of the protractor muscles, though they are just visible in microscopic sections. There appears to be but little contortion of the free edge of the mesentery, and the traces of any organs resembling acontia are rare. This, however, may be due to deficiency of material, which has much hampered my investigation of this form.

Both primary and secondary mesenteries appear to be united to the stomatodæum for its whole length; those of the third order become disconnected from it very high up, and do not run deep down into the colony, the cavities in which they lie disappearing among the other perforations of the theca.

The number of pairs of mesenteries right and left of the "directives" is not necessarily equal. Complete systems both of mesenteries and septa (1, 3, 2, 3, 1, in notation) are generally found only at the ends of the long axis of the calyx, *i.e.* in the neighbourhood of the directives. This has been noticed in many other corals.

That the almost exact correspondence of costæ with septa, and of the external lamellæ (*M'* in the figures) with the mesenteries, adds to the probability of the correctness of v. Koch's view is undeniable. But it is to be noted that no muscles are to be recognised on the mesoderm plates of these lamellæ, as would probably be the case had they once been part of the mesenteries; nor in the highest section of the decalcified polyp are any cases of decaying tissue visible where the growing theca is supposed to have cut them.

iii. *Histology*—This is of such a simple character as to hardly require comment. The ectoderm is composed of simple columnar cells, the endoderm of similar but more cubical cells. Calyco blasts are present, but in small numbers in comparison with *Flabellum*. Nematocysts are of two forms and sizes, of which, as in *Flabellum*, the smaller is the only one occurring on the tentacles. Of the mesenterial filament, as unusual in outline, a sketch is given in fig. 16.

In conclusion, I have to acknowledge my obligations to Professor Moseley for much kind assistance and most of my material; to Pro-

fessor Milnes Marshall for valuable advice; to Mr. John Murray, of the "Challenger" office, for several specimens of *Flabellum*; and lastly, to the anonymous donor of the Berkeley Fellowship, whose generosity has enabled me to pursue the investigation.

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CLASSIFICATION OF THE ZOANTHARIA (HEXACORALLA.)

1. Actiniaria (Malacodermata).
  - i. Hexactiniæ ..... *Actinia.*
  - ii. Edwardsiæ.
  - iii. Zoanthææ.
  - iv. Cerianthiæ.
2. Madreporaria (Sclerodermata).
  - A. Imperforata (Aporosa).
    - i. Turbinolidæ ..... *Flabellum.*  
*Caryophyllia.*
    - ii. Oculinidæ ..... *Stylophora.*
    - iii. Pocilloporidæ ..... *Pocillopora.*  
*Seriatopora.*
    - iv. Astræidæ ..... *Cladocora.*
  - B. Fungida.
    - i. Fungidæ ..... *Bathyactis.*
  - C. Perforata.
    - i. Eupsammidæ ..... *Stephanophyllia.*  
*Rhodopsammia.*

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#### DESCRIPTION OF PLATES.

*b.w.* Cut edge of internal body-wall. *ch.* Calycolblast layer. *Cœl.* Coelenteron. *C.* or *Col.* Columella. *Cos.* Costa. *D.* “Directive” septum and mesenteries. *Ect.* Ectoderm. *En.* Endoderm. *En.S.* Entoseptum. *Ex.S.* Exoseptum. *M.* or *Mes.* Mesentery. *M’* “Peripheral” part of mesentery. *M.D.* Mouth-disc. *Me.* Mesoderm. *Me’.* Mesenterial muscles. *M.F.* Mesenterial filament. *m.long.* Longitudinal muscles. *m.obl.* Oblique muscles. *n.* Nematocyst. *Pe.* Pedicle. *S.* Septum. *St.* Stomatodæum. *Te.* Tentacle. *Th.* Theca.

Fig. 1.—Section through two quarters of Flabellum, diagrammatic, the right half showing the primary and secondary mesenteries attached to the stomatodæum, taken along the line *b*, Fig. 2, the left being lower down in the polyp, where the mesenteries have all developed filaments, taken along the line *c*, Fig. 2. *A.* Exocœle. *B.* Entocœle. *i, ii, iii,* Orders of septa and mesenteries, wrongly numbered in the figure. The numbers should run, reckoning from the central directive septum (*D*), 1, 4, 3, 4, 2, 4, 3, 4, 1, &c. Corallum coloured deep black throughout the figures.

Fig. 2.—Diagrammatic section along the line *a*, Fig. 1, *i.e.* in an exocœle, so that the external face of the mesentery is seen flat, while the mouth-disc and internal body-wall are cut. The contortions of the free edge of the mesentery are omitted.

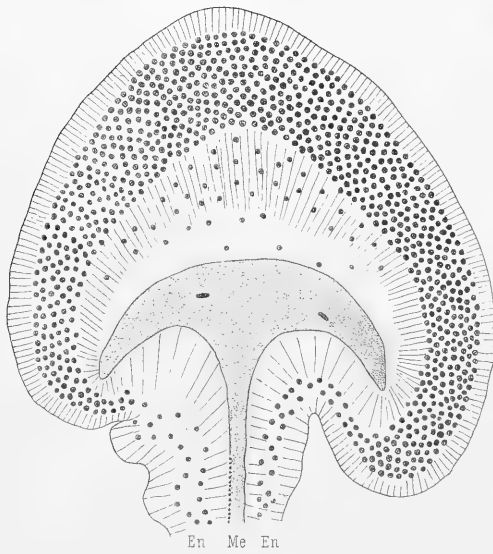
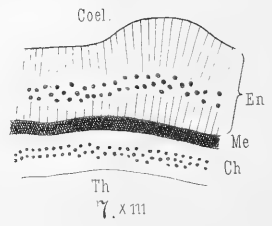
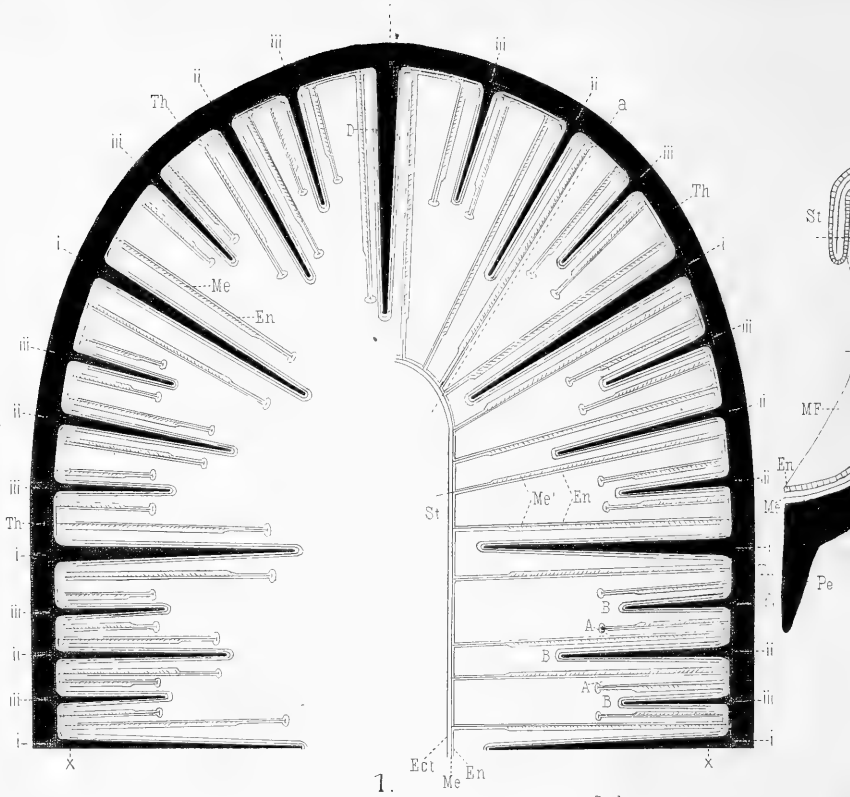
- Fig. 3.—View of half of the corallum of Flabellum, showing the relations of pedicle, theca, and septa, and the incomplete union of the septa marked *x*, in Fig. 1, into a columella.
- Fig. 4.—Portion of the lip of the calyx of Flabellum, viewed from the exterior by transmitted light, to show the grooves, *i*, *ii*, *iii*, corresponding to the septa of those orders, with the lines of growth of the theca curving upwards at those points.
- Fig. 5.—Primary mesentery and base of primary tentacles of Flabellum, showing the direction of the muscles, contortions of the free edge omitted.
- Fig. 6.—Section along the line *a*, Fig. 2, from a full-grown specimen, with the layer of calyco blasts between mesoderm and theca.
- Fig. 7.—Similar section through the internal body-wall of a younger polyp, in which the calyco blasts are much better marked.
- Fig. 8.—Section through the tissues clothing the septum of a young Flabellum.
- Fig. 9.—Section through the wall of a tentacle, including one complete "battery."
- Fig. 10.—Section through a mesenterial filament of Flabellum.
- Fig. 11.—Transverse section through part of a mesentery, to show the mesodermal pleatings on which lie the muscles.
- Fig. 12.—Transverse section of an acontium of Flabellum.
- Fig. A. Transverse diagrammatic section of Caryophyllia (after v. Koch). *a*. Septa. *b*. Mesenteries.
- Fig. B. Similar section through Caryophyllia, in a lower plane than A (after v. Koch). *a*. Septa. *b'*. Central, *b''*. peripheral parts of the mesentery. *c*. Costa. *th*. Theca.
- Fig. 13.—Diagram of a longitudinal section of Rhodopsammia, considerably shortened in the longer axis; the right half of the figure taken along the lines *c. c.* in Figs. 14 and 15, *i. e.* in an exocœle; the left along the lines *d. d.* in the same figures, and therefore cutting through a septum and a tentacle. *c*. Cut edge of external body-wall. *d*. Cut edge of tissues clothing theca and columella. *B*. Tissue clothing the entoseptum, which is seen projecting from behind the mesentery. On the left side the inner face of a mesentery (*m*) is seen similarly projecting from behind the septum. The costæ being in this form rows of spines, appear as projections in both transverse (Fig. 14) and longitudinal (Fig. 13) sections.

- Fig. 14.—Transverse section of half of the calyx of *Rhodopsammia*, along the plane *a*, Fig. 13 (camera drawing). The numerals 1, 2, 3 are placed in the entocœles formed by a pair of mesenteries of those orders. Complete systems, 1, 3, 2, 3, 1, are only found in the region of the directives. The dashed numerals, 1', 2', 3', are placed in the external chambers which correspond to the entocœles. *ext.b.v.* External body-wall. Corallum deep black, soft tissues in lighter black lines.
- Fig. 15.—Similar section along the plane *b*, Fig. 13. The septa have fused into the columella, and are numbered 1, 2, 3, according to their orders. *f.* Cavity filled with sponge spicules. *g.* Line of tissue which may belong to the polyp.
- Fig. 16.—Mesenterial filament of *Rhodopsammia* in transverse section.
- Fig. 17.—Part of Fig. 14, enlarged to show the relations of the three body-layers. Mesoderm black, corallum grey. *c.* Thecal canal system in transverse section. *c'.* External chambers, corresponding to exocœles and entocœles, in each of which lies a costa.
- Fig. 18.—Part of the thecal canal system of *Rhodopsammia*, after removal of the corallum by decalcification.
- Figs. 19, 20, and 21.—Diagrams of the relations of a complete system of septa of *Rhodopsammia* at different heights. The numerals are placed at the bases of the entosepta.
- Fig. 22.—Calyx of *Rhodopsammia*, viewed from above. From a specimen in the British Museum.
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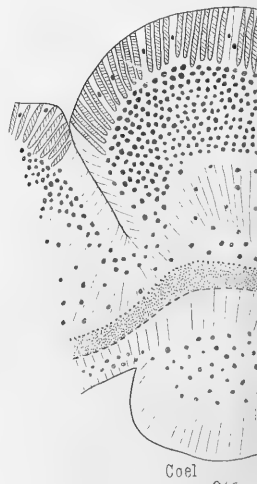




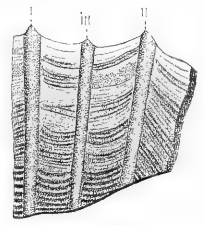
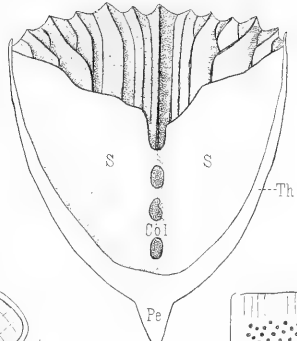
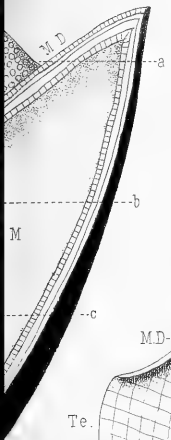




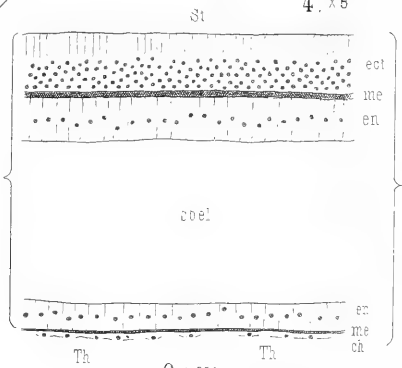
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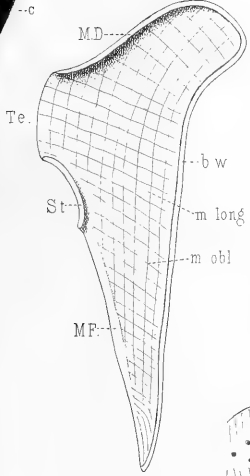
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4. x 5



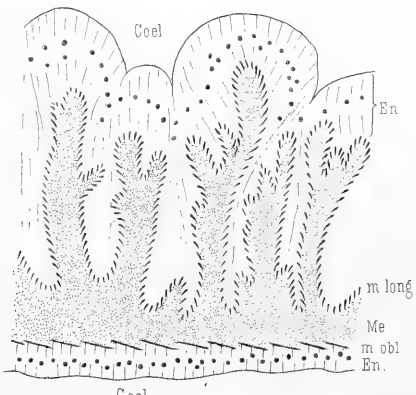
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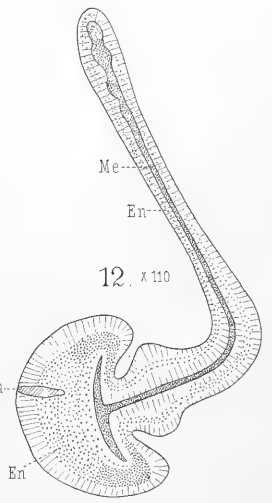
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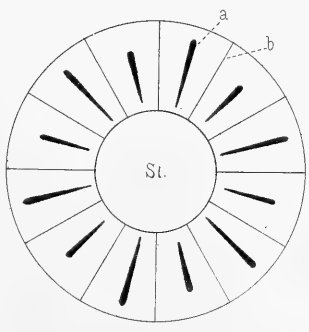
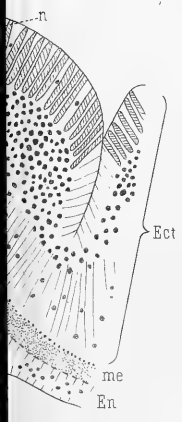
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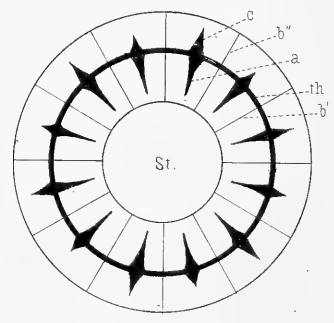
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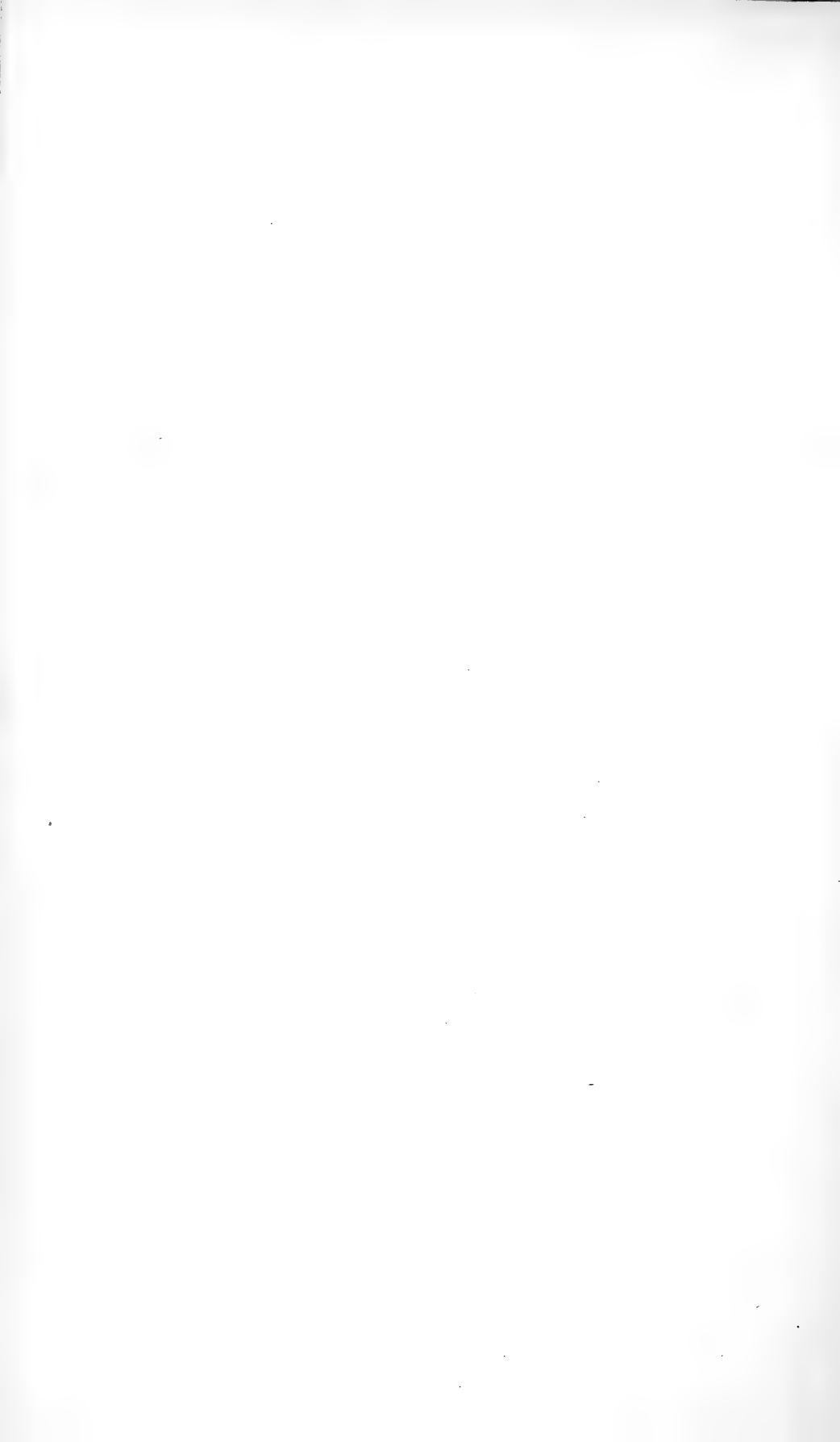
12. x 110



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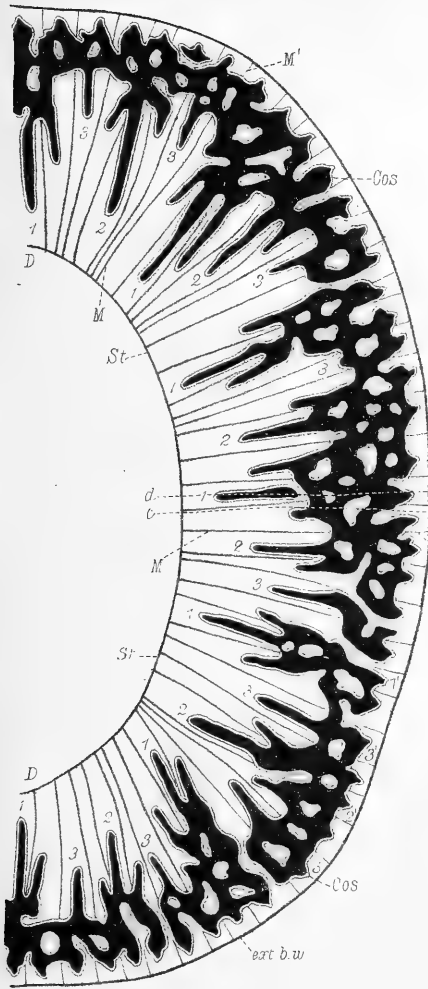


B.

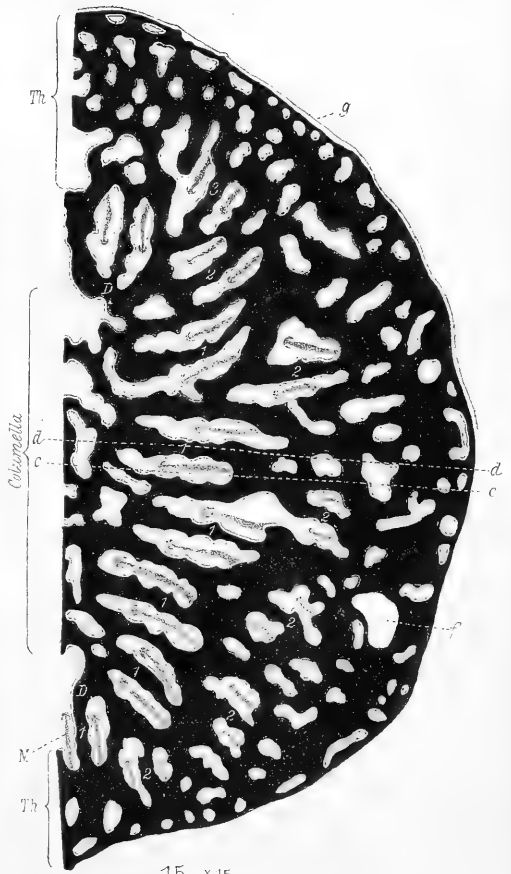




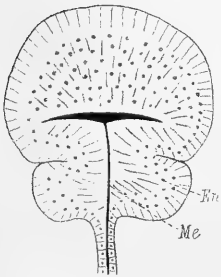




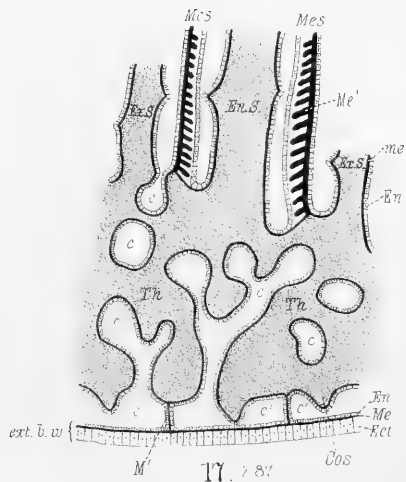
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15. x 15



16. x 550

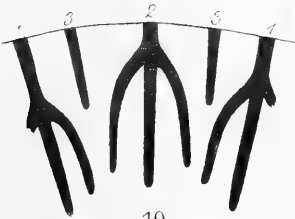




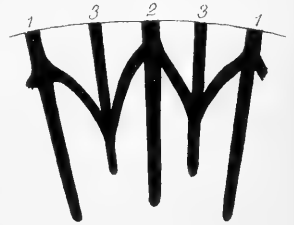




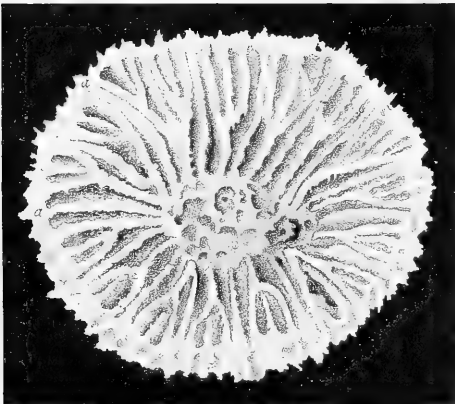
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19.



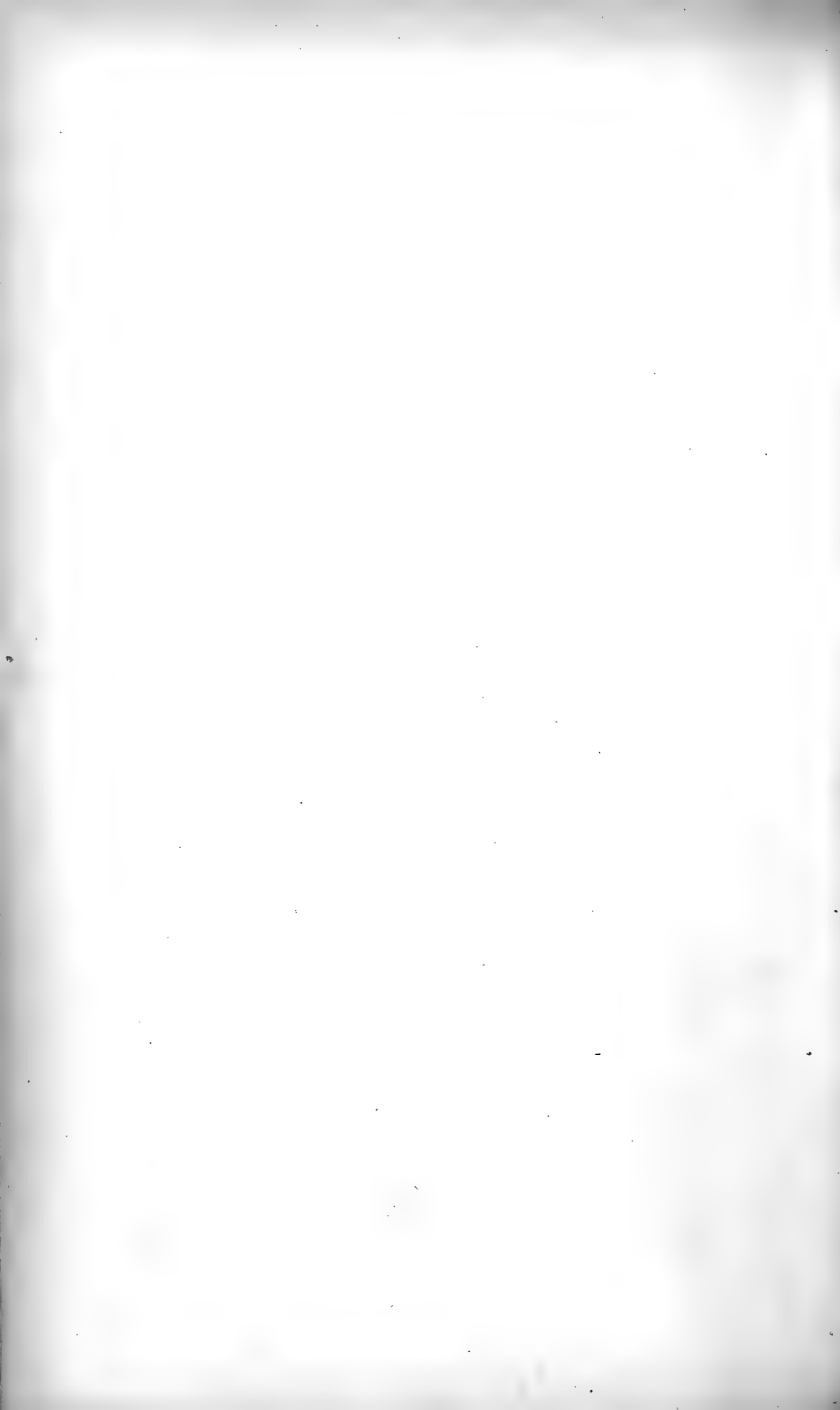
20.



22. x 11



21.



## ON THE NERVOUS SYSTEM OF *ANTEDON ROSACEUS*.

By A. MILNES MARSHALL, M.D., D.Sc., M.A., *Fellow of St. John's College, Cambridge; Beyer Professor of Zoology in Owens College.*

[PLATE XIV.]

During a recent visit to the Zoological Station at Naples, I devoted some time to an investigation of the nervous system of *Antedon*, with the object of testing by actual experiment the validity of the rival doctrines which have been advanced concerning it of late years.

I propose in the present paper to give (1) a brief sketch of the general organisation of *Antedon*, in order to define the terms employed, and to make the following descriptions more readily intelligible; (2) a short historical account of the controversy regarding the nervous system of *Antedon*, including the present position of the question; (3) an account of my own experiments and observations; and (4) a discussion of certain points of morphological interest affected by the conclusions arrived at in the preceding section.

### I. GENERAL DESCRIPTION OF *ANTEDON*.

*Antedon*<sup>1</sup> consists of a central *disc* from which radiate five pairs of long *arms*, fringed with *pinnules*.

<sup>1</sup> For fuller descriptions, *vide* Carpenter "Researches on the Structure, Physiology, and Development of *Antedon rosaceus*," part i, 'Phil. Trans.,' 1866; and Ludwig, 'Morphologische Studien an Echinodermen,' Bd. I, Abth. i; and for a very excellent summary of recent researches, *vide* P. H. Carpenter, "The Minute Anatomy of the Brachiate Echinoderms," 'Quart. Journ. Micr. Sc.,' vol. xxi.

The disc consists of a calcareous cup or *calyx* (*vide* fig. 1), and of the *visceral mass* which is lodged within the cavity of the calyx, and contains the whole of the alimentary canal and important parts of the vascular, sensory, and other systems.

The surface of the visceral mass covered by the calyx is commonly called the *dorsal* or *aboral*, the opposite one being the *ventral* or *oral* surface. In, or near, the middle of the latter is the mouth (fig. 1, *r*); this leads into a convoluted alimentary canal (*s*) ending in an anus placed at the top of a conical chimney-like projection, which arises from the oral surface of the disc not far from its edge, and interradially, *i.e.* between two pairs of arms.

To the dorsal surface of the calyx are attached from twenty to thirty jointed filaments or *cirri* (fig. 1, *p*), by which the animal attaches itself to foreign bodies. The calyx itself consists of a number of calcareous plates arranged as follows (cf. figs. 1 and 3):—In the centre is a single pentagonal *centro-dorsal plate* (C.D.), to the dorsal surface of which the cirri are attached, while the ventral surface is hollowed out in its centre to form a cup-shaped cavity closed above by a thin calcareous plate—the *Rosette* (R.); more peripherally the centrodorsal plate supports a ring of five plates called *First Radials* (R.<sub>1</sub>). To the outer surfaces of these are connected five *Second Radials* (R.<sub>2</sub>) which overlap and almost entirely conceal the First Radials from the dorsal surface (fig. 1), and beyond the second comes a set of five *Third Radials* (R.<sub>3</sub>).

Each *Third Radial* bears distally a pair of *First Brachials* (figs. 1 and 3, Br.), which are the first of a series of short calcareous joints placed end to end and extending the whole length of the arms.

The spaces between the radials and between the basal joints of the arms as far as the fourth brachials are filled up by uncalcified portions of the perisome or body wall, which thus complete the calyx.

The several joints of the arms are moveable on one another. Movement towards the oral or ventral surface, which will be called *flexion*, is effected by muscles (figs. 1 and 2, *u*) running between the successive segments; *extension* or movement towards the dorsal surface is on the other hand almost entirely due to the action of elastic ligaments placed nearer the dorsal surfaces of the segments.

The dorsal and lateral surfaces of the arms are covered by an extremely thin layer of integument, but along the ventral surface the soft parts are much thicker and exhibit considerable complexity of

structure. Running along the ventral surface of each arm is a longitudinal furrow, the *ventral* or *ambulacral groove* (fig. 2, *i*), bordered on either side by a fold of perisome, the edge of which is notched into a series of concentric leaflets, at the base of each of which is a group of three hollow *tentacles* (*k*).

The ambulacral groove is lined by a special *ambulacral epithelium* which is columnar and ciliated and much thicker than the non-ciliated epithelium covering the rest of the body. Beneath the columnar cells is a fibrillar layer (fig. 2, *h*), spoken of as the *subepithelial band*. This consists of very slender fibrils arranged for the most part longitudinally, and so appearing as fine dots in transverse sections of the arm: interspersed among the fibrils are very small nucleated cells. The subepithelial band in *Antedon rosaceus* is continuous with the ambulacral epithelium, of which it may be described as forming the deepest layer; it is traversed vertically by strands which are continuous on the one hand with certain cells of the columnar epithelium, and on the other with a connective tissue stratum underlying the band. In other species the subepithelial band appears from the descriptions of Ludwig and others to be separated from the ambulacral epithelium by a very thin connective tissue lamella.

At the bases of the arms the ambulacral grooves are continued on to the disc; those of each pair of arms unite together and so give rise to five radial grooves which run over the surface of the disc to the mouth, where they meet. Round the mouth the subepithelial bands of the five radial grooves unite to form a pentagonal ring.

The tentacles, as described above, are hollow; their cavities communicate with a longitudinal canal (fig. 2, *l*) which runs along the arm just below the subepithelial band. These *radial ambulacral canals* are continued into the disc and open into a circular canal round the mouth, (fig. 1) from which short branching canals are given off ending in open mouths communicating with the body cavity.

Besides the radial ambulacral canal, each arm contains also three diverticula of the body cavity or coelom. Of these the most ventrally situated (fig. 2, *m*) is called the *subtentacular canal* and is commonly divided, as in the figure, by a median vertical partition; the most dorsally placed canal (fig. 2, *n*) is called the *coeliac* and communicates at the end of the arm with the *subtentacular*. The third or *genital canal* is placed between the other two and lodges the cord-like genital gland; it is very small in the arm, but much larger in the pinnules.

In the centre of the visceral mass is a plexiform structure (fig. 1, *g*), the real nature of which has been much disputed, but which, according to Ludwig and P. H. Carpenter, is part of the vascular system from which branches are given to all parts of the body, and among others a radial ventral vessel down each arm in the substance of the subepithelial band. This *central plexus* passes down through the central canal formed by the First Radials, passes through a hole in the middle of the rosette, and enters the cavity in the centrodorsal plate, where it expands to form a sac divided by vertical septa into five radial compartments, and hence called the *chambered organ* (fig. 1, *f*).

The chambered organ is surrounded by a thick fibrillar investment (*d*) known as the *central capsule*, and this is in connection with a system of fibrillar bands which run down the arms in the substance of the calcareous joints, and are hence called *axial cords* (figs. 1, 2, 3, *a*).

The connection between the central capsule and the axial cords is rather complicated; but it is necessary to describe it in some detail, as it is with these parts that we shall be specially concerned later on.

The central capsule is lodged as we have seen in the hollow of the centrodorsal plate and is covered on its ventral surface by the rosette; it forms a complete investment to the chambered organ (fig. 1) excepting where it is perforated by the central plexus in the middle of the ventral surface. The dorsal and lateral walls are, as shown in the figure, thicker than the ventral.

From the dorsal surface are given off processes to the cirri (fig. 1, *e*), each of which is traversed down its centre by a vessel derived from the central plexus.

From the margin of the central capsule arise five short interradial processes (figs. 1 and 3), which, passing ventralwards and slightly outwards, bifurcate into right and left branches between the centrodorsal plate and the First Radials. These branches, diverging from one another, enter the substance of the First Radials and then unite in pairs, the right branch of one interradial stem uniting with the left branch of the one next to it, to form five stout radial nerves (fig. 3) which run outwards in the substance of the First and Second Radials. On reaching the boundary line between the Second and Third Radials each of these radial cords divides into two branches right and left, which, traversing the Third Radial, enter the right and left arms respectively of the pair, along which they pass as the axial cords

(figs. 1, 2, 3 *a*) in the substance of the brachials or calcareous segments of the arms.

Besides the connections described above there are certain others which must be noticed. A pentagonal commissure (fig. 3) connects all the branches together immediately after they have entered the First Radial. There is also a further connection in each Third Radial between the branches into which the radial cord divides to supply the two arms of the pair; this connection, as shown in fig. 3, consists of a transverse commissural band of fibres, and a chiasma formed by two obliquely placed bands which cross one another and furnish additional communications between the right and left axial cords.

In the arms the axial cords lie in tubular channels perforating the calcareous joints (figs. 1 and 2). Each cord gives off alternately right and left stout branches, which enter the pinnules (fig. 2, *c*), in which their relations are the same as in the arms themselves. Besides these, finer branches are given off, both from the axial cords themselves and from the pinnule branches, which, passing through the calcareous joints, can be traced into very intimate relation with the muscles moving the arm-joints on one another, with the tentacles and the crescentic leaflets bordering the ambulacral groove, and with the tegumentary covering of the arms generally.

Histologically, the central capsule and the various cords in connection with it consist principally of very delicate fibrils, arranged for the most part longitudinally, and having interspersed among them very small nucleated cells, both the cells and fibrils closely resembling those of the subepithelial bands of the ambulacral grooves. Other fibres, of more irregular size and distribution, which traverse both the capsule and cords in various directions, appear to be of the nature of connective tissue, and to correspond to the vertical strands in the subepithelial bands. Externally both the capsule and the cords are invested by a layer of cells, which are much larger than the small ones found in the substance of the cords, stain deeply, and give off branching processes which are in very close relation with the reticulum forming the organic basis of the skeletal parts. This external layer of cells appear to me to be a mere investment of the cords and to be no part of their real substance.

The pinnules of each arm arise alternately from the right and left sides, each of the brachials except the first bearing one pinnule. The structure of the pinnules is, with certain exceptions, the same as that

of the arms, each having an ambulacral groove, subepithelial band, tentacles, ambulacral, subtentacular, genital and coeliac canals, a branch of the axial cord, &c.; the genital rachis, however, which is only a slender cord in the arms, dilates in the pinnules to form the genital glands. The proximal or *oral pinnules*, *i.e.* those borne by the Second Brachials, differ markedly from the others; they are longer than the rest, and habitually bend inwards, so as to arch over and cover the disc; they have no tentacles<sup>1</sup> and no ambulacral grooves, the ciliated epithelium of the grooves and the subepithelial bands being both absent;<sup>2</sup> they possess, however, like the other pinnules, branches of the axial cords of the arms.

## II. HISTORICAL SKETCH.

I propose, in this section, to notice briefly the principal views that have been advanced concerning the nervous system of *Antedon*.

Müller,<sup>3</sup> in 1841, gave the first account of the genital rachis in the arms of *Antedon*, but mistook it for the nervous system, and described it as such; he also mentioned the axial cords, but described them as vessels.

In 1865 Dr. Carpenter, in his 'Memoir on the Structure, Physiology, and Development of *Antedon rosaceus*,' corrected Müller's mistake concerning the genital rachis; and, with regard to the axial cords, stated:<sup>4</sup> "It will be shown, in the second part of this memoir . . . that a system of branching fibres proceeding from the solid cord that traverses the axial canal of each calcareous segment of the rays and arms is traceable on the extremities of the muscular bundles, and reasons will be given for regarding these fibres as probably having the function of nerves, though not exhibiting their characteristic structure."

In 1872 Baudelot<sup>5</sup> called attention to the anatomical and histological resemblances between the axial cords of *Antedon* and the radial nerve-cords of other Echinoderms. He mentioned the pentagonal commissure in the calyx and the branches of the axial cords to the pinnules, and described the cords as consisting of fibrils cemented together by a finely

<sup>1</sup> Carpenter, 'Phil. Trans.,' 1836, p. 702.

<sup>2</sup> P. H. Carpenter, "Remarks on the Anatomy of the Arms of the Crinoids," part ii, 'Journal of Anatomy and Physiology,' vol. xi, p. 90.

<sup>3</sup> J. Müller, "Ueber den Bau des *Pentacrinus Caput Medusæ*." 'Physikalische Abhandlungen der Königlichen Akademie des Wissenschaften zu Berlin.'

<sup>4</sup> Carpenter, 'Phil. Trans.,' vol. 156, 1866, p. 705.

<sup>5</sup> Baudelot, "Études Générales sur le Système Nerveux," 'Archives de Zoologie Expérimentale,' tome 1, 1872, p. 211.



granular substance and with very small cells imbedded in the fibrillar tissue. He appears to have been unacquainted with Dr. Carpenter's work, and, in spite of the resemblances he points out so clearly, denies absolutely the nervous nature of the axial cords without stating definitely his reasons for so doing.

Semper, in 1874,<sup>1</sup> published an independent refutation of Müller's error as to the genital rachis, and suggests, concerning the nervous system, in ignorance of Dr. Carpenter's statement quoted above, "It might even be possible that the cord in the interior of the calcareous skeleton (*i.e.* the axial cord) is a nervous cord; and if so, then the so-called heart situated in the calyx would certainly have to be looked upon as a ganglion." Semper also suggests that a fibrous cord described by Perrier<sup>2</sup> as lying above, *i.e.* on the ventral side of the tentacular canal, may also belong to the nervous system. He confirms the existence of this cord, and refers to it as *x* in a diagrammatic transverse section of an arm.

In an addendum to the translation of Semper's paper<sup>3</sup> and in a second communication to the Royal Society on the structure, physiology, and development of *Antedon rosaceus*,<sup>4</sup> Dr. Carpenter further develops the theory that the axial cords "are really nerve-trunks, and that the five-chambered organ in the centrodorsal basin is their centre." He refers to the "quickness and consentaneousness" with which the coiling and uncoiling of the arms are effected, and to the fact that irritation of the oral pinnules causes the whole circlet of arms to close together as strong evidence of the presence of a definite nervous system, and suggests that the histological simplicity of the axial cords may "be related to the fact that as the muscles are all flexors the nerves have only one function to perform, and that there is consequently no need of the insulation which they require where nerve-fibres of very different functions are bound up in the same sheath."

He further supports his theory by the following experiment made at Oban in 1867, and which for convenience of reference I shall describe as:—

*Experiment A.*—The entire visceral mass was removed from a living specimen so as to leave nothing but the calyx with the central capsule

<sup>1</sup> Semper, "Kurze Anatomische Bemerkungen ueber Comatula," 'Arbeiten aus dem Zool.-Zoot. Institut in Würzburg,' Band i, 1874, p. 259. Translated in the 'Annals and Magazine of Natural History,' 1875, p. 202.

<sup>2</sup> Perrier, 'Archives de Zoologie Expérimentale,' tome ii, 1873, p. 55.

<sup>3</sup> Carpenter, 'Annals and Magazine of Natural History,' 1875, p. 206.

<sup>4</sup> Carpenter, 'Proceedings of the Royal Society,' 1876, p. 226.

and its prolongations and the arms. A needle was then passed down the canal surrounded by the First Radials (cf. fig. 1) so as to irritate the chambered organ. "All the ten arms then suddenly and contemporaneously closed up. On the withdrawal of the needle the arms gradually straightened themselves again, and again coiled up as before when the irritation of the central organ was renewed."

In January, 1876, Greef<sup>1</sup> called attention to the thickened epithelium forming the floor of the ambulacral grooves both of the arms and disc. He pointed out the close correspondence both in position and histological structure between this ambulacral epithelium of *Antedon*, and the radial nerves and circumoral commissure of a Starfish, and suggested that the former, like the latter, was nervous in function. At the same time he denied the nervous character of the axial cords.

In the following month Ludwig,<sup>2</sup> without being acquainted with Greef's work, described for the first time a "delicate fibrillar band" immediately beneath the ambulacral epithelium, *i.e.* what we have named above the subepithelial band (fig. 2, *h*), which he regarded on histological and morphological grounds as the true nervous system of *Antedon*, and as the representative of the radial nerves of other Echinoderms.

In April of the same year P. H. Carpenter<sup>3</sup> confirmed Ludwig's description of the subepithelial bands which he had himself independently discovered, and agreed with him in regarding them as nervous. He also showed that the cord *x*, described by Semper, was not identical as he had supposed with Perrier's fibrous cord, and that neither of these structures corresponded to the subepithelial band, Semper's cord being merely a pigmented cellular thickening between the ambulacral and subtentacular canals, while Perrier's fibrous cord is a muscular band in the ventral wall of the ambulacral canal. P. H. Carpenter, however, differed from Ludwig in regarding not only the subepithelial bands, but the axial cords also as nervous, and he was the first to distinctly maintain the existence in *Antedon* of this double nervous system, in spite of the morphological difficulties involved in this view. He brought forward as additional evidence in favour of the nervous character of the axial cords the fact that in the

<sup>1</sup> Greef, "Ueber den Bau der Crinoideen," 'Sitzungsb. d. Gesellsch. z. Beförd. der gesam. Naturwiss. zu Marburg,' No. 1, 1876, pp. 16-29.

<sup>2</sup> Ludwig, 'Nachrichten v. der Königl. Gesellschaft der Wissenschaften, und der Universität zu Göttingen,' No. 5, Feb. 23rd, 1876.

<sup>3</sup> P. H. Carpenter, "Remarks on the Anatomy of the Arms of the Crinoids," 'Journal of Anatomy and Physiology,' April, 1876.

arms of Actinometra the cord enlarges in the centre of each ossicle and gives off branches to both dorsal and ventral surfaces, some of the latter reaching "the bases, or in some cases even the tips of the respiratory leaves." He even suspected a connection between some of those branches of the axial cord and the subepithelial bands; some, he says, "appear to enter into the plexus of tissue forming the organic base of the skeleton, others seem to become connected with epidermic structures."

In a supplemental note<sup>1</sup> Dr. Carpenter also confirms the existence of the subepithelial band, and considers that it is "by no means improbable, looking alike to its position and to its histological character, that this band is a nerve." On account mainly of its position he suggests that it is "an afferent rather than a motor nerve." He also brings forward the following extremely important additional experimental evidence in support of the nervous nature of the central capsule and axial cords.

*Experiment B.*—The visceral mass was removed from a large and vigorous Antedon, leaving the calyx with the central capsule and the arms intact. On replacing the animal in the water *it executed the usual swimming movements as perfectly as the entire animal had previously done.*

*Experiment C.*—From a second active specimen the entire centro-dorsal basin with its contents and appendages were removed. On replacing the animal in the water *all the arms were rigidly straightened out, apparently by the action of the elastic ligaments which the muscles were powerless to antagonise.*

*Experiment D.*—In an active specimen the soft parts of one of the arms were divided down to the calcareous segments. On replacing the animal in water *all the arms worked as usual without the slightest disturbance of regularity.*

*Experiment E.*—By means of nitric acid applied with a fine brush, the dorsal half of one of the arms was dissolved away until the axial cord was reached and destroyed. On replacing the animal in the water *the injured arm remained rigidly stretched out, while all the other arms worked as usual.*

From these experiments Dr. Carpenter concludes that the central capsule is the co-ordinating centre of a nervous system whose peripheral portion consists of the axial cords of the rays, arms and pinnules; also

<sup>1</sup> Carpenter, 'Proceedings of the Royal Society,' vol. xxiv, 1876, p. 651.

that the subepithelial band, if a nerve at all, has no immediate relation to the swimming movements of the arms.

In 1877 Ludwig<sup>1</sup> published a more detailed account of the subepithelial band in *Antedon*, in which he describes the band and the columnar epithelium covering it as being sometimes directly continuous with one another and sometimes separated by a delicate horizontal lamella. This lamella he finds to be a more constant and evident structure in *Antedon Eschrichtii* than in *A. rosaceus*. He considers that the subepithelial band is alone to be regarded as the nerve, and points out that the close histological similarity between this band in Crinoids and the radial nerve of an Asterid, which latter, from the position of the eyes, must certainly be nervous, is a strong argument in support of his view. He also discusses the claim of the axial cords to rank as parts of the nervous system; but, while admitting the great importance of Dr. Carpenter's experiments, considers that the case is not yet satisfactorily proved, and that the morphological difficulties involved in the possession by Crinoids of a nervous system altogether unknown in other Echinoderms, are too great to permit the acceptance of Dr. Carpenter's views. According to Ludwig, the axial cords are parts of the connective tissue basis of the skeleton, which persist in an uncalcified condition, and are probably nutritive in function.

P. H. Carpenter, in a further paper on the arms of Crinoids,<sup>2</sup> and in a monograph on the genus *Actinometra*,<sup>3</sup> brings forward strong additional evidence in support of the nervous nature of the axial cords. He shows that in *Antedon rosaceus* the oral pinnules differ from the other pinnules, not only in being destitute of tentacles (as pointed out by Dr. Carpenter in 1865), but also in having no ambulacral groove, no thickened ambulacral epithelium, and *no trace of the subepithelial band, i.e.* that they are totally devoid of what Ludwig considers to be the sole nervous system of *Antedon*; and yet these oral pinnules are peculiarly irritable, a slight touch being sufficient to cause all ten arms to be suddenly coiled up over the disc.

He further finds that in *Antedon Eschrichtii* this absence of ambulacral groove and epithelium, and of the subepithelial band, occurs not only in the oral pinnules, but at the distal extremities of the

<sup>1</sup> Ludwig, 'Morphologische Studien an Echinodermen,' Heft i, Abh. i; 'Separat. Abdruck aus der Zeitschrift f. wissenschaftliche Zoologie,' Bd. 23.

<sup>2</sup> P. H. Carpenter, "Remarks on the Anatomy of the Arms of the Crinoids," part ii, 'Journal of Anatomy and Physiology,' vol. xi, October 1876.

<sup>3</sup> P. H. Carpenter, "On the Genus *Actinometra*," 'Transactions of the Linnean Society,' 2nd series Zoology, vol. ii, part i, 1879.

arms and other pinnules. The allied genus *Actinometra* is still more remarkable, for here entire arms may be completely devoid of ambulacral groove and epithelium, and of the subepithelial band, and yet such arms, though on Ludwig's theory possessing no nerves at all, are described, on Semper's authority, as exhibiting as regular and active movements while swimming as the other arms. On the other hand, the axial cords or their branches extend along all the arms and pinnules, whether possessing ambulacral grooves or not.

In all cases the absence of ambulacral grooves is associated with the absence of tentacles. Non-tentaculiferous arms are met with in a large number of species of *Actinometra*, no less than twenty-three out of the forty-eight species collected by the "Challenger"<sup>1</sup> having more or fewer of such arms, the number of which varies greatly in different individuals.

In a short paper published in 1883 Perrier<sup>2</sup> adopts very definitely the views of the Carpenters concerning the nervous system. He traces branches of the axial cords into connection, through the intermediation of stellate cells, with the muscle fibres. Other branches were traced by him into the tentacles. He gives no figures, however, and his descriptions leave some doubt as to whether the stellate cells do not rather belong to the connective tissue investment of the nerve or muscle than to the nerves themselves.

P. H. Carpenter<sup>3</sup> has recently described tripolar cells intercalated in the course of the axial cords and their branches in *Antedon*. He has also traced in three species of *Antedon* a fibrillar plexus, derived from the axial cords, into the connective tissue of the perisome forming the ventral surface of the disc, and is "strongly inclined to believe that extensions of this plexus are in direct connection with the fibrils of the subepithelial bands."

Finally, Dr. Carpenter has very recently<sup>4</sup> given a summary of the investigations concerning the nervous system of the Crinoids which have been published since his former paper in 1876. He points out that the evidence accumulated in this interval is most strongly in

<sup>1</sup> P. H. Carpenter, "Preliminary Report upon the Comatulæ of the 'Challenger' Expedition," 'Proceedings of the Royal Society,' No. 194, 1879, p. 395.

<sup>2</sup> Perrier, "Note sur l'organisation des Crinoïdes," 'Comptes rendus,' tome, xvii, 1883, pp. 187-189.

<sup>3</sup> P. H. Carpenter, "Notes on Echinoderm Morphology," No. 6, 'Quarterly Journal of Microscopical Science,' 1883.

<sup>4</sup> Carpenter, "On the Nervous System of the Crinoidea," 'Proceedings of the Royal Society,' 1884.

favour of his view, which, on the other hand, is opposed merely "by a theoretical homology, a preconceived notion of what Crinoids ought to be." He concludes with some important observations on the morphological aspects of the question, which will be noticed in a later section of this paper.

The present position of the question may be briefly described thus. The Carpenters and Perrier, on the one hand, maintain that the central capsule and axial cords, with their branches, constitute the essential and principal part of the nervous system, both motor and sensory, while the subepithelial bands, if nervous at all, are of very subordinate functional importance. On the other hand, Ludwig and the German morphologists generally maintain that the subepithelial bands constitute the sole nervous system. The former school cite in support of their views a large mass of anatomical and histological observations and certain direct experiments; while the latter school rely entirely on theoretical morphological objections to the views of their opponents.

### III. EXPERIMENTAL INVESTIGATION OF THE NERVOUS SYSTEM OF *ANTEDON ROSACEUS*.

This section of the paper, containing the account of my own investigations made at Naples last April, I propose to subdivide under the following heads:—A. The movements of uninjured specimens. B. The effects of removal of the visceral mass. C. The power of regeneration. D. The functions of the central capsule. E. The functions of the axial cords. F. The functions of the subepithelial bands.

#### A. *The Movements of Uninjured Specimens.*

The normal position of *Antedon rosaceus*, the species on which all my experiments were made, is a fixed one, the animal being attached by the dorsal cirri to some foreign body, and the arms spread out horizontally with their tips slightly flexed. The oral pinnules are bent over the disc, crossing one another above it; the other pinnules are spread out nearly at right angles to the arms.

In an aquarium containing a large number of specimens the great majority will be found attached either to the bottom or sides of the tank, *i.e.* with the oral surface directed either upwards or more or less obliquely; some specimens, however, are almost certain to be found, if there be foreign bodies in suitable positions for attachment, inverted, with the oral surface downwards.

An Antedon when once attached exhibits very little tendency to alter its position, and may remain fixed in the same place for weeks. If detached, either spontaneously or by force, it can, and usually does, swim actively until it reaches a suitable place of rest, to which it anchors itself by its cirri. The normal swimming movements, which are peculiarly graceful, consist in strong flexion of the proximal half of the arm, which is raised vertically over the disc, and then extension of the whole arm, the distal half of which is thrown out something like a whiplash or the line of a flyrod. During flexion the pinnules are folded alongside the arm; during extension spread out so as to expose as great a surface as possible. Usually two or three arms are raised simultaneously, sometimes as many as five, and the only rule I have noticed is that the two arms of each pair are always flexed alternately and not simultaneously.

When attached by its cirri, the arms of Antedon exhibit but very slight movements; they are usually spread out widely, apparently to expose as large a surface as possible for the entanglement of food particles, which, if they once come in contact with the ambulacral epithelium, get carried by the action of its cilia to the mouth.

Irritation of the ambulacral groove at any part causes the adjacent pinnules to be at once turned forwards, *i.e.* with their tips towards the free end of the arm, and folded alongside the irritated part, apparently to protect it from further injury. Slight irritation of a pinnule or of an arm causes correspondingly slight and local movements; stronger irritation causes movements of the whole arm, which may spread to other arms, or lead to the animal detaching itself and swimming freely. Irritation of the oral pinnules, however slight, causes them to be firmly closed over the disc, and stronger or prolonged irritation causes the arms to be flexed strongly, so as to cover the disc, or else the whole animal to detach itself and swim away.

If an Antedon be detached and placed with its oral surface downwards, it will right itself almost at once. If the surface on which it is placed be a rough one, the righting movement is effected in a few seconds or almost instantaneously. In a glass vessel it takes longer to perform, but with an active specimen I have never seen more than two minutes spent over the operation. In righting itself an Antedon first flexes all the arms slightly, so as to raise the disc a little above the ground; then follows a moment of apparent uncertainty as to which arm to use. One arm is then flexed more strongly than the

others, so as to slightly lift the disc on that side, the pinnules of the flexed arm being extended and apparently used to push against the ground. Then after another pause, a rather sudden and violent flexion of the arms immediately adjacent to the already flexed one causes the animal to turn on its side, when a few energetic swimming movements place it right way up. An active animal has apparently the strongest objection to being placed mouth downwards, and will right itself again and again if so inverted. When attached by the cirri, however, they may, as noticed above, remain in the inverted position for days or weeks.

If an arm be cut off from an active Antedon, the detached arm will retain its vitality for many hours. It will at first exhibit strong movements of flexion, lasting from a few minutes to as long as a couple of hours, the arm being alternately coiled up spirally, and then extended with great force and rapidity.

Antedon, if kept in captivity, requires the water to be frequently changed, or else very efficiently aerated. Specimens left over-night in a small basin of sea-water were found dead the next morning. In dead specimens, owing to the unopposed action of the elastic ligaments, the arms are very strongly extended.

#### B. *On the Effects of Removal of the Visceral Mass.*

In a living specimen the visceral mass can be removed from the calyx with great ease, as was pointed out long ago by Dr. Carpenter. If the visceral mass be grasped with forceps an exceedingly slight pull suffices to remove it. In such eviscerated specimens the central capsule with its prolongations and the axial cords remain in the calyx intact, excepting, of course, the branches of the cords described by P. H. Carpenter as distributed to the oral perisome: the ambulacral grooves and other soft parts, on the other hand, are torn across at the bases of the arms, and the subepithelial bands consequently isolated from one another.

*Experiment 1.*<sup>1</sup>—A large and vigorous specimen was eviscerated without removal from the water. On being released it remained quiescent for about a minute, and then swam about the tank actively and in a perfectly normal manner. After a short time it came to rest on the bottom in a perfectly normal position. Half an hour later,

<sup>1</sup> For convenience of reference I propose to number the various experiments consecutively. It will be understood that they were not made in the order given here, and that only those which seem of distinct importance are recorded. No experiment is described from a single observation only, and in most cases the experiments were repeated several times.



without the slightest disturbance or irritation of any kind, it began spontaneously to swim again actively and normally. Coming in contact with a piece of stick, it attached itself to it by the dorsal cirri, and remained there for more than a week.

The above experiment is the same as Dr. Carpenter's Experiment B described above. It is extremely important as proving that the co-ordinating mechanism which regulates the complex swimming movements of the arms is entirely without the visceral mass. As the direct connection between the subepithelial bands of the several arms is also destroyed, the experiment renders it extremely doubtful whether these bands have any part in regulating the swimming movements of the arms.

*Experiment 2.*—An active specimen was eviscerated, and allowed to come to rest. The ventral surface of one of the arms was then irritated gently with a needle; active movements both of the irritated arm and of the others resulted. The same effect followed irritation of one of the ordinary pinnules; while irritation of the oral pinnules caused immediate and strong flexion of all the arms.

This shows that the effect of irritation of the arms or pinnules is practically unmodified by the removal of the visceral mass; the only difference I have noted being that the response is slightly quicker and more extensive in an eviscerated than in an uninjured specimen. The nervous connection between the sensory epithelium of any one of the arms or pinnules and the muscular system, not only of that arm, but of all the others as well, must, therefore, be without the visceral mass.

As a source of irritation in this and other experiments I employed at first scratching with a sharp needle. I found afterwards that nipping with forceps was preferable, as the needle is apt to shake the whole animal, and so cause disturbance of parts other than those it is desired to irritate. The nip should be a sharp sudden one, and the irritated part released at once. In all the experiments here recorded, except when otherwise specified, both needle and forceps irritation were tried. In some instances the application of acid by a fine brush was made use of as an irritant; but this can only be done satisfactorily on specimens removed from the water.

*Experiment 3.*—An active specimen was eviscerated and allowed to come to rest in the normal position. It was then inverted and placed mouth downwards on the bottom of the tank. After a short rest it righted itself in the normal manner, but rather more slowly than usual,

the interval between inversion and completion of the righting manœuvre being about two and a half minutes. This experiment was repeated many times with different specimens. Some righted themselves instantaneously, others took a longer or shorter time, but the general average of the times taken by eviscerated specimens to right themselves was about half a minute longer than that of uninjured ones.

This affords strong additional evidence that the co-ordinating centre of the complex muscular movements of which an Antedon is capable is situated not in the visceral mass, but in the calyx.

C. *On the Power of Regeneration of Eviscerated Specimens.*

It has been stated above that an eviscerated Antedon not only attaches itself by its cirri in a perfectly normal manner, but that it may remain so attached for a week or more. On experimenting one day with a specimen that had been eviscerated about a fortnight previously, I noticed that it righted itself when inverted rather more readily than is usual in eviscerated specimens; and on examination I found that very considerable regeneration of the visceral mass had occurred. The soft tissues lining the calyx were of some thickness; a mouth was already present in the centre of the oral surface, and ambulacral grooves had formed converging from the arms to the mouth. I at once took steps to secure a complete series of specimens, showing all stages of this regeneration, and I hope to be able shortly to describe the process in detail.

That Antedon possesses this very extensive power of regeneration, greatly exceeding even that of Holothurians, was an entirely new fact to me. Dr. Carpenter tells me that he was led to suspect this long ago, and he has very kindly shown me specimens that have been in his possession for many years, which seem to me to be clearly cases in which regeneration has been partially effected. Dr. P. H. Carpenter also tells me he has known this fact for some time, though I believe no notice of it has yet been published. It is only fair to add that while at Naples the possibility that an eviscerated Antedon might regenerate its visceral mass was suggested to me in conversation by Dr. Örley, of Buda-Pesth. I made very light of the suggestion at the time, and was much astonished when a few days later I found the specimen described above.

The influence of the nervous system on the regeneration of lost parts is a point concerning which we know very little; but the apparent

ease with which this extensive regeneration is effected in Antedon would certainly be still more surprising were the main centre of the nervous system to be lodged in the part lost, and so far may be regarded as an argument against such a location.

D. *On the Functions of the Central Capsule.*

*Experiment 4.*—A specimen was eviscerated and allowed to come to rest ; a needle was then passed from the oral surface down the canal surrounded by the First Radials (fig. 1) so as to irritate the central capsule ; the result was immediate flexion of the arms, and in many cases active swimming movements of the whole animal.

*Experiment 5.*—A specimen was eviscerated and then cut into two parts, one having two pairs of arms and the other three. The central capsule, which was divided and freely exposed by the operation, was then irritated by a needle. The slightest irritation caused very active and violent flexion of the arms.

*Experiment 6.*—An active uninjured specimen was held under water, and the dorsal half of the centrodorsal plate removed by a single snip with a large pair of scissors so as to expose and partly remove the central capsule (cf. fig. 1). On being released the animal fell to the bottom with the arms very strongly extended, but in about twenty minutes gradually righted itself and assumed the normal position. The exposed central capsule was then irritated, first with a needle and then with strong nitric acid applied by a small brush ; the effect of irritation was to cause very strong and spasmodic flexion of the arms, which in the first case ceased on removal of the stimulus, but in the case of the acid persisted for several hours.

The three preceding experiments show that irritation of the central capsule, whether mechanical or chemical, causes strong flexion of all the arms, which persists as long as the stimulation is continued. Experiment 4 is the same as Dr. Carpenter's Experiment A, though the results are not quite identical ; for while Dr. Carpenter describes sudden and consentaneous flexion of the arms as following irritation of the central capsule from the oral surface, I have found that swimming movements quite as often result. The difference is a slight one, and may, I believe, be accounted for by the oral pinnules being accidentally irritated in some of the experiments. If these were clipped off I found that swimming movements of the arms almost invariably followed irritation of the central capsule from above.

The experiments prove in the most positive manner that the central capsule is in direct physiological connection with the muscles of the arms; and the further fact that the experiments yield identical results, whether performed on eviscerated or unmutilated specimens, proves that the subepithelial bands form at any rate no part of the central mechanism.

*Experiment 7.*—The centrodorsal plate of an active specimen was removed with scissors and the central capsule carefully scooped out with a small scalpel. The animal on being released fell to the bottom of the water, where it lay on its side with the arms very strongly extended; it remained in this position for several hours without any attempt to move. If taken from the water and thrown in again the arms moved fairly actively, but there was no attempt at swimming, each arm apparently acting quite independently of the rest. Finally, if placed on its oral surface it remained there for an indefinite time without making the slightest attempt to right itself.

*Experiment 8.*—The preceding experiment was repeated on an eviscerated specimen, the results being in all respects the same.

These two experiments are of very great importance. They show that removal of the central capsule completely destroys the coordinating mechanism between the arms, as tested (*a*) by the power of executing the normal swimming movements, (*b*) by the power of righting itself when inverted; both these powers being permanently destroyed by the operation. To obtain definite results it is necessary to completely remove the central capsule, and this I have found cannot be effected by simply cutting away the centrodorsal plate; besides this the capsule must be either scraped out with a fine scalpel or else destroyed by free painting with strong acid. Specimens in which the centrodorsal plate has been simply snipped off, though they lose temporarily the power both of swimming and of righting themselves, yet regain these more or less completely after an interval of half an hour to an hour. If, however, sufficient care has been taken to entirely destroy the central capsule the loss of power is absolute and permanent.

*Experiment 9.*—The centrodorsal plate of an active specimen was removed, and the central capsule entirely destroyed; the cavity was also very freely painted with nitric acid so as to expose and destroy the pentagonal commissure connecting the axial cords together at their roots (cf. fig. 3). After being left at rest for an hour the arms were irritated one by one; each arm responded readily and extensively to

the stimulation, but the movement was limited to the arm directly irritated, none of the other arms sharing in it, except sometimes the other arm of the pair to which the irritated arm belonged.

This experiment shows that the physiological connection between the arms can be destroyed by removal of the central capsule and of its branches, including the pentagonal commissure. After this operation the several arms, with the exception of the two of each pair, are physiologically isolated from one another. The experiment yields identical results whether the visceral mass be present or not.

I have found it very necessary after severe operations to allow sufficient time for recovery from shock before experimenting further, and through failure to observe this precaution I obtained at first several very contradictory and perplexing results. From half an hour to an hour I usually found to be sufficient.

#### E. *On the Functions of the Axial Cords.*

Experiments on the functions of the axial cords and their branches fall naturally under two heads, *i.e.* those concerned with the relations of these structures to sensation and to motion respectively.

I propose to commence with the former of these, though, as it sometimes happens that the same experiment is concerned with both sensation and motion, it will not be advisable to draw too sharp a line between the two divisions.

*Experiment 10.*—Various parts of the surface, both of the disc and the arms, of active uninjured specimens were irritated, both mechanically and chemically, in order to determine the normal distribution of sensation. All parts of the surface were found to be sensitive, but in very unequal degrees. Irritation of the dorsal surface of the calyx caused only slight movements of the arms, unless the irritation were severe or prolonged. Irritation of the dorsal or lateral surfaces of the arms, where the layer of integument is very thin, caused flexion of the arms, with extension of the pinnules close to the irritated spot. The response was usually ready, but the movement only slight. Prolonged or more violent irritation caused exaggeration of the movement, together with approximation of the adjacent arms towards the irritated arm, as though to remove the source of irritation, and in some cases active movement of the whole animal in a direction away from the irritated arm. Irritation of a pinnule causes, according to the degree and duration of the stimulation, movement of the pinnule,

movement of the whole arm, approximation of the adjacent arms to the affected one, or active movement of the whole animal away from the source of irritation. Irritation of the oral pinnules causes, as already noticed, immediate and very active flexion of all the arms, so as to close in over the disc.

The epithelium of the ambulacral grooves is extremely sensitive, and the results of stimulation are very definite. The slightest irritation causes instantaneous movement of the four or five pairs of pinnules immediately adjacent to the irritated spot, the pinnules being folded alongside the ambulacral groove so as to close it in and grasp the needle or other source of irritation. If the stimulation be continued the arm is actively flexed and the adjacent arms applied to it, and rubbed along the affected part, as though to remove the source of irritation. Finally, irritation of the ventral surface of the disc between the ambulacral grooves causes movements of the arms, but not nearly so active as when the oral pinnules are touched.

*Experiment 11.*—An active specimen was eviscerated, and left for half an hour. The calyx, arms, and pinnules were then successively stimulated, as in the preceding experiment. The results were exactly the same, showing that the communication between the sensitive surface of any part of the calyx, arms, or pinnules, and the motor mechanism of all the arms, is placed elsewhere than in the visceral mass.

*Experiment 12.*—An active specimen was taken, and all the soft parts scraped away with a knife from the ventral surface of one of the arms, the scraped portion being about a quarter of an inch in length and one inch from the disc. The pinnules were immediately folded closely alongside the wound, and the animal on being released swam actively in a direction away from the injured arm. It soon came to rest in the normal position, and about six minutes after the operation the distal end of the injured arm was nipped with the forceps. The distal part of the arm, beyond the injury, was at once flexed actively, the proximal part less actively, and the other arms did not move. After a twenty minutes' interval the distal end of the injured arm was again nipped, when active movement of all the arms at once resulted, the animal moving rapidly away from the source of irritation.

The above experiment shows that the communication between the sensitive surface of an arm or pinnule and the motor mechanism of all the arms is not effected by the subepithelial band. The practically

negative result obtained when the stimulation was applied very shortly after the operation is, I think, most certainly to be ascribed to the shock of the operation, which, as already noticed, must always be kept in mind as a disturbing element.

If the communication is not effected by the subepithelial band, nor by any of the soft parts of the ventral surface of the arm—all of which were scraped away in the operation—it must take place either through the integument of the dorsal and lateral surfaces of the arm, or through the calcareous segments, or through the axial cords, for these are the only parts left uninjured. To determine which of these is the real path of communication the following crucial experiment was made.

*Experiment 13.*—A large and vigorous specimen was taken, and a quarter of an inch of one of the arms, about an inch from the base, thoroughly scraped with a scalpel all round so as to remove the soft parts as completely as possible. The pinnules of the affected part and for a quarter of an inch on either side of the wound were cut away to prevent any possibility of contact communication between the parts on either side of the injury. The injured part was then painted all round very freely with strong nitric acid, the operation being repeated until fully half the thickness of the calcareous segments had been dissolved away. The wound was then washed freely with sea water and the animal returned to the tank. It fell at once to the bottom on its side with the injured arm and the other one of the pair stretched straight out horizontally, and the other arms rather strongly extended. After a few minutes it began to move slowly, and in six minutes had completely resumed the normal position. After half an hour's interval the distal end of the injured arm was sharply nipped with forceps, when strong active movements of all the arms at once resulted, the animal moving rapidly away from the source of irritation.

The above experiment, which was repeated several times, both on entire and eviscerated specimens, proves conclusively that the communication between the distal end of the irritated arm and the motor mechanism of the arms is effected by the axial cord; in other words, that the axial cord plays the part of an afferent or sensory nerve, conveying impulses centripetally. Furthermore, that it is the normal path of communication of such impulses is, I think, evident from the response to stimulation being as ready when it alone remains as in the uninjured animal. It remains, however, to show whether it is the only path of communication. To test this I attempted several times

to divide the axial cord between two of the segments by a fine scalpel, but I failed, as Dr. Carpenter had done previously, owing to the fact that as soon as the knife reached the axial cord the arm was at once thrown off, usually at a point two or three segments nearer the disc than the injury. I then tried the plan adopted by Dr. Carpenter,<sup>1</sup> *i.e.* burning away the dorsal half of the arm with nitric acid so as to expose and divide the axial cord, and with the following results.

*Experiment 14.*—An active specimen was removed from the water, the dorsal surface of one of the arms carefully dried, and strong nitric acid applied with a fine brush to the dorsal surface of the sixth and seventh radials, which were dissolved away until the axial cord was exposed and destroyed. If the arm were held during the operation it was usually thrown off, but if the disc only were held and the arm allowed merely to rest on the fingers, the operation was always successful. The animal was then returned to the water, where it assumed almost at once the normal position. After half an hour's rest, the distal end of the injured arm was nipped sharply with forceps; active movements of the irritated arm beyond the injury ensued, but no movement whatever of either the proximal part of the injured arm or of any of the other arms.

This experiment also was repeated several times on both entire and eviscerated specimens, the results being without exception as recorded above. It is difficult to limit the action of the acid to the dorsal surface of the arm, but by sufficient care it can be done, and on several occasions the ambulacral epithelium, including of course the subepithelial band, was left absolutely uninjured, responding to stimulation in a perfectly normal manner. The experiment must, I think, be considered, when taken in conjunction with Experiment 13, as proving that the axial cord is the sole afferent communication between the arm and the central motor mechanism, for the former experiment shows that the communication is still perfect when it alone remains, while the latter shows that division of the cord, other parts remaining intact, destroys the communication absolutely.

*Experiment 15.*—One further and very obvious experiment is worth recording. One of the arms of an active specimen was cut across about its middle, and the animal held in the tank so that the stump of the amputated arm was just above the surface of the water; the cut end of the axial cord could then be very readily seen with the naked eye.

<sup>1</sup> Carpenter, 'Proc. Royal Soc.,' 1876, p. 654.



The stump was carefully dried and the axial cord touched with a fine needle or with a finely-pointed brush charged with nitric acid, very violent movements of all the arms at once resulting. Similar stimulation of the ambulacral epithelium or of other parts of the section produced but very slight and local movements.

This concludes my experiments as to the afferent functions of the axial cord, excepting certain points relating to the commissural connections between these cords, which will be dealt with later on. I propose now to enquire into the motor function of the axial cords.

*Experiment 16.*—As in Experiment 12, the soft parts were scraped away from the ventral surface of about a quarter of an inch of one of the arms, an inch from its base. On being returned to the water the animal swam actively, all the arms moving vigorously and normally, including the injured one, which, however, was rather less active than the others, and a little stiff at the scraped part, probably from direct injury to the muscles.

This experiment, which was repeated on eviscerated specimens with identical results, shows that the path by which motor impulses are conveyed to the muscles of the arms is neither the subepithelial band nor any part of the soft structures on the ventral surface of the arm.

*Experiment 17.*—The operation was the same as in Experiment 14, the dorsal half of one of the arms, about an inch from the disc, being dissolved away by nitric acid until the axial cord was exposed and divided. The animal was then returned to the water, where it remained quiescent for a few seconds, and then commenced to swim actively and spontaneously, all the arms moving perfectly normally, except the injured one, the proximal end of which moved slightly, while the distal part beyond the injury was perfectly motionless and flexed spirally into a coil. After a short time the animal came to rest in a perfectly normal position, but for the spiral coiling of the distal part of the injured arm, which persisted. After a quarter of an hour's rest one of the uninjured arms was irritated, causing at once active movements of the uninjured arms and of the proximal part of the injured arm, but none whatever of its distal part.

*Experiment 18.*—In a fresh Antedon two injuries, similar to that in Experiment 17, were made in one of the arms at spots about an inch and a half apart. Stimulation of the arm itself, or of the pinnules, between the two wounds caused movements of the middle portion of the arm, but none whatever of the proximal or distal portions.

The two preceding experiments show that division of the axial cord destroys the motor communication between the parts on either side of the section as completely as we have already found it to destroy the afferent or sensory communication. When combined with Experiment 16, which shows that the motor communication is not effected by any other of the soft parts, the inference is irresistible that the sole motor communication is that afforded by the axial cords. One additional experiment may be mentioned in support of this conclusion.

*Experiment 19.*—One of the arms of a vigorous specimen was amputated by a snip of the scissors. The detached arm exhibited extremely active movements for about a quarter of an hour, coiling and uncoiling with great force and rapidity. After a time it became quiescent. It was then held in the tank with the proximal end just out of water. The end was carefully dried and the exposed section of the axial cord touched with a needle and a fine brush charged with nitric acid. The slightest irritation, whether mechanical or chemical, caused violent and repeated flexion of the arm. Stimulation applied to other parts of the cut end produced but very little effect.

It still remains to inquire into the functions of the commissural bands which connect the axial cords together, for if the axial cords are really nerves these connecting bands, which are identical with them in histological structure, must be nerves also, and experiment ought to throw light on their purpose. These commissures are of two kinds (cf. fig. 3): there is, firstly, the great pentagonal commissure in the First Radials which connect together the roots of the radial cords; and, secondly, we have in each Third Radial a rather complicated connection, by means of a transverse commissure and a chiasma, between the two axial cords into which each radial cord divides. I propose to deal with these two sets of fibres separately, taking the great pentagonal commissure first.

*Experiment 20.*—A specimen was eviscerated, and a needle passed down from the oral surface into the chambered organ, and worked about so as to destroy as completely as possible the central capsule and chambered organ (cf. fig. 1). The animal was then returned to the water, and left at rest for half an hour. One of the arms was then suddenly nipped with forceps, when all the arms exhibited active movement, though the animal did not attempt to swim.

This experiment shows that the central capsule does not form the sole physiological connection between the axial cords (nerves) of the

several arms. Figs. 1 and 3 show that the pentagonal commissure, which is lodged in the First Radials, would not be touched by the operation, and, as it furnishes an anatomical connection between the axial cords, it was naturally suspected to be the physiological connection as well. To test this the following experiment was made :

*Experiment 21.*—The same specimen employed in the preceding experiment was taken, and the inside of the canal surrounded by the First Radials freely painted with nitric acid, until the pentagonal commissure was exposed and destroyed. The animal was then returned to the water and left for half an hour on its oral face, where it remained without any attempt to right itself or to swim. The arms were then strongly nipped with forceps one by one ; each arm when irritated responded by active movements, but none of the other arms stirred except the other arm of the pair to which the irritated arm belonged, which moved sometimes slightly, sometimes actively.

This last observation shows that there is a physiological connection between the two arms of each pair still remaining after the several pairs are isolated from one another by destruction of the pentagonal commissure. There is, as we have seen, an anatomical connection in the Third Radial (fig. 3), and the following experiments were made to test whether this furnishes also the physiological connection in question.

*Experiment 22.*—A pair of arms was cut off a specimen, the section passing between the First and Second Radials. After half an hour's interval one of the arms was stimulated, when both arms moved actively.

*Experiment 23.*—Another specimen was eviscerated and a pair of arms removed, the section passing between the Second and Third Radials (cf. fig. 3). All the soft parts were scraped from the basal portions of the arms, the basal pinnules were cut off, and the Third Radial and basal joints of the arms freely scraped and painted with nitric acid, so that the sole connection between the two arms was through the substance of the Third Radial. After half an hour one of the arms was sharply nipped ; the irritated arm moved freely, and the other arm slightly but distinctly. The experiment was repeated with a second specimen, and an interval of three hours allowed between the operation and stimulation of the arm. In this case active and extensive movements of both arms followed on irritation of either one.

As the radial cord (fig. 3) divides into the two axial cords before entering the Third Radial, the sole anatomical connection between the axial cords of the two arms in the above experiment is afforded by the transverse commissure and the chiasma, one or other of which, or both, must therefore furnish the physiological connection which the experiment proves to exist. From the anatomical relations of the parts, and from the fact that the proximal ends of the chiasma must almost certainly have been injured in the operation, I think it probable that the transverse commissure is the real connecting link in this instance. As to the chiasma, the disposition of the fibres suggests that it may be connected with the alternating movements of the two arms of each pair which we have seen to occur in the act of swimming.

F. *On the Functions of the Subepithelial Bands.*

The subepithelial bands are supposed by Ludwig, as we have seen above, to constitute the sole or main nervous system of *Antedon*. The experiments detailed above demonstrate the incorrectness of this view. They show that the central connection of the subepithelial bands on the oral disc is in no way essential to, in fact, has nothing whatever to do with the complicated and co-ordinated movements of swimming, and of righting when inverted; they show, further, that division or destruction of the subepithelial band at any place does not destroy or even disturb either the sensory or motor communications between the parts on either side of the injury. In fact, they not only prove conclusively that these structures are not the sole nervous system, but even raise doubts as to whether they belong to the system at all.

I think, however, that the close histological resemblance between the subepithelial bands and the axial cords, coupled with the close correspondence as regards their relations to the ambulacral epithelium which exists between Crinoids and other Echinodermata in which, as in Asterids, they are most certainly nervous, must compel us to consider these bands in *Antedon* as nervous in nature, though what their exact function is has yet to be determined. The ambulacral epithelium is extremely and exceptionally sensitive, and irritation of it is responded to in a definite and peculiar manner, *i.e.* by the sudden folding of the pinnules alongside the irritated spot. The ambulacral grooves are structures of great importance to the animal, for it is by them that food particles are captured and swept along by the ciliary currents

to the mouth. Furthermore, the subepithelial band is in very intimate relation with that most characteristic Echinoderm system, the ambulacral vessels and their prolongations into the tentacles.

Seeing, then, that there are along the ventral surface of the arms structures of great importance in very close anatomical relation with these subepithelial bands, which agree histologically with what are undoubtedly nerves, it seems probable that these bands form a special part of the nervous system connected with one or other, or perhaps all of these special structures.

That the connection between the subepithelial bands and the ambulacral epithelium and tentacles is a very intimate one is shown by P. H. Carpenter's observation, alluded to above, that in both *Antedon* and *Actinometra* all three structures disappear together, both in the oral pinnules and in those arms or portions of arms which are devoid of ambulacral grooves.

#### G. *Summary of Results.*

1. The central capsule, and its prolongations the axial cords and their branches, constitute the main nervous system of *Antedon*.
2. The central capsule is specially connected with the complex co-ordinated movements of swimming and of righting when inverted.
3. The axial cords act as both afferent and efferent nerves.
4. The subepithelial bands are probably also nerves, but their exact function, probably a special and subordinate one in connection with the ambulacral tentacles and epithelium, is not yet ascertained.
5. Evisceration apparently causes but little inconvenience to the animal, and the visceral mass is regenerated completely in a few weeks' time.

These results are in complete accordance with the views so steadfastly advocated for many years past by the Carpenters, and recently adopted by Perrier; while, on the other hand, they are in direct opposition to the tenets of the German school.<sup>1</sup>

#### IV. *Morphological Considerations.*

Certain points of very considerable morphological interest arise in

<sup>1</sup> Since this paper was written Dr. Jickeli, of Jena, has published an account of experiments made on the nervous system of *Antedon*, which lead him to strongly uphold the correctness of Dr. Carpenter's views. Many of Jickeli's experiments are identical with ones described above, and his paper, ('*Zoologischer Anzeiger*,' 23rd June, 1884) although at present incomplete, contains much valuable information.

connection with the results detailed above, and I propose in this concluding section to notice briefly a few of the more important of these.

In the first place the morphological difficulty arising from the possession by *Antedon* of an antambulacral in addition to the typical ambulacral nervous system of Echinoderms must be considered. This objection has been strongly urged by Ludwig, and constitutes indeed the real ground of his dissent from Dr. Carpenter's views; and it must be admitted that the presence of a complicated nervous system in Crinoids, which is apparently altogether unrepresented in other Echinoderms, is a feature which a morphologist might well shrink from accepting until the fullest proof was forthcoming. This proof I have attempted to supply in the preceding section; the morphological puzzle, however, still remains to be considered.

Tiedemann<sup>1</sup> was the first to describe the ambulacral nervous system of Echinoderms, and since his time the five radial bands with their connecting circumoral commissure have been universally accepted as constituting the typical Echinoderm nervous system. This nervous system, as was pointed out by Tiedemann, is differently situated in the different groups: in Asterids it is quite superficial, while in Ophiurids, Echinids, and Holothurids it is much more deeply placed, being separated from the surface by a thick layer of cutis which in the two former groups is firmly calcified. Agassiz<sup>2</sup> urged this difference of position as an objection to the homology of the radial bands in Asterids and Echinids, but the objection was not sustained. More recent researches, while confirming the presence and the nervous nature of these radial bands and oral commissure, and adding much to our knowledge of their minute structure and relations,<sup>3</sup> have, however, tended to show that they only form a part of what is really a very widely-spread and diffuse nervous system.

Thus in Asterids it is very easy to demonstrate that the nerve-layer, which is perfectly continuous with the epidermis, of which indeed it forms the deepest stratum, is not confined to the floor of the ambulacral groove, but extends, though as a thinner layer, over the

<sup>1</sup> Tiedemann, 'Beobachtungen ueber das Nervensystem und die sensiblen Erscheinungen der Seesterne,' Meckel's 'Archiv. für Physiologie,' Bd. i, 1815; and 'Anatomie des Rohrenholothuries, des Seesterns und Steineigels,' Landshut, 1816.

<sup>2</sup> Agassiz et Desor, "Catalogue raisonné des familles des Echinodermes," 'Annales des Sciences Naturelles,' 1846.

<sup>3</sup> Ludwig's researches on the nervous system of Echinoderms, embodied in his 'Morphologische Studien an Echinodermen,' are of especial value and importance; and a recent paper by Hamann, referred to below, contains many new points of great interest.

tube feet.<sup>1</sup> The nervous layer can also be recognised with little difficulty in the epidermis of the dorsal or antambulacral surface, and Hamann<sup>2</sup> has shown that it really forms a continuous sheath over the whole dorsal surface of the animal, which, though exceedingly thin over the greater part of the back, thickens considerably at certain places, notably at the bases of the respiratory processes. Hamann describes the epidermis of Echinoderms as consisting of elements of four kinds—(1) supporting cells, columnar cells whose deeper ends are produced into fibres which pass down into the underlying dermis; (2) sensory cells, columnar and sometimes ciliated cells whose deeper ends are continuous with (3) the nerve-fibrils, delicate bands whose direction is mainly parallel to the surface of the epithelium and which are in places aggregated into bundles; and (4) ganglion cells, small nucleated cells connected with the nerve-fibrils. Of these structures the two latter form the nervous elements, which in Asterids are directly continuous with the more superficially placed columnar cells.

In Ophiurids the radial nerves, though having the same histological structure as in Asterids, are quite distinct from the epidermis, and separated from it by a thick layer of calcified dermis. Here also, however, branches from the radial nerves can be traced into the tube feet, where they form a layer immediately beneath the epidermis. Whether an epidermic nerve-sheath or plexus is present on the ambulacral surface has not, I believe, yet been demonstrated. I have been led to suspect the existence of such a sheath on physiological grounds, but have not yet seen it.

According to Baudelot,<sup>3</sup> in *Ophioderma longicauda* each of the nerves to the tube feet gives off a branch, which "se portait en haut et en arrière, et m'a paru se perdre dans la région dorsale du bras."

In Echinids Krohn<sup>4</sup> was the first to show that the radial nerves, which, like those of Ophiurids, are separated from the external epidermis by a thick layer of calcified dermis, give off branches, which accompany the tube feet through the pores in the ambulacral plates, and run in the substance of the tube feet as far as their free ends.

<sup>1</sup> I believe Greef was the first to show this in 1871.

<sup>2</sup> Hamann, "Beiträge zur Histologie der Echinodermen," "Zeitschrift für wissenschaftliche Zoologie," Bd. xxxix, 1883.

<sup>3</sup> Baudelot, "Etudes Générales sur le Système Nerveux," Archives de Zoologie Expérimentale, tome i, 1872, p. 208.

<sup>4</sup> Krohn, "Ueber die Anatomie der Nervensystem der Echiniden und Holothurien," 'Archiv für Anatomie,' 1841, translated in 'Annales des Sciences Naturelles,' tome xvi, 1841.

Lovén<sup>1</sup> describes the branches which accompany the tube feet as spreading out on the external surface of the test to form a network of fibres with numerous ganglion-cells. He figures a part of this external nerve plexus in *Brissopsis lyrifera*, and says concerning it: "On conçoit que tous les rameaux du tronc nerveux se divisant de cette manière, il y aura, répandu à la surface du corps, un système nerveux périphérique extrêmement développé, fournissant des nerfs aux radioles, aux pédicellaires, aux clavicles des fascioles, et en général à toutes les parties externes."

Fredericq<sup>2</sup> was led, from a series of experiments on *Echinus* and *Toxopneustes*, to suspect the presence "d'un plexus nerveux situé dans l'épaisseur de la peau qui recouvre le test à l'extérieur," but did not succeed in demonstrating its existence anatomically.

More recently Romanes and Ewart<sup>3</sup> have described experiments on living *Echini*, which lead them to believe in the existence not only of an external nerve plexus outside the test, but also of an internal plexus on its inner surface; they further believe that the two systems are connected by nerve-fibres running through the plates of the test. The external plexus they figure<sup>4</sup> and describe "as lying almost immediately under the surface epithelium, and extending from the shell to the spines and pedicellariæ;" and in a postscript they state that they "have been successful in obtaining full histological demonstration of the internal nervous plexus of *Echinus*," and promise full descriptions of "its character, distribution, and mode of communication with the external plexus."<sup>5</sup>

Concerning Holothurids, both Krohn and Baudelot describe, in the memoirs cited above, branches from the radial nerves to the tube feet. More recently Hamann,<sup>6</sup> in the paper already quoted, has added valuable details concerning the distribution of these branches. He shows that the branches to the tube feet, which are at first situated, like the radial nerves from which they arise, beneath the dermis, soon

<sup>1</sup> Lovén, "Etudes sur les Echinodées," 'Kongl. Svenska Vetenskaps Academiens Handlingar,' Bandet ii, No. 7, Stockholm, 1874, p. 8, and pl. ii, figs. 30 and 31.

<sup>2</sup> Fredericq, "Contributions à l'étude des Echinides," 'Archives de Zoologie Expérimentale,' tome v, 1876, p. 433.

<sup>3</sup> Romanes and Ewart, "On the Locomotor System of Echinodermata," 'Phil. Trans.,' 1881, part iii.

<sup>4</sup> Romanes and Ewart, loc. cit., p. 836, pl. 80, figs. 16-18. These figures are very different to Lovén's, which, however, were drawn from another genus.

<sup>5</sup> Loc. cit., p. 832.

<sup>6</sup> Hamann, loc. cit., p. 168, and pl. ii, figs. 51, 52, 53.



pass through this, and expand to form nerve sheaths around the tube feet and immediately beneath the external epidermis.

From the above descriptions it follows that the ordinary text-book accounts of the Echinoderm nervous system, which mention the radial nerves and the circumoral commissure, but nothing more, require very considerable modification.

We have in addition to the Crinoids four well-marked groups of recent Echinoderms, the Asterids, Ophiurids, Echinids, and Holothurids. Of these four there is, I think, no doubt that the Asterids must be regarded as the most primitive group, while the apodous Holothurids are perhaps the most modified. This primitive character of the Asterids is well illustrated by their nervous system, which as we have seen above, is in the form of a continuous nerve-sheath, enclosing the whole body, and directly continuous with the external epidermis of which it forms the deepest layer. This nerve-sheath is thickened at certain places, notably along the ambulacral grooves, where it forms the five radial or ambulacral nerves. Such a condition of the nervous system there is very strong reason for regarding as a very primitive one. It occurs in a slightly modified form in many Cœlenterates; it occurs in that primitive group of Nemertines which Hubrecht proposes to call Palæonemertini; it occurs also in the young of Sagitta and in several other cases. Even in Vertebrates the central nervous system really remains throughout life continuous with the epidermis, for the epithelium lining the central canal of the cord and the ventricle of the brain, was originally part of the surface epidermis.<sup>1</sup>

The fact that the Asterid nerve system remains in this primitive condition is of considerable importance from two points of view; in the first place it shows us the parent form from which the more modified nervous systems of other Echinoderms must have sprung, and thereby throws great light on the mutual relations of these several forms; in the second place it is of special interest in connection with the subject of the present paper, as showing that the Asterids are, in at any rate one extremely important respect, far more primitive than the Crinoids. I propose to say a few words on each of these points.

Starting with the Asterid nervous system it is easy to derive from it, theoretically, the nervous systems of other groups. The sinking down of the radial nerves in Ophiurids and Echinids may possibly be

<sup>1</sup> Attention has recently been directed to this point by Sedgwick in the 'Proceedings of the Cambridge Philosophical Society,' vol. iv, pl. vi.

connected with the development of the protective calcareous plates on the ambulacral surface, while the similar position they hold in Holothurids is probably due to the descent of this group from mailed ancestors provided with calcareous ambulacral plates, a line of descent for which there is a considerable amount of evidence forthcoming. That the radial nerves of Ophiurids, Echinids, and Holothurids are really the same things as the radial thickenings of the nerve-sheath in Asterids, in spite of their difference of position, is practically proved by the identical relations of the branches of these nerves or thickenings to the tube feet, which branches in all cases alike form sheaths immediately beneath the epidermis. The external plexus of Echinids may clearly be viewed as a somewhat modified nerve-sheath; and the internal plexus of Romanes and Ewart, which is said to be connected directly with the external plexus through the substance of the test, may be explained as due to this nerve-sheath having commenced to shift inwards, just as the radial nerves have done, but at present remaining entangled in the substance of the calcified dermis.

As regards the origin of the Crinoid nervous system, I think that the Asterid again gives us an important clue, though much yet remains to be explained. It is commonly assumed that the subepithelial bands of the Crinoid are homologous with the radial nerve-bands of an Asterid, and I think the homology must be accepted when we consider how absolutely identical the relations of these two structures are to what is perhaps the most characteristic feature in an Echinoderm, *i.e.* the ambulacral system. The histological identity is an additional argument, though of less weight, on the same side.

Accepting this homology as proved, the fact that Crinoids possess part of a nerve-sheath in a primitive and unmodified condition is, to my mind, strong reason for viewing them as descended from forms which agreed with the recent Asterids in possessing a complete nerve-sheath (though possibly very unlike Asterids in other respects); and I am, therefore, disposed to regard the antambulacral nervous system of a Crinoid, *i.e.* the central capsule and axial cords with their branches, as being derived from the antambulacral part of the primitive nerve-sheath, and not as an entirely new set of structures possessed by no other Echinoderms. A certain amount of evidence can be adduced in support of this view. Dr. Carpenter has shown<sup>1</sup> that in an early stage of development of Antedon the radials do not

<sup>1</sup> Carpenter, 'Phil. Trans.,' 1868.

enclose the radial cords, but merely form calcareous plates between the cords and the integument, which later on thicken, grow round, and enclose the cords completely.<sup>1</sup> In this early stage the relations of the radial cords are very similar to those of the ambulacral nerves of an adult Ophiurid or Echinid,<sup>2</sup> and as the latter have certainly acquired their adult condition by becoming detached from the epidermis and shifting inwards, so also may the same process be supposed to have occurred in the Crinoid. The subepithelial bands of the Crinoid retain their primitive positions, but the delicate connective-tissue lamella that sometimes separates them from the overlying epithelium in *Antedon rosaceus*, and is a far more evident structure in *Antedon Eschrichtii* and in *Actinometra*, probably represents the earliest stage in the process by which the nerve becomes detached from the epidermis and shifted inwards. Again, the external and internal plexuses of Echinus, with their connecting fibres in the substance of the calcareous test offer us a condition of things in some respects approaching that of the Crinoid.

Concerning the morphology of the central capsule, I feel in much more doubt. Dr. Carpenter's observations lead to the belief that, at any rate in its present form, it is connected with the change from the pedunculate to the free-swimming condition; and it is worthy of notice that the two actions with which it has been found to be specially concerned physiologically, *i.e.* the movements of swimming and of righting, are ones that the pedunculate form, from the very nature of things, can never exercise.

While, however, this theory of the derivation of the system of the central capsule and axial cords of a Crinoid, by concentration from the antambulacral portion of a continuous nerve-sheath, renders a comparison between the Crinoids and the Echinoderms possible, it still leaves the gap between the two groups a very wide one. Crinoids are sometimes compared with Asterids or Ophiurids, but they differ from both these groups in a great number of points of fundamental importance.

In the absence of any representatives of ambulacral ossicles, the convoluted character of the alimentary canal, the position of the anus, the permanent communication between the ambulacral system and the coelom, the replacement (functionally if not morphologically) of the

<sup>1</sup> That this condition is a primitive one is shown by its occurrence in some of the Palæocrinoids in which the axial cords often lie in grooves, and not in canals in the calcareous plates (Carpenter).

<sup>2</sup> Of course they do not correspond to these.

madreporic plate by a number of ciliated openings, we have, quite apart from the entirely exceptional features of the nervous system, a list of characters, which could be very easily added to, which mark off the Crinoids as a group widely separate from the other Echinoderms.

When we bear in mind that in a number of these points the Crinoid condition is not only not a primitive one, but a very highly specialised one, the gap becomes wider still.

I do not propose at present to pursue further this point, which has recently been noticed by both the Carpenters, and will close my paper by venturing to call attention to the great importance of supplementing morphological and histological inquiries by direct experimental investigations. In this age of specialisation there is a very real danger of men confining their attention too exclusively to one side of the problems they attack, to the entire neglect of others, which are not only of equal importance, but which would in many cases yield them far more ready clues.

Comparative physiology is a phrase which has become well-nigh extinct ; but it is the name of a very real and very necessary science, which only requires better opportunities for development, such as we hope shortly to see forthcoming in this country, in order to yield results of first-rate importance to morphologists and physiologists alike.



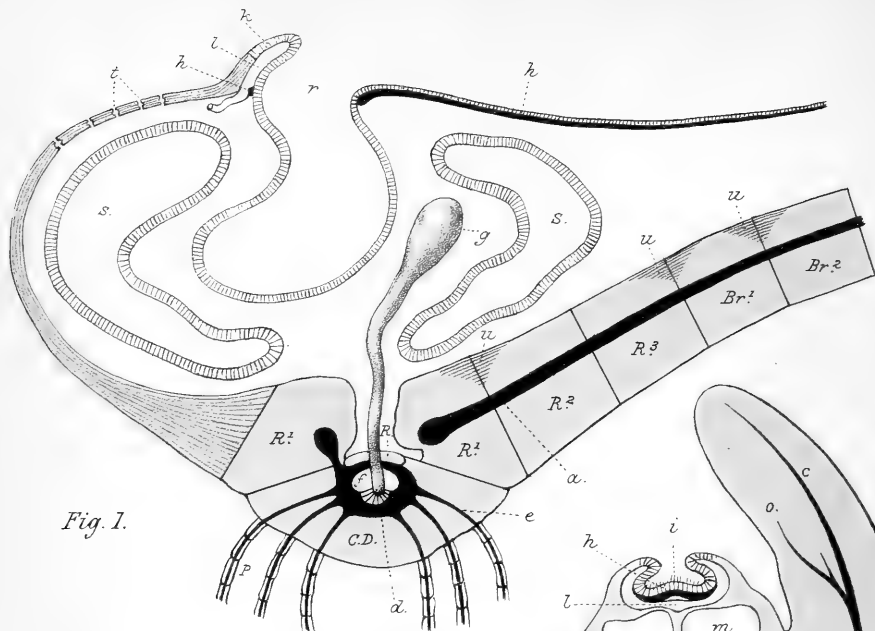
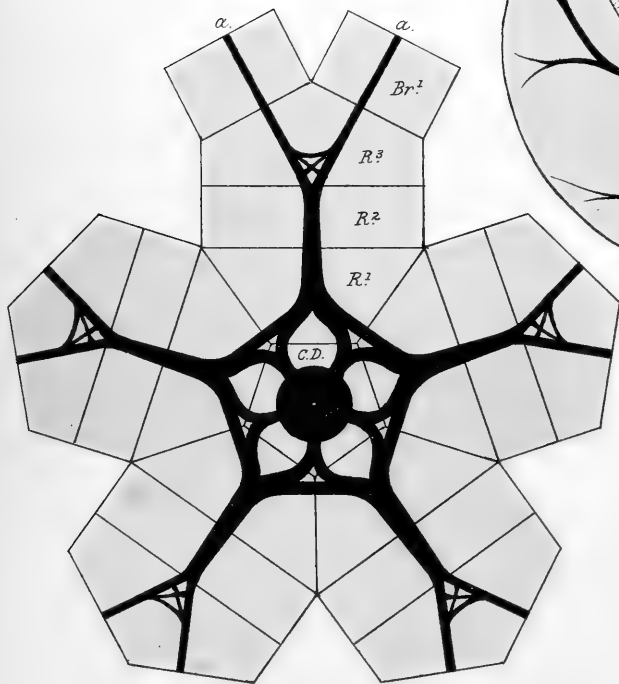


Fig. 1.



Fig. 2.

Fig. 3.



## DESCRIPTION OF PLATE XIV.

The figures are diagrammatic, and are intended merely to show the position and relations of the nervous system; the other systems being omitted, either wholly or in part. The nervous system is coloured black in all three figures. In figures 1 and 3, I have borrowed ideas from figures given by Ludwig ('Morphologische Studien'): all the figures are, however, constructed from camera drawings of my own preparations, and the two mentioned will be found to differ in some important points from the corresponding ones of Ludwig.

*Alphabetical List of References.*

*a.* Axial cord. *b.* Branches of axial cord. *c.* Branches of axial cord to pinnule. *d.* Central capsule. *e.* Branches from central capsule to cirri. *f.* Chambered organ. *g.* Central plexus. *h.* Subepithelial band (ambulacral nerve). *i.* Ambulacral groove. *k.* Tentacle. *l.* Ambulacral canal. *m.* Subtentacular canal. *n.* Coeliac canal. *o.* Pinnule. *p.* Cirri. *r.* Mouth. *s.* Intestine. *t.* Ciliated openings in body-wall. *u.* Muscle. *C.D.* Centrodorsal plate. *R.* Rosette. *R*<sub>1</sub>. First Radial. *R*<sub>2</sub>. Second Radial. *R*<sub>3</sub>. Third Radial. *Br*<sub>1</sub>. First Brachial. *Br*<sub>2</sub>. Second Brachial.

Fig. 1. Diagrammatic vertical section through the disc and base of one of the arms of *Antedon rosaceus*, showing the relations of the central capsule, axial cords, and subepithelial bands. The section is interradiar, *i.e.* passes between two pairs of arms, on the left side, radial on the right.

Fig. 2. Transverse section of an arm of *Antedon rosaceus*, passing on the right side through the base of one of the pinnules. The figure is diagrammatic as regards the branches of the axial cord, which are filled in from a considerable number of sections.

Fig. 3. Diagrammatic plan of the central capsule and its branches in *Antedon rosaceus*.





ON THE REGENERATION OF THE VISCERAL MASS  
IN *ANTEDON ROSACEUS*.

By ARTHUR DENDY, B.Sc., *Associate of the Owens College.*

It has been known for a long time that the visceral mass of certain species of *Comatulæ* is separable from the calyx with great ease. Thus Fabius Columna in 1592, speaking of the visceral mass of *Antedon*, says: "ipsum vero et facillime disjungitur a stella."<sup>1</sup> This passage is quoted by Dr. W. B. Carpenter in his memoir on *Antedon rosaceus*.<sup>2</sup> The separation of the visceral mass is easily brought about artificially, as mentioned by Professor Milnes Marshall in his paper on the nervous system of *Antedon rosaceus*;<sup>3</sup> and in some species it has been observed, on the Challenger expedition,<sup>4</sup> by Dr. Carpenter,<sup>5</sup> and by myself, that the animals are not infrequently dredged up without any visceral masses at all, these latter, in like manner, being sometimes dredged up by themselves.

Not only do these *Comatulæ* thus easily part with their visceral masses, but it has been shown that *Comatulæ* which have lost their visceral masses have the remarkable power of developing new ones (this has been proved for *Antedon rosaceus* alone). This fact, suspected by Dr. Carpenter many years ago,<sup>6</sup> was observed by Professor Marshall

<sup>1</sup> *Phytobasanus, sive Plantarum aliquot Historia*, by Fabius Columna, Neapoli, 1592.

<sup>2</sup> *Phil. Trans. Royal Soc.*, 1866.

<sup>3</sup> "On the Nervous System of *Antedon rosaceus*," *Quart. Journ. Micr. Sc.*, July 1884.

<sup>4</sup> *Challenger Report, Narrative of Cruise*, vol. I., Pt. I., pp. 310-311.

<sup>5</sup> *Proc. Royal Soc., Lond.*, vol. 24, p. 215.

<sup>6</sup> *Phil. Trans. Royal Soc.*, vol. 24, p. 215.

at Naples in 1884, and is referred to by him in the paper already cited.

Professor Marshall at once took steps to secure a complete series of specimens, showing all stages of this regeneration, and on his return placed his specimens in my hands for description. The greater part of my work in connection with the subject has been done in the laboratory of the Owens College, under the direction of Professor Marshall, to whom my best thanks are due. Unfortunately the series of specimens proved incomplete in many important respects, and in the summer of 1885, in the hopes of completing it, I went up to continue my investigations at the Scottish Marine Station for Scientific Research, at Millport on the Clyde, being enabled to do so through the kindness of Dr. Murray, Director of the Challenger Commission, to whom I am much indebted for the valuable material which I was able to collect there.

The following is a short account of the main features in the processes of evisceration and regeneration as observed in *Antedon rosaceus* (*Comatula mediterranea*), a species found very abundantly in the Firth of Clyde.

Before proceeding, it may be well to say a preliminary word or two with regard to the relations of the visceral mass to the arms and calyx in an uninjured *Antedon*, although I shall have to deal with this question more fully later on. The entire body of *Antedon* may be divided roughly into three main parts, the calyx, the arms, and the visceral mass. Of these parts the calyx appears to be the most important, for when an arm is cast off it can easily be regenerated,<sup>1</sup> and the same is now known to be the case with the visceral mass. But no cases are known of an arm or a visceral mass having been able to grow again into a perfect animal after separation from the calyx. This fact, as suggested by Professor Marshall,<sup>2</sup> is to be connected with the fact that the most important part of the nervous system is located in the calyx. The concave surface of the calyx is lined by a thin layer of connective tissue, on which the visceral mass rests and to which it is very closely attached. The visceral mass when removed from the calyx, is a roundish ball, from a quarter to half an inch in diameter and somewhat flattened on the ventral surface. This forms such a complete

<sup>1</sup> Perrier, "Sur l'Anatomie et la Régénération des bras de la *Comatula*," *Archives de Zoologie expérimentale*, Tome II., p. 68.

<sup>2</sup> Loc. cit., p. 22 (reprint).

thing in itself and looks so little like a mere part of an organism that a specimen of the isolated visceral mass of one species of *Comatula* was once described as a distinct animal. The dorsal surface of the visceral mass, that is the one resting on the calyx, is covered by a compact layer of connective tissue, which has a silky appearance on the outside. In the centre of the dorsal surface is a minute hole which serves for the passage of the central plexus of blood vessels from the chambered organ into the visceral mass. The ventral surface is covered by epidermis overlying connective tissue, and has the mouth in the centre and the anal cone at one side. This layer of epidermis and connective tissue, covering the ventral surface of the visceral mass, has been called by Carpenter the "oral perisome," and is continuous with a similar layer of tissue clothing the ventral surface of each arm.

THE PROCESS OF EVISCERATION.—In some specimens of *Antedon rosaceus* the visceral mass is very easily removed from the calyx by pushing it aside with a blunt needle ; but it is a noteworthy fact that this evisceration cannot be effected with equal ease in all cases, for, while in some specimens the visceral mass comes away almost with a touch, in others it adheres firmly to the calyx and cannot be brought away at all except by tearing it to pieces.

While at Millport, I observed that specimens were dredged up by the steam launch "Medusa," which had already lost their visceral masses. At first I was inclined to think that the animals cast them out of their own accord on being irritated, as some Holothurians cast out their intestines, but this must be regarded as very doubtful, for, owing to the slight manner in which they are generally attached to the calyx the visceral masses might have been torn out by rough treatment in the dredge. This view is perhaps supported by the fact that isolated visceral masses have themselves occasionally been dredged up.<sup>1</sup>

But although this evisceration is probably in most cases effected by the dredge, yet I have very strong reason for believing that evisceration and subsequent regeneration of the lost visceral mass occasionally occur quite independently of dredging operations. I have in my possession a specimen of *Antedon rosaceus*, from the Firth of Clyde, in which the visceral mass shows clear traces of having been regenerated ;

<sup>1</sup> *Challenger Report, Narr. of Cruise*, vol. I., Pt. I., p. 311.

this specimen was almost certainly dredged in its present condition; it was certainly not intentionally eviscerated, and it is hardly possible that it could have regenerated to such an extent in the aquarium before I came to examine it, for owing to the great heat of the weather and other causes I found it impossible to keep the *Comatulæ* alive in the aquaria for more than a very few days. The specimen referred to has arrived at the stage represented by one of the intentionally-eviscerated specimens from Naples which had been regenerating for about nineteen days. In fact, although this *Comatula* in many respects differs from a normal one in appearance, yet regeneration is fairly complete. The differences may be enumerated as follows: (1) the disc is of a very light colour, while in normal specimens it is usually considerably pigmented; (2) the disc is flat, while in normal specimens it is usually strongly convex; (3) the lappets bordering the ambulacral grooves around the mouth are very slightly developed; (4) the anal cone is very small; (5) the line of tear, caused by the removal of the old visceral mass is still marked by the cessation of the pigment spots bordering the ambulacral grooves. In all these particulars this specimen agrees with a regenerated specimen of about nineteen days and differs from a normal one.

It has been already stated that in some specimens the visceral mass may be very readily removed from the calyx, while in others it adheres to it very tightly. I happened to be at Millport at what seemed to be the height of the breeding season for *Antedon rosaceus* (July), the pinnules being commonly much distended with ova which were frequently discharged into the surrounding water in great numbers.

On examining my specimens after my return I noticed that out of all which I had preserved of the specimens dredged without their visceral masses, hardly one had the pinnules distended to any considerable extent with genital products. It appears to me also that usually those specimens which most readily part with their visceral masses are those in which the pinnules are least distended-with genital products, while, if the pinnules are much distended, the visceral mass is more firmly attached to the calyx. It is perhaps possible to trace a connection between these facts, for, when the energies of the animal are being devoted to reproduction it would obviously be much less capable of repairing the loss of such a large amount of tissue as is represented by the visceral mass, and if evisceration were to occur at this period it would be likely to result in the death of the animal from

starvation, the whole alimentary canal being, of course, removed. In order to avoid this danger we may imagine that the visceral mass at this period becomes more firmly attached to the calyx. If this view be correct, we must suppose that the loss of the visceral mass is by no means an uncommon occurrence amongst *Comatula*, and I am strongly inclined to believe that such is the case. What is the meaning of this process, and whether it plays any special part in the economy of the animal must for the present remain undecided; I have, however, thrown out a suggestion on this point at the end of the paper.

EFFECTS OF EVISCERATION.—Removal of the visceral mass appears to affect the animals but little. At first they fold their arms over the calyx, but this condition does not generally last for long, and in an hour or two, when they have once recovered from the shock, their vigour seems to be unimpaired.

It is evident that evisceration involves tearing of the epidermis and dermis, which together form the oral perisome, in a more or less regular circle all round where the arms join on to the disc, and that the ambulacral grooves and nerves, together with the ambulacral vessels, must also be torn across. The central plexus of blood vessels must also be broken across at some part of its course. The visceral mass, as it appears after its removal from the calyx, has been already described. Isolated specimens of the visceral mass of one species of *Comatula*, dredged on the Challenger expedition, were observed by Sir Wyville Thomson to perform slow creeping movements;<sup>1</sup> but I have noted nothing of the sort in *Antedon rosaceus*—on the contrary, the visceral masses appeared to remain quite still, and slowly decay.

The central capsule, together with the nerves radiating from it to the arms, is uninjured by the operation. It will be convenient for purposes of reference to speak of the shallow, empty cup, left by the removal of the visceral mass, as the "visceral basin."

The earlier stages in the process of regeneration will perhaps be rendered clearer if I first of all give a somewhat more particular account of the appearance presented by a freshly eviscerated specimen. The following description is taken from a specimen which was dredged in the eviscerated condition, but will apply equally well to a specimen upon which the operation has been intentionally and successfully performed. The line of tear, along which the oral perisome has been

<sup>1</sup> *Challenger Report, Narr. of Cruise*, vol. I., Pt. I., p. 311.

separated from the integument of the arms, is very distinctly marked. Each ambulacral groove is torn sharply and suddenly across; generally at about the level of the base of the first (oral) pinnule. Between any two adjacent arms, or pairs of arms, is a tightly stretched, translucent membrane, bounded by a free, incurved edge which marks the line of tear interradially and radially. These membranes form an important part of the floor of the visceral basin, and assist in supporting the visceral mass. It will be seen that the line of tear forms the edge of the visceral basin. This edge does not maintain an even curvature all the way round, but is deeply notched. There are ten of these notches, or bays, one between the two arms of every pair (radial), and one between each two adjacent pairs of arms (interradial), the latter being a good deal deeper than the former.

The floor of the visceral basin is lined by an exceedingly thin, transparent membrane, of connective tissue, through which a series of muscles is very distinctly visible. These are the muscles between the calcareous plates of the calyx, and they are arranged in two concentric circles. The inner of these two circles is made up of five pairs of muscles, connecting the first and second radial plates in pairs. The outer circle is composed of ten pairs of muscles, which connect in similar manner the third radials with the first brachials. The degree of distinctness with which these muscles are visible, beneath the overlying regenerating tissues, forms, during the earliest stages, a good indication of the amount of regeneration which has taken place.

On examining sections of a freshly eviscerated *Antedon*, and comparing these with sections of an uninjured specimen and of the isolated visceral mass, it is fairly easy to determine the exact region in which the separation of the visceral mass occurs. In sections of a freshly-eviscerated *Antedon* the surface of the visceral basin, including the muscles above described, is seen to be covered by a thin, smooth layer of connective tissue, excepting in the centre, within the inner circle of muscles, where this layer is incomplete. Here we have a deep pit, the sides of which are formed by the first radial plates and the floor by the perforated rosette. This pit is in the narrowest part a little over half a millimetre broad and, in the one specimen I have measured, four fifths of a millimetre deep. The central plexus sticks up into it through a hole in the middle of the rosette and is surrounded by strands of connective tissue, which, attaching it to the walls of the pit, support it. In the specimen measured the central plexus projects

above the surface of the rosette plate to a height of three-fifths of a millimetre. The strands of connective tissue which support it appear to be continuous with the organic basis of the first radials.

In sections of the isolated visceral mass we find that the aboral surface is covered by a thin layer of connective tissue, which has on the outside a very distinct outline, caused by an extremely thin, deeply staining layer which is possibly epithelial. In sections of an uninjured *Antedon* this layer is seen to rest on the layer of tissue already described as forming the floor of the visceral basin, and is connected with it by very delicate bands of connective tissue which run across from the one to the other.

Thus, in the process of evisceration the separation of the visceral mass takes place between two layers of connective tissue—the one lining the surface of the visceral basin and the other covering the aboral surface of the visceral mass.

THE REGENERATION OF THE LOST VISCERAL MASS.—Unfortunately the series of specimens at my disposal is still very incomplete and it is as yet impossible to give a complete account of this process. For the sake of convenience I shall divide the description into several parts, according to the length of time for which the specimens treated of have been regenerating; but it must not be thought that all specimens which have been regenerating for the same length of time have arrived at the same stage in the process; this is by no means the case, and I find that different specimens of the same date present considerable variations. The regenerated specimens of forty-three hours are from the Firth of Clyde, those of later dates from Naples.

*Forty-three hours.*—The connective tissue lining the floor of the visceral basin has begun to thicken. As a result of this the two circlets of muscles already described are much less distinct than in a freshly-eviscerated specimen, although still plainly visible. The thickening is more marked around the edges of the injured area than in the centre, so that the inner circle of muscles is much more distinctly visible than the outer one; in one specimen of this date the outer circle of muscles is nearly hidden by the ingrowing mass of connective tissue. The line of tear, along which the visceral mass has been separated from the calyx, no longer appears sharp and distinct.

*Two days.*—The new growth of connective tissue, which had commenced at forty-three hours, has proceeded further. The circlets of

muscles on the floor of the visceral basin are both completely hidden by the newly formed tissues. The line of tear is marked by the sudden stopping of the lappets and pigment spots along the borders of the ambulacral grooves.

In sections we see that the surface of the regenerating cushion of tissue is covered by a thin, deeply staining layer, which is apparently formed by the arching over, and ingrowth of the edges of the injured area. This layer is formed of two parts, an upper layer, which is probably epidermal, and a lower dermal layer. The deeper, connective tissue layer is continuous with strands of the same substance which have grown out from the floor of the visceral basin. In the centre, above the chambered organ, the superficial layer of this regenerating visceral mass is still incomplete; so that on looking at it from above two or three little holes are visible in the centre, leading down to the chambered organ. The regenerated cushion of tissue is still very thin, averaging in thickness only about half a millimetre.

Regeneration, then, appears to commence in two chief ways; (1) by a series of outgrowths from the thin layer of connective tissue which forms the floor of the visceral basin; (2) by an ingrowth of connective tissue and epidermis from the edges of the injured area, forming a roof to the visceral basin.

*Three days.*—Of this date I have two specimens, of both of which I have sections, but the one is so much further advanced in regeneration than the other that I shall treat them as two separate stages, taking the less advanced one first.

(a) The new visceral mass is seen to have grown and has thickened considerably. The openings leading down to the chambered organ are now closed over. In the centre of the regenerating visceral cushion is a small opaque papilla, probably due to thickening of the epidermis preparatory to the formation of a mouth, but unfortunately the sections are not sufficiently well preserved to decide this point. Sections show that regeneration has proceeded as far as the formation of a cushion of loose connective tissue, much thicker than at two days and covered by the deeply staining epidermic layer before mentioned. The growth of connective tissue is especially strong underneath the point where the mouth is subsequently to be formed; so that we have here a pillar of denser connective tissue, reaching from the top of the rosette plate to the roof of the visceral basin and thickest just beneath the epidermic layer. The central plexus is very well shown, but in



these sections, and also in those of later date, I have no means of telling how much of it, if any, has regenerated and how much was left after evisceration.

The ambulacral system has begun to regenerate, as is shown by the presence of new ambulacral pores. On examining one of these more closely, it is seen to be vesicular, lined by columnar epithelium and opening to the outside. No internal opening is yet visible.

(b) This specimen<sup>1</sup> is much more advanced than the preceding one, in that mouth and alimentary canal are already present; in other respects it agrees with it, so that it will only be necessary here to speak of the alimentary canal. In the centre of the visceral mass is a depression; the epidermis covering the depressed area is thickened and in the centre is a small mouth leading obliquely downwards into a feebly developed alimentary canal. The alimentary canal has already the characteristic form found in normal specimens; that is to say it takes about one turn in an almost horizontal plane and ends in an anus placed interradially. The most remarkable feature about it is the rudimentary condition of its walls. In histological characters the walls of the alimentary canal are almost indistinguishable from the roof of the visceral basin, and to all appearance have been formed by an invagination of this roof. The roof of the visceral basin is composed of two layers; externally a thin layer of minute, rounded, deeply staining cells which are probably epidermic, and beneath this a thicker layer of connective tissue which stains less deeply. In the floor of the central depression already mentioned, the epidermic cells show a slight tendency to become columnar. The walls of the alimentary canal are composed of exactly the same two layers, a layer of more deeply staining cells which corresponds to the epidermic layer of the roof, but is now, of course, on the inside, and outside this a layer of connective tissue. The cells of the inner layer show a tendency to become columnar, and this layer is more distinct than the epidermic layer of the roof. The lumen of the alimentary canal is very small and its walls are very thin and but slightly folded. There is no trace of an anal cone; the anus itself is represented by a minute perforation in the roof of the visceral basin. The walls of the last part of the

<sup>1</sup> The great amount of regeneration which has taken place in this specimen compared with the other of the same date suggests the possibility that some mistake may have been made, either by myself or at Naples, with regard to the time of regeneration. There seems, however, to be no doubt that considerable variation in this respect does occur.

intestine, just before reaching the anus, are quite indistinguishable from this roof.

I have thus no decisive evidence to bring forward as to the manner in which the alimentary canal is formed; but there appears to me to be a strong probability in favour of the view that it is formed by invagination, the inner, glandular layer of its walls being formed from invaginated epidermis and the outer connective tissue layer from the underlying dermis.

*Five days.*—This specimen agrees closely with the last. The alimentary canal appears to be in nearly the same condition. It is very small and almost solid, the inner layer of its walls being composed of minute, deeply staining, nucleated cells, more or less rounded in shape and showing a slight tendency to become columnar in places, and being histologically indistinguishable from the tissue covering the general surface of the disc. Here again the alimentary canal appears to have been formed by invagination, accompanied by rapid proliferation of the cells of the epidermis. In the specimen described this proliferation has formed a thick mass of minute cells projecting on the surface at one side of the mouth. No anal cone is as yet visible. The terminal portion of the intestine touches the roof of the visceral basin, but I found no definite opening through; but it must be remembered that the anus, on its first appearance, is so minute a structure that it might easily escape observation, especially in imperfectly preserved sections.

No ambulacral nerves or canals are as yet visible round the mouth. A number of new ambulacral pores have been formed, apparently by invagination. The epithelium lining them is at first not distinctly columnar.

*Nine days.*—Externally the boundaries of the ambulacral grooves are seen to have met and formed a pentagon around the mouth. They enclose a slightly depressed area with the mouth in the centre, and appear as thickish white ridges, in some places notched, showing where lappets are beginning to regenerate.

It appears to me that the ambulacral grooves are, from the first commencement of regeneration, left as areas along which the thickening of the regenerating tissue is not so great as elsewhere. This thickening takes place centripetally, in five distinct areas, one in each interradius; as these grow inwards the ambulacral grooves and the central depression around the mouth are left as less thickened portions.

The mouth is oval, runs obliquely downwards, and is now for the first time very distinctly bounded. The beginning of the anal cone is visible as a small conical papilla placed interradially.

In sections the alimentary canal is seen to be fairly well developed; it contains food refuse, indicating that it has begun to be used again. The epidermis covering the depressed area round the mouth is now composed of columnar, nucleated cells, and passes gradually into the inner lining of the œsophagus, the cells of which are still more columnar. The walls of the stomach are also composed of the usual two layers, an inner layer of columnar, nucleated cells, and an outer layer of connective tissue; this outer layer is connected with the loose connective tissue filling the body cavity. In the specimen now described the cells forming the inner wall of the alimentary canal have become columnar right up to the anus. The anus is very minute and situated on the top of a small papilla. The columnar cells lining the cavity of the anus are very small and pass gradually into the epidermic cells covering the rudimentary anal cone; the epidermic layer at this point is thickened. In the region of the stomach the walls of the alimentary canal are beginning to become folded, especially on the axial side, and on this side also the glandular layer is thicker.

In another specimen of this date, which appears not to have advanced quite so far in regeneration, the alimentary canal, as it approaches the anus, narrows very much; its inner wall, which in the first part of its course is composed of the usual columnar cells, here consists of a layer of very minute cells, such as line the whole of the canal at an earlier date, and identical with the cells covering the general surface of the disc. In thickness this inner layer is very irregular and the cells composing it appear to be rapidly proliferating. At its extreme end they block up the lumen of the canal, so that the latter appears solid, and the end of the canal fuses indistinguishably with the layer of similar cells covering the general surface of the disc. At the point of fusion there is a thick mass of these minute round cells, forming a little elevation on the surface of the disc. This indicates the position of the future anal cone. In this specimen I have been able to find no distinct anal opening. These facts appear to me to support the view that the alimentary canal is formed by invagination from its oral end.

*Twelve days.*—No great advance has been made on the condition presented by a nine days specimen. In one specimen the pentagon

round the mouth, formed by the lips of the ambulacral grooves, has become notched into lappets all the way round and in sections the alimentary canal is seen to have become very considerably complicated and folded upon itself. In sections of another specimen of this date the ambulacral epithelium and canals can be traced across the disc very nearly to the lip of the mouth. They present the same features as in normal specimens; thus the canals exhibit the transverse muscle fibres found in ordinary specimens. The anal cone is still very small.

*Nineteen days.*—Little advance is to be noted except in the further growth of the anal cone, which is now fairly well developed. Sections show a large body cavity in the anal cone, surrounding the terminal portion of the intestine, which latter is attached to the body wall by strands of connective tissue.

*Twenty-one days.*—In sections of a specimen of this date a blind diverticulum is visible, given off from the alimentary canal at the junction of the stomach with the œsophagus, as described by Ludwig in the normal animal.<sup>1</sup> There is little to distinguish a regenerated specimen of this date from a normal *Antedon*, excepting the smaller size of the visceral mass and the want of pigment upon it.

COMPARISON WITH OTHER ECHINODERMS.—So little has as yet been written on the regeneration of lost parts in the *Echinodermata* that it is difficult to make any comparison between *Antedon* and other forms in this respect. The most important paper as yet published on the subject appears to me to be that by Professor Ernst Haeckel on Comet Forms.<sup>2</sup>

It is a noteworthy fact that comet forms are, so far as I know, only described as occurring in Asteroids (*e.g.* *Linckia*, *Brisinga*, *Asteracanthion*), none being described in Ophiuroids. Schizogony, on the other hand, is known to occur in both groups (*e.g.* *Asteracanthion* and *Ophiactis*).

In the formation of a comet form a single arm of a star-fish, after separating itself from the disc, produces, by budding at the proximal end, from the wounded surface, new arms; then a new disc appears and thus the perfect condition is again attained. The mouth is at

<sup>1</sup> Ludwig, *Morphol. Stud. an Echinoderm.*

<sup>2</sup> "Die Kometenform der Seesterne und der Generationswechsel der Echinodermen," *Zeit. für wiss. Zool.*, Bd. 30, Suppl. 1878.

first formed simply from the open end of the gut diverticulum which extends into the arm.

The non-occurrence of this phenomenon in Ophiuroids appears to me to be due to the absence of any gut diverticula in the arms of the latter, so that a separated arm is entirely without any portion of the alimentary canal, by the growth of which the remainder can be regenerated. Schizogony, however, occurs in both Asteroids and Ophiuroids, probably because, in this case, the disc itself divides into two parts and hence each of the resulting halves will contain a portion of the alimentary canal, whether or not the latter be produced into the arms.

It thus appears that regeneration, so far as it is known, in Asteroids and Ophiuroids is very different from that which takes place in *Antedon*, for, in the two former groups regeneration never appears to occur when the alimentary canal has been entirely removed.

Unfortunately Professor Haeckel has only been able to examine the external characters of the comet forms described by him; further research will probably throw much light on the subject, and, if comet forms should ever be found to occur in Ophiuroids, it may be possible to institute a closer comparison in respect of regeneration between *Antedon* and Ophiuroids than between *Antedon* and Asteroids.

On the regeneration of lost individual arms in Asteroids and Ophiuroids it is needless to dwell; this phenomenon finds a parallel in the regeneration of the arms of *Antedon*.

As yet little is known concerning the ejection and regeneration of the viscera, including the alimentary canal, in Holothurians, beyond the mere fact that it does occur, and this was shown to be the case by Dalyell.<sup>1</sup> It is possible that we have here a more exact parallel to what takes place in *Antedon* than in Asteroids or Ophiuroids.

I have stated above that the meaning of the process in *Antedon*, supposing it to be a normal occurrence, must for the present remain undecided; suffice it here to offer, as a suggestion, the only explanation which seems at all probable. Crinoids, unlike most Echinoderms, have no selective power over their food supply; all sorts of food, good or bad, wholesome or poisonous, are carried into the alimentary canal by the action of the cilia in the ambulacral grooves. If any irritating or poisonous particles, or even any dangerous parasite, were conveyed by

<sup>1</sup> *The Powers of the Creator, displayed in the Creation*, London, 1851, vol. i., p. 49 et seq. It was only after considerable difficulty that I obtained this reference, for which I am indebted to Professor F. J. Bell, who also pointed out to me Semper's remarks on the subject; *Reisen in Archipel der Philippinen*, vol. i., Holothurien, p. 200 et seq.

this means into the alimentary canal, as might easily be the case, the only way in which the animal could rid itself of the obnoxious matter would be to cast out the alimentary canal, and this, owing to the structure and relations of the parts concerned, which have probably been specially adapted for the purpose, is most simply and readily effected by the rejection of the entire visceral mass. This appears to me to be a not improbable hypothesis, and the only one that will explain the facts.

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SOME INVESTIGATIONS ON THE PHYSIOLOGY OF THE  
NERVOUS SYSTEM OF THE LOBSTER.

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Owens College.*

The following investigations were commenced in the Physiological Laboratory of the Owens College during the past winter, under the direction of Professor Gamgee and Professor Milnes Marshall, with a view to determine the following points in the Physiology of the nervous system of the Lobster :—

1. Are there distinct motor and sensory roots to the nerves arising from the central nervous system, similar to those by which the spinal nerves arise in vertebrates ?
2. Is there any marked decussation of the nerve fibres in the central nervous system ?

The determination of these points in any invertebrate animal is a matter of considerable morphological importance.

No definite results were obtained from the Lobsters experimented upon at the Owens College, for the reason that they were not in a fit state for experiment by the time they reached the laboratory. However, through the kindness of Mr. J. T. Cunningham, I was enabled during last August to continue my researches at the Scottish Marine Station at Granton, a spot which is eminently suited for carrying on experiments of this kind. There I was able to obtain perfectly fresh lobsters which were kept in tanks through which sea water was constantly circulating. I must here express my thanks to

Mr. Cunningham for much advice and help in conducting the experiments, nearly all the more important of which were witnessed by him.

As this paper does not profess to be a complete account of the physiology of the nervous system of the lobster, I shall not attempt to give an exhaustive account of the literature of the subject, but only refer to such papers as bear directly on the questions stated above. The most important paper dealing with these questions is one by Emile Yung, entitled "Physiologie de la chaîne ganglionnaire chez les crustacés."<sup>1</sup> This paper I was unfortunately unable to obtain access to till after my experiments had been completed. Although many of my results were anticipated by Yung, yet, since my methods of investigation differ for the most part from those employed by him, I think it worth while publishing them. Confirmatory results arrived at independently are always of value in experimental physiology.

Two other papers, one by Richet, "Contribution à la physiologie des centres nerveux et des muscles de l'écrévisse,"<sup>2</sup> and another by Fredericq et Vandeveldé, "Physiologie des muscles et des nerfs du homard,"<sup>3</sup> deal only with the phenomena of muscle and nerve and the various conditions of muscular excitability, and do not bear on the subject of this paper. Mr. J. Ward has written a short paper "Some Notes on the Physiology of the Nervous System of *Astacus Fluvialtilis*."<sup>4</sup> His results were obtained by cutting the nerves and replacing the animals in running water and observing the subsequent movements. Mr. Ward came to the conclusion that there was no evidence of decussation of nerves in the central nervous system of the Crayfish. The most recent contribution is a short note by Reichenbach, "Beobachtungen über die Physiologie des Nervensystems vom Flusskrebs."<sup>5</sup> This I was unfortunately unable to obtain.

I shall now give a detailed account of my investigations dealing first with the anatomy and then with the physiology, and compare my results with those obtained by Yung.

#### A. ANATOMY.

1. *Thoracic Nerves*.—Arising from each of the thoracic ganglia, with the exception of the supra- and sub-oesophageal ganglia, there are two

<sup>1</sup> *Archives de Zoologie*, t. VII, 1878.

<sup>2</sup> *Archives de Physiologie normale et pathologique*, t. VI, 1879.

<sup>3</sup> *Bulletin de l'Académie royale de Belgique*, 2<sup>e</sup> série, t. 47, 1879.

<sup>4</sup> *Journal of Physiology*, vol. II., 1879-80.

<sup>5</sup> In *Humboldt*, Bd. I., p. 26-27, 1883.



nerves on each side of considerable size which pass to the limb of the corresponding side. Besides these there are other smaller nerves distributed to the wall of the thorax, but these do not concern the present question. Of these two nerves the one is anterior and the other posterior with regard to the normal position of the animal: but both nerves arise in the same horizontal plane, *i.e.* one is not dorsal to the other. The anterior nerve is much the smaller in the great chelae and first and second ambulatory legs, where the posterior nerve is of great thickness. In the last two ambulatory legs the two nerves are nearly the same size. Throughout this paper the anterior will be spoken of as the "small nerve" and the posterior as the "large nerve."

On tracing these two nerves to their distribution in the large chelae in specimens hardened in alcohol, the small nerve seemed to consist of two parts, one of which could be traced to the divaricator muscle (adductor of Huxley<sup>1</sup>) which opens the claw. The other part of the nerve appeared to go to the skin. But no such division into two parts was found in the fresh specimen. The small nerve also supplies the extensor muscles of the various joints of the limb.

The large nerve gives off branches to the flexor muscles of the limb, but is chiefly distributed to the large ocluser muscle (adductor of Huxley<sup>1</sup>) which closes the claw. Also there are numerous branches to the skin of the claw.

In the case of the first or sub-oesophageal ganglion the nerves are so crowded together that it is difficult to determine whether the nerves to the masticatory appendages which arise from the ganglion are similarly arranged.

2. *Abdominal Nerves.*—From each abdominal ganglion two nerves arise on each side in a similar manner to the nerves of the thorax. The anterior of these is distributed to the abdominal appendage of the same side, and in specimens hardened in alcohol appeared to consist of two parts, but no such division was found in fresh specimens.

The posterior nerve, after passing between the abdominal muscles, terminates in the skin along the side of the abdomen.

The abdominal muscles themselves are supplied by nerves which arise chiefly from the abdominal nerve cord between the ganglia: but according to Yung the superior (posterior) nerve ramifies in the muscle of the abdomen.<sup>2</sup>

<sup>1</sup> Huxley: *The Crayfish*, p. 93.

<sup>2</sup> *Loc. cit.*, p. 487.

In the case of the last abdominal ganglion there are two nerves on each side passing to the telson, corresponding to the two divisions of the nerves to the other abdominal appendages. There is another nerve on each side passing to the skin corresponding to those from the other abdominal ganglia, but differing from them in being placed anterior to the nerves to the appendage. There are also several nerves arising from the posterior end of the ganglion.

#### B. PHYSIOLOGY.

All experiments were performed on the lobster immediately after removal from the tanks. A "holder" consisting of a vertical block of wood fixed in the centre of a board was employed, on which the animal was secured by means of pieces of tape attached to screws placed in convenient positions. The chief difficulty experienced was the rapidity with which the nerves when exposed lose their power of transmitting the nervous impulse, the protoplasm of the nerve appearing to disintegrate very rapidly. Another difficulty was the presence of the large quantity of blood pouring out of the wounds which rapidly coagulated and so hindered the operations.

The first difficulty was overcome after some practice by opening the limbs and exposing the nerves as rapidly as possible. Also by opening the thorax and letting out some of the blood, the amount pouring out of the limbs was much diminished. For purposes of stimulation, a Du Bois Reymond Induction coil with Magnetic Interruptor and a single Daniell Cell were used. The electrodes used were of Platinum wire. In most cases silk ligatures were used for the purpose of lifting the nerves on to the electrodes.

I found very little trouble from the animal's claws, because from their great weight they cannot be moved quickly when out of water.

#### 1. EXPERIMENTS ON THE NERVES IN THE LARGE CHELAE.

The chief experiments on the investigation of motor and sensory roots were performed on the nerves of the large chelae, since from their large size they are most suitable for experiment. The nerves are most easily exposed on the ventral surface of the third joint of the limb, where they lie close to the anterior border. The animal in these experiments was in the supine position.

*Experiment A.*—The small nerve being intact, the large nerve was ligatured and cut. On stimulating the distal end of the latter nerve

the limb was sharply raised and the claw closed. On stimulating its central end there ensued sharp reflex movements of the thoracic appendages, flapping of the abdomen and slight movements of the antennae and masticatory appendages. Also in the most lively animals there was a distinct opening of the claw.

This experiment shows: (1) that the large nerve contains the motor fibres to the muscles which raise the limb and close the claw; (2) that this nerve contains many afferent fibres and is hence mixed; (3) that it contains special afferent fibres which cause opening of the claw by reflex action through the small nerve which alone supplies the divaricator muscle of the claw.

*Experiment B.*—The large nerve being intact the small nerve was ligatured and cut. On stimulating its distal end the claw was sharply opened and the limb extended. On stimulating the central end the claw was closed and the limb raised. In some cases slight movements of the thoracic and abdominal appendages were observed, but in most cases there were no appreciable general movements.

This experiment shows (1) that the small nerve contains motor fibres which supply the extensor muscles of the limb and especially the divaricator muscle of the claw; (2) that it contains afferent fibres which cause reflex contraction of the claw through the large nerve which supplies the oclusor muscle. This nerve is therefore mixed also. Thus these two experiments show that each of the two nerves is mixed, and hence separate motor and sensory roots do not exist in the lobster.

*Experiment C.*—The following experiment shows that there is a distinct sense of touch in the claws. In an active lobster the eyes and antennae were removed and the animal again placed in the tank. The animal then moved round and round in circles and appeared unable to walk in a straight line unless guided by the side of the tank. When a stick was placed in front of it, it did not grip the stick as it did before the operation, but directly the stick was placed within the claws the latter closed upon it; thus showing that there is a distinct sense of touch in the claw, in spite of the great thickness and hardness of the calcareous cuticle. This fact one would presuppose from the large number of nerves which pass to the skin of the claw and which are chiefly derived from branches of the large nerve.

*Experiment D.*—Mr. Cunningham found that on placing his finger in the claw after the small nerve was cut he could feel a distinct

closing of the claw. This was repeated several times. This experiment again shows that the large nerve contains both afferent and efferent fibres: for we have seen above that the skin is sensitive to external stimuli, and these passing up the large nerve must have caused closing of the claw by reflex action through efferent fibres in the same nerve, because the small nerve was cut.

*Experiment E.*—In an active lobster the circum-oesophageal commissures were cut on both sides. This operation was performed by making an aperture in the thorax and cutting with scissors where the commissures were supposed to lie. That the commissures were really cut was confirmed subsequently by *post mortem* examination, a precaution the necessity of which is evident. The connection between the cerebrum and the rest of the nervous system was thus severed.

The experiments A and B were then repeated. On cutting open the limbs there ensued general movements much more marked than before, the opposite limb being even brought over to the place of operation manifestly for the purpose of removing the operating instruments.

On stimulating the distal end of the small nerve the claw was sharply opened, and on stimulating the central end of the same nerve the claw was sharply closed by reflex action through the large nerve.

In the opposite limb on stimulating the central end of the large nerve the claw was sharply opened and the limb extended by reflex action through the small nerve: and on stimulating the distal end of the large nerve the claw was closed and the limb raised.

All these actions were much more strongly marked and more regular than in the case of the animals in which the connection with the cerebrum was intact. This experiment shows that all the effects described above are due to reflex action, and are more regular in action and more strongly marked when the cerebrum is cut off from the rest of the nervous system; doubtless because inhibitory impulses which might pass down from the cerebrum are stopped when the oesophageal commissures are cut. This no doubt explains the fact that in some of the animals experimented upon the effects described in A and B did not all take place.

*Experiment F.*—In another lobster the nerve chain was cut both in front of and behind the second thoracic ganglion: the latter ganglion which supplies the chelae was thus isolated (confirmed by *post mortem* examination). The experiments A and B were performed on this animal with the same results (excepting, of course, the general movements of

the body and appendages). But the effects were much less marked than in the case of E. The reflex divaricator action on stimulating the central end of the large nerve was slight. The reflex closing of the claw on stimulating the central end of the small nerve, however, was distinct, and the effects on stimulating the distal end of each nerve the same as usual. The animal in this case was soon exhausted.

This experiment shows that the second thoracic ganglion acts as a special reflex centre for the great chelae; since the reflex closing and opening of the claw on stimulating the central end of the small and large nerve respectively take place when it is isolated. This is presumably the case with the other thoracic ganglia.

Yung came to a similar conclusion with regard to these ganglia; he says:—"Chaque ganglion est un centre de sensibilité et de mouvement pour le segment du corps auquel il appartient; mais la sensibilité est inconsciente et les mouvements réflexes lorsque le ganglion est séparé de ceux qui le précèdent" (pp. 492, 493). "Les ganglions thoraciques se comportent comme les ganglions abdominaux pour les membres de leur segment respectif. Leur destruction entraîne l'abolition des mouvements volontaires dans les appendices situés en arrière" (p. 525). These results were obtained chiefly by cutting the chain at various points and observing the subsequent effects.

## 2. THE NERVES IN THE AMBULATORY LIMBS.

Experiments on the nerves in the other ambulatory limbs were not carried on in the same detail as in the case of the chelae, for the reason that the nerves are much more difficult to expose.

On stimulating either nerve movements took place in the limb and also general reflex movements of the body and appendages; the latter movements being strongly marked in the case of the large nerve, but slight when the small nerve was stimulated. When the large nerve was stimulated the limb as a whole was flexed, and extended when the small nerve was stimulated.

Thus each nerve is mixed as in the case of the chelae.

## 3. THE ABDOMINAL NERVES.

The nerves arising from one of the abdominal ganglia were exposed and cut. Sharp flapping of the abdomen took place when the central end of either nerve was stimulated, but the effects were stronger in

the case of the posterior nerve which supplies the skin. On stimulating the distal end of the latter nerve no effect was observed. On stimulating the distal end of the anterior nerve which supplies the abdominal appendage, the latter contracted. Hence the anterior nerve would seem to be mixed, but the posterior nerve purely sensory.

These results differ somewhat from those obtained by Yung. He states that mechanical excitation of the two nerves produce the same effect, each nerve being mixed (p. 487).

With regard to the investigation of motor and sensory roots, Yung does not appear to have experimented upon the nerves arising from the thoracic ganglia. He says:—"Les racines des nerfs irradiant de la chaîne ventrale sont à la fois motrices et sensitives" (p. 525); but this result was obtained only on the nerves arising from the abdominal ganglia (p. 486). This result, as stated above, differs slightly from the result I obtained from the abdominal nerves, one of which I found to be mixed and the other purely sensory. Most of Yung's experiments on the thoracic ganglia seem to have been performed with a view to investigate the statement previously made by some investigators that the superior and inferior surfaces of the ganglia held a different function with regard to motion and sensation. On page 525 we find:—"L'opinion classique, que la face inférieure de la chaîne est sensitive, tandis que la face supérieure serait motrice, est infirmée par nos expériences."

#### 4. EXPERIMENTS ON DECUSSATION.

(A) The cesophageal commissure of one side was cut and the anterior end stimulated; the antennae and antennules of the same side moved sharply, those of the opposite side slightly. The same effect took place when the other commissure was cut. Also the effects were similar when the stimulation was performed on the other side.

On stimulating the posterior end of each commissure, the thoracic appendages moved mainly on the side stimulated, but slightly also on the opposite side. The same effects were observed when the commissures joining the separate ganglia were stimulated.

These experiments show that there is no marked decussation of the fibres as in vertebrates, for if it were so the effect would be more marked on the opposite side of the body to that stimulated.

Yung came to the following conclusions with regard to decussation (pp. 525, 526): "Chaque moitié droite et gauche du cerveau agit

sur la partie correspondante du corps." "Chaque portion de la chaîne agit également d'une manière directe sur le côté du corps qui lui correspond. Il n'y a pas d'entrecroisement dans le parcours des fibres nerveuses." These results were obtained by stimulation with a needle and by chemical stimulus. Electric stimulation was not employed.

Although the normal passage of the nervous impulse is along the same side of the nerve chain as that stimulated, the following experiments show that when the normal path is interrupted by section, the impulse can pass across the chain.

(B) The nerve chain was cut completely across in front of the fourth thoracic ganglion, and the interganglionic commissure on the *right* side was cut just behind the same ganglion. On stimulating the stump of the *right* commissure in front of and connected with the ganglion, the fourth and fifth ambulatory limbs on the *left* side moved sharply; this shows that the impulse not being able to influence the limbs of the same side, owing to the commissure being cut on that side, travelled across the ganglion and acted on the limbs of the opposite side.

(C) One of the thoracic ganglia was isolated by section of the cords in front and behind. The large nerve to the limb of one side was cut and its central end stimulated; the limb of the opposite side moved sharply, the impulse having passed directly across the ganglion.

##### 5. GENERAL EXPERIMENTS ON THE NERVOUS SYSTEM.

(A) Stimulation of the cerebrum or supra-œsophageal ganglion caused sharp movements of the antennae, thoracic appendages, and abdomen. The animal crushed one claw within the other. The same effects were observed when the œsophageal commissures were stimulated, but the movements were even more violent.

Yung obtained the same result by stimulating the cerebrum with a needle and he points out that "le cerveau ou ganglion sur-œsophagien est sensible sur toutes ses faces comme les autres ganglions de la chaîne nerveuse, et contrairement à ce qui a lieu chez les insectes et les vertébrés" (p. 525).

(B) Stimulation of the sub-œsophageal ganglion caused movements of the masticatory appendages and maxillipedes; also flapping of the abdomen and movements of the thoracic appendages.

(C) Stimulation of each thoracic ganglion caused movements, mainly of the appendages which it supplied, but also movements of the appendages behind, and flapping of the abdomen.

In all these cases the movements of the appendages in front of the ganglion stimulated were slight: hence the normal passage of the nervous impulse is down the cord: but if the cord was cut behind the ganglion stimulated, the appendages in front of the ganglion moved more strongly when the latter was stimulated than before.

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#### SUMMARY OF RESULTS.

1. Motor and sensory roots analogous to those by which the spinal nerves of vertebrates arise do not exist in the lobster.
2. There is no marked decussation of the nerve fibres in the central nervous system: but nervous impulses readily travel across the ganglia from one side to the other.
3. Each ganglion is a reflex centre for the appendages which it supplies.
4. There is a distinct sense of touch which can be exercised through the thick cuticle of all parts, especially in the large claws.
5. The cerebrum or supra-oesophageal ganglion is the seat of origin of inhibitory impulses: reflex actions are much more marked when the connection between this ganglion and the rest of the nervous system is severed.
6. All the ganglia, including the cerebrum, are sensitive, *i.e.* respond to stimulation.
7. The normal passage of the nervous impulse is down the cord, but when this path is interrupted, it will pass up the cord.

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It will be seen that the two most important results of these investigations are negative ones. There is no marked decussation and there are no separate motor and sensory roots. Nevertheless it is important to have determined these points in such a highly organised invertebrate animal as the lobster. It would be interesting to investigate these points in other highly organised invertebrates, such as the *Insecta*, *Arachnida*, and *Cephalopoda*.



Motor and sensory roots are now recognised throughout the vertebrate kingdom (with the exception of the *Ascidians*); they are now even supposed to be present in *Amphioxus*, and it is a curious point if this condition is confined to them alone and does not exist in any invertebrate.

Concerning decussation, I believe complete crossing of the nerves has been found to exist in every vertebrate in which this point has been investigated. Now from the experiments described above,—especially experiment B,—it appears that although there is no marked decussation, yet partial decussation does exist in the lobster, at any rate functionally, contrary to the opinion of Yung; but whether this is a decussation of nerve fibres only, or whether nerve cells are concerned in it, is not proved. This is of interest as affording a possible clue to the condition found in vertebrates in which the crossing is complete and in which nerve cells are concerned.

My thanks are due to Dr. Michael Foster and to Professor A. Milnes Marshall, who have kindly examined the results of my experiments and also suggested valuable alterations in the paper.

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## THE MORPHOLOGY OF THE SEXUAL ORGANS OF HYDRA.

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Hydra stands alone, or almost so, among Hydrozoa, inasmuch as its reproductive organs, whether ovaries or testes, develop and ripen in the body-wall of the animal instead of in special buds or gonophores. Concerning the relationship in this respect between Hydra and other Hydrozoa two diametrically opposite views have been held, one being that Hydra exhibits the simplest and most primitive condition of the reproductive organs, prior to the evolution of special sexual buds; the other that the condition in Hydra is one of extreme degeneration, the sexual buds that were previously present having become completely aborted.

A short time ago, Professor Weismann of Freiburg published some extremely interesting and valuable researches on the development of the sexual products in Hydrozoa,<sup>1</sup> and it is the object of the present paper to enquire into the bearing of these results on the problem stated above concerning Hydra.

In one of the typical hydroid colonies such as *Podocoryne* or *Bougainvillea* the sexual products, whether ova or spermatozoa, are contained in medusoid buds, and do not ripen until these medusæ have attained full development, and detached themselves from the colony so as to lead a free-swimming existence. In many cases, however, the sexual products ripen before the medusoid bud has completed its development, in which case the bud remains attached to the colony in

<sup>1</sup> Weismann, *Die Entstehung der Sexualzellen bei den Hydromedusen.* Jena, 1883.

a more or less immature condition. In some instances the gonophore is a fully-formed medusa, which, however, never detaches itself from the colony, such a gonophore being called an attached medusa; in other cases development stops at a still earlier stage, giving rise to a disguised medusa, in which all the essential parts of the medusa are present, but in an unexpanded condition; and, finally, development may go no further than the production of a hollow diverticulum of the body-wall of the parent known as a sporosac or sporophore, within the walls of which the ova or spermatozoa are matured.

It is worthy of notice that the free medusa in the course of its development passes through in succession the stages of sporosac, disguised medusa, and attached medusa; so that these latter may be regarded as due to arrested development of the medusa at an earlier or later stage. That this view is correct rather than one which would regard the sporosac, disguised medusa, and attached medusa as representing stages in the gradual progressive evolution of the free medusa, is evident from the consideration that the disguised medusa and attached medusa, which have all the parts of the free medusa fitting it for independent existence, but never have an opportunity of employing them, could never have arisen by a process of natural selection from the sporosac, for the possession of a swimming bell that is never opened could clearly be of no advantage.

Hence the forms with free-swimming medusæ must be regarded as the most primitive, and those with attached or disguised medusæ, or with sporosacs, must be viewed as derived from these by abortion, more or less complete, of the various parts of the free medusa, such abortion being intimately associated with the early or premature ripening of the sexual products.

Weismann, in the work alluded to above, has shown that the genital cells of Hydrozoa may arise in parts other than those in which they are ultimately lodged, and indeed before the appearance of these latter, into which they migrate later on. In some cases this may be carried so far that the genital cells arise in the body-wall of the primary zooid not only before the commencement of the development of the gonophore, or sexual bud, but even before the first trace of the appearance of the branch on which the gonophore will subsequently be borne. A good example of this is afforded by the fresh-water genus *Cordylophora*, in which the ova arise in what Weismann calls the germinal zone of the primary zooid, then migrate into the lateral branch of the

zooid when this is formed, and later on shift again into the gonophore which arises as an offset from this lateral branch.

The explanation of this curious migration is probably to be found, as Weismann suggests, in the advantage derived from commencing the development of the sexual products as early as possible. The formation of the ovum, especially, is a long and complicated process, which in most animals is commenced at a very early date; in the highest mammals, for instance, the ovary contains either at or very shortly after the time of birth all the ova that will ever be developed in it. The development of spermatozoa is a more rapid and less elaborate process than that of ova, and we find accordingly that the date of their appearance is not thrown back so far as that of the ova. For instance, in *Eudendrium* the ova arise in the primary zooid before the appearance of the lateral branches; the male cells, however, are not formed till later, and appear first in the lateral branches, from which, like the ova, they migrate into the gonophores.

The suggestion I would make with regard to *Hydra* is that it represents one step further in the process of migration beyond the stage reached by *Cordylophora* or *Eudendrium*; *i.e.* that in *Hydra* the genital products not only make their first appearance in the wall of the primary zooid, but remain and undergo their whole development in the same position, no lateral bud or gonophore being formed.

Weismann<sup>1</sup> himself takes the directly opposite view that *Hydra* represents a primitive, and not, as I believe it to be, an extremely modified condition. He considers that in *Hydra* there has been no shifting of the place of origin of the sexual cells, but that *Hydra* represents in this respect the primitive and original condition.

In support of the contention that *Hydra* is, as regards its generative organs, a modified and not a primitive form I would submit the following arguments:—

1. *Hydra* is hermaphrodite, being in this respect almost unique among Hydrozoa. A hermaphrodite condition is altogether exceptional among Hydrozoa, and there is not the slightest evidence for regarding it as primitive in them; while there is very strong reason for viewing it as secondary and acquired, wherever it occurs in other groups of animals.

2. *Hydra* is fresh water, differing in this respect also from almost all other Hydrozoa. It is very generally accepted that fresh water forms

<sup>1</sup> Weismann, *op. cit.*, p. 254.

are in the great majority of cases derived from marine forms, and also that they are very liable to undergo modification in consequence of their change of habitat.

3. The other fresh water Hydroid, *Cordylophora* affords very interesting evidence. In the first place, there is very strong reason for regarding it having only recently migrated from the sea and adopted a fresh water habitat.<sup>1</sup> Then as regards its sexual organs *Cordylophora* is in an extremely modified condition. The genital cells, whether ova or spermatozoa, are lodged when fully developed in gonophores which never reach even the disguised medusa condition, but are arrested at a stage very little in advance of the sporosac.<sup>2</sup>

This, however, is not all, for Weismann has shown<sup>3</sup> that the genital cells of *Cordylophora* do not arise in the gonophores, but in the body walls of the primary zooids, where they may be recognised long before the gonophores have commenced to develop, though on the appearance of these latter they migrate into them.

These facts seem to me to speak very strongly in support of the view advanced above concerning *Hydra*. *Cordylophora* is a genus which has only recently become a fresh water one, and in which the condition of the reproductive organs is such that *were the genital cells to remain and ripen in the position in which they first appear, i.e. in the body wall of the zooid, they would agree exactly in all essential points with those of Hydra*. It is surely more reasonable to suppose that in *Hydra*, whose fresh water habits have been longer established, a peculiarity which we find already very highly developed, in *Cordylophora* should be carried just one step further, than that the two forms should represent the opposite extremes of the series, for on Weismann's view *Hydra* is the most primitive, *Cordylophora* the most modified of Hydroids.

4. Additional evidence of considerable value is afforded by the structure of the ovary itself in *Hydra*. In its early stages of development<sup>4</sup> this consists of a plate-like mass of interstitial cells which at first are all of about equal size, but of which one only, situated in the centre of

<sup>1</sup> Concerning this migration, Semper states:—"It is, so far as I know, the only example of an animal that can be proved to have originally lived in the sea or in brackish water, and which, within our own time, has gradually accustomed itself to live in pure fresh water." *Animal Life*, pp. 151-153.

<sup>2</sup> For good figures of these gonophores, vide F. E. Schulze, *Ueber den Bau und die Entwicklung von Cordylophora lacustris*. Pl. III & IV.

<sup>3</sup> Weismann, *op. cit.*, 29-33.

<sup>4</sup> For an account of the development of the ovum in *Hydra*, vide Kleinenberg, *Hydra*, 1872.

the plate, develops into an ovum, the remaining cells supplying it with nutriment. This must be regarded as a very highly specialised condition, for all the cells of the ovary are at first alike and must be supposed to be of equal value. It very usually happens among animals that of the cells composing the ovary, certain ones alone develop into ova, the others serving to feed them, but it is altogether exceptional that only a single ovum should attain maturity: other instances are indeed known, such as *Moina* and perhaps some other Entomostraca, and also some Ascidians, such as *Salpa*, but such cases are rare.

I am disposed to lay stress on this point, for if it be granted, as I think it must, that the ovary of *Hydra* is in an exceptionally specialised condition, it becomes very difficult to believe, as Weismann would have us do, that it is also from another point of view in a far more primitive condition than that of other Hydroids.

5. Kleinenberg and others have denied that any direct comparison is possible between the reproductive organs of *Hydra* and the gonophores of an ordinary Hydroid on the ground that the former consist of ectoderm alone, while the latter even in their most degenerate condition involve endoderm as well as ectoderm; and it must be admitted at once that the objection is a valid one.

However, it does not in the least affect the position I have attempted to establish, which is that the reproductive organs of *Hydra* correspond not to the gonophore of *Cordylophora* but to the *zone of germination* round the necks of the zooids in which the genital cells arise in *Cordylophora* and in which they both arise and ripen in *Hydra*.

I would in conclusion point out that the above argument only concerns the sexual organs of *Hydra*.

As regards its general morphology I fully agree with Weismann<sup>1</sup> that *Hydra* has strong claims to be regarded as having departed very little from the condition of the ancestral form from which all *Hydro-medusæ* may have sprung.

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<sup>1</sup> Weismann, *op. cit.*, p. 254.











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