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C. O. WHITMAN,

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EDWARD PHELPS ALLIS, JR.,

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INTRODUCTION.

THE zoölogical literature of this country is found in the various publications of the Smithsonian Institution, in voluminous reports of government commissions, in the memoirs and proceedings of societies and academies, in the bulletins and memoirs of a few universities, and in numerous periodicals devoted to the natural sciences.

With such varied ways and means of publication what more can be required? The answer must be brief: diversity in these respects is not an evidence of efficiency, but of weakness. Concentration is our need. How shall we effect it? Can any one or more of our present media of publication be converted into a strong central organ, devoted exclusively to the presentation of original research in animal morphology? Unfortunately no one of them appears to be capable of undergoing such a radical metamorphosis. Every attempt in this direction has failed, and for reasons too obvious to require notice here; and every combination scheme has found an effectual barrier in the rivalries of different institutions.

Our scientific publications are miscellanies, and such they are destined to remain. No one of them can make any pretension to fulfilling the functions of a morphological journal. Nowhere in this entire country is there a single efficient serial publication offering to extend its privileges to zoölogists in general, without regard to local restrictions. The result is that valuable papers have been shelved for years; some have been published with illustrations of an inferior quality; and not a few have been brought to the light through the aid of foreign journals.

Much, then, as we owe to our scientific societies for what they have done and are still doing for the biological sciences, and earnestly as we may desire to sustain and strengthen their resources, we recognize needs which such organizations have never undertaken to supply.

Our system of publication, even if it were not limited in means and burdened with local restrictions, would still suffer from defects of method that admit of no remedy. The inaccessibility of our literature — scattered as it is among the publications of so many societies and institutions, and mixed up with a mass of heterogeneous matter that has no value for a zoölogist — is notorious. The mixed character and scattered sources of our publications are twin evils that have become intolerable both at home and abroad. The establishment of the JOURNAL OF MORPHOLOGY may not be the deathblow to these evils; but there is hope that it will, at least, relieve the more embarrassing difficulties of the present situation.

It is unnecessary to expatiate on the advantages offered by such a medium of publication. They have long been acknowledged, appreciated, and enjoyed by those who have occupied themselves with the biological sciences in other countries. Germany, France, England, Austria, Holland, Belgium, Italy, Sweden, Norway, and Switzerland have their morphological journals; and the number supported in each country may be taken as an index of its productivity in morphological research.

We have not hitherto followed the example of other nations in this particular; but we venture to say that the time has come when at least one morphological journal should and can be creditably maintained. Our confidence is based on the fact that we now have several flourishing morphological laboratories established in this country; on the hearty assurances of support given by those who represent the principal centres of research in the United States and Canada; and on the character and number of the contributions offered for the first volume.

As previously announced, the JOURNAL OF MORPHOLOGY will be devoted principally to *embryological*, *anatomical*, and *histological* subjects. Although limited in a general way to *animal* morphology, it has not been thought necessary to make this fact prominent in the title.

The Journal will be issued in numbers, each containing from one hundred and fifty to two hundred or more pages, and from eight to ten lithographic plates. The second number, completing the first volume, will appear in November.

It is hardly practicable, and perhaps it is not desirable, to

have stated times of publication. It is more important to provide for the early appearance of papers than for regularity in issue; and accordingly the plan has been adopted of publishing numbers as often as the requisite material is furnished.

A partial compensation for the unavoidable delays that have attended the issue of the first number will be found in the fact that it has been made much larger, and more expensive in illustration, than was promised in the original announcement.

C. O. WHITMAN.

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JOURNAL

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MORPHOLOGY.

SPHYRANURA OSLERI: A CONTRIBUTION TO AMERICAN HELMINTHOLOGY.

BY R. RAMSAY WRIGHT AND A. B. MACALLUM,

PROFESSOR IN UNIVERSITY COLLEGE, TORONTO. | FELLOW OF

Introductory.

IN 1879 an ectoparasitic Trematode attacking the skin of the interesting Menobranch of our northern lakes (*Necturus lateralis*, Raf) was described by the senior author[1] from some specimens mounted in glycerine as microscopic preparations by Prof. W. Osler, now of Philadelphia, who, at the same time, furnished some notes of his observations on the living worm. Although the specimens at the author's disposal were not such as to permit a full account of the form, enough was made out to determine its systematic position and to justify the formation of the new genus Sphyranura for its reception. With this there was associated the specific name *Osleri*, in compliment to the original discoverer.

It was then shown that Sphyranura is a form intermediate between Gyrodactylus and Polystomum, an interesting confirmation being thus furnished of v. Willemoes-Suhm's suggestion[2]

[1] R. Ramsay Wright, contributions to American Helminthology. Proceedings of the Canadian Institute, Toronto, 1879.

[2] Zeit. wiss. Zoöl., XXII., 38.

that the two latter forms are genealogically connected, which is further strengthened by Zeller's[1] account of a two-suckered (or Sphyranura) stage of the developing Polystomum.

It was further urged that, when the hosts of the forms mentioned are taken into account, a certain parallelism between the phylogeny of both is apparent, for Necturus occupies a half-way position between the fishes and the frogs, and the thought lies upon the surface that both the frog and Necturus have inherited their parasites from fish-like ancestors, the gills of which were infested by Gyrodactylus-like forms. Such a direct transmission of parasites from one generation of the host to another is less likely to have been disturbed in the case of monogenetic than of digenetic Trematodes, for the development of the latter provides for a wider dispersion of the species than does that of the former.

When the description of Sphyranura was originally published, Van Beneden's[2] arrangement of the families of digenetic Trematodes was accepted as the best expression of their relationship. He recognized five families, — Tristomida, Polystomida, Octocotylida, Udonellida, and Gyrodactylida. The second of these was formed for the reception of the genera Polystomum, Onchocotyle, and Erpocotyle; while in the fifth were included Gyrodactylus and Dactylogyrus, Calceostoma, Tetraonchus, and Diplectanum. The existence of a form, however, intermediate between Gyrodactylus and Polystomum, as well as the development of the latter, seemed to point to a closer relationship between these two families, and it was suggested that both might be included in a family Gyrodactylida, Gyrodactylus being obviously nearest the ancestral form.

In the same year (1879) Taschenberg[3] suggested a somewhat different grouping of the monogenetic Trematodes. He recognizes two families, Tristomeæ (incl. Tristomidæ, Monocotylidæ, Udonellidæ) and Polystomeæ (incl. Octobothriidæ, Polystomidæ, Microcotylidæ, Gyrodactylidæ), the sub-families, Polystomidæ and Gyrodactylidæ being coextensive with Van Beneden's similarly named Families. If this grouping be accepted (which may be done provisionally until a more complete knowl-

[1] Zeit. wiss. Zoöl., XXII., Tab. I, Fig. 7.

[2] Recherches sur les Trématodes marins, p. 64. Van Beneden & Hesse.

[3] Zeitsch. für die gesammt. Naturwiss., LII., p. 235.

edge of the anatomy of the monogenetic Trematodes renders possible a better representation of their phyletic relationship) Sphyranura ought to be associated with Polystomum under the sub-family Polystomidæ, as it is clearly more closely related to that genus than are the Selachian ectoparasites Onchocotyle and Erpocotyle, or the Diplobothrium of the Sturgeon, which three genera, on the other hand, form a very natural group. It is interesting to note that, leaving Aspidogaster out of the question, the five genera constituting this sub-family are the only monogenetic Trematodes which do not occur on Teleosts. The following slight alteration of the diagnosis, furnished by Taschenberg, is necessary to admit of the genus Sphyranura:—

Sub-family Polystomidæ.

Without accessory anterior suckers, caudal lamina armed with two strong hooks often carried on a special appendage. Suckers, sometimes two, generally six, in two parallel rows on the ventral surface of the caudal lamina. Sexual apertures in the ventral middle line. Two vaginæ opening on the right and left borders, or absent (or a single one opening ventrally? [1]). Egg-shells with or without polar threads.

Access to fresh specimens has rendered possible some emendation of the diagnosis previously furnished for Sphyranura.

(Fam. Polystomeæ, Taschenberg.)

Sphyranura, char. emend.

Caudal lamina, *considerably wider* than the slender body, with *two* immersed suckers, two large hooks behind these, and sixteen small hooks (seven along each side of the lamina, and one in each sucker). Two contractile bladders anteriorly, each with a dorsal excretory pore. No lateral vaginæ. Oviparous.

S. Osleri.

Body 2.6–4 mm. in length, 0.7 mm. in breadth, narrowed at each end, especially where it joins the caudal lamina, which is 1.2 mm. broad, and about 0.6 long. Large hooks, 0.24 mm. long. Lobules of testis, 12–16 in number, occupying the interval between the intestinal cœca, which anastomose posteriorly. Uterus with single mature egg, oval, with brownish-yellow shell, 0.4 × 0.2 mm.

Since the publication of the original description no favorable opportunity occurred to verify it till the spring of the present

[1] We share the doubt expressed by Ijima, that the tube in Onchocotyle, here referred to, is a vagina. Zoöl. Anz., vii., 638.

year, when the laboratory aquarium was fully stocked with full-grown Necturi. It was soon observed that these would furnish abundant material for a thorough study of the organization of Sphyranura, and this was accordingly undertaken, with the results given in the following pages.

Methods of Study.

Specimens of Sphyranura are rarely too large to prevent a complete study in the fresh condition. The weight of a thin cover-glass is alone necessary to render the whole body accessible to high powers. Of course the opacity of the yolk-glands interferes with the examination of the nerve cords, and some of their commissures; but these glands are so little developed in young examples as to offer no obstruction. Without abundance of fresh material many points, such as the ciliation of the water-vascular system, its openings, the course of the genital products within the ducts, the presence of tactile hairs, and of the smaller nerve-commissures, would have been overlooked or remained obscure. The thorough study of certain of the cellular elements in the living condition likewise permitted the selection of the fixing reagent, which gave us the nearest approach to the natural condition of the cells. This precaution appeared to us particularly desirable, as some of the more important recent memoirs dealing with the anatomy of Trematodes are based upon material preserved in alcohol and other inadequate fixative reagents. With such delicate elements as are to be found in the Trematodes the utmost care is required in all the preliminary operations of microtomy to obviate shrinkage and alteration of the cell-structure. Various fluids which have been recently recommended by histologists for fixation were experimented with, and it was found that here, as in higher animals, no one fluid is equally successful with all tissues. It was necessary to select that giving the best general results, and the fluid which satisfies this requirement is one which, as far as we are aware, has hitherto only rarely been employed for the study of invertebrate tissues, viz., Flemming's chrom-osmio-acetic mixture.

The following is the method of procedure employed: an example of Sphyranura is placed on a slide with merely sufficient water to cover it; a drop of the reagent is placed beside

that in which the specimen lies, and the two are allowed to mingle, with the result that in five or ten seconds death, but not immediate fixation, ensues. The greater part of the fluid is now drained away, and the worm straightened out gently with a needle; when this is done — it should take but a few seconds, as the flexibility of the specimen allows it to be arranged in any way — a second drop of the reagent is put on the worm and allowed to stand for two or three minutes, after which the form of the body is fixed. It can now be transferred to a larger quantity of the reagent, where it must remain for thirty minutes, and thereafter be passed through alcohols, gradually increasing in strength from 30% to 90%.

Lang's Planarian Fluid, as well as other solutions containing corrosive sublimate, gave us less satisfactory results than Flemming's fluid. With its use much greater shrinkage occurs during fixation, as is especially well seen in the contents of the ovary and testes; but the preparations are of some value for purposes of orientation, as the tissues generally are more brilliantly stained than when Flemming's fluid has been employed. Although this is the case, the cell-structure is, with the exception of few tissues, much more easily studied in a Flemming's fluid preparation.

Solutions containing picric acid have not given preparations offering any specially advantageous features; indeed it is difficult to avoid distortion, as well as shrinkage, with such media, while Perenyi's fluid has the additional drawback of interfering with the selective capacity of the cells for staining reagents.

We have also used Delage's osmic carmine.[1] It offers no advantage over Flemming's fluid, when specimens hardened by the latter are subsequently stained in alum-cochineal. This procedure gives to the cytoplasm of ganglion-cells and their processes a characteristic brownish-red hue, which throws these cells into a very effective contrast with the surrounding tissues. All nuclei take a reddish-purple stain under this procedure, in which the cytoplasm rarely participates, and then only very slightly.

No successful results were obtained in attempts to demonstrate the nervous system with gold-chloride, all the tissues having a strong tendency to take a violet tint with this reagent.

[1] Arch. de zoöl. exper., IV., 120.

The subsequent removal of this from all except nervous tissues, by means of potassium cyanide, as recommended by Cybulsky and Delage, was attempted unsuccessfully, perhaps on account of the slow penetration of the cyanide, which, while decolorizing the outer tissues, leaves the central portions unaffected. It is quite possible that a larger number of experiments would have yielded some successful preparations, but we were satisfied with the unfailing results of the method described above.

The process of imbedding selected was the chloroform-paraffine method, the substitution of chloroform for turpentine having been found to obviate shrinkage in the delicate cells, to be afterwards described in connection with the excretory system.

Various aniline colors were used in addition to alum-cochineal for staining the specimens *in toto*, but none gave such uniformly good results as that medium. Many series of sections were prepared horizontal, transverse, and sagittal, and mounted by Schällibaum's method in balsam or dammar.

We have thought it best to outline the details of the technique employed in the present research, not only because the histological results arrived at diverge in many respects from received opinions, but also as a protest against the use of material inadequately preserved by alcohol or other means as the basis of histological descriptions.

General Description of Appearance and Habits.

On account of the dark color of the skin of the Menobranchs there is no difficulty in recognizing the presence of their colorless ectoparasites. These are frequently found all over the skin, especially on the inner surfaces of the legs and along the back, and, indeed, on all places from which they cannot easily be dislodged by the efforts of the host to free itself from its tormentors. During the winter they have been observed to be more frequent among the gill-filaments, and such specimens do not seem to wander so freely as do the larger summer individuals. That the Menobranchs are tormented by the worms one realizes from their actions when infested by several individuals, although their efforts to rub them off can only be successful when the parasites attack the

body. The worms by no means confine themselves to any one spot of the integument, but may move rapidly from one part of the body to another, especially if disturbed. When the mouth-sucker is not applied to the skin of the host the creature exhibits most lively changes in form, becoming extremely slender, and waving from side to side, or retracting itself into a short, rounded clump.

If surprised, the worms are readily removed from the host by means of forceps, or even on the point of a needle; but if the first attempt be not successful it becomes a matter of greater difficulty to remove them intact. Should they become free in a large quantity of water their swimming powers are soon seen to be considerable, and they can progress, leech-like, along the wall of a glass vessel by alternate fixation of the lips and caudal suckers. Placed in a drop of water on a glass side the worm soon fixes its caudal lamina firmly to the glass, and it becomes difficult to detach it for examination from the ventral surface, unless one has first placed the creature on its back, and secured it in that position by a thin cover-glass.

The generic name Sphyranura was suggested by the shape of the caudal lamina, which is somewhat like a double-headed hammer, the handle being formed by the long slender body. (Fig. 1.) When the caudal lamina is not fixed to any surface it rarely rests in this shape, the two suckers being frequently approximated to each other, so as to bring the opposite thin, hook-bearing edges of the lamina in contact. Just as little does the anterior end constantly retain the form represented in the figure. The upper lip is at one time protruded in almost cylindrical form; at another retracted so as to form with the lower lip a circumoral sucker.

One can readily distinguish the worms in which the single ripe egg is present, its size being such, especially when the body assumes its slenderest form, as to cause a distinct swelling opposite the uterus; the opaque yolk glands are readily detected; they partly conceal the intestinal tubes, but leave uncovered the central transparent testicular area, between which and the opening of the genital sinus are the ovary, uterus, and genital ducts. In front of the genital sinus can be seen the bowl-shaped pharynx in constant activity, having free play in the surrounding prepharynx into which the mouth opens. On first exami-

nation also, the contractile bladders of the excretory system and the receptacula seminis are likely to strike the observer, but the appearance of the various organs in the living animal may be more conveniently considered under the systematic headings which follow.

The Investing Membrane.

Zoölogists are familiar with the different theories which have been propounded as to the nature of the superficial layer of the skin in the Trematodes. It is unnecessary to detail the history of these here, as they may be found collated in any of the recent German papers bearing on the subject;[1] but it may be said summarily, that it has been considered, 1, a basement membrane, from off which the epithelium has been lost (a view defended by Minot in his study of *Distomum crassicolle*)[2]; 2, a cuticula derived from a subjacent epithelium in which the cellular elements are more or less distinct (Leuckart *olim*, Sommer, Taschenberg, etc.); and, 3, a syncytium resting directly on the circular muscles, from which the nuclei have generally disappeared (Ziegler, Looss, etc.). The developmental evidence supplied by Leuckart and Schwarze[3] appears to us to establish the third view; but we are unable to furnish any account of the origin of the investing membrane in Sphyranura, which is, however, in the adult certainly homogeneous, destitute of nuclei, and rests directly without the intervention of a subcuticular layer on the circular muscular coat.

The investing membrane in Sphyranura is very elastic, and thus measurements of its thickness vary with the condition of contraction or extension of the body. In a carefully fixed specimen its thickness is about 4.2 μ, except where, as in the caudal suckers, a local thickening occurs. In specimens fixed during violent contraction the investing membrane is thrown into transverse wrinkles, a condition which can also be seen during life, and is no doubt due to the arrangement of the circular muscular fibres.

If a fresh specimen be examined under a high power there will be seen projecting to 12 or 13 μ beyond the free surface a great

[1] Very completely in that of Looss Zeit. wiss. Zoöl., XLI., 391.
[2] Mem. Bost. Soc. Nat. Hist., Vol. III., 2.
[3] Zeit. wiss. Zoöl., XLIII., 41.

number of conical bodies, which we regard as tactile organs, and the appearance of which is represented in Fig. 2. At such places one recognizes that the deep surface of the investing membrane does not rest immediately on the circular muscles, but that there intervenes a narrow space containing fluid (Fig. 10), which is enlarged to form the cavity of the tactile cone. In examples that have been under observation for some time granules are seen floating in this fluid; but in perfectly fresh specimens such granules are not present. The wall of the tactile cone is formed by the investing membrane, which is, however, perforated at the apex of the cone to allow of the passage of a tactile hair of 13 or 14 μ length, to which we have frequently traced a delicate fibril traversing the axis of the cone. We have not succeeded in satisfying ourselves that these fibrils proceed from subcutaneous nerves, but we consider such an origin probable.

The free surface of the cone is, furthermore, beset with very short and delicate hairs; but we have not succeeded in fixing either these or the principal tactile hairs, although the cones themselves are quite well preserved. Twin cones, such as are also represented in Fig. 2, are not uncommon.

Sphyranura leads such an active life in comparison with the other Trematodes that one might well look for some nervous structures specially adapting it for this. No eyes are present, such as are evident in larval Amphistomes, and such as have been described in young Polystomes and *P. ocellatum*, so the development of a series of tactile organs may be regarded as compensatory. The only observations known to us as to tactile organs in Trematodes are on certain structures described by Fischer[1] in the neighborhood of the sexual aperture in Opisthotrema.

These are possibly of the same character as our tactile cones, although Fischer describes them (from preserved specimens) as local thickenings in the investing membrane, which are penetrated by an axial fibril terminating in a knob.

Structures somewhat similar to the tactile cones of Sphyranura were observed in a peculiar free-swimming Sporocyst, described by the senior author[2] from a single example met

[1] Zeit. wiss. zoöl., XL., 12.
[2] Amer. Naturalist, XIX., 310.

with by chance in a small aquarium. No note was made of tactile hairs during the observation of the living Sporocyst, nor are such to be detected in a series of sections prepared from it, although the papillæ themselves are well-preserved. The latter will probably soon be described by Prof. Leuckart, to whom the series was transferred on account of its exceptional interest, and to whom the senior author is much indebted for kind assistance in the study of the anatomy of the Trematodes and Cestodes.

In connection with the above it may be recalled that various marine Cercariæ have been described with bunches of setæ (*Cercaria setifera*, Müller)[1], and it is extremely probable that all the structures referred to belong to the same category.

The tactile cones of Sphyranura are especially numerous on the upper lip, where they must have an abundant nerve supply from the plexus, to be afterwards, described in that region.

Nothing remains to be stated as to the investing membrane except that certain of the parenchymatous muscles are attached to its deep surface, Figs. 2 and 10, which produces in many specimens a slight irregularity of its inner contour. In a specimen which has been subjected to examination for some time, and is in a moribund condition, certain protrusions of the investing membrane are brought about by the accumulation of fluid in the subjacent space. Such protrusions are apt to take a rounded outline, and are seen on examination to be bounded on either side by a muscular attachment; these, therefore, tie down the investing membrane, which would otherwise be lifted off by the osmotic process.

The Organs of Attachment.

In describing the general appearance of Sphyranura we have made reference to the obstinacy with which it can attach itself to any surface; this is rendered possible by the hooks and suckers borne on the ventral surface of the caudal lamina. Bearing in mind the development of Polystomum we shall first describe the hooks as phyletically the older organs. As in Polystomum, Gyrodactylus, and Dactylogyrus two of the

[1] Fewkes, Amer. Jour. Sci., Feb., '82.

hooks exceed the others in size and functional importance, those of Sphyranura resembling the figures given by Wagener for some species of Dactylogyrus more closely than do the hooks of the other genera. Each hook is formed of a splint-like shaft, striated on both of its flat surfaces for the attachment of one of the three muscles to be afterwards described (*c*, Fig. 4); the shaft becomes rounded and stronger as it passes into the curved part of the hook, and at the junction of its flattened and rounded parts is a strong muscular process, with protuberances for the two remaining muscles attached here (*a* and *b* in Fig. 4). The curvature of the hook is not only sharper than in the other genera, but at the beginning of the curvature there are two barbs, one on the surface of the other, which no doubt increase the efficiency of the sharp point. Wagener has represented in several of his figures[1] a transverse chitinous piece, which joins the muscular processes of the two large hooks. This answers in position to the transverse muscle (*b*, Fig. 4) in Sphyranura, the ends of the fibre of which seem, in certain instances, to have undergone a change in consistency. In specimens flattened under a cover the position of the large hooks varies much according to which of the three pairs of muscles has contracted most violently.

The hooklets are situated seven on each side of the caudal lamina, as indicated in Fig. 4, and one in the centre of each sucker; all of them have their shafts turned towards the middle line of the lamina. They resemble the large hooks in miniature, having a shaft and muscular process just like these; but at the point of projection through the investing membrane each is surrounded by a chitinous "eye," in all respects similar to that figured by Zeller for Polystomum.[2] The "eye" is thinner at one spot (Fig. 5), so that it assumes an oval or circular outline, according as it is or is not pressed against by the hooklet which projects through it. Slender muscular bundles pass to the shaft and muscular process of the hooklets; but these hardly attract attention, except in the case of the acetabular hooklets, where they have to perforate the centre of the sucker to reach their attachment (*m*, Fig. 3).

[1] Beiträge zur Entwickelungsgeschichte der Eingeweidewürmer; Haarlem, 1857, Pl. XV., Fig. 5.

[2] Zeit. wiss. Zoöl., XXII., Tab. II., Fig. 2.

It is interesting to note that here, as in Polystomum, each sucker is developed around a hooklet. In the two European species of Polystomum (*P. integerrimum* and *ocellatum*) different hooklets serve as starting-points for the suckers, if we may judge from the figures of Zeller and v. Willemoes-Suhm;[1] but in *P. oblongum*, described by the senior author (loc. cit.), from the urinary bladder of the Musk Turtle (*Aromochelys odoratus*) the arrangement of the hooklets is similar to that in *P. integerrimum*. The hooklets of Sphyranura are persistent structures; we have never observed them to be absent, even in the largest examples, and are inclined to doubt that they are ever larval organs, as asserted by v. Willemoes-Suhm (loc. cit., p. 37) for Polystomum.

In examining the suckers from the ventral aspect the observer is struck by the reticulation of the surface of the investing membrane (Fig. 4); by the subdivision of the cavity into concentric zones, and by the thick, strong, doubly contoured line which surrounds the hooklet. These appearances are explained by examination of a vertical section through the centre of a sucker. (Fig. 3.) From such it is seen that the substance of the sucker is bounded on all sides by a membrane continuous in the cavity of the sucker with the investing membrane, and resembling that in appearance, although much thinner, where it bounds the convexity of the sucker. Instead of the substance of the sucker being formed of muscular fibres disposed in three directions, and capable of modifying the shape of the cavity, as in the Distomes, it is not possessed of contractility in Sphyranura (and probably in Polystomum), and is formed of prismatic fibres, rather of a supportive than of a muscular character, arranged perpendicularly between the concave and convex limiting membranes of the sucker. The fibres measure 2-3 μ in thickness; they do not stain, are easily isolated, appear to be homogeneous, and present between them no nuclei or large cells, such as are characteristic of the Distome sucker.

The appearance of reticulation in the concavity of the sucker referred to above is due to the superficial ends of these prisms, and is heightened by the dipping down of the investing membrane around each. It is to be noted that the investing membrane is not only considerably thicker (6 μ) here than else-

[1] Zeit. wiss. Zoöl., XXII., Tab. I., 9; Tab. III., 1.

where, but that it has apparently undergone a chemical change, assuming a bluish-green stain with iodine-green, which is not present elsewhere. As the wall of the sucker is itself destitute of contractility another arrangement exists for modifying the shape of the cavity. Its wall is really divided into three concentric zones, which, by special extrinsic muscles, can be worked independently. The two circular lines which separate these zones are marked by an infolding of the investing membrane, which forms a sort of joint, permitting the independent movement of the zones. These lines mark off the concentric bands seen from the ventral aspect, while the doubly contoured line surrounding the central hooklet is the expression of the investing membrane, lining the funnel-shaped perforation in the wall of the sucker occupied by the hooklet, and the soft parts on which it rests.

The movements of the sucker are best seen when the worm is uncovered, and able to curl round the edges of the caudal lamina. One thus has a side view of the sucker, and readily sees the independent movements of the second and third tiers in altering the shape of the cavity. These movements are effected by extrinsic fibres in the substance of the caudal lamina, which are partly disposed equatorially near the aperture of the sucker and meridionally over its convexity, while more distinct bundles penetrating the dorsal wall of the caudal lamina (Fig. 3) are attached to the second and third tiers of the sucker and to the central hooklet. Some of these fibres appear to penetrate the supportive framework of the sucker. Other muscular bands which act upon the suckers will be referred to under the musculature of the caudal lamina.

The Musculature.

We have little to note here as to the general arrangement of the musculature, except that the diagonal fibres so abundantly present in the larger Distomes are hardly represented here, so that we have merely to do with an external circular, a deeper longitudinal layer of fibres, and the somewhat scattered parenchyma fibres. The longitudinal fibres are much more developed on the ventral than on the dorsal aspect; instead of forming there a single layer they are disposed in two strong sheets, right and left of the middle line, this local development being due to the

fact that certain important groups of fibres destined for the caudal lamina have their origin forward on the ventral surface of the body.

The bulk of the fibres referred to converge on each side into a stout, rounded bundle as they enter into the caudal lamina, are there confined by a series of looped fibres (*d*, Fig. 4), and then diverge to their attachment on the chief prominence of the muscular processes of each of the large hooks. The other muscles of the hooks are confined to the caudal lamina, the transverse muscle being a strong bundle attached to the small prominence of the muscular process, while the fibres which are attached to the flat, striated shafts originate near the middle line of the lamina, where those destined for opposite sides decussate.

It is obvious that the three sets of muscles described provide for complicated movements of the large hooks; that those coming from the ventral surface of the body are chiefly engaged in "striking;" while those attached to the shaft, when acting alone, can disengage the hooks from the host. The looped fibres, above referred to, can alter the direction of action of the great muscular bundles by approximating or divaricating them as they enter the lamina. Again, it is to be noted that when the caudal lamina is attached, and the body free, the movements of the latter are largely carried out by the ventral muscles described.

Apart from the muscles of the hooks certain other groups of fibres enter or are differentiated within the caudal lamina, which are chiefly destined for the movement of the suckers. Some fibres from the ventral musculature, instead of entering the rounded bundles attached to the hooks, diverge into the anterior margins of the caudal lamina; these must tend to approximate the opposite edge of the lamina, but this is chiefly effected by the strong, transverse bundle which lies directly behind the junction of the body and caudal lamina. On the other hand, the opponents of these muscles are the bands already referred to, which penetrate the dorsal wall of the caudal lamina, and are attached to the convexity of the suckers.

Concerning the minute structure of the muscle fibre in Trematodes some few exact statements are to be met with. According

to Salensky[1] every muscular fibre belonging to the parenchyma of Amphilina represents a muscle cell in which two elements can be distinguished; a cortical layer (Rindenschicht) and a medullary substance (Marksubstanz). The first forms the greater part of the fibre, at the extremities of which it splits up into fine fibrillæ, difficult to follow, but terminating in the peripheral muscular layers. The medulla forms on the course of the fibre a large swelling, which, when viewed from certain directions, is spoon-shaped, and which contains a granular protoplasm and a nucleus, the latter being larger than the surrounding nuclei. The cortical layer, except at the swollen part, encloses the medulla; at that point, however, to which the medulla is confined, the latter protrudes through a longitudinal, slit-like opening in the cortex.

Chatin[2] describes each muscular element as consisting of a central protoplasmic body, with a distinct nucleus from which irregular processes arise; one of the latter becomes considerably prolonged and strongly developed in such a way that the protoplasmic body appears of accessory or minor importance. Chatin asserts that these muscular fibres are like those of Nematodes and of Amphilina.

Poirier[3] found, in the parenchyma of young Distomes, cells presenting all stages, transitional from that of the ordinary cell to that of a fully formed muscle fibre. These cells have a delicate envelope and a granular protoplasm which stains feebly, like muscle fibre, with picrocarmine. They elongate gradually, and, when in groups, in a direction parallel to each other. During this elongation the walls of the cell thicken, and the granular protoplasm condenses in the axis of the prolongation and finally disappears. The same fate is eventually shared by the central part of the cell and its nucleus.

The caudal muscular bands of Sphyranura, and especially the longitudinal ones, which are generally over two millimeters in length, offer favorable material for the study of the individ-

[1] Salensky. Ueber den Bau und die Entwickelungsgechichte der Amphilina. Zeit. wiss. Zoöl., XXIV., p. 306.

[2] Structure des Éléments musculaires chez les Distomiens. Bull. Soc. Philomath. Paris, Tome 6, 1882, p. 200–202. Abstract in the Zoölogischer Jahresbericht for 1883, Abth. I., p. 160.

[3] Arch. Zoöl. Experiment. 2ieme Series, Tome III., p. 492–4.

ual fibres. They were treated with various reagents, in order to determine the presence of nuclei, and to elucidate the structure of the fibres; but no nuclei could be detected in them, nor could any be demonstrated in the course of the transverse bands. We are not, however, inclined on this account to adopt Poirier's view as to their complete disappearance. The ends of many of the muscular fibres have been traced into the so-called subcuticular layer, and, as we have observed circular and longitudinal fibres of the investing musculature as well as parenchymatous fibres in connection with cells of this layer, we are inclined to believe that these bands, which are local developments of one or the other of these sets of fibres, have similar connections, although we have not been able to trace them. The connections referred to are illustrated in Fig. 10, *e*, from which it will be apparent that many of the cells of the subcuticular layer are in reality the central protoplasmic elements of the muscular fibres, the contractile elements of which form the musculature on which the investing membrane directly rests. Such connections may be most easily demonstrated in the parenchyma fibres, but suitable sections permit of recognition of similar relations in the circular and longitudinal fibres.

These muscle cells have generally been interpreted either as formative cells of the cuticula, or as unicellular glands, or as ordinary connective tissue elements. Fischer alone appears to have hinted at their real nature. This observer denies the presence of a subcuticular layer of matrix cells in Opisthotrema, but describes the occurrence of fusiform cells in a layer below the subcuticular muscles. These fusiform cells were observed to be in close connection with the muscular fibres, and were always found to be the most numerous where the muscle fibres were most abundant. Leuckart regarded these structures as a species of tendon fibres, or as peculiarly formed parenchyma cells.

These cells are most favorably observed immediately beneath the subcuticular muscle fibres. They measure about seven μ, are fusiform, with their long axis directed obliquely to the muscular layers, and do not resemble connective tissue elements in any respect. Usually there are two fibrils arising from each cell, one always being more strongly developed than the other; when there is only one fibril the cell has a pear-shaped

appearance. The nucleus occupies the greater part of the cell-body, the whole staining vividly, the nucleus somewhat more so than the rest. The fibrils, however, lose the capacity for staining at a short distance from the central body, and on this account the study of these structures is less easy.

Longitudinal sections are the most favorable for a study of the relations of the subcuticular cells to the muscular layers. In this case the subcuticular cells give origin to fibres which usually run in an oblique direction, and enter the longitudinal muscle layer. Immediately under a tactile papilla several of the fibrils may run, slightly curved, upwards through the longitudinal muscular fibres to the circular layer, and, curving again, are continued as circular fibres. It is to be noted that sections of all the circular fibres under the tactile papilla always appear thicker than elsewhere, owing to the fibres being here directed obliquely, and consequently cut obliquely; and this, coupled with the fact that the point of origin of such circular fibres as we determined was beneath a tactile papilla, has led us to believe that all or nearly all of the fibres of the outer muscular layer originate in this position.

The structure of the muscular fibre could not well be determined during life. In specimens killed by chloroform the fibre is seen to be made up of two parts, — a membrane and a medulla. (Fig. 9, *a*.) The membrane is hyaline in appearance, and distinct from its contents, which are finely granular and apparently fluid. This condition cannot be demonstrated distinctly in fibres not relaxed by reagents. On the other hand, one finds this to be the case in hardened preparations, although the granules are much less numerous.

A fine branching of the fibres at their terminations, such as Salensky found in the parenchyma muscles of Amphilina, was detected in the upper lip only of Sphyranura. (Fig. 6.)

Only one observer, Poirier, describes the contracted condition of muscle fibre in Trematodes. According to him the fibre, when contracted, is swollen at regular intervals along its course; that is, alternate lengths of the fibre are thickened and contracted. Our observations are opposed to this, the contraction appearing rather as due to the fibre being thrown into a wrinkled or zigzag course. Sometimes it seemed as if the fibre assumed a spiral course; but of this we cannot be certain. The

appearance, ordinarily, which the contracted fibre gives is that represented in Fig. 9, *b*. This mode of contraction seems to find its closest analogy in the stalk of Vorticella.

The Connective Tissues.

Leuckart, in the first edition of his great work,[1] described two forms of connective tissue in Trematodes, the one forming a granular reticulum with nuclei distributed through it, the other composed of large vesicular cells which resemble the cells of vegetable parenchyma. Both kinds of tissue are also recognized by recent observers, the first being resolved into a reticulum of branched cells, while the nature and function of the second have been variously interpreted. Looss,[2] *e. g.*, speaks of them as remains of the original formative cells, with a distinct nucleus surrounded by a thin layer of protoplasm, which gradually gives out peripherically. Ziegler[3] attributes an osmotic function to them; but Schwarze (pp. 58–71), who describes both their development and adult structure in *Distomum endolobum* (*Cercaria armata*), devotes most attention to them.

According to this author these vesicular cells are early differentiated from the ordinary parenchyma cells, and soon attain from twenty to thirty times the size of these. Their plasma becomes hyaline and loses its staining capacity, although an extremely fine granulation may be detected with Hæmatoxylin. Their function is to lend to the body that liquidity which enables the cercaria to escape from the narrow aperture of the sporocyst, but simultaneously, by virtue of the pressure dependent on their own turgescence, to give to the elastic investing membrane and the underlying musculature that tenseness without which no movement is possible. They may become altered in the adult through the assumption of this osmotic function by other cells, notably those of the intestine.

We have studied these peculiar cells in *Amphistoma subclavatum*, both in fresh specimens and examples prepared by the method detailed above; but we have found no cells in

[1] Die menschl. Parasiten, p. 457.
[2] Loc. cit., p. 398.
[3] Zeit. wiss. Zoöl., XXXIX., p. 550.

Sphyranura which can be considered as similar to them, and it was consequently with interest that we turned to the account of the body-parenchyma in other monogenetic Trematodes to ascertain whether they had been detected there. Taschenberg's account of that tissue in Tristomum is the most satisfactory one, and he states that his later observation of Onchocotyle confirms his views. According to this author[1] the parenchyma of the adult is a reticulated matrix, containing protoplasm formed at the expense of the plasma of certain cells, the nuclei of which are found scattered in the matrix; while other cells, whose plasma has not undergone this change, are found free within cavities of the matrix. He considers that the latter elements are the plant-like cells of Leuckart, and that what has been interpreted as the membrane of the plant-like cell is in reality the boundary of the matrix cavity. It is possible that these plant-like cells of Taschenberg are the vesicular cells of the recent authors, but their description does not correspond to anything we have met with in Sphyranura.

The connective tissue or parenchyma in Sphyranura is composed of branching cells, forming a mesh-work, the size of the meshes varying in different parts of the body. In the caudal region these cells are closely packed together, and leave between them but little interspace, except in the neighborhood of the looping of the larger excretory trunks posterior to the intestinal anastomosis. These cells answer to those of the first kind described by Looss and Kerbert. Their processes, evidently elastic, are homogeneous, not stainable, and the relations of these can only be determined with highly magnifying powers. The cells themselves vary slightly in size, measuring 12–22 μ, and have either an oval or spherical shape or an irregular form. The nucleus fills out the greater part of the cell-body, there being but little or no protoplasm surrounding it; it stains slightly as a whole where its granular character is not pronounced; in this case there is one or more chromatin nucleoli and a nuclear net-work is commonly visible. This network is sometimes of a coarse character, especially if granules are absent, when the chromatin is gathered into one or two large nucleoli. When the nucleus stains vividly its contents are finely granular; at the same time the protoplasm about

[1] Beiträge, p. 12. Weitere Beiträge, p. 8.

the nucleus occurs in greater quantity. A membrane is present to each cell, but it is sometimes detectable with difficulty.

Scattered amongst the connective tissue cells, and chiefly near the posterior termination of the intestine, are numbers of cells, which measure 10–12 μ in diameter, and whose significance cannot be determined at present. (Fig. 10, *f*.) They are unlike the ordinary connective tissue elements, appearing polyhedral, sometimes fusiform, and when they are found in groups the adjoining faces of neighboring cells are in contact. The nucleus in each is somewhat shrunken, and contains one or two chromatin nucleoli. The cell protoplasm is clear, homogeneous, and unstainable. From their position these structures appeared to be muscular in their nature; but a connection with muscular fibres, or with fibres of any sort, was not demonstrated. They may possibly represent the structures which have been interpreted as cutaneous glands in other Trematodes, but they apparently have no glandular character.

These comprise all the forms of connective tissue cells, but there are mingled with them the central bodies of the muscular fibres, as described above, and large, clear cells throughout the body and muscular pharynx, which are fully described under the Excretory System below.

The Excretory System.

The course and manner of opening of the excretory vessels have not been accurately studied in many of the monogenetic Trematodes; but Sphyranura agrees with Polystomum, as described by Zeller, in the possession of two anterior contractile bladders, each of which opens by a dorsal pore towards its anterior end. Onchocotyle, which, as we have seen, is generally associated with Polystomum under the sub-family Polystomidæ, has its contractile bladders in the bifid appendage of the caudal lamina; whereas the Tristomeæ which have been examined have the bladders anteriorly, as in Sphyranura.

The dorsal pores of the pulsating bladders escaped us in preserved specimens, but they are readily seen in the fresh worm when it is lying on its ventral surface. The transparency of the tissues overlying the bladders offers a favorable opportunity for observing, in their natural condition, the large ganglion cells which are applied to the wall of the bladders, and, presumably,

control their pulsations. These are effected by the muscular fibres which line the bladders, and which, like the parenchymatous muscles, are provided with oval nuclei.

Contraction of both bladders was rarely observed simultaneously; nor did there appear to be any constancy in the rate of pulsation, at any rate under the artificial conditions of observation. It was observed that the dilatation of the vascular trunks, resulting from pressure under a cover-glass, was accompanied by prolongation of the interval of pulsation from half a minute to a minute and a half. The systole is somewhat slower than the diastole; it does not affect the complete obliteration of the cavity of the bladder, but merely reduces its diameter from 0.1 mm to 0.02 mm.

From each bladder there runs back towards the caudal lamina a strong lateral stem, which gives off numerous twigs to the lamina, and turns sharply upon itself so as to course forwards towards the head. This it does in close association with the before-mentioned part, being sometimes twisted round it, only parting from it at the contractile bladder, where it runs forward to terminate in the capillaries of the upper lip, first forming an anastomosis with that of the other side.

The walls of the vascular trunks are highly elastic; whether they are provided with muscular fibres throughout it is difficult to say, although such are undoubtedly present in some parts of their course. An outer coat of elastic or muscular fibrils contains here and there an elongated or flattened nucleus, and surrounds a hyaline inner coat; no distinct longitudinal muscular fibres, such as described by Poirier for *Distomum clavatum* and *megnini*, are demonstrable.

Although the walls of the finer excretory capillaries are thinner, for they rarely exceed 1 μ in thickness, it is more difficult to give a satisfactory account of their structure. Only one coat formed of a homogeneous refracting substance appears to be present, and this is often so delicate in the finest tubes as to cause one to doubt the existence of a membrane in fresh specimens. After a Sphyranura has been under a cover-glass for half an hour the smaller channels become injected with very minute granules, which in the finest capillaries are arranged in a single row, only accumulating in several rows at the anastomoses. In such very fine tubes the lumen appears to be bounded merely

by neighboring cells, an appearance which is of interest in connection with the ultimate terminations of the excretory system, which we now proceed to discuss in the light of the important modern researches on this point.

According to Fraipont[1] the finest canaliculi terminate in ciliated funnels (*entonnoirs ciliés*). The base or expanded end of the funnel is covered by a cap formed of a cell, which often extends over the edge of the funnel on to its lateral surfaces. The covering cell is convex superficially, and concave internally, where the ciliated brush is inserted. An oval opening in the lateral wall of the funnel allows a communication between it and the lacunar spaces. The nucleus of the covering cell is large, provided with a large nucleolus, and a part of the protoplasm of the cell on its concave surface is differentiated in the form of a small disk, which does not stain, and on which the ciliary brush is inserted. Fraipont made these observations on *Distomum squamula* and *Diplostomum volvens*.

According to Looss[2] the finest capillaries terminate blindly in funnel-shaped expansions, which are formed of the capillary wall. In the interior of this closed funnel, on the expanded end, is inserted a brush of very fine cilia. He could observe no openings in the lateral wall of the funnel. For these observations he employed *Polystomum ocellatum*. In a Distomum he, however, found branched cells, resembling the "Geisselzellen," described by Pintner as occurring in Cestodes. These covered the funnels, or rather the latter appeared to lie in the cells, but the contour of the funnels could not be determined. Consequently Looss accepts Lang's view as to these structures, as they are in Planarians; they are hollow cells, with cilia in their interior. Poirier[3] also found these funnels in *Distomum clavatum* and other species, but observed no lateral orifice in them, such as found by Fraipont. Covering these funnels is a spherical body, probably a cell, and the interior of the funnel is filled with granular contents. It is also to be noted here, that Looss corroborates the statements of the earlier observers, as to the

[1] Recherches sur l'appareil excreteur des Trématodes et des Cestoides. Arch. de Biologie, Tome I., p. 427.

[2] *Op. cit.*, p. 409.

[3] *Op. cit.*, p. 590.

presence of cilia in the larger excretory vessels at certain points.

Our own observations on the presence of funnels and ciliary movements are the following: The finest capillaries, which possess apparently a definite membrane, present at certain points funnel-shaped expansions where the membrane terminates. In the interior of this funnel, which is not closed, are cilia, which appear to be inserted on or at the rim of the funnel and hanging down into it. The funnel is itself a gradual expansion of the capillary membrane, and is somewhat elongated. (Fig. 12, *d.*) Beyond the mouth of the funnel a net-work of fine intercellular canaliculi is rendered visible by means of the fine granules which inject them when the specimen has been subjected to pressure for some time. Sometimes a nucleus can be seen in a mesh of this net-work; oftener not; so that it is difficult to determine if a mesh represents the boundaries of a cell of connective tissue. No covering cell was found, although several fresh specimens of Sphyranura were studied day after day for weeks under a one-twelfth-inch oil-immersion of Leitz, and a No. 10 water-immersion of Hartnack. These funnels lie in the interior of a connective tissue-cell, and the fine canal which leads into the broad mouth of the funnel passes through the cell substance. The outlines of these cells can be very distinctly seen after the addition of acetic methyl-green and glycerine. The cilia of the funnel absorb the color till they take a very deep tint in contrast with the surrounding structures, while the outlines of the excretory capillary in both directions from the funnel disappear, leaving no trace of their presence. (Fig. 12, *d.*) The nucleus of the containing cell is always placed on one side of the funnel and excentrically in the cell. The most favorable position for the observation of these relations is in either of the lateral edges of the caudal lamina.

The funnel, as well as the capillary into which it empties, always has a distinct wall up to the rim of its broad mouth. The cilia hang over this rim into the funnel from the protoplasm of the containing cell, and are so inserted that the series of origins correspond exactly with the rim of the funnel.

It is only after pressure that the ciliated funnels of Sphyranura become visible; possibly the conditions of the tissues are

thereby so altered as to call forth the most vigorous activity of the cilia of the funnels, thereby rendering them more easily seen. When the specimen is dead, but before the tissues become clouded, the movements of the cilia slacken very much, and one is then permitted to study its nature. A single movement of each cilium consists of a wavy motion passing from its inserted to its free end; *i.e.*, down the cavity of the funnel.

We have observed the presence of bunches of cilia in the larger trunks, especially immediately in front of the contractile bladders. Sometimes these bunches are long and slender, at other times quite thick. From what they take their origin it is difficult to say. In two cases they appeared to arise from a cell, or protoplasmic structure, embracing the canal. Sections of hardened specimens have not, in the most diligent search, betrayed the presence of cilia in the larger canals, so that nothing definite can be said corroborative of what fresh specimens show. On the other hand, in the same preparations, one sometimes sees an excretory vessel apparently perforate a cell; but no cilia could be observed in the cavity of this perforation, although their presence is extremely easy to overlook.

As in the ciliated funnels, so in the larger trunks of the excretory system, the ciliary action is best seen only under extraordinary conditions. It is noticeable, also, under like circumstances, that the larger trunks, although fully dilated, yet contain very rarely any of the granules which gorge the smaller vessels. An explanation of this is possibly that pressure from the cover-glass prevents the entrance of these granules into the larger trunks. On the other hand, it is not certain whether the distention of these trunks is due primarily to the excess of fluid which they contain, or to the relaxation of their elastic walls. In the so dilated vessels were several times observed monad-like organisms, always with a rotatory movement.

We have now to describe structures of paramount interest in connection with the excretory system of Sphyranura, and which have not, we believe, been observed in other Trematodes. These bear most resemblance in form to the plant-like cells of certain Trematodes, as described by Leuckart and others, but beyond this point the similarity ceases.

These structures are cells of the character and appearance presented in Figs. 12, *a*, *b*, *c*, which represent them both in the

fresh and in fixed conditions. A large number are to be observed in the caudal region in the middle line anterior to the acetabula; but they are not confined to this point, being more or less scattered throughout the body. They are usually of a polyhedral shape, sometimes with short processes at the angles, and they measure generally 37–50 μ, while the nucleus, when it is observed in a spherical condition, measures 18–20 μ. The cytoplasma forms coarse trabeculæ, usually radiating from the centre of the cell to the periphery, Fig. 12, *c*, and containing a system of communicating spaces empty in the fixed, but often unobservable in the fresh, condition. The trabeculæ themselves contain a quantity of granules. The cell has a definite membrane, and applied to it are the united ends of the trabeculæ. Nine-tenths of the nuclei have a curved or crescent shape, fitting into the concave side of which is a large, clear space of varying outline, sometimes circular; but the nucleus loses its crescentic form and becomes spherical in specimens which have been subjected to pressure for some time. The cell usually does not stain, while the nucleus takes a feeble red color, sometimes, however, a gray tint. A nuclear recticulum can be observed, having large meshes, and containing one to several chromatin nucleoli of variable size. Each cell has a process at one pole, with an axial wavy channel connected with one of the neighboring excretory capillaries, Fig. 12, *a*, the wall of which passes insensibly into the membrane of the cell.

The structure of these cells was determined only after the various methods of preparation were employed; the only method which we found satisfactory being that detailed above, p. . Treated with solutions of corrosive sublimate and acetic acid, of chromic acid, or of picric acid, they appear like branched connective tissue-cells, or like unipolar ganglion-cells, and in the caudal region, where they are most abundant, they, with the excretory trunks, give the appearance of retiform tissue with great lacunar spaces. We first believed these cells to be analogous to those in *Planaria torva*, described by Lang[1] as perforated by the large excretory channels. Some support was given to this opinion when cells were observed apparently similar to

[1] Der Bau von *Gunda segmentata*, etc. Mitth. aus der Zoöl. Stat., Zu Neapel., Bd. III., p. 187.

those of Lang, except that the cytoplasma was strongly reticular, and when these were compared with the cells described above we were inclined to think that the former were in a stage of transition to the latter. When a careful study of a number of both kinds of cells was made it was seen, however, that they were essentially unlike. As mentioned above, the cells which appear perforated give origin to the brushes of cilia found in the larger excretory trunks, and, therefore, these may correspond to the cells described by Lang.

The fact that an extension of the cell in the form of a process is not seen in preparations made with the chrom-osmio-acetic mixture is probably due to the reagent, and partly to the sectioning. In respect to the latter it is to be expected that a unipolar cell variously placed in the tissue would be cut in various planes, and that sections involving both the body of the cell and the process would be rare. When the long axis of the cell occurs in sections its demonstration is not distinct, for the structure of the pole or neck of the cell is formed of material indifferent to staining. We made experiments with different reagents in order to determine which one would afford the best demonstration of the presence of a pole to the cell. Such a reagent we found to be Lang's fluid, which enhances the staining power of cells, at the same time altering their cytological structure. It so affects the cells in question that both cell and nucleus stain a red tint with alum-cochineal. The polar process in this case stains also, but the less so the farther from the body of the cell. Observed in this way these cells are extremely similar in shape to unipolar ganglion-cells. They do not now reveal such an internal structure as is described above, the clear central space is often absent, the nucleus is large and spherical, the cytoplasma gives slight or no trace of radiating trabeculæ, and the neck of the cell has slightly granular contents. Such preparations have, however, given the clearest results as to the presence of an axial channel in the polar process, and as to the connection of this process with excretory vessels. This connection suggests that they have a depuratory action; that is, that they are renal in the strict meaning of that term.

To this class we also assign the cells which are found in the muscular pharynx. These are in their greatest measurements 35 μ, and their nuclei vary in diameter between 10 μ and 20 μ.

The cell-body and the nucleus rarely take any stain at all in chrom-osmio-acetic preparations, with the exception of one or more nucleoli, which may be present. The limits of the cell are often quite indefinite, and this, with the somewhat large trabeculæ, makes them resemble the figures which Looss has given of them. Where the cell limits are clear and distinct the cytological structure is usually obscure for some reason, and in these cases their complete independence of the muscular elements lying around them is definite and decisive. (Fig. 6.) They sometimes have a concavo-convex nucleus, which may often be shrunken, while the cell in the fresh condition has in several cases been observed to have a process like that possessed by the renal cells. These cells in Sphyranura are, doubtless, the homologues of the cells described by Looss as found in the muscular pharynx of *Distomum trigonocephalum*, and the question naturally arises whether the cells, as Looss represents them, are not artificially affected by the reagents he has used to demonstrate them. Treatment with nitric acid, such as he used, however clearly it may bring out the coarser details of these cells, cannot certainly be depended on to preserve with any degree of accuracy the finer cell-structure, and we must, therefore, doubt if the coarse reticulum which he finds in it and the passage of muscular fibres through the meshes of this reticulum represent a natural condition.

The nature of these cells has been variously interpreted. According to the extreme and very absurd view of Villot[1] they are not really cells, but transections of vascular dilatations of the excretory system, which by an optical illusion have come to be considered as cells. All the other observers, with the exception of Looss, Fischer, and Leuckart, who have referred to these structures, regard them as ganglion-cells. Lang[2] described in *Tristomum molæ* the connection of these structures with the ganglion cells. Leuckart regards them as glandular; Fischer is doubtful of their nature; while Looss considers them to belong to connective tissue.

In order to determine as carefully as possible the nature of these cells we compared them with the cells found in the oral and caudal suckers of *Amphistomum subclavtum*. Speci-

[1] Ann. de Zoöl., 1878, p. 14.
[2] Mitt. Zoöl. Stat., Neapel. II., p. 44.

mens of this form were hardened in Flemming's fluid, stained with alum-cochineal and sectioned in the manner already detailed. The cells in the oral sucker are few in number, not more than five or six, there being a much larger number, on the other hand, in the caudal sucker. Those of the oral sucker have usually a definite globose or spherical form, while those of the caudal sucker are pear-shaped, elongated oval, or more or less flattened by the parallel muscular fibres in which they are imbedded. The average diameters of the cells in both localities and their nuclei are, respectively, 55 μ and 20 μ. The cell membrane is in all forms quite distinct. The cytoplasma in the cells of the oral sucker is made up of a fine reticulum, definitely visible only with high powers, in the meshes of which no formed elements can be detected; and its staining power is very feeble, possessing in cochineal preparations a colorless appearance, while the muscular fibres and the cells lining the excretory vessels of the sucker take a deep tint. The cytoplasma in the cells of the caudal sucker is likewise unstainable, but very coarsely meshed, and present here and there cavities of considerable size, sometimes comparable in this respect to the central cavity of the renal cells in Sphyranura; but these are by no means regularly placed. Sometimes, also, one can find here the radiate arrangement of cytoplasmic trabeculæ so common in the renal cells. The nucleus of the cells in the sucker is spherical, with a fine caryoplasma and one or more chromatin nucleolar spherules; while the nuclei of the caudal cells are often collapsed to the size of the chromatin body which each may contain. In cells possessing nuclei of the latter description the cytoplasmic reticulum is very coarse, somewhat hyaline, and its meshes are completely free from any formed element. It is in such cells as these that are found an almost complete resemblance to the renal cells in Sphyranura.

In Amphistomum the oral and caudal suckers, especially the latter, are richly supplied with excretory vessels of varying diameters. We could not determine whether these are connected with the large cells of the oral sucker, because the number of the latter is very limited, and thus a favorable chance of determining their relations to the vessels did not occur. In the caudal sucker, however, in two or three cases, the membrane of one of the finer excretory vessels was seen to be continuous with

that of one of the cells in question, the cavity of the former passing into the interfilar spaces of the cell. The reason why the connection of the vessels and cells is not more often seen is the same as that advanced above in the case of the renal cells of Sphyranura, — that a series of sections rarely takes the plane of the connecting parts of the excretory vessels and cells.

There can hardly be any reasonable grounds advanced against accepting the view that the cells of the oral and caudal suckers in Amphistomum are homologous with those of the muscular pharynx in Sphyranura; and as the former, from their connection with the excretory system, are in all probability renal in function, it appears to us to be a correct conclusion that the similar cells in Sphyranura are renal also.

It seems strange that it is in the oral and caudal suckers only of Amphistomum that all, or nearly all, of these cells are to be found; while in Sphyranura they are scattered through the body as well, although mainly present where the musculature is most prominent, — in the caudal lamina and in the region immediately anterior to this. It is probable that in the muscular system of these forms the greater part of their metabolism occurs, and in this way we may account for the presence of a large number of renal cells in such organs. This view is supported by the fact that in Sphyranura the caudal suckers (acetabula) completely lack this sort of cells, together with excretory vessels; but at the same time muscular fibres are absent. The latter are probably present in young or embryonic forms, but in the adult Sphyranuræ become changed into those rods which at first sight present a strong resemblance to muscular fibres. These rods being skeletal, or merely for the purpose of support, can exercise no metabolic activity; hence the absence of renal cells and excretory vessels.

The presence of excretory vessels in the caudal sucker of Amphistomum has been held to imply the passive inflation of its frame-work through injection of the vessels with water. The connection of these vessels with the large cells and the structure of the latter point decidedly to a physiological function which must be seriously interfered with if pressure is allowed to act on the internal structure of the cell, which must naturally be subjected to the same mechanical conditions as the vessels.

The Nervous System.

We have found Sphyranura particularly favorable for the study of the nervous system during life. In no other Trematode have we seen the fibrillation of the plasma of the ganglion-cells so distinctly as in this; and as it is easy to study living young examples under one-eighth inch or even one-twelfth inch oil-immersions, the lateral nerves, their commissures and branches can be followed with care.

Fig. 7 is intended to represent, diagramatically, the general arrangement of the nervous system. The ganglion-cells are disposed in two masses, which lie right and left of the muscular pharynx, but extend both in front of and behind that organ. It is to be noted that they are not grouped *around* the pharynx, but lie well to its sides, none being found on the dorsal or ventral aspects of that organ. Single cells straggling out of the ganglionic area are to be found in the upper lip, and isolated cells are applied to the walls of the contractile vesicles and receptacula seminis, but we have found none behind this line.

The lateral ganglia are connected by two commissures, the stouter of which is supra-pharyngeal, and connects the ganglia near the middle of their lengths, while the slenderer is infra-pharyngeal and crosses the anterior arch of the intestine, connecting the ganglia at the point of origin of the lateral nerve-stems. These are four in number, two to each side, and may be described as lateral and ventro-lateral. Their position relative to each other and to the organs of the body may be best gathered from Fig. 14. Of the two the lateral nerve-cord is the stouter, and is more directly connected with the lateral ganglion, while the ventro-lateral receives many of its fibres from the infra-pharyngeal commissure.

The transparency of the body of *Distomum isostomum*, enabled Gaffron[1] to detect an arrangement of the nerve-cords, and commissures much more complicated than had been supposed to occur in Trematodes. He describes six nerve-stems, four of which agree in position with those referred to above, but two of which, dorsal in position, are unrepresented in Sphyranura. The ventral nerves unite posteriorly, as do the

[1] Zoöl. Anz., VI., 508.

dorsal; but the lateral break up and fuse with the others. All are connected by commissures.

We find that in Sphyranura the lateral and ventro-lateral nerves converge to a single cord before entering the caudal lamina. Where they unite there is a strong transverse commissure (see Fig. 7); but the strongest commissure is that in the caudal lamina itself, between the united cords, from which various branches are given off to the various parts of the lamina. The two commissures mentioned are not the only ones, for at least two further delicate connecting strands between the lateral and ventro-lateral nerves were observed in the position indicated in the diagram. From the anterior extremity of each lateral ganglion there projects forwards a bundle of nerve-fibres, interspersed with ganglion-cells; these give rise to a very complex nerve-plexus in the upper lip, many of the fibres of which may be seen to originate directly from the scattered ganglion-cells. After a Sphyranura has been subjected to pressure for some time the fibrillated contents of the ganglion-cells and their processes may frequently be seen to ooze out through those branches which come nearest the surface, into the blisters formed by the raising of the investing membrane.

The nerve-cells have as limits of measurement, 20 μ and 42 μ and their nuclei, 8 μ and 11 μ. They are unipolar or bipolar, multipolar ones not being common, having been observed only at the ends of the commissures. The nucleus possesses one or several irregular chromatin-nucleoli, and is generally placed eccentrically. The contents of each cell bear a strongly-marked fibrillated appearance. In the case of unipolar cells these fibrillæ, if they are such, are seen, at the blunt end of the cell, to curve around concentrically with the periphery of the cell, and in the case of bipolar cells the fibrillæ of one pole can be followed distinctly into the other. Transections of one of the poles, show these fibrillæ as granules of the tint which is that of the ganglion-cells generally, the granules or points being circumscribed by a clear interfibrillar substance. (Fig. 8 *b*.)

The method of hardening with Flemming's fluid and staining with alum-cochineal gives these nerve-cells a peculiar color, somewhat like chocolate-brown. This tint is also present in the poles, but diminishes in intensity the farther the polar process

is followed from the cell-body. The nerve-fibres acquire no color by this method of treatment; each fibre appears to be a simple tube filled with a clear homogeneous jelly-like fluid, to which only gold chloride gives a color. The crossing and intertwining of these fibres in a nerve-cord give the appearance usually known as "spongy,"—an appearance quite common in Trematodes and Cestodes. As to the internal structure of these fibres, beyond what is said, we have determined nothing.

The Intestinal Canal.

Reference has been made above to the extremely mobile character of the upper and lower lips, and to their acting as an oral sucker. The mouth leads into a prepharynx, such as has now been observed in so many Trematodes, and in this cavity the muscular pharynx has free play, alternately opening to swallow the contents of prepharynx, and closing to discharge these backwards into the intestine. Neither the prepharynx nor muscular pharynx call for special remark. The histology of the latter appears to be very similar to that in other Trematodes. What we have to say specially as to the large cells present between the contractile fibres will be found above.

There is no œsophagus, the muscular pharynx opening directly into the anterior arch of the intestine. The lateral intestinal canals are simple without cœca, and they terminate by the formation of a simple posterior arch. It will be remembered that a similar arch is present in *Polystomum integerrimum*, complicated, however, by giving off numerous cœca into the caudal lamina, whereas in *P. ocellatum and P. oblongum* the intestinal branches end cœcally without such an anastomosis.

The wall of the intestinal branches is composed of a muscular and an epithelial layer, the thickness of the latter varying widely, while that of the former is never greater than 2.5 μ.

The muscular fibres are circular, longitudinal ones having been observed only anteriorly. The circular fibres have elongated oval nuclei, measuring 20 $\mu \times 3.5\ \mu$ which curve with the fibres on the epithelial layer. Capillaries of the excretory system appear frequently to pierce the muscular layer.

The epithelial layer is sometimes 60 μ in thickness, at other times it is found as thin as 3.5 μ, while the breadth of each cell in this layer does not vary much, being about 20 μ. The nuclei are oval or flattened in the thinner epithelial layer, rounded in other conditions, and measuring less than 11 μ. The shapes of the free ends of the cells are different even in the same section, being wedge-like, flattened, or rounded. The nucleus is usually at the base or fixed end of the cell, contains several chromatin bodies, and a distinct reticulum. The cytoplasma is granular, but in some preparations, notably those made with Flemming's fluid, a delicate reticulum is observable with trabeculæ stretching toward the free surface of the cell. (Fig. 11.) The basal border has a serrated appearance, probably due to the presence of the so-called prickles. The free border of the cell is usually covered with a layer of granules, this layer exhibiting a fine striation, which may be due to the presence of permanent cilia or to amœboid processes, quite unlike the strong, stiff cilia, which project from the intestinal, epithelial cells of *Amphistomum subclavatum*. The greater part of these granules doubtlessly belong to the swallowed food of the parasite, although they do not betray the characteristics of the granules found in the intestinal fluid. In preparations made with the chrom-osmio-acetic fluid there are in the neighborhood of the nucleus a number of blackened granules, probably of a fatty or zymogenic nature. (Fig. 11.) They are not pigmentary, for granules of this sort are distributed more evenly in the half of the cell next its free surface, and have a quite different tint and size.

The epithelial cells are observable with difficulty in the living condition, and then only at certain points, in front of the yolk-glands, and sometimes in the posterior arch of the intestine. They have a clouded, granular appearance, which prevents a definite ascertainment of their conditions of activity while fresh. No amœboid movements of the free ends of the cells were observed.

Structures answering to the "Kolben" of Kerbert were not found here. All the cells of the intestinal epithelium are alike, only differing in form at their free ends. The latter characteristic gave at first some support to the opinion that the

shapes are due to amœboid movements; but the facts cited in the following paragraphs seem to show that it is brought about by functional waste and decay.

The observations of Sommer on *Distomum hepaticum* and Kerbert on *Dist. Westermanni* tend to show that digestion in these forms is intracellular. The intestinal cells in the first named Trematode seem to throw out processes which draw in the particles of food into the interior of the cells.

This may be the case in the forms mentioned, but intracellular digestion plays at the most only a subordinate rôle in Sphyranura.

The food of Sphyranura consists almost wholly of structures derived from the epithelium and blood of *Necturus*. Cells from all the layers of the cutaneous epithelium of the host are present in the intestinal contents, and with the cells of the basal epithelial layer are swallowed those wandering cells or leucocytes quite common amongst the latter, more especially when inflammation of the subjacent tissues is induced by the irritation of the parasite. All these cells can be observed in a healthy vigorous specimen of the Sphyranura. Besides these formed elements there are an immense number of granules, consisting principally of the disintegrated remains of the cells of the host. Many of these granules, and all the larger ones, have, after treatment with the chrom-osmio-acetic mixture and staining with alum-cochineal, a vivid stain, and they are, therefore, the chromatin elements of the nuclei of the swallowed cells. The larger granules have characteristic shapes, sometimes round, sometimes crescent-shaped, and at other times they manifest that peculiar shape so common in the nuclei of the wandering cells of Necturus. All the stages of the disintegration of the cells can be fully seen, which give rise to these chromatin bodies as the ultimate visible product. First, the cytoplasma disappears gradually, leaving the nucleus intact; then the nuclear membrane breaks down, followed by the solution of the nuclear net-work, setting free the chromatin elements. In those cases where the nuclei are observed free and intact one is surprised to find the chromatin in them appear clear and brilliant, while the caryoplasma is not visible when treated with Flemming's fluid and stained. The reason for this is not far to seek; it is well known that the chromatin will dissolve and dis-

appear quickly in those nuclei which are subjected to the action of alkaline or neutral fluids, and that it is easily preserved when the fluid is acid. This evidently points out that the intestinal fluid in Sphyranura is acid, the acidity preserving the form of the chromatin of the nuclei, even when the latter are broken down.

Now, the chromatin portions of the nuclei, if they are swallowed by the intestinal cells, ought to give the latter an intense red hue when stained with alum-cochineal. Instead of that they are very lightly stained, and the staining is a diffuse one, not localized towards the free borders of the cell. On the other hand, the free chromatin granules become welded into large masses of amœboid form and of a gelatinous consistency, and float about in the intestinal fluid, appearing in stained preparations, very deeply colored. They assume various amœboid shapes passively, owing to the contraction of the intestinal walls, and contain, at times, several epithelial nuclei and even epithelial cells, besides a number of vacuoles. They measure variously up to 120 μ. We at first considered these structures to be true Amœbae, but careful observations on them after they were ejected from the intestine showed that they exhibit no vital phenomena, and it was then seen that they were agglomerated masses of nuclein or chromatin.

From all these facts, — the gradual disintegration of the epithelial cells of the host, their final solution, leaving the nuclear chromatin and the agglomeration of the latter into amœboid masses in the intestine, with the fact that the intestinal epithelium swallows but few, if any, of the granular contents, — one is led to conclude that the greater part of the digestive process in Sphyranura is accomplished by a soluble ferment diffused in the fluid contents of the intestine, and, further, that the reaction of these contents, as pointed out above, is acid.

Frequently the cells of the intestine show, in their outer halves, traces of pigment, which we supposed at first to be swallowed by the cell. It is possible, however, that these pigment granules arise from the metabolic processes of the cells themselves.

As to the origin of the soluble ferment the conclusion at once is that it is derived from the cells of the intestinal epithelium. Reference has already been made above to granules in the

neighborhood of their nuclei, which blacken in the osmic acid of Flemming's fluid, and which may be either fatty or zymogenic in composition; they are never abundant, and are easily overlooked. (Fig. 11.) Apart from this the epithelial cells show all the changes of size, which are exhibited by normal secreting-cells during their life history. The varying thickness of the cells has been given already. This variation is seen sometimes even in the same section; but in the same specimen it is usual to find that all the epithelial cells from the pharynx to the anastomosis of the intestinal branches have a like height. If, now, the intracellular process of digestion occurred in these cells one should hardly expect to find such a variety of thickness when the intestinal contents in every case alike is copious. One would rather believe the difference in thickness to be due to a greater or less waste of the cell-structure, such as is observed in cells secreting a soluble forment.

In some large forms of Sphyranura, evidently old individuals, the epithelial layer was very thin, so much so that its presence was detectable with difficulty. In our series of sections it was not possible for us to say that those which exhibit a thin epithelial layer were made from such old individuals; nevertheless, we believe that this decrease in thickness goes on gradually with advancing age, and that the cells are not renewed, but persist throughout life. For, although we have often determined the presence of cell-division in all the other organs of the body, yet not one case of this was observed in the intestinal epithelium.

The Reproductive Organs.

The diagram (Fig. 13) will serve to elucidate some points in the arrangement of the sexual organs not clearly shown in Fig. 1. Sphyranura is hermaphrodite; but the male and female organs are quite independent of each other, although there exists a tube, interpreted by Zeller as an internal connecting tube between the two, the true nature of which has, however, only recently been explained by Ijima.[1]

The male organs comprise the testes, vas-deferens, ejaculatory bulb, and its terminal part, with the coronet of spicules. The female organs comprise the ovary, oviduct, and ootype, from which there lead the ducts to the right and left recep-

[1] Zoöl. Anzeig., VII., 635.

tacula seminis, the overflow-tube to the intestine (on the same side as the ovary), and the uterine tract of the oviduct.

We have referred above to the position of the testicular lobules between the intestinal branches. Fig. 14, which illustrates half of a transection through the middle of the body, shows well the relation of such a lobule to the intestine and vitellogen. Near the anterior end of the testicular area the vas deferens can be made out near the middle line of the dorsal surface (v. d.), but the short tubes which lead from it to the lobules, and the more posterior parts of the vas deferens itself, do not exhibit the same epithelial lining which may be detected anteriorly.

Occupying the same position towards the dorsal surface as the vas deferens, its anterior end or ejaculatory bulb exhibits little difference except in regard to size and to musculature. When filled with sperm it is very often strongly curved to one side (Fig. 1), and in such conditions forms a very prominent feature in the Sphyranura, viewed as a transparent object, on account of the opacity of the sperm. When the bulbus arrives nearly to the level of the anterior arch of the intestine it changes its course, running directly towards the ventral surface to its aperture in the genital sinus. (Fig. 16, *b*, *e*.) In this part of its course it is suddenly constricted before being expanded into the terminal globular part, which is crowned with a circlet of spicules, and which can be everted into the genital sinus, if not out of the external aperture thereof. While the main part of the bulbus is merely provided with circular muscular fibres, this terminal globular part or cirrhus is provided with longitudinal nucleated fibres, whose duty is to evert the spicules, and thus open the aperture of the cirrhus. Retraction and eversion of the whole cirrhus is effected by specially modified parenchyma fibres, which are only partly indicated in Fig. 16. In form the individual spicules of the coronet closely resemble those of the genus Polystomum.

We now proceed to detail the results we have obtained as to the structure of the testis, and the development and form of the spermatozoa.

The lobules of the testis number from twelve to fifteen, and are of a different form, according as they are seen in transverse or horizontal sections of the body. In horizontal sections they

have a polygonal outline, while in transections they have an elongated oval shape, the longest diameter being directed dorso-ventrally (Fig. 14.) They are separated from each other by fibrous tissue, which forms a basement-membrane to the cells lining the lobules.

Highly magnified, each lobule is seen to consist of a central and a parietal part. The latter is formed of a single layer of cells of uniform character and size, while the remaining contents of the lobule are varied in structure, and present all the stages of the development of spermatozoa. The parietal cells of 16 μ in diameter form a continuous lining for the wall of the lobule, and even extend into the cavity, and mingle there with the other elements; their nuclei measure 12–15 μ and are surrounded by a sparing cytoplasma which does not stain, while the nucleus itself stains deeply; and in summer specimens of Sphyranura is generally in one of the stages of division. (Fig. 15, *a*.) The chromatin, when it is in the skein form, is usually accompanied by a nucleolus of the same nature, but not of the same staining capacity. These cells we regard as the mother-cells of the spermatozoids. There are in some series of sections a few cells, on the average not more than half a dozen, which lie in the parietal layer of cells, and are widely different from those just described. They measure 16–20 μ, and their nuclei about 6 μ. The cells of this class are somewhat irregular in form, have a feeble staining capacity, the cytoplasma being formed of delicate, wavy fibrils, the meshes of which contain a hyaline substance. The nuclear cavity is filled with a clear homogeneous substance, in which is a single nucleolus very feebly stained. These cells we consider to be the persistent remnant of those out of which the more abundant parietal cells have arisen.

In the cavity of the lobule are, as already mentioned, all the stages in the development of the spermatozoa. Of these the one which always attracts attention at first is the sphere or ball of cells (Fig. 14, Fig 15, *c*, *d*, *e*) which is the result of the final division of the cells of the lobule, and is usually termed a spermatogemma. There may be as many as fifteen of these spheres in a single lobule at one time, and the average diameter of each is about 38 μ. The cells forming them number

over forty, and in the newly developed sphere are cylindrical in shape and radiate from the centre to the periphery adjacent to which the nuclei are placed. (Fig. 15, *c*.) When the nuclei of these cells are round they do not measure more than between 3 and 4 μ. On the examination of many of these spheres it is found that they present various stages of development, the final outcome of which is the fully-developed spermatozoids. This is represented in Fig. 15, *c*, *d*, *e*, and *f*. In *c* we have the initial stage with a small central cavity; in *d* the latter and the cell outlines have vanished, while the nuclei are elongated; in *e* this elongation of the nuclei is carried still further, so that they now extend from the periphery to the centre of the sphere. In all these stages the cytoplasmic element of each cell degenerates more and more, till finally it becomes fluid, and is probably partly absorbed in that condition by the developing spermatozoid. In *f* we have briefly represented the different stages of the development of the spermatozoid out of the round nucleus. Of great importance is the arrangement and character of the chromatin in these; at first it is in the form of delicate looped fibrils which are interwoven with each other, but in the next stage these fibrils are shorter; then they take the form of excessively fine rodlets, the axes of which are nearly parallel with the elongation of the nucleus, while in the next stage again these become dissolved, and now the elongated nucleus stains uniformly and homogeneously. Finally, the outer or peripheral end of the elongated body becomes pear-shaped, the remainder attenuates to a fine fibril, which forms the tail of the spermatozoon, and the chromatin is transferred to the pear-shaped head, which is the only part that now stains with coloring reagents. The cytoplasma becomes, as already stated, a fluid, and this, coupled with the fact that there is no formed membrane proper to the original sphere, easily permits of a rearrangement of the spermatozoids with respect to one another. This rearrangement is a gradual one, resulting in a sheaf of about 12 μ in thickness, in which the heads of the spermatozoids are all directed one way.

In the cavity of a lobule also are irregular masses formed of dividing cells. (Fig. 15, *b*.) As the nuclei of these present various sizes, intermediate between those of the parietal cells (15 μ) and those of the fully formed spheres (4 μ), the

masses with the largest number of cells having the smallest nulcei, and as the number of cells present in the different masses varies in arithmetical proportion, we have come to the conclusion that a single parietal cell, when it falls into the cavity of the lobule, gives rise by repeated division to a sphere or spermatogemma, each division being represented by a clump or mass of cells, which vary in number with the number of divisions the cells have undergone. The irregular masses of cells resulting from the final division become spherical, the individual cells arranging themselves radially round a sort of segmentation-cavity, and then moving toward the periphery of the sphere.

Our observations as to Spermatogenesis in Sphyranura agree with the main details of the brief sketch given by Schwarze[1] of this process in *Distomum endolobum*, but are at variance with this observer's in one important respect, namely, as to the share which the cytoplasma takes in the formation of the spermatozoid. This author describes the head of the spermatozoid as arising out of the nucleus while the cytoplasma gives origin to its tail. The structures in question in Sphyranura and their mode of development may be so easily observed that we cannot permit ourselves to doubt the conclusion indicated above, — that the spermatozoids arise wholly from the nuclei of the sphere or spermatogemma.

Kerbert's and Looss' descriptions of spermatogenesis in Distomes appear to us inexact in some points. They find crescentic cells, the concave face of each of which may contain the heads of a number of spermatozoids. Sommer found the heads inserted in the substance of the crescentic cells. We have observed appearances like those figured by Looss and Kerbert, but they are accidental rather than natural. For instance, the nuclei of the parietal layer of the lobule, and the nuclei like them in its cavity, are very frequently in that initial stage of division in which a part of the nuclear membrane is free from contact with the chromatin-filament, this part answering to the "Pole" of Rabl. Oblique views of this Pole often give the appearance of a crescentic cell, and if the heads of a sheaf of spermatozoids are seen over against one of these we get the appearances figured by the above-named observers.

[1] Zeit. Wiss. Zoöl., XLIII., 73.

Zeller had given such a detailed account of the process of sexual union, and of the copulatory cushions and lateral vaginæ in *Polystomum integerrimum*, and structures of so entirely similar a character had been described by the senior author in *P. oblongum*, from the musk-turtle, that we were prepared to find similar conditions in Sphyranura. Here, however, no lateral vaginæ exist, or rather they are only represented by the two receptacula seminis, and the ducts which lead from them to the central ootype. The sperm with which these receptacles are generally packed is consequently introduced through the genital sinus, and inwards to the ootype through the uterine tract of the oviduct, the direction of the ciliary movement in which must favor its inward passage. We at first observed that the investing membrane was often incomplete in sections opposite the receptacula, and supposed that this pointed to the existence of a direct communication with the outside; but repeated attempts to demonstrate the existence of such apertures failed, both with fresh and preserved specimens, so that we concluded that the fragility of the wall of the body there was owing to the distention of the underlying cavity.

We conclude that these receptacula, whose walls are muscular and under control of certain large isolated ganglion-cells similar to those which we have spoken of in connection with the contractile bladders of the excretory system, serve as reservoirs of sperm which is introduced through the genital aperture before the uterine cavity is occupied by a mature egg, and is discharged thence as the ripening of the eggs demands. What the source of the sperm in the receptacula is we are unable to say; we have never seen any individuals in copula; and it is obvious, from Fig. 16, that self-impregnation would be rendered quite possible by the simple closure of the aperture of the genital sinus.

Fertilization takes place, not in the ootype, but within the ovary itself. We have frequently observed spermatozoids between the ripe egg and the ovarian wall, and, indeed, have detected in the ripe egg a male pronucleus, with the surrounding disturbance of the cytoplasma. Some cases of polyspermy were likewise noted; but we have never seen segmentation taking place, and conclude that the egg leaves the ovary for the uterus on the completion of the act of fertilization.

It will be observed that the ootype is here formed of two distinct parts,—one which receives the ducts from the receptacula seminis, and another in which centre the ovarian and uterine tracts of the oviduct, as well as the overflow-tube to the intestine. The latter part hardly affords accommodation for much more than the large ovarian ovum, so we gather that the arrangement of the food-yolk balls around the egg takes place in the uterus.

The slight pressure of a cover-glass is no doubt sufficient to bring about changes, which would perhaps not occur in the natural condition; but it is very interesting to watch the effect of such pressure upon the receptacula and the vitellogen. Masses of sperm, and especially of yolk, are forced into the first part of the ootype, whence they are discharged by a spasmodic movement into the second. Here they are at once caught in a strong ciliary current, and are rapidly swept into the intestine through what may be termed the overflow-tube. This tube and the ovarian tract of the oviduct occupy approximately the same transverse plane; the oviduct originating near the dorsal surface from the ovary, and running straight toward the ventral surface to its opening in the ootype, from which there likewise diverges the short, wide overflow-tube to the intestine. We had not observed the discharge of the sexual products into the intestine until we had puzzled a good deal over the ending of this overflow-tube, and had satisfied ourselves that its epithelium and the intestinal epithelium became continous at the spot marked * in the diagram. We regard this as an interesting confirmation of Ijima's discovery of the true nature of the "internal vas deferens" of Polystomum, especially as we were hunting for such an internal connecting tube, and had forgotten his note upon the subject. How such an economical method of disposing of surplus yolk can have been arrived at, whether by the modification of a Laurer's canal or otherwise, we are unable to say. It is probable that further careful researches on other monogenetic and digenetic Trematodes will throw light upon the subject.

The fact that the surplus material is conducted off by this channel may probably be more easily observed in Sphyranura than in any other monogenetic form, owing to its accessibility to high powers. We have noticed, after prolonged pressure, a

stagnation of yolk in the overflow-tube, with the result that the new yolk-balls forced out are pressed into the uterus and even into the ovarian cavity. The latter, of course, never takes place in ordinary conditions, and we have never seen the former occur except after prolonged pressure.

We now proceed to give some details as to the minute structure of the female organs.

As already stated the lobules of the vitellogen are chiefly disposed on the lateral, anterior, and posterior faces of the intestinal branches; they average about 48 μ in diameter, and are composed of cells and nuclei of 28–32 μ and 11 μ respectively. In preparations made with Flemming's fluid the cytoplasma of these is obscured by the yolk-granules, which are blackened, while the nucleus contains its chromatin in the form of short threads, nucleoli, and granules. In corrosive sublimate preparations, on the other hand, further information is to be obtained as to the cytoplasma, which is disposed in a reticulum, as represented in Fig. 17, from the meshes of which the yolk-granules have been dissolved, probably by the acetic acid employed with the sublimate.

The Ovary is an oval body, the long axis of which, in fixed preparations, is directed dorso-ventrally, and measures on the average, 100 μ by 160 μ. Of the two poles, that which contains the ripe and maturing eggs is dorsal, so that the oviduct originates near the dorsal surface, as already explained. In fresh specimens under the cover-glass, the pressure of the latter causes the long axis to be horizontally placed. There is a distinct thickened membrane, formed by the fibrous tissue surrounding the organ, and in this membrane one can find sometimes a flattened and elongated nucleus, usually less than 2 μ in thickness, which may be regarded as belonging to the fibres forming the membrane.

In the ovary itself there are cells of two sorts, namely, those which line the internal surface of the membrane, and which therefore may be named the parietal cells, and those which closely fill the cavity, and represent every stage of the ripening ovum. (Fig. 18, *a*, *b*, *c*.) The parietal cells (*a*) vary considerably in size, averaging in thickness, however, about 6–8 μ; their nuclei measure 6 μ in thickness by 11 μ in length, and have their

chromatin disposed in granules in the nuclear cavity. The cytoplasma is also slightly granular, and either takes a feeble stain in cochineal or none at all. These cells are quite distinct from the structures in the fibrous membrane, and may be compared to the parietal cells of the testicular lobules, for out of them arise by increase in size and division, the cells filling the cavity of the ovary. Such a division is represented in Fig. 18, *b*. and a number of examples of the different stages of this division can be made out in each ovary. One of the resulting two cells falls into the cavity of the ovary and takes there, through pressure of the adjacent cells, a wedge-shaped form. Owing to the fact that the single ripe ovum is placed at one pole of the ovary, while the newly formed ova are added at the other pole, the intervening cells have their long axes parallel to each other and directed transversely across the cavity of the ovary. Their cytoplasma is granular, has a certain amount of staining capacity, and is limited peripherically by a thin membrane, on the inner face of which is a layer of definitely placed granules, often giving the impression of striation to the membrane. The nuclei are oval, and vary in diameter from 12 μ to 60 μ, according to the age and condition of ripeness of the ovum. The chromatin is disposed either in nucleoli, which may measure as much as 8 μ in thickness, or in granules arranged in festoons throughout the nuclear cavity. The nucleoli are spherical, contain a vacuole, and, when they are of the size given, only one is to be found in each nucleus.

The ripe ova measure about 55–60 μ, and their nuclei about 35–40 μ. The latter, in the fresh condition, are large, clear structures, enclosing one or more nucleoli, which may or may not have vacuoles in their interior, but in the fixed condition there are found besides the same festoons of chromatin granules exhibited in the unripe ova. The cell-substance, clear and homogeneous in the fresh condition, in the fixed state is seen to contain granules disposed in the meshes formed by wavy fibrils of the cytoplasma. The cell-membrane is thick and striated, the striations being due to delicate canals traversing it.

With regard to the minute structure of the oviduct and the other sexual ducts we do not find anything worthy of remark, except the strong ciliation of the uterine tract of the oviduct, the central part of the ootype and the overflow-tube. Unlike the

ovarian tract of the oviduct, the uterine tract is considerably folded, and undergoes a marked constriction before it passes into the uterus. This organ is imbedded in a mass of unicellular shell-glands, a great many of which empty their contents into the oviduct at the above-mentioned constriction, while others pierce the dorsal and ventral wall of the uterus, towards which their slender ducts converge. (Fig. 16, *sgc.*) The shell-gland cells extend out over the intestinal branches, and consequently their narrow ducts, converging toward the uterus, produce a characteristic coarse striping over that organ, especially if they are loaded with the shell-material, which is likely to be the case if a mature egg has just been discharged and the glands are about to furnish the shell for the next egg. On the other hand, the ducts of the cells are not conspicuous unless they are filled with the yellow granules of shell-stuff, and generally a good deal of difference may be traced in the cells themselves according to the state of their functional activity.

We have frequently observed formless masses of shell-material within the uterus, perhaps ovum or egg as centre, which has not been enveloped in food-yolk balls. How the shell is moulded upon the surface of the latter we are unable to say. It is possible that the epithelium of the uterus plays an active part in this process. In the empty uterus these lining-cells are tall (60 μ), and have a markedly reticular cytoplasm, the threads of which are chiefly disposed in the long axis of the cell (Fig. 16), toward the free end of which is to be seen a nucleus of 12 μ in diameter; when, on the other hand, the uterus is dilated with an egg the epithelial cells are flattened into a very different form.

The uterus never contains more than a single egg, resembling in this respect that phase of *Polystomum integerrimum* which occurs within the gill-chamber of the tadpole, but unlike the ordinary form from the urinary bladder of the frog.

It will be remembered that *Polystomum oblongum* and *ocellatum* differ from *P. integerrimum* as to the development of the egg, which takes place entirely outside the body in the latter, but gives rise to a Gyrodactylus-like larva within the uterus in the former. We have not observed development advance so far in Sphyranura but we have reason to believe that this genus stands midway between the above-mentioned forms in regard to the extent of embryonic development within the uterus. We pro-

pose to return to this subject after we have obtained more definite information as to this point.

Zeller has pointed out that the rounder and smaller Polystomum eggs bear a short hook-like process at the hinder end. A similar process is to be frequently seen on the hinder, more pointed ends of the eggs in Sphyranura. These are probably to be interpreted as persistent rudiments of the polar threads so common in other monogenetic Trematodes.

Explanation of Plate.

Minute histological details are represented as seen with Leitz's $\frac{1}{12}$ inch oil-immersion, although the same enlargement, as will be seen from the figures, has rarely been retained.

FIG. 1. — Sphyranura, from ventral surface; × 35; *m*, mouth; *pp*, prepharnyx; *mp*, muscular pharynx; *lg*, area of lateral ganglion; *ai*, anterior arch of intestine; *ga*, aperture of genital sinus; *cb*, contractile bladder of water-vascular system; *be*, bulbus ejaculatorius; *u*, uterus with a mature egg; *rs*, one of the receptacula seminis; *ov*, ovary; *, point of entrance of overflow-tube into intestine; *vi*, vitellogen. [The ducts from the female sexual organs may be seen to converge to a central ootype.]; *t*, testicular lobules; *pi*, the posterior arch of the intestine.

FIG. 2. — Portion of investing membrane, with tactile organs fresh; × 300.

FIG. 3. — Section through centre of caudal sucker, from a sagittal series stained with iodine-green; *c*, *c'*, the cuticularized limiting layers of the sucker; *m*, the muscle of the central hooklet; × 155.

FIG. 4. — Caudal lamina from ventral surface, showing the form of the hooks and the position of the hooklets; *a*, *b*, *c*, the muscles of the large hooks; at *d*, some of the muscles of the suckers; × 100.

FIG. 5. — Hooklets and eyelets; × 550.

FIG. 6. — From a horizontal series prepared with Flemming's fluid and alum-cochineal. The section is in the plane of the suprapharyngeal commissure, *sc*, and the dorsal wall of the

muscular pharynx, *mp*. It passes through the anterior arch of the intestine; *m*, radial muscles of the upper lip; *g*, ganglion-cells; *r*, a renal cell; × 165.

FIG. 7. — Diagram of the nervous system of Sphyranura from the ventral surface; *lg*, lateral ganglion, opposite the suprapharyngeal commissure; *ic*, infrapharyngeal commissure; *l*, lateral; *vl*, ventro-lateral nerve-cords with their commissures *c'*, *pcc*, the precaudal, and *cc*, the caudal commissures.

FIG. 8. — *a*, ganglion-cell from Flemming's fluid preparation; *b*, transverse section of process of a ganglion-cell; × 1000.

FIG. 9. — *a*, muscular fibre from caudal lamina of a fresh specimen treated with chloroform; *b*, muscular fibre fixed in contraction; × 1000.

FIG. 10. — From a horizontal preparation, like Fig. 6, near the caudal lamina; *a*, *b*, superficial and deep layers of the investing membrane; *c*, circular; *d*, longitudinal muscles; *e*, muscle-nuclei; *f*, and *h*, cells of the parenchyma; *g*, renal cell; × 600.

FIG. 11. — Intestinal epithelial cell from similar section; × 1000.

FIG. 12. — *a*, renal cell fresh in connection with a vessel filled with granules; *b*, renal cell, fresh; *c*, from a Flemming's fluid preparation; *d*, ciliated funnels; × 800.

FIG. 13. — Diagram of the female genital ducts; *rs*, the receptacula seminis, the ducts from which are joined by those from the vitellogen before they communicate with a reservoir in the middle line, which opens by a constricted passage into the strongly ciliated ootype (*oo*). Into this there open the ducts communicating with the uterus (*u*) and ovary (*ov*), as well as the overflow-tube into the intestine, the mouth of which is indicated by the *. The arrows show the direction of the ciliary movement.

FIG. 14. — From a transverse section; *d*, dorsal; *v*, ventral surface; *vn*, *ln*, ventral and lateral nerve-cords; *vd*, vas deferens; *vi*, vitellogen; *p*, parietal cells of a testicular lobule, which is filled with sperm-cells in various stages of development; × 220.

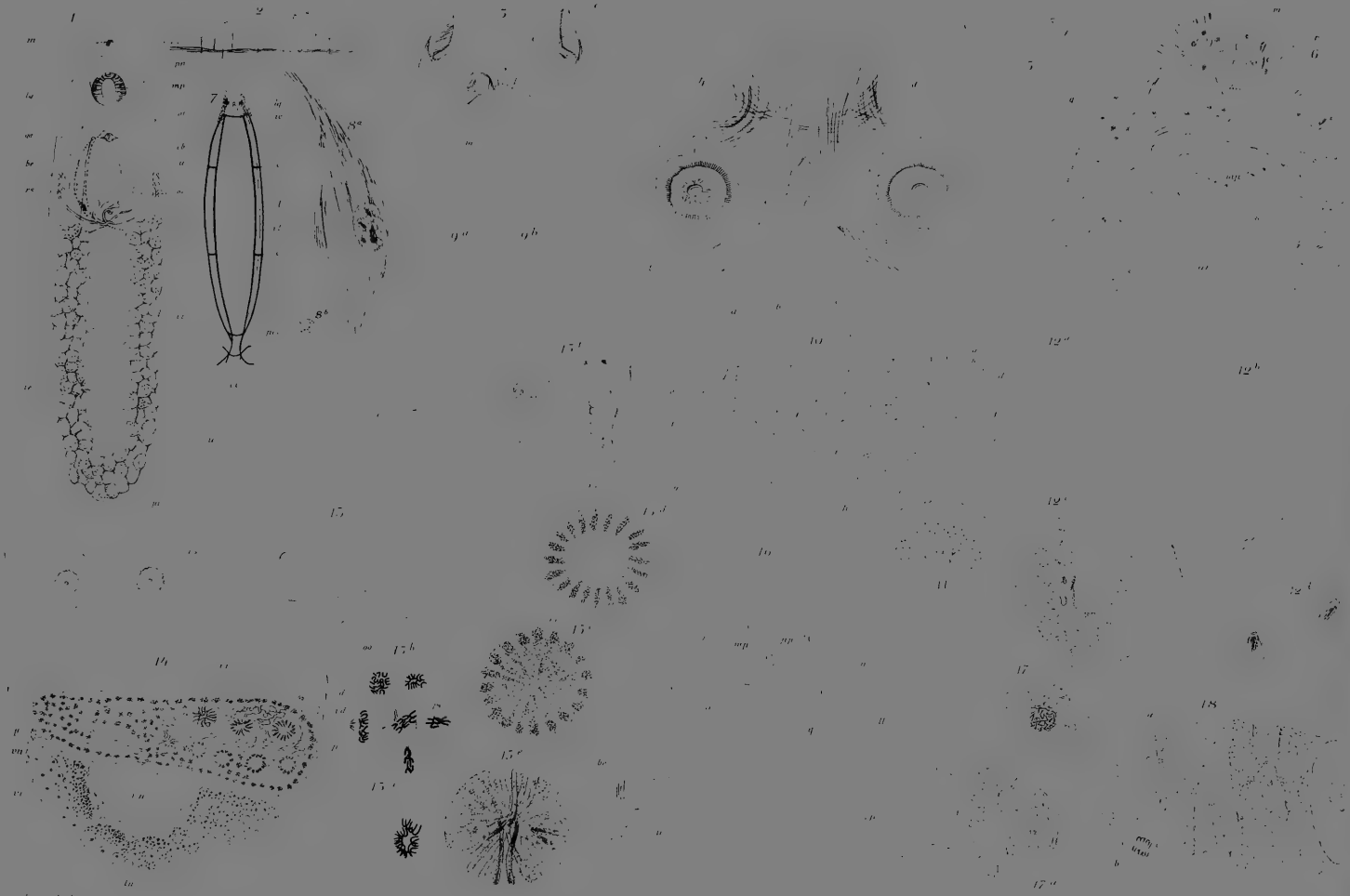
FIG. 15. — *a*, parietal cells of testis; one of these is dividing; *b*, first stage in formation of a sperm-sphere; *c*, a sperm-sphere with segmentation-cavity; *d*, a sperm-sphere with elongated

nuclei, and fused protoplasm; *e*, nuclei further elongated; *f*, stages in conversion of nuclei into ripe spermatozoa; *b*, *c*, *d*, *e* × 590; *a*, *f* × 1000.

FIG. 16. — From a sagittal series prepared with corrosive sublimate; *u*, upper, *ll*, lower lip; *m*, mouth; *pp*, pre-, and *mp*, muscular pharynx; *ai*, anterior arch of intestine; *gs*, genital sinus; *be*, bulbus ejaculatorius; *u*, uterus without an egg; *sgc*, shell-gland cells; × 165.

FIG. 17. — Cell of the vitellogen with corrosive sublimate; *a*, lobule of the vitellogen with Flemming's fluid; × 650.

FIG. 18. — Ovary from a Flemming's fluid preparation; a, parietal cells; *b*, dividing cell; *c*, a ripe ovum; × 375.



THE DEVELOPMENT OF THE COMPOUND EYE OF CRANGON.

J. S. KINGSLEY, SC.D.

FOR several reasons I have thought it best to present my observations on the development of the compound eye in the common shrimp, *Crangon vulgaris*, in advance of my complete paper on the embryology of that form. This seems the more desirable since I differ from Reichenbach, the most recent writer on the development of the compound eye, in several important points.

Methods.

The eggs were hardened by means of Perenyi's fluid, followed by alcohol of increasing strength, a process which works well with almost all arthropod tissues. In most instances they were stained entire with Grenacher's alum-carmine, though in some instances Kleinenberg's hæmatoxylin or Grenacher's borax-carmine were employed. In the later stages, where the deposition of pigment in the eye interfered with a clear vision of all the structures concerned, the following course was followed: The eggs were sectioned as usual, the sections being fastened to the slide with Mayer's albumen fixative. After melting the paraffin and allowing the sections to drop into the adhesive mixture, the imbedding material was dissolved in turpentine, and this in turn was washed away with alcohol (95%). The sections were then covered with a mixture of equal parts of nitric acid and 95% alcohol, which was allowed to remain until the pigment was removed, — a process requiring from ten to fifteen minutes. The slide was next washed with strong alcohol, and the sections stained deeply with Kleinenberg's hæmatoxylin, and the excess then removed with acid alcohol in the usual manner. The sections were then mounted in balsam.

In the figures which illustrate this article no artistic or diagrammatic latitude has been allowed, except where expressly stated, each nucleus being drawn by the Oberhäuser camera. While in surface-views of the early stages the cell-boundaries seem very distinct, they are but very rarely visible in the sections of embryonic stages (possibly the result of Perenyi's fluid), and hence the following account deals almost wholly with nuclei rather than with cells.

Development.

The first appearance of the compound eyes is shown in Fig. 1, which represents the egg of Crangon very soon after the closure of the blastopore. In the median line is shown the, as yet, undifferentiated germinal area of the body while at *ol*, on either side, are the optic lobes, or better, optic discs. These three regions indicated are readily recognized by the character of the cells. These in superficial area are smaller than those of the rest of the blastoderm; but in section they are much deeper. Each optic lobe is connected with the central area by a row of but slightly larger cells, the ultimate fate of which is to enter into the composition of the brain, though all parts of this organ are not derived from them. As seen in a side view, the optic lobes are oval, the major axis being about one and one-third the length of the minor. The distance between the optic lobes differs somewhat in different eggs, being usually a little less than in the specimen figured. This may, however, be a result of development, for not all the eggs taken from the same shrimp seem to be of exactly the same age. A transverse section of one of the optic lobes at an early stage is shown in Fig. 2. The cell-boundaries are not visible, but the arrangement of the oval nuclei clearly indicates that here the epiblast is to be regarded as but a single cell in thickness. Immediately beneath the slightly stained protoplasm comes the yolk. Near the centre of the lobe is seen the first stage of the optic invagination. Seven of the nuclei are sunk below the rest, and form in outline a shallow cup (*oi*), the concavity of which is directed outward. The larger nucleus at one side is about to divide, although no karyokinetic figures are visible.

This pit rapidly grows deeper, and, extending outwards, downwards, and forwards, soon comes to occupy a position

beneath the anterior and outer part of the optic disc, before any striking changes are visible in the external appearance of the embryo. The relations are shown in Fig. 3. The cavity of invagination is small, but comparatively wide, and it retains its size until the lips begin to grow together, when it becomes more flattened. The posterior part of the optic lobe, together with the cord of small cells reaching back to the rudiments of the embryo (Fig. 1), give rise to the brain, and need not be followed further here; the anterior part of the lobes and the invaginated pit alone concern us. The separation of the pit from the parent epiblast is completed at about the time of the budding of the first pair of appendages, and the appearance of the stomodeum. At this time the relation of the invaginated sac to the surrounding parts is shown in Fig. 4.

We have now three layers to deal with, all of which are concerned in the development of the optic apparatus. The external one is the epiblast or ectoderm (*e*), while the two others are derived from the invaginated portion of the same primitive layer. The inner of these, the layer which lies against the yolk of the egg, gives rise, as will be seen later, to the chain of ganglia and nerves which lies within the stalk of the adult eye, connecting the optic apparatus with the brain. Hence the name *gangliogen* (*g*) may be appropriate for it. The other wall of the optic cavity becomes closely pressed against the ectoderm, and from the fact that it subsequently develops all the retinal portions of the eye, I have called it the *retinogen* (*r*). At the stage figured one may notice a correspondence in number, size, and position of the nuclei of all three layers. This equality lasts but a comparatively short time. The optic cavity soon becomes flattened, so that the walls nearly touch, but absolute contact is never reached. At the same time the nuclei of both gangliogen and retinogen are undergoing division, so that the extent of both layers is increased, without, however, losing their primitive character of being one cell in depth. The epidermis does not divide as rapidly, and hence there results a lack of agreement between the nuclei of the retinogen and those of the overlying layer. This is shown in Fig. 5, which represents the eye at the period when two pairs of appendages are outlined.

There is another feature in this figure which needs a moment's

attention. The nuclei of both gangliogen and retinogen have become elongated at right angles to the surfaces of the layers, and several nucleoli are visible in each. The subsequent stages increase this peculiarity until the nuclei attain a length of three or four times their shorter axis. In this process those of the gangliogen are in advance of those of the other layer, and in them division takes place first. Then the retinogen follows through almost exactly the same phases, which can be understood by a reference to Fig. 6, which represents the eye in an embryo, in which seven pairs of appendages have become outlined. Here the nuclei of the retinogen are elongate, but those of the gangliogen have divided transversely, each giving rise to a series of nuclei arranged in a single row, and directed towards the centre of the egg. This structure does not show so plainly in the figure as it would were the figure more diagrammatic, but it is readily recognizable on one side of the drawing, which is an exact copy of the section. It is next to impossible to cut the sections so exactly as to pass precisely in the desired plane, but a study of the consecutive sections clearly shows this radial arrangement, and also that all the nuclei in each row arise from the corresponding nucleus of the gangliogen, and extend inwards. This process of division continues until each ganglionic row consists of six nuclei before any other change in this region occurs. When these ganglionic rows are formed the nuclei of the retinogen have reached their greatest length, but have not yet begun to divide transversely, nor is there as yet any indication of an arrangement into groups. The general appearance, as shown in sections, can be seen from Fig. 6. The pyriform shape of some of the nuclei is due to their being cut obliquely; it is further noticeable that the more dorsal nuclei are more elongate than those nearer the ventral surface. The ectoderm is a thin layer with scattered nuclei.

At this stage still another element begins to enter the eye. Fig. 12 is a section from the same embryo as that shown in Fig. 6, but is taken a short distance further back. Here all the features described above are clearly seen, but in addition there are some others. In the lower centre is the cephalic ganglion with the fibrous portions already developed. Immediately above this is shown a thin layer of tissue with elongate or fusiform nuclei, easily distinguishable from all the rest. This is the

anterior extension of the mesoderm, and at the left side it may be seen extending itself down between the cephalic ganglion and the gangliogenic tissues towards the optic cavity. Later sections show that it then turns upwards, grows into the cavity, but retains its primitive character and its single-celled thickness until after the young shrimp escapes from the egg. I have not fully traced its later stages, but have evidence to show that in the adult it forms the thick layer of pigmented connective-tissue, which sheathes the nerve-fibres between the ommatidia and the outer ganglion of the eye-stalk of the adult.

The next series of changes are shown in Fig. 7, which represents a portion of the eye of an embryo much further advanced. Only seven appendages are as yet outlined; but these show plainly the distinctions between maxillæ and maxillipeds. The abdomen is cylindrical, and is terminated by a bifurcated telson armed with the typical fourteen spines; the ganglia of the abdomen are outlined, the heart is formed, and has begun to beat, and the deposition of pigment has begun in the eye, though this is not shown in the figure under discussion.

First it is to be noticed that the ectodermal nuclei have increased in number, and have come to correspond in position with those of the underlying layer. The nuclei of the retinogen which, in the stage last described, were elongate, have divided just as did those of the gangliogen, and each has given rise to five nuclei arranged in a row. These rows are arranged in sets. In the sections two will be seen closely appressed to each other, and separated from the adjacent pairs by a rod of apparently structureless material. As subsequent development shows, this intervening substance is the rudiment of the crystalline cone, and the adjacent rows of nuclei in reality belong to different ommatidia or optic elements. Horizontal sections show that there are four of these rows to each ommatidium, but only two of these can be shown in one section. The optic cavity retains its early shape, and the mesoderm (exaggerated in thickness in the drawing) shows no change. In the ganglionic layer a change is visible. The rows of nuclei have broken in twain, and have formed the rudiments of two ganglia, one retaining four, and the other two of the nuclei of the last stage. Between these two a fibrous area has arisen, in which occasional nuclei, staining less deeply than those of the surrounding parts, can be seen.

The origin of these, I have not determined. Beyond the second of these ganglia is a second fibrous portion, which connects the visual organs and the optic ganglia with the brain. This connection did not exist in the earlier stages, but I am unable to say whether the fibres grow out from the supra-œsophageal ganglion or from the nervous rudiments of the eye. It should be mentioned that the rows of ganglionic nuclei at this stage are opposite the crystalline cones, and alternate with the rows of retinal nuclei.

Fig. 8 is a portion of the eye of the same stage still further enlarged, but taken a little further back. It shows the first appearance of the pigment, which is plainly not of mesodermal but of epidermal origin, arising from the retinogen. The cell-outlines are very indistinct; but it is evident that the pigment is deposited first in the inner ends of the inner cells of the retinal rows, or those which form the outer wall of the optic cavity. The same figure also shows more clearly the distinctness of the epidermal layer, and also that the crystalline cone is divided through the middle. At this stage, however, one cannot see which of the cells arising from the retinogen are concerned in the secretion of the cones; but this point becomes apparent at a later stage, when the embryo is nearly ready to leave the egg.

Figs. 11, 13, and 10 will explain the various processes involved. They are taken from an embryo in which the yolk has become nearly absorbed, and in which the optic stalks have begun to bud out. Fig. 11 is an actual section, which is diagrammatic only in that it omits, for clearness, a part of the pigment. Fig. 10 is drawn from stained and teased sections, and shows the shapes of the cells of the retinogen. Fig. 13 is a diagram constructed from both of them. It contains, however, nothing which I have not clearly seen. At this point it is necessary to add some terms for the various portions, and I have adopted the terminology introduced by Patten (*'86*). The epidermis cells (*c*) are now distinct from those of the retinogen, and have begun the secretion of the cuticula, which is modified into lenses (*l*) over each crystalline cone. The extension of the cell protoplasm across the end of the crystalline cone, as represented in Fig. 13, is not certain. It does, however, take such a position in later stages (after hatching), and so I have introduced it into the diagram. In the rows of retinal nuclei there has

been a development and a differentiation. Each of the cells (Fig. 10) has become greatly elongated, the protoplasm extending out to a considerable distance from the nucleus in a thread-like prolongation, which apparently (Fig. 11, 13) reach from the wall of the optic cavity to the epidermis. The nuclei are placed at different heights in these cells, and the tail-like prolongations are arranged in layers around the crystalline cone. Exactly which of the cells are inside and which outside I am not able to determine, except in the case of the distal member of the retinal row (*r*). This is clearly the one which abuts against the crystalline cone, and is the crystalline cone-cell or retinophora. Of these there are four surrounding the cone, and their walls touch so that they form a cup or calyx in which the cone is situated, and from which it is secreted. Below the calyx the ends of the retinophoral cells unite to form a slender pedicle (*pd*), which traverses the whole of the pigmented layer as it at present exists. This pedicle is clearly the rhabdom of Grenacher, and it is as plainly formed, as was first demonstrated by Patten, by the retinophoræ, and not as a secretion from the surrounding pigment-cells. On this point I am fully convinced of Patten's accuracy.

The retinophoral nuclei still retain their position (as they do throughout life) close to the epidermis, but among the others of the retinal rows a separation is visible. Of these cells, which we may call the pigment-cells, two remain in close contact with the retinophoral nuclei, (p^1, p^2, Fig. 11) while the two remaining nuclei (p^3, p^4) are separated from the rest and from each other by a considerable interval. A comparison of Figs. 8 and 11 will show what has taken place. Pigment-cell 4 has retained its position at the margin of the optic cavity, but between it and pigment-cell 2 a considerable interstitial growth has occurred, mostly accomplished by an elongation of the protoplasm of the cell. The deposition of pigment, which, in Fig. 8, had just begun at the proximal surface has now covered the entire nucleus, and has extended thence up the filiform prolongation of the cell (see also Fig. 10) until it has reached the level of the nucleus of pigment-cell 2. These prolongations are the well-known rods or bacilli (*b*) so characteristic of the arthropod eye. The mesoderm still retains its primitive character at this stage, and as yet no connection is made between the

ommateal and the ganglionic portions of the eye. Fig. 13 shows these features well, but it must be borne in mind that in it the region of the crystalline cone is exaggerated in order to show the surrounding cells.

The subsequent growth of the ommateum can readily be understood without a figure. The retinophoræ continue the secretion of the crystalline cone and with the growth of this portion of the eye the nuclei of the cells are forced from their present position. Those of the retinophoræ are driven to occupy a place on the ends of the crystalline cones while pigment-cells 1 and 2 are forced to take a much deeper position and the filamentary terminations of all the pigment-cells (the bacilli) become much more slender. This is especially the case with the outer or distal ends of these organs. The deposition of pigment continues and extends in each cell as far as the outer layer of nuclei. In the lower ends of the retinophoræ, where they unite to form the pedicle the changes are obscure. The pedicle becomes gracefully swollen and is separated from the calyx by a slender style. In the interior of the pedicle appear (how, I know not) curious cross bands (vide Patten, *'86*, Pl. xxxi., Fig. 72.) In the ganglionic regions the changes are but slight and of the same character as those already described. There is an increase in the number and size of both ganglia and the intermediate fibrous portions; and in some way fibres come to cross the optic cavity, a filament going from each ganglionic row to the corresponding pedicle. How these arise I am not able to say, nor have I seen that complex arrangement of the fibrillæ and the nerve terminations described by Dr. Patten. I have, however, been able to trace the axial nerve through the pedicle and style and into the distal portion of the crystalline cone. The character of this axial nerve, together with theoretical considerations, render it probable that it is an outgrowth from the ganglionic portion of the eye.

The changes which occur in the optic cavity are considerable, but I have not satisfactorily traced the steps. The cavity becomes greatly expanded, so that it eventually measures half the width of the ommateal portion of the eye. The mesoderm contained in it becomes correspondingly developed and apparently forms a neurilemma which sheathes each nerve-fibre crossing the cavity, and in which there is a considerable deposition of pig-

ment. The nuclei are scattered, but I should not regard the figure given by Claus (*'86*, Pl. vii., Fig. 6) of the connective tissue elements in the eye of Branchipus, as representing the corresponding portion of Crangon. In the latter there exists, besides the thin neurilemma, long club-shaped and knobbed masses which follow the general course of the nerve-fibres and which are deeply pigmented.

The differences between the adult eye of Crangon and that of Peneus, as described by Patten, are considerable, but they cannot well be elucidated without illustrations. Still the homologies between the two can be readily traced, even to details, and my sections I regard as confirming in every respect, except nerve terminations, those of Patten. The finer ramifications of the nerve fibrillæ I have not seen, nor have I used proper means to do so.

The development of the compound eye has been studied by several authors. In the older works we frequently find allusions to it, but usually as a mere mention of the deposition of pigment in this region. Dohrn (*'70*, p. 121 of Separate) gave as good a description of the processes involved as could have been obtained from surface views. He used no sections and unfortunately he does not figure any of the features he describes.

Bobretzky (*'73*) was the first to give any account of the growth of the compound eye as revealed by sections. He studied it in Astacus and Palæmon, but as his paper is written in Russian I have been obliged to depend on the abstracts given in Hoyer (*'75*) and in Balfour (*'81*). This author did not see the invagination, and hence his whole account is modified. He derives the crystalline cone-cells (retinophoræ) and "Semper's nuclei" (the corneal epidermis) from the epidermis, some of the pigment from intrusive mesoderm, and thinks that the retinulæ, etc., arise from a portion of the supraœsophageal ganglion, which early becomes separated from the rest. The plates illustrating the article clearly show that if we suppose an invagination, the whole development of the eye of these two genera is clearly reconcilable with that given above.

We shall recur again to the work of Reichenbach, but here must mention that in his earlier paper on Astacus (*'77*) he saw the optic invagination, although he interpreted it as contributing

to the development of the brain, and did not recognize its connection with the retinal elements.

The observations of Sedgwick and Kennel upon the development of the eye in Peripatus may be referred to in this connection, although the present author does not believe this form to be an arthropod. Sedgwick (*'85*, p. 461) first described the eye as arising by an invagination, and says that the outer wall of the vesicle forms the epithelium outside the lens, while the inner, from which the retina arises, remains continuous with the cerebral ganglion, and hence the eye of Peripatus is a "cerebral eye," an expression the force of which I fail to perceive. Kennel, not quite a year later, gives an account of the development of the eye in the two South American species studied by him, illustrating it with three figures. (*'86*, pp. 31–33, and 83, Pl. iii., Figs. 32–34.) The account agrees essentially with that of Sedgwick, except that Kennel maintains that the inner wall of the vesicle is converted into only the retinal elements, and that it does not unite with the cerebral ganglion until a late date. The eye of Peripatus, as was first shown by Balfour (*'81*, p. 395, and *'83*, Pl. xvii., Fig. 24) is totally unlike that of any arthropod. Balfour compares it with the eyes of molluscs, but a far more perfect parallel is to be found in the eyes of the Syllid worm Autolytus. In this genus sections show an almost exact reproduction of Balfour's figure, cited above, except that the nuclei of the ganglionic layer are visible all the way around the pigment until they merge in the prelenticular epithelium of the optic vesicle (Kennel's Fig. 34 shows a similar relation), and except that the layer of rods and cones in the eye of Peripatus is represented as divided by transverse bars or partitions which I cannot recognize in the eye of the worm. In Autolytus the region between the lens and the pigment is occupied by crystalline slightly staining bodies, in the centre of which the nerve-fibre can be traced, much as is represented in Patten's figure (*l, c,*) 142. These crystalline cones abut directly upon the spherical laminated lens.

To Locy (*'86*) is due the credit of first recognizing the existence of an invagination in the development of the arthropod eye. In the Arachnid Agelena he finds that both the median and the lateral eyes originate by an invagination, and, excepting the inner of the three resulting layers, the subsequent processes,

though not followed out in detail, correspond with the foregoing account of the development of the eye of Crangon. The epidermal cells secrete the lens, those of the retinogen give rise to the rods and cones, while the fate of my gangliogen was not traced. Dr. E. L. Mark has in press some further observations on the eyes of this form, in which the fate of the gangliogen is traced, and light thrown upon the nerve-supply. Mr. Locy also suggested a comparison of the eye of spiders with that of vertebrates, to which we will return later.

Claus, describing the development of the stalked eyes of Branchipus and Artemia ('*86*, pp. 307–324), gives far more details than any previous writer on the development of the crustacean eye, but I find it difficult to bring his account into correspondence with the processes seen in Crangon. His figures are lacking in histological distinctness, so that one cannot readily see the exact state of affairs. Claus does not recognize an invagination (if one exists), but says the first appearance of the eye is a broad ridge-like ectodermal thickening in the metanauplius stage which narrows beneath, where it contains the rudiments of the optic ganglion which is not yet separated from the "secundären Gehirnlappen." This ridge splits transversely, its outer layer forming the cuticula and the crystalline cones, and the deeper, nerve-rods and pigment. The figure (*l*, *c*, Pl. vii., Fig. 1.) quoted to illustrate this is as intelligible on the supposition that an invagination has taken place, and the relations of the optic ganglion are somewhat intermediate between those of Crangon and those of Astacus, as described by Reichenbach (*vide infra*). From this point on the account is not easy to understand. The author seems to have no suspicion of the errors of Grenacher (*vide* Patten), and as he has not deprived his sections of pigment, it is not clear how they are to be explained. In all except one of his figures he shows no nuclei beyond the pigment-zone. In that one he has two at the end of each crystalline cone which he interprets as hypoderm nuclei. Whether this be true, or whether he has missed the true epiderm-cells, and these are the nuclei of the retinophoræ, it is not easy to say, though, from the relations of the calyx to the crystalline cones, the latter seems the more probable view. The same figure (Pl. vii., Fig. 7) seems to afford additional evidence that the "rhabdom" of Grenacher is, in reality, formed

by the proximal ends of the retinophoræ, as maintained by Patten.

Carrière (*'86*) led by Locy's observations, publishes some results of studies on the eyes of Chrysides and Ichneumoids, which, in the absence of illustrations, are absolutely unintelligible. The hypodermis cells elongate, and then divide into two layers, and at the edge of this patch a pouch-like invagination begins, going obliquely downward, and carrying with it both layers in their "normalan Lage." Here arises the difficulty; how many layers result? The outer layer is described as the lens-building — the inner, as the potential retina forming; but which "inner" and which "outer" layer is referred to of the *six* which may exist is left in doubt. Next, some of the invaginated cells are represented as extending to the surface and sharing with the superficial cells in the formation of the lens. In the meantime the retinal cells have separated from the hypodermis, and form the pigment and rods, while the lens-forming cells have never severed their connection with that layer. Further, the invaginated pouch is never separated from its parent layer.

The last author to be mentioned on the development is Dr. Reichenbach, who has given us by far the most complete account of the development of a compound eye which has yet appeared. He describes (*'86*, pp. 85–96) the development of the eye of Astacus, and, in the earlier stages, there is a striking similarity in our results, when one bears in mind the considerable difference in the size of the eggs investigated. One difference is, however, to be noticed: I could not see the cell-divisions so plainly shown on the beautiful plates of the Frankfurt naturalist; and, besides, gastrulation takes place in Crangon before, not after, the formation of the optic lobes. Beyond this we agree perfectly in the position and direction of the optic invagination; but in Astacus the invagination is solid. This portion is represented as becoming separated from the parent layer and coming to lie beneath a patch of epiblast in front and a little outside the place of invagination. This patch, interpreted as the rudiment of the crystalline-cone layer, soon thickens, and becomes several layers of cells or nuclei deep, while the invaginated portion folds so as to inclose a cavity with an inner and outer wall. Here come our differences, which naturally affect all the subsequent stages.

I am strongly of the opinion that Reichenbach's layer of "krystallkegelzellen" is not a simple layer, formed by a proliferation of epidermal cells, but is really compound, as it is in Crangon. Then, if we recognize in his "Augeneinstulpung" not the whole of the optic invagination, but only my gangliogen, it is comparatively easy to make many of our results agree with as few differences as might be expected with individuals belonging to different orders. Then from the compound layer will arise, as in Crangon, the epidermal cells (Semper's nuclei), the crystalline cone-cells (retinophoræ), and the pigment cells. Thus, too, can be explained the inwandering mesodermal pigment-cells into the optic cavity. The resemblance is even closer. The four Semper's nuclei cap each crystalline cone, and below them are the nuclei of the four crystalline cone-cells, the bodies of which unite to form the calyx. Dr. Reichenbach does not say definitely whether the proximal ends of the retinophoræ form the pedicle, but his figures (especially Figs. 224 and 225) warrant the conclusion that here, as in Crangon and Peneus (Patten), they do. The history of rentinophoræ and pigment-cells is not traced with that detail that it is in the present paper.

Reichenbach describes a folding of his invaginated tissue into inner and outer walls, which certainly does not exist in Crangon. The outer wall forms, as in Crangon, rows of ganglionic nuclei, which divide into three, instead of two, portions at first (compare Reichenbach, Pl. xiv., Fig. 224 *Rl*, with my Fig. 7). It, however, seems to me that our author must be clearly wrong when (p. 92) he regards the outer of these layers as a retinula and the middle one as composed of rhabdoms, and claims that the primary invagination was for their production. The inner wall unites with the "optic" (my cerebral) ganglion.

As to the optic ganglion of Reichenbach, he regards it as of segmental value, and as distinct from the supra-œsophageal ganglion. Certainly this is not wholly true in Crangon, for the corresponding ganglion (it arises from the same region and in exactly the same way as in Astacus) is the supra-œsophageal ganglion, and those of the two sides do not become united by commissures until a comparatively late stage of development. Hence I do not regard it as an optic ganglion, but have restricted that term to the ganglia lying in the stalk of the adult eye. Concerning the segmental value of the eye and its being homodynamous

with the other appendages I see no reason to change the view I have always held that it is not. If it is we must allow all arthropod eyes arising from invaginations (*e.g.*, spiders) to be appendages, and in this way we should find ourselves in no end of trouble. In Astacus the eye attains the dignity of a stalk at a very early date; in Crangon, at the time of hatching, the constriction which is to make it a mobile organ has hardly begun. Some remarks on this point will be found in my paper on Limulus ('*85*, pp. 545, 546).

Last in order I must refer to Patten's valuable paper ('*86*), which has thrown a flood of light upon our knowledge of the arthropod eye, and which, so far as I have tested it, is accurate in all its details of structure. It treats, however, only of the adult arthropod eye. Still it has some speculations which must be noticed. Dr. Patten (*l. c.*, p. 688) mentions the question as to an invagination in the compound eye, and dismisses it with the remark that, though possible, it is not proven, and further states that the rods are not inverted. Still, on p. 680 he is driven to the conclusion that the ancestral arthropod eyes consisted of closed optic vesicles, formed by invaginations lying close beneath the hypodermis, which formed a continuous layer over them. He further constructs a figure (Pl. xxxii., Fig 141) to illustrate his idea, but regards the deeper wall of the enclosed vesicle as forming the layer of rods and cones, while the outer one either disappears or forms the so-called vitreous body. In this he was doubtless influenced by his belief that the layer of rods was not inverted (and in this belief he was warranted as can be seen from the foregoing review of the literature). Had he recognized even the possibility of this inversion he would doubtless have modified his theory and diagram.

That the layer of cones is inverted was first pointed out by Locy, and can readily be traced in the figures illustrating the present paper. The deeper ends of the retinal structures touch the optic cavity, which, of course, was primarily a portion of the external surface of the body, while the outer or distal ends of the retinophoræ were primitively turned from the surface. This fact has some weight, and Carrière ('*86*, p. 499) is hardly warranted in his criticism of Locy's comparison of the eye of a spider with that of a veterbrate. In both the layer of rods and cones is developed from the outer wall of an invaginated vesi-

cle and in both the light traverses these organs in exactly the same direction. Further, as Patten has shown, there is a similarity in the nerve-supply. In the vertebrate the optic nerve enters the eye in a mass, passes between the retinal elements and the lens, and gives off its fibres to the distal ends of these elements. In the Crustacea, on the other hand, as shown by Patten for various genera, the nerve to each ommatidium enters the optical portions of the eye separately, but ultimately becomes distributed to the distal portions of the organ. In the anterior median eyes of the spiders, on the other hand (I am informed by my friend, Dr. E. L. Mark), the optic nerves enter the eye in a cord much as in vertebrates, and are thence distributed to the distal ends of the ommatidium.

Still these similarities, interesting as they are, do not prove homologies, though I am not yet ready to discuss this point in all its bearings. One prominent difference must, however, be noted. In the arthropod eye the rods and cones are turned toward, in the vertebrate eye away from, the lens.

The development of the compound eye, as here given, must be regarded as having great weight in settling the question whether the compound eye has arisen by a concrescence of ocelli in the negative. Both ocelli (Locy) and compound eye arise by a single invagination. Were the other alternative true, we should expect the compound eye to have an invagination for each ocellus composing it.

The observations as yet recorded are not sufficient to throw any great light upon the phylogeny of the arthropod eye, still one or two points may be spoken of. The mere fact of invagination must be regarded as indicating an ancestral condition; but what this condition was is uncertain. The pit or groove must have had sensory functions, and either wall must, for a time, have been like its fellow, as is shown by its having similar nuclei, and by the similar development of rows of nuclei. The position of the eye, too, at the extreme end of the nervous cords would indicate that it was differentiated as a part of the primitive nervous system; but whether the invagination was originally confined to the eye alone, or whether it is the remnant of a condition which formerly extended throughout the whole length of the cords, is another problem. It may be well, however, to call attention to the fact that in all arthropods that have been inves-

tigated, the supra-œsophageal commissure develops much later than the optic cords, and the same is true of many of the worms.

MALDEN, MASS., October, 1886.

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EXPLANATION OF PLATE.

Reference Letters.

b, bacilli.
c, crystalline cone.
e, ectoderm or epidermis.
g, gangliogen.
gt, rows of ganglionic nuclei.
h, entoderm or hypoblast.
l, corneal lens.
m, mesoblast.
o, ommatidium.
oc, optic cavity.
og, optic ganglion.
oi, optic invagination.
ol, optic lobes.
*p*1–*p*4, pigment-cells.
pd, pedicle.
r, retinogen.
rp, retinophora.
rt, rows of retinal nuclei.
sg, supra-œsophageal ganglion.
x, undifferentiated ventral surface of the body.

The figures were all drawn with the Hartnack microscope and Oberhäuser's camera, unless stated to be diagrammatic. The amplification is stated in most instances.

FIGURE 1. Surface view of a stained egg at the time of the appearance of the optic lobes. (× 258.) Every nucleus was drawn with a camera, except those on the extreme margin.

FIG. 2. Transverse section through an optic lobe of Fig. 1, the left side being toward the median line. (× 550.)

FIG. 3. Optic lobe at about the time of closure of the optic invagination. (× 840.)

FIG. 4. Optic cavity and surrounding portions at a stage when one appendage is outlined. (× 575.)

FIG. 5. Same when two appendages are outlined. (× 550.)

FIG. 6. Eye at time when seven appendages are outlined. The upper is the more dorsal portion. (× 550.)

FIG. 7. Section through half the head at the time of first deposition of pigment, showing the relationships of optic and cerebral ganglia and eye.

FIG. 8. Retinal and epidermal elements of same eye still more enlarged.

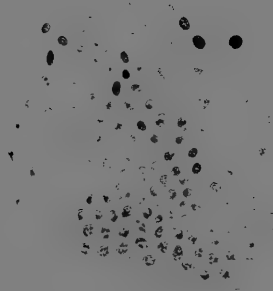
FIG. 9. Surface view of pigment at time of first appearance in the same eye. Drawn from living embryo.

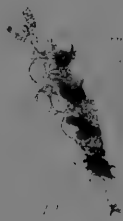
FIG. 10. Three retinal elements (one retinophora and two pigment cells) teased from a section of an eye in the stage of Fig. 11. Free-hand drawing.

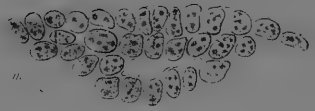
FIG. 11. Portions of three ommatidia of an embryo nearly ready to hatch. (× 700.)

FIG. 12. Half of cephalic region of embryo, same age as Fig. 6, showing the mesoderm, etc. (× 260.)

FIG. 13. Diagram of a single ommatidium of Fig. 11, to show the relations of the pigment-cells, retinophoræ, crystalline cone, and pedicle. The length of the crystalline cone and the distances between the outer pigment-cells are exaggerated for sake of clearness.







EYES OF MOLLUSCS AND ARTHROPODS.

WILLIAM PATTEN, PH.D.

DURING the year 1885 it was my good fortune to enjoy a prolonged stay at the Zoölogical Station in Naples, that Mecca to which all good disciples of zoölogy hope to make at least one pilgrimage.

My observations there made on the eyes of Molluscs and Arthropods were published in full in the sixth volume of the *Mittheilungen aus den Zool. Stat. zu Neapel.* . The more important of those observations are described in the following summary, which has been prepared for the *Journal of Morphology*, at the suggestion of Dr. Whitman.

I found the retina of Molluscs, as well as of Arthropods, to be composed of circles of pigmented cells surrounding central, colorless ones, characterized by constant and remarkable structural features. Believing that these groups of cells constitute the structural elements of most, if not all eyes, I have called them *ommatidia;* but it must be borne in mind that, according to my observations, their structure is quite different from that which Carrière, who first suggested the term, supposed them to have in the compound eyes of Arthropods.

The simplest ommatidia that I have seen are to be found in the pigmented areas of epithelial cells distributed over the exposed parts of the body of Lamellibranchiata, especially upon the mantle and sipho. They consist of a single circle of four to six pigmented cells surrounding a colorless, central one (Fig. 6); the latter is the most important part of the ommatidium, for it is mainly upon this element that those structural improvements are consummated that lead to the formation of the most perfect eyes. This central body of the ommatidium is a double cell whose broad outer end contains two nuclei, one of which (Fig. 6, n, rf^2) is often difficult to see, stains faintly and, at first sight, has little resemblance to a nucleus; an axial nerve-fibre passes through the centre of the cell, and issues from

its pointed inner end (*ax.n*). The inner portion of the double cell is filled with refractive and colorless globules. (Figs. 7 and 8, *ag.*)

In the undifferentiated epidermis of the mantle edge of Molluscs the nerves extend along the lateral walls of the cells. (Fig. 18, IV. and V.). The fibrillæ are applied to the surface of the cells, and usually cling so closely to it that they appear to, and probably do, penetrate the wall of the cell, and stand in direct communication with its protoplasmic contents. The nerve-fibres are therefore inter-cellular. But if two cells, whose lateral walls are well supplied with nerve-fibres, unite, and the apposed walls disappear, those nerve-fibres which were originally *between* the cells would then lie *in the centre* of a double cell. The central cells, or *retinophoræ*, of the ommatidia in Lamellibranchiata have been formed in this way, by the fusion of two cells whose apposed walls have disappeared, allowing the inter-cellular nerve-fibres to form *intra-cellular*, or axial nerves. In some cases the outer ends of the two cells composing the retinophora have failed to unite; and, as each end then contains a perfectly normal nucleus, we can clearly see the double nature of the retinophoræ. When the union is complete, as in the normal retinophoræ, one of the nuclei degenerates and often disappears. The retinophoræ are surrounded by a circle of pigmented cells, or *retinulæ*, whose inner ends are often reduced to slender hyaline stalks or *bacilli* (Figs. 7 and 8, *bc*). The retinulæ [1] are never double, and therefore never contain an axial nerve-fibre. The *cuticula*, which is often slightly thickened over the pigmented areas containing ommatidia, usually consists of two layers: a thin and structureless outer one devoid of nerve fibres, the *corneal cuticula* (Fig. 6, *c.c.*), and an inner, thicker layer, the *retinidial cuticula*. The latter contains a part of the network of nerve-fibrillæ, or *retia termi-*

[1] One meets serious difficulties in attempting to designate the pigmented cells surrounding the retinophoræ. If we regard the ommatidia as little retinas, then retinula-cells would include the retinophoræ as well as the pigmented cells. I have used, provisionally, the term *retinulæ* to designate in a general way the pigmented cells surrounding the retinophoræ, while in the Arthropods I have used it interchangably with the term retinula-cells of Grenacher, in contradistinction to those pigmented-cells surrounding the calyx. In most cases, I believe, the reader will not be misled. The term, however, as I have used it, cannot be recommended, and it is to be hoped that a better one may be suggested.

nalia, produced by the ramification of the inter-cellular nerve-fibres. Each cell, therefore, of these simple ommatidia is capped with a double cuticular layer, which may be continuous over all the cells, or divided more or less distinctly into hexagonal areas corresponding in size and shape with the outer ends of the cells.

Now we find that in *Arca* the simple ommatidia described above tend to collect in well-defined groups, forming, according to their arrangement, optic cups or convex, faceted eyes. In the formation of these eyes the ommatidia become more highly developed, the nerve-supply is increased, while the inner cuticular layer thickens and divides into distinct blocks overlying each cell. The retia terminalia extend into these blocks, which are subsequently converted into hexagonal, cuticular columns, or *rods*. These rods, which correspond to the rods found in the retina of all other eyes, contain, therefore, a specialized part of the *retia terminalia*, or a *retinidium*. Since the *retinophora* of the Molluscan ommatidium is always double, its overlying rod is also double, and contains an axial nerve-fibre like the retinophora itself, while the rods of the retinulæ are always single, and contain no axial nerves.

The *retia terminalia* form an irregular network of very fine fibrillæ, *continuous* with each other in all directions; the fibrillæ are most numerous around the outer ends of the epithelial cells, and they are arranged so that most of the fibrillæ are parallel with the surface of the cuticula. It is undoubtedly this network of nerves which gives the whole surface of the body its sensitiveness to light.

There is reason to believe that, in order to produce the greatest effect upon the fibrillæ, the rays of light must fall upon them at right angles. This result is obtained by arranging the fibrillæ in superimposed layers, and by regulating the direction of the rays of light. Axial nerves can give off radiating fibrillæ arranged in this way more easily than external nerves. The double rods of the retinophoræ, therefore, have an advantage in the possession of axial nerves, in virtue of which they gradually assume the most important rôle, while the retinulæ become modified in other directions. We therefore find in the simpler eyes of the Mollusca, as in *Haliotis* and *Patella*, that both single and double rods are present; while, in the more

highly specialized eyes of Cephalopods and of *Pecten*, the double rods of the retinophoræ have alone been retained. In *Arca* the optic cups possess both single and double rods; in the convex faceted eyes found side by side with the optic cups, there are, however, only double rods, while the cells bearing single rods have been modified to serve secondary purposes.

The *compound eyes* of *Arthropods* consist of two parts: a thin outer layer of cells, the *corneal hypodermis* (Fig. 14, *c.hy.*), which secretes the corneal facets; the remaining portion of the eye, or *ommateal hypodermis*, although it is often extremely thick, represents but a single layer of cells. These facts are of importance in determining the homologies of the compound eye. While Grenacher and his followers have either overlooked or misunderstood the corneal hypodermis, they have maintained that the crystalline-cone cells and the surrounding pigmented ones constitute a distinct outer layer, and the retinulæ and so-called rhabdoms an equally distinct inner layer of the ommateum. That the ommateum proper, which does not include the corneal hypodermis, is not a double layer is shown by the fact that the retinulæ and other pigmented cells extend through the whole thickness of the ommateum; and, above all, by the fact that the so-called rhabdom is not produced by the retinulæ, but by the inward prolongation of the crystalline-cone cells. It follows, therefore, that generalizations founded upon the supposition that the ommateum is two-layered are no longer tenable.

We find good reasons for believing that the ocelli are also composed of ommatidia having essentially the same structure as those of the compound eye. The ocelli of spiders consist of groups of cells, each cluster containing a double colorless cell with either double apical rods, as in Molluscs, or with double axial rods and overlying nuclei. The rods in the latter case coincide essentially with the crystalline cones of the compound eye, and we therefore consider them as homologous structures.[1] The compound cells, or retinophoræ, of the Arachnid ocellus, like those in the compound eye, are surrounded by circles of pigment-cells. Although the retinophoræ of spiders, as far as

[1] My recent observations on the eyes of *Phalangium* show that its ommateum is composed of ommatidia in all essential points like those of the compound eye. The resemblance of the threefold conical rods of *Phalangium* to the fourfold crystalline cone is especially evident. *Vide* my preliminary account in this journal.

known, contain double rods only, the crystalline-cone cells, or retinophoræ, of the compound eye are usually quadruple, although in Amphipods and related Crustacea they are double. On the other hand, the rod-bearing cells, or retinophoræ, of *Scorpio* and *Limulus* are respectively five and ten or fifteen fold.

It is probable that ommatidia are present in various modifications in all eyes. In Vertebrates the axial nerve-fibre of the rods, and the presence of two nuclear-like bodies in the rod-bearing cells, afford good reasons for supposing that the rods, and the cells which bear them, are double. The ommatidia are, therefore, essential elements, and a classification of eyes must be founded mainly on the modifications which they have undergone. I distinguish three principal kinds of light-sensitive layers, according to the modification of the ommatidia.

A *retineum* is a collection of ommatidia in which the rods of both retinulæ and retinophoræ, or of the latter alone, form a continuous layer, the retinulæ retaining their pigment and primitive arrangement around the retinophoræ; *e.g.*, invaginate eyes of all Molluscs, except *Pecten*, and worms (?).

An *ommateum* is a group of ommatidia in which the rods, produced only by the retinophoræ, are completely isolated; *e.g.*, faceted eyes of Molluscs and Arthropods, and some Arthropod ocelli.

A *retina* is composed of ommatidia whose retinulæ, having lost their rods, are transformed into colorless ganglionic cells; *e.g.*, Pecten and Vertebrates.

Part I.

Molluscs.

Arca Noæ is extremely sensitive to slight changes in the intensity of light. In a normal condition it never fails to close its shell when any shadow is cast upon it. This perception of light gradations may be so delicate that if a small object, such as a lead-pencil, is brought with extreme caution within two and a half or three inches of the open shell, and in such a manner that no perceptible shadow falls upon the animal, it at once closes its shell, and with the same energy as when a deep shadow is cast upon it. On examining the exposed parts of the mantle

edge, one readily sees numerous dark spots of various sizes, which, upon closer inspection, prove to be highly-organized eyes. They are, undoubtedly, the light-sensitive organs which the foregoing experiment showed must be present in *Arca*. The mantle edge of *Arca*, as well as all other Lamellibranchiata that I have examined, is divided into three longitudinal folds, — an outer one, or *shell fold*, an inner one, the *velar fold*, or *velum*, and a median one, or *ophthalmic fold*. At the base of the furrow, separating the shell fold from the ophthalmic one, is the gland secreting the cuticular-like covering of the shell. The whole mantle edge of *Area* is well supplied with patches of pigment which are especially abundant on the inner face of the ophthalmic fold. These pigmented areas are composed of columnar, pigmented cells, among which are a number of colorless cells provided with two nuclei and an axial nerve-fibre. These colorless cells are usually surrounded by a circle of four pigmented ones, distinguished from the surrounding cells by their color and sharp configuration. These pigmented patches, with their clusters of cells, or ommatidia, and with no special thickening of the cuticula, belong to the simplest light-sensitive organs known. Along the summit of the ophthalmic fold, the ommatidia are collected into well-defined groups to form either the *pseudo-lenticulate*, the *invaginate*, or the *faceted* eyes.

The *pseudo-lenticulate* eyes, of which there are about two hundred in each individual, are scattered irregularly over the surface of the ophthalmic fold. They consist of groups of ommatidia, over which the cuticula is thickened to form a lens-like body. The latter is composed of a number of cuticular rods, each one overlying the cell by which it is secreted. The whole cuticular mass is richly supplied with prolongations of the nerve-fibres found between the ommatidial cells, and the whole network of nerve-fibre in the cuticular mass is simply an extended and modified part of the *retia terminalia* of the simple epithelial cells.

There are about eight hundred *invaginate* eyes in each full-grown specimen of *Arca Noæ*. The groups of ommatidia which constitute these eyes are sunken beneath the surface to form minute cups, the mouths of which may be reduced to narrow slits. The rods of the ommateal cells form a thick cuticular floor for each cup.

The *faceted eyes*, of which there are about two hundred in each individual, are the most highly developed of all; and they are of special interest, since they possess all those characters which distinguish the so-called compound or faceted eyes of Arthropods. The faceted eyes of *Arca* are collections of about eighty highly specialized ommatidia to form minute hemispherical projections, lineally arranged along the summit of the ophthalmic fold, at the anterior and posterior portion of the mantle edge. In these eyes, the colorless cells, or *retinophoræ*, are quite large (Fig. 8), and contain two nuclei, and an axial nerve-fibre; on their outer ends is a large, double rod which projects slightly above the surface. Carrière mistook these refractive rods for minute lenses. The inner part of the conical retinophora is filled with a mass of brilliantly refractive globules which act as reflectors, causing the light to pass a second time through the rods. Both nuclei are situated at the outer end of the retinophora; one is large, stains deeply, and contains a well-marked nucleolus (*n. rf.*^{1}); the other (*n. rf.*^{2}), is usually smaller, and seldom absorbs coloring matter.

One often finds, however, cases in which both nuclei are quite alike, while the outer end of the cell is strongly bifurcate, proving beyond doubt that the retinophora is formed by the fusion of two cells. Each retinophora is surrounded by eight retinulæ, arranged in two circles of four cells each. In one circle the four retinulæ are pigmented only at their inner ends, which form a complete sheath for the inner ends of the retinophoræ (*pg.*^{2}) The outer third of each cell is reduced to a very thin and colorless membrane, which unites with the similar prolongations of the three other cells to form a delicate sheath around the outer ends of the retinophoræ. In the other circle (*pg.*^{1}), the inner ends of the four retinulæ are reduced to slender and colorless stalks, or *bacilli*, (*b.c.*^{1}) while their swollen outer ends form a complete sheath of pigment around the double rod. It is important to notice that the retinulæ of the evaginate eye have lost their rods, and now serve simply as a covering to exclude lateral rays of light from the highly developed rods of the retinophorae. The first step towards the formation of the eyes in *Arca* is the collection of the isolated ommatidia into groups. At the same time there is a thickening of the retinidial cuticula, to form over each cell a cuticular column, or rod, which contains a part of

the "*retia terminalia*." The corneal cuticula remains as a thin membrane covering the outer ends or the rods. (Fig. 7.) If the rods formed a lens-like thickening over such a cluster of ommatidia there would be formed a *pseudo-lenticulate* eye. If such an eye were invaginated an optic cup would be formed; if it were evaginated, and the ommatidial cells slightly modified, one of the faceted eyes would be the result.

Arca Noæ, therefore, is a valuable subject for the study of the origin of the eyes; for there we have a complete series of transitional forms between highly specialized visual cells and simple epithelial ones; but, what is of still more value, we have all stages in the development of the nerve-fibres of the undifferentiated epithelium up to those supplying the most specialized visual cells. In Arca we have conclusive proof that the so-called rods of the eye are derived from cuticular thickenings over sense-cells, and that the cuticula serves no other purpose than as a support for a system of minute nerve-fibrillæ, which are the real sensitive elements of the eye.

We have in Arca a sluggish, and for the most part fixed, animal lavishly supplied with over twelve hundred well-developed light-sensitive organs, not to mention the innumerable isolated ommatidia scattered everywhere over the surface of the mantle. The presence of all these organs in such an animal may well excite surprise especially when we consider that *Avicula*, a related genus, and one that is not provided with specialized eyes (so called), is exactly as sensitive, if not more so, to changes in the amount of light as is Arca.

Pectunculus has about twenty-five faceted eyes, similar in structure to those of *Arca*, upon the right mantle edge, and twenty-two on the left. No invaginate or pseudo-lenticulate eyes are present.

Anatomy of the Eyes of Pecten.

The eyes of *Pecten*, since Poli first described them as such, in 1795, have attracted the attention of many zoölogical students, not a few of whom have made them the object of special study. The general structure of the organs in question is, therefore, well known.

I have shown that the control of the dioptric apparatus was more perfect than had been supposed. The curvature of the

cornea and of the outer surface of the lens can be modified by radiating and circular contractile fibres. The size of the pupil may be modified by increasing the curvature of the cornea. The lens may be raised bodily, or lowered, by the combined action of what I have called the *ciliary muscle*, and of an elastic cushion, the *septal membrane*. That the body in question is a true lens, and that its change of shape and of position is to modify the position of an image, is shown by placing the eye in such a position that one may observe the inverted image of any neighboring object formed by the lens upon the retina.

On focusing between the *argentea* and the place where the image formed by the lens is seen with the greatest distinctness, one sees a double image, less distinctly toward the argentea, but increasing in sharpness toward the focal point of the eye, where the two images coincide. The only explanation I have to offer for the origin of the second image is that it is a reflected one of the first, formed by the curved surface of the argentea.

The eyes of *Pecten*, like the faceted ones of *Arca*, are disposed more or less distinctly in pairs, and show several peculiarities in arrangement and coloration.

The eyes of the flat, left valve are larger and more numerous than those of the curved, right one. In *Arca* there is a difference between the eyes of the right and left sides, but none in the shape of the valves. In most *Pectens* the maximum difference in the shape of the valves is accompanied by a maximum difference in the size and number of the eyes on both sides. One occasionally finds an eye, in those species in which eyes are especially numerous, — *Pecten varius* and *P. opercularis*, — the pupil of which is entirely covered with pigment. I have taken especial pains to examine these organs, which could no longer function as eyes, and have found that the retina with its rods and nerve-fibres is perfectly developed. The corneal cells are provided with median transverse teeth, and with longitudinal folds at their inner ends. The teeth and folds fit into corresponding indentations of neighboring cells, giving firmness and flexibility to the cornea. The longitudinal folds are continuous with fine fibres which cross the pseudo-cornea and unite with the outer surface of the lens.

Beneath the iris there is a nucleated layer of fibres, some of

which terminate at the edge of the cornea in an outward curve as though attached to the epithelium at that point, forming what I have called the *ciliaris;* other fibres are continued onward between the cornea and lens, forming an almost structureless layer, the *pseudo-cornea*, in which nuclei are seldom seen.

The lens is held in place by a *suspensory ligament* attached to the periphery of its outer surface, which is supplied with a layer of concentric, circular fibres superimposed by a layer of radiating ones.

The inner surface of the lens is sparingly supplied with branching fibres, which, in *Pecten opercularis*, accumulate near the centre to form a nucleated mass of fibres connecting the lens with the septal membrane.

In *Pecten opercularis* there is a special accumulation of circular fibres to form two contractile rings, situated close together, one on the outer and the other on the lateral surface of the lens. The inner surface of the lens is much more convex than the outer.

The posterior portion of the eye consists of a concave disc, completely enclosed within a membranous sac. The thick anterior wall of the sac, or the *septal membrane*, serves to protect the retinal cells, and as an elastic cushion for the lens. The inner wall constitutes a tough, double-layered *sclerotica*. At the confluence of these two membranes the wall of the sac is much thinner, and is perforated by innumerable passages for the entrance of nerve-fibres from the axial branch of the optic nerve. The cells within the ommateal sac constitute a closed vesicle whose anterior and posterior walls touch each other, thus obliterating the central cavity. The wall of the vesicle was originally composed of a single layer of cells, an arrangement which is subsequently obscured by the division of both anterior and posterior walls into several secondary layers. The posterior wall of the ommateal vesicle consists of four layers,— the *vitreous net-work*, the double-layered *argentea*, and the *tapetum*. The anterior wall is likewise composed of four layers,— an *outer ganglionic layer*, an *inner ganglionic layer*, the *retinophoræ*, and the *rods*.

The *retinophoræ* (Fig. 15) are long, bent cells, one end supplied with an inwardly directed rod, while the other is drawn out to a slender tube continuous with the axial nerve. Each retinophora contains a large, oval nucleus and a small, faintly stain-

able one. The flanged walls of the inner ends of the retinophoræ unite at the same level with those of neighboring cells, producing a sharp division between the rods and the inner ends of the cells. This *pseudo-membrane* was named the "*sieve membrane*" by Carrière. A delicate wall, the *terminal membrane*, separates the retinophora from its rod; on the edge of the retina are many slender and rodless retinophoræ.

The rods are cylindrical, and consist of a refractive cap or sheath, surrounding a pyramidal, axial core. The axial nerve is continued through the distal end of the rod, and immediately divides into two branches, one of which unites with a similar branch from a neighboring rod, while the other is bent over and distributed in fibrillæ over the surface of the rod. I have called these nerves the *axial nerve-loops*. That portion of the axial nerve within the rod gives rise to successive *êtages* of radiating fibrillæ which unite with the nerve-fibres upon the surface of the rod. These cross fibrillæ constitute the *retinidium*, which is composed of fibrillæ similar to those seen in the rods of *Haliotis*, but arranged in a more systematic manner. There are also circular fibrillæ arranged around the axial core of each rod, and connecting the radiating fibrillæ.

Above the retinophoræ is the *outer ganglionic layer*, which consists of large ganglionic cells (*g. c.*$^{1-5}$), terminating at either end in a varying number of fibrous prolongations; those of the outer end are continued into the ganglionic branch of the optic nerve, while those of the opposite extremity extend along the walls of the retinophoræ to the rods, over the surface of which they form a net-work of fibres. This layer contains cells in instructive stages of ganglionic perfection.

The *inner ganglionic layer* consists of a single row of minute ganglionic cells (*g. c.*^{5}), which, when seen at all by previous writers, have been mistaken for the nuclei of the retinophoræ. Each cell, which is nearly filled by its nucleus, is provided with several fibrous prolongations, one of which is directed outwards, passing into the ganglionic nerve-branch, while five or six others extend inwards to help form the net-work of fibres on the surface of the rods. Many of the nerve-branches from both ganglionic layers terminate upon the walls of the retinophoræ in one of two ways, — either a single fibre impinges directly upon the cell-wall, and there divides into several short fibrillæ, con-

nected at their distal extremities with a circular fibril enclosing the whole (*y.*), or a nerve-fibre follows the cell-wall for some distance, giving off at intervals smaller, lateral branches, and finally becoming so minute as to disappear (*z.*).

The outer prolongations of the ganglionic layer form, beneath the septal membrane, a mass of free fibres, which were mistaken by *Carrière* for nucleated cells, and by *Hensen* for fibres pulled out of the retina by shrinkage. The layer of free nerve-fibres is, however, a normal condition, and is necessarily so in order to give the lens space for focal adjustment without injury to the retina.

A system of circular fibres surrounds the periphery of the inner face of the retina, forming a *membrana circularis.*

Beneath the rods there is a thin layer of a vitreous substance forming a net-work, the meshes of which constitute a hexagonal crown for the inner end of each rod. On the periphery of the retina the *vitreous net-work* is transformed into a thin plate, pierced by numerous and irregularly-shaped holes.

The *argentea* is formed by the modification of two cell layers into refractive membranes. Each membrane is composed of minute square plates, whose edges are bevelled in such a manner that their outer faces are smaller than the inner, which rest upon the undifferentiated, under surface of the membrane by which all the plates are held together. In passing inwards the membranes become thinner, less distinct, and refractive, while the plated structure entirely disappears. The thick outer layer of the *argentea* in the adult never contains nuclei, although one or two may occasionally be found in the inner layer.

The *tapetum*, the red-pigment layer of previous writers, usually consists of a single layer of cells, decreasing in thickness from the axial part of the eye toward the periphery, and terminating with the argentea at the entrance into the retina of the fibres from the axial branch of the optic nerve.

The thickened central part of the outer layer of the septum, a little to one side of the optic axis, is perforated by the ganglionic nerve-branch. The peripheral part, gradually diminishing in thickness toward the edge of the retinal sac, consists of nucleated connective-tissue cells modified into circular fibres.

The inner layer of the *sclerotica* is marked with short par-

allel cross-lines; the thick outer layer consists of longitudinal fibres, which may contain a few nuclei.

There are numerous refractive fibres which arise from the periphery of the eye-stalk, and, converging toward the base of the eye, penetrate the sclerotica and the superimposed layers as far as the inner ends of the rods. In the sclerotica they expand into refractive spindle-shaped bodies, often of a faint pink color.

Development of the Eyes of Pecten.

On the branchial wall of the ophthalmic fold of *Pectens*, 2 mm. long, are a few minute, pigmented and transitory cups, undoubtedly homologous with the invaginated eyes of *Arca*.

The stalked eyes first appear as oval optic thickenings at the base of the ophthalmic fold. By a continued proliferation of the cells on the outer side of the optic thickening, an oval knob-like papilla is formed, containing a solid core of hypodermic cells. At first the core is ill-defined; several of the more deeply situated cells separate from the rest to form the ganglionic cells, which later provide the eye with nerve-fibres. The whole papilla then elongates, and a disc-shaped cavity appears in the centre of the core, transforming it into an *optic vesicle*.

In the following stages the posterior wall becomes more sharply defined, and there, for the first time, the cells of the optic vesicle are provided with distinct cell-walls. The inner wall of the optic vesicle divides into two layers, — the inner one giving rise to the *tapetum*, and the outer one to the *argentea*. The latter is formed by the transformation of cells into superimposed plated membranes, the nuclei being retained, in some cases, until the eye has completed its development. The outer wall of the optic vesicle divides into three zones, consisting of the fibrous, the ganglionic, and the retinophoric layer of the retina.

Some of the connective-tissue cells surrounding the optic vesicle give rise to the retinal-sac, a nucleated membrane, the anterior wall of which develops into the *septum*, and the posterior into the *sclerotica*.

The connective-tissue cells above the septum give rise to the *lens*. The cells of the *tapetum* are at first filled with coarse,

colorless granules, which subsequently acquire a characteristic red color. The nuclei of the retinophoræ, which at first are situated in a peripheral thickening of the retina, gradually push their way towards the centre of the eye. It is not till quite late in the devolopment, after the appearance of the rods, that their cell-walls become visible.

The only difference between the rods, when first seen, and those of the adult, was the large size of the axial core, and the extremely thin shell, or sheath, scarcely visible except at the tips of the rods. As soon as the rods could be clearly distinguished they were seen to contain an axial nerve-fibre. The nuclei of the argentea decrease in size until they finally disappear, with the exception of those in the inner layers, where, in the adult even, one or two aborted nuclei may occasionally be seen.

The *vitreous net-work*, in contrast with its subsequent condition, forms at first a thick homogeneous and structureless layer.

The innumerable isolated fibres which, even in the earlier stages innervated the eye, subsequently unite to form a single, loose bundle of nerve-fibres, — the primitive optic nerve, which divides later into the *axial* and *ganglionic* branches of the definite optic nerve. All the nerve-fibres supplying the optic vesicle are not collected to form the optic nerve; for many entering the base of the vesicle retain their primitive arrangement, and appear to penetrate the sclerotica, tapetum, and argentea, as far as the rods. The circumpallial nerve contains as many ganglionic swellings as there are optic nerves. In many, if not all, of these ganglia there is a peculiar infolding dividing them into halves.

The free edge of the ophthalmic fold contains, at regular intervals, large ova-like cells, which may be seen in preparations of the whole mantle edge, as well as in sections. In the neighborhood of the hinge the branchial wall of the mantel of younger specimens is thrown into a variety of thick ciliated folds, the nuclei of which are, in most cases, several rows deep. In some cases one of the folds becomes especially enlarged at its extremity, the walls thickening to form a kidney-shaped body with a great many small, deeply-stained nuclei. The surface is covered with a cuticula, provided with minute

papillæ, from each one of which arises an enormously long cilium.

The *sense-hair papillæ*, which originate at any place along the outer surface of the velum, first appear as thickenings of the hypodermis which soon become conical, and provided with a tuft of stiff sense-hairs at the apex. The inward proliferation of the cells at the apex of the thickening gives rise to an ectodermic core, which becomes transformed into the longitudinal nerve with which every tentacle is provided. As the papillæ increase in length, tufts of sense-hairs are formed on the sides, each connected with one or two ganglionic cells. In those papillæ which do not develop into tentacles no distinct nerve is formed; but two or three cells separate from the summit of the papilla and wander into the underlying tissue, there forming ganglionic cells, the nerve-like ends of which may terminate in a small number of sense-hairs; or, if the cells are more highly specialized, the sense-hairs may be absent, while the terminal fibres divide into numerous fibrillæ which supply the adjacent cells.

We have found the same sensitiveness to changes in the intensity of light in *Ostrea*, *Mactra stultorum*, *M. solidissima*, *Pinna*, and *Avicula*, that was so marked in *Arca*. In these cases, however, there were no well-defined eyes, but large pigmented and shallow grooves, or slightly depressed areas over which the cuticula was but little thickened. In these pigmented patches were numerous ommatidia having essentially the same construction as those in the pseudo-lenticulate and invaginate eyes of *Arca*. They undoubtedly belong to the simplest of light-sensitive organs; but, in spite of their simple structure, they are in some cases wonderfully delicate organs. In *Avicula*, for instance, the simple and diffuse ommatidia, the only visual organs present, are able to perceive the difference in light produced by holding such a small object as a pencil between them and the source of light. Under such circumstances a shadow so faint as to be imperceptible to the experimentor caused immediate contractions of the whole animal.

At the end of each siphonal tentacle of *Cardium edule*, beneath a semicircular band of pigment is a minute eye. It

consists of a roughly spherical mass of large cells, which emit, when living, a faint red color, reflected by the underlying argentea. I have counted, with a pocket lens, fifty-one eye-bearing tentacles on one individual.

The extremely simple retina, which is oblong in shape, — the short diameter being at right angles to the pigmented covering, — consists of five or six rows of cells, the ends of which, being directed inwards, rest upon the mass of connective-tissue fibres which serves at once as a capsule and tapetum. The opposite extremities of the retinal cells, where the large oval and sharply stained nuclei are situated, appear to terminate in single nerve-fibres, which pass out of the capsule and, bending at right angles, extend along the axis of the tentacle. At the angle of each of these cells, nearly opposite the large nuclei, is a small and poorly defined cell containing a minute but deeply stained nucleus.

The argentea is similar to that of *Pecten*, and consists of nucleated connective-tissue cells, the bodies of which are flattened into membranes, composed of minute refractive squares. The argentea envelops the whole eye, but is thickest on the sides next the pigment, and toward the base of the tentacle. Whether the inner ends of the retinal cells are provided with rods similar to those of Pecten could not be determined with certainty.

The round cellular body situated in front of the retina is composed of large, characteristic cells, which, however, are not confined to this region alone, but extend thence, in a double row, nearly half the length of the tentacle.

In *Cardium tuberculatum* the tentacles are also provided with eyes, although the pigmented patches at the tips of the tentacles are absent.

In *Cardita sulcata* isolated ommatidia are present, but no tentacular eyes.

The most important fact obtained by studying the eyes of *Haliotis* was that the colorless cells are not gland-cells or of secondary importance to the retina, but are true retinophoræ, having double rods, two nuclei, and an axial nerve fibre. The pigmented cells, or retinulæ, are provided with single, club-shaped rods, which contain a net-work of fibrillæ formed by the ramification of intercellular nerve-fibres. The most satisfactory

proof of the intercellular nature of the nerves and of the nervous structure of the rods was obtained by dissolving the cuticula, leaving the net-work of nerve-fibrillæ free and uninjured. It is then seen that the fibres of the optic nerve penetrate the basal membrane and pass outwards between the cells of the ommatidia. As they pass beyond the outer end of the cells the nerves break up into innumerable branches, which do not end freely, but unite with each other in all directions to form a network of continuous fibrillæ.

On the upper side of each tentacle of Haliotis is a longitudinal groove, the floor of which consists of thick columnar cells, filled with a dark-brown or black pigment; the cuticula is not especially developed, neither could any colorless cells be seen. The similarity of these pigmented bands to those on the sipho and mantle edge of the Lamellibranchiata is apparent.

II.

Crustacea.

Each square corneal facet of *Pinœus* is secreted by two underlying oblong cells belonging to the corneal hypodermis.

The centre of each ommatidium is occupied by four colorless cells, — the retinophoræ, — united to form an inverted pyramid, whose base abuts against the corneal hypodermis, while the apex rests upon the basal membrane. The inner end of the pyramid is reduced to a slender, hollow stalk, — the *style*, — whose inner end enlarges into a solid, pyramidal thickening, the *pedicle;* the latter rests upon the basal membrane by a delicate stalk composed of the attenuated, inner ends of the four retinophoræ, two of which are united with each other. Each leg of the stalk is divided at its inner end into several fibres by which it is united to the basal membrane. This fact is important, for it shows that the segments of the so-called rhabdom of Grenacher are not secretions of the retinulæ, but merely the inner ends of the retinophoræ (or crystalline-cone cells), which terminate in the same root-like fibres seen in nearly all hypodermic cells.

The pedicle, whose abaxial walls are very thick, entirely obliterating the central canal, is composed of plates varying in thickness in different parts. Each plate is marked by a set of

parallel lines at right angles with a similar set in the adjacent plate. The plates of the pedicles resemble, in some respects, the plates in the argentea of *Pecten* and other Molluscs, and I have suggested that they may have a similar function; *i.e.*, they act as reflectors to intensify the light impressions in cases where a great deal of light is necessary, or when there is little light at the animal's disposal. The fact that the pedicles are usually present in nocturnal insects harmonizes with this interpretation. The rounded outer ends of the retinophoræ are capped with protoplasmic thickenings, in which the nuclei are situated.

Below the nuclei is an enormous crystalline cone nearly half as long as the ommatidium. Near the centre of the eye, almost at the inner ends of the crystalline cone, the opposite halves of the calycal wall develop granular thickenings, sickle-shaped in cross-sections, which increase in size as the diameter of the retinophoræ diminishes.

Surrounding the retinophoræ are seven oddly-shaped retinulæ of different apparent lengths, four of which are nearly black, while the remaining three are filled with light-brown pigment.

The retinulæ seem to terminate at the apex of the pedicle in the knob-like swellings containing the nuclei; this, however, is not so, for they are continued outwards as extremely delicate membranes, similar to those of the retinulæ of *Arca*. Toward the outer surface of the eye the united terminal membranes of each group of retinulæ form a delicate sheath, loosely surrounding the style and calyx. Toward the outer surface of the eye the sheath divides into seven hyaline thickenings, which abut against the inner face of the cornea, to form, at the corner of each facet, regular four-armed figures. Each thickening of the sheath represents the outer end of a retinula.

One of the retinulæ is remarkable for its great size and peculiar shape. At the beginning of the laminated structure of the pedicle the axial wall of this cell becomes scalloped, each fold projecting into the end of a plate.

The pigmented collar of the retinophoræ is formed by a circle of four cells arranged in two pairs. Each cell is continued inward as a slender colorless rod, or *bacillus*. The outer edges of the collar cells contain refractive granules, which, in reflected light, are yellowish-white and perfectly opaque. The cells are

continued outwards as four delicate fibres, which produce four minute impressions at each corner of a corneal facet.

In the spaces between the inner ends of the ommatidia is a third group of cells, the boundaries of which cannot be distinguished, and it is therefore difficult to determine the exact number belonging to each ommatidium. The nuclei are arranged at various niveaux around the inner ends of the pedicles. These cells contain yellowish fat-like crystals which form, at the inner surface of the ommateum, a narrow and intensely white band. The crystals are insoluble in absolute alcohol, clove-oil, creosote, chloroform, or ether. But a very dilute solution of caustic potash dissolves them at once, with the formation of a purple solution.

In the spaces between adjacent facets which have been treated with caustic potash, may be seen four groups of fibres, or impressions of the same. They are probably the outer ends of the basal cells just described, although I have not been able to trace any connection between the structures in question. This supposition, if correct, would fix the number of these cells at four, which agrees very well with what appears to be present.

The *basal membrane* is composed of Greek-cross-shaped masses of connective-tissue. From the centre of the inner surface of each cross a group of fibres projects inwards and unites with the connective-tissue cells underlying the basal membrane. The squares enclosed by the crosses are bridged by a bundle of diagonal fibres. A series of cross-sections of the inner ends of the ommatidia enable us to determine the position that each cell occupies upon the basal membrane. Beyond the base of the pedicle the retinulæ suddenly separate, and the stalk of the pedicle dissolves into two groups of fibres, which become attached to the outer surface of the cross. One group is formed of two separate bundles, while the other is also composed of two bundles, but so closely placed as to form one figure, the outline of which indicates its dual composition. These four bundles are the inner ends of the retinophoræ and the fibres are their root-like terminations.

To each basal-membrane square are also attached, in regular and constant order, the inner ends of the bacilli and retinulæ. Thus each cross of the basal membrane furnishes the support

for a single ommatidium, and both these structures correspond in number.

In longitudinal sections one sees that a bundle of pigmented nerves-fibres passes to each of the openings leading into the square spaces enclosed by the crosses; just before reaching the basal membrane it breaks up into smaller branches, one of which goes to each cell attached to a basal-membrane cross. Besides the pigmented fibres there are four colorless ones which, arising from as many main nerve-branches, ascend the four angles of the cross, and extend along the outer surface of the four cells composing the retinophora. Lastly, a single colorless branch enters the base of the cross and issues from the centre of the opposite surface, to be continued straight upward through the centre of the style to the crystalline cone. Although the basal-membrane crosses, and the enclosed squares, as well as the principal nerve-bundles, coincide in number, each ommatidium is supplied with nerve-fibres from four different bundles. It is probable that the superficial fibres distributed over the wall of the calyx communicate with the axial nerve by means of cross fibrillæ, just as in *Pecten* and *Arca*. In the outer ends of the crystalline cone, in that part which is densest and most hyaline, I have not been able to demonstrate anything like cross lines or fibres.

In *Galathea*, *Palæmon* and *Pagurus*, one may easily observe the corneal hypodermis the general characters of which differ but little from that of *Penaeus*. In both *Palæmon* and *Pagurus* there are two peripherally placed nuclei for each quadrilateral facet. In *Galathea* there is a remarkable modification of these cells to form, for each ommatidium, an iris with a slit-like contractile opening, the walls of which may be expanded by means of radiating contractile fibres. In *Galathea* I have followed the external longitudinal nerve-fibres of the style, as well as the central, axial fibres, up to the calyx, where the latter nerves extend into the centre of the crystalline cone, and the former give rise to branching fibres spread over the wall of the calyx. In *Galathea* there are four lateral thickenings of the calycal wall. In *Galathea*, *Palæmon*, and *Pagurus*, the pedicles are composed of two sets of plates similar to those of *Pinæus*, the markings in one set being at right angles to those of the other.

In *Branchipus* the nuclei of the retinophoræ are situated in protoplasmic thickenings over the outer ends of the rods. A corneal hypodermis is present, composed of indefinitely arranged cells.

The *style* is a flattened tube, containing an axial nerve-fibre.

In *Mantis religiosa* there are two corneal cells beneath each facet. Each ommatidium has seven retinulæ and at least six light-brown pigment-cells surrounding the calyx. There are also two large black cells enclosing the neck of the calyx. Their inner ends terminate abruptly at the outer end of the style. Three of the retinulæ are longer and more deeply pigmented than the others.

The *basal membrane* is a thick layer of nucleated connective-tissue, permeated by canals corresponding in number with the ommatidia; through each canal passes a bundle of pigmented nerve-fibres.

Just beyond the narrow neck of the calyx the four axial nerves contained in the style break up into four bundles, one entering each chamber of the calyx. There each fibre gives rises to innumerable horizontal fibrillæ, which unite with each other to form a complete nervous net-work.

The style is surrounded by six nerve-fibres, which appear in cross-sections as so many small dots. They may be followed as far as the calyx, where they break up into numerous smaller branches, continuous with those inside the calyx, by means of minute cross fibrillæ. The outer ends of the retinulæ are reduced to structureless membranes which unite to form a sheath around the calyx. The abaxial face of each retinula is provided with longitudinal nerve-fibres connected with each other by circular fibrillæ. Around the retinulæ are several, probably eight, bacilli. The nerve-fibres surrounding each bacillus supply the outer pigmented ends of the same.

The various stages in the formation of ganglionic cells out of sensory ones may be studied in the mantle edge of Molluscs. They arise in the following manner: The nucleus of a slender sense-hair cell, which terminates inwardly in a long fibre exactly similar to the prolongations of the neuro-epithelial cells of Coelenterates, wanders below the basal membrane (Fig. 18, III.), while the outer end of the cell is reduced to a fine fibre, still terminating in one or more sense-hairs.

The outer end of the fibre then gives rise to minute cross-fibrillæ, which either adhere to the wall of the neighboring sense-cell, or unite with similar fibrillæ from older nerve-fibres; lastly, the tuft of sense-hairs disappears, and the conversion of the sense-cell into a bipolar ganglionic one is complete. (IV.) Subsequently the body of the bipolar cell gives rise to numerous secondary fibres, which unite with those from other cells, and so convert the bipolar cell into a multipolar one, whose primitive, outer end still retains its original position between the epithelial cells. (V.) This process of nerve formation may occur at any part of the mantle edge, and is not confined to the larval stage, but takes place also in the nearly full-grown individual. Here, then, is the explanation of the intercellular nerve endings in the Mollusca; and unless degeneration of the outer ends of the nerves has taken place, they should always extend to the cuticula. In no case do nerves from the central nervous system unite directly with the sense-cells of the epidermis. All the nerve-ends in the hypodermis mark approximately the places where ganglionic cells originated. The latter alone are directly united on the one hand with the hypodermis and on the other with the central nervous system.

The *basal membrane* of Molluscs is formed by the union of the root-like ends of ordinary epithelial cells. I consider that the latter cells and the basal membrane represent the myo-epithelial cells and the underlying layer of fibres of Coelenterates.

My observations on the structures of the compound eye have led me to the conclusion that it is a modified ocellus. The primitive Arthropod ocellus I regarded as a closed optic vesicle, the inner wall forming the retina whose rods are therefore upright. The outer wall of the optic vesicle in most cases is not visible. The hypodermis overlying the optic vesicle is represented by the "*vitreous-layer*," or what I have called the *corneal hypodermis*. In the compound eye the same layer is present, which I have also called the corneal hypodermis, as a thin stratum of cells over the crystalline cones. The crystalline-cone cells are, therefore, not the homologue of the vitreous layer of the ocelli, but of the colorless rod-bearing cells, or retinophoræ, with which they have a common function. If this be true, then the crystalline cones are not dioptric organs, but the

essential, light-sensitive elements. If this also be true, it must of necessity follow that by far the majority of compound eyes are not adapted for "*mosaic vision*," as commonly understood, but for the perception of inverted images formed by the corneal facets upon the crystalline cones.

I also claimed, on theoretical grounds, that neither the ommateum nor the retina of Arthropods could have arisen as an outgrowth of the brain, and I may add that my recent observations on the development of the eyes of *Vespa* have confirmed this conclusion.

Certain facts in the anatomy and distribution of the simplest kinds of eyes, as well as other sense-organs, lead me to suppose that their function was originally not that of sense-organs; that is, organs by means of which their possessor became cognizant of changes in external conditions; they were rather the receivers and transmitters of external changes which had in themselves a stimulating and beneficial effect upon the organism. The constant association of certain sensory impressions with changes in external condition finally led to the so-called "recognition" of such changes, and the organ which recorded those changes then became a true sense-organ. This supposition may explain the multiplication of highly complicated "sense-organs" in animals which can apparently make no use of so many to *perceive* objects. The great number of sense-organs present in some animals is intelligible when we assume that they have a phagous function; and as, on any supposition, they are especially affected by *changes* in external conditions, I have called them *Dynamophagous* organs.

Milwaukee, April 11.

EXPLANATION OF PLATE III.

ax.n., axial nerve.
bc., bacillus.
b.m., basal membrane.
c.c., corneal cuticula.
c.c.c., crystalline-cone cells.
c.hy., corneal hypodermis.
ex.n., external nerve-fibres.
g.c., ganglionic cells.
l.ax.^{1} and *l.ax.*^{2} axial-nerve loops.
n f., nerve-fibres.
n.rf., nuclei of retinophoræ.
n.rf.^{1}, nucleolated nucleus of retinophoræ.
n.rf.^{2}, aborted nucleus of retinophoræ
pd., pedicle.
pg., pigment cells.
pg.$^{1-3}$, first, second, and third circle of pigment cells.
rf., retinophoræ.
rf.^{1}, innermost ends of crystalline cone cells, or retinophoræ.
rh., rod, or crystalline cone.
rt., retinula.
rt.$^{1-3}$, hyaline continuation of the retinulæ.
st., style of the retinophoræ, or crystalline-cone cells.
v.l., vitreous cell layer.
v.b., vitreous body.
v., crystalline cone, or vitrella.

1. Ancestral arthropod eye.
2. Same of larval insect.
3. Ocellus of *Scorpio;* only one ommatidium is represented.
4. Posterior ocellus of spiders.
5. Diagram of compound eye, to illustrate its origin as a modified ocellus.
6. One of the isolated ommatidia from the hypodermis of a Mollusc.
7. An ommatidium from a Molluscan retineum.
8. Ommatidium from the compound eye of *Arca* or *Pectunculus*; the retinulæ *pg.*$^{1\,2}$ have lost their rods, as is the case in all the succeeding diagrams, and serve only to protect the rod of the retinophoræ, or become transformed into ganglionic cells.
9. Same, with cross-section from the anterior ocellus of a spider.
10. The same, from the ocellus of Scorpio.
11. The same, from posterior ocellus of a spider.
12. The same, from the compound eye of Insects and Crustacea.
13. Two ommatidia from a vertebrate retina, without the outer ganglionic layers.

13*a*. Is a cross-section of the rods.

14. Diagram of an ommatidium, with the corneal facet and its cells, from a compound arthropod eye. The pedicle, walls of the retinophoræ, and the style, have been drawn in red for the sake of clearness; in all other cases the red indicates nerve-fibres; *x* is the refractive division between adjacent facets; *a*, that between the halves of each facet; *y*, thickening, sometimes present, of the abaxial walls of the calyx; the crystalline cone may be present or absent, but it can never fuse with the facet, as is supposed to be the case in *Lampyris*.

14*a* and 14*b* are cross-sections through the calyx and middle of the style, respectively.

15. Two retinophoræ with their ganglionic cells, from the retina of *Pecten*, showing the loops of the axial, and external nerves of the rods, the two nuclei of the retinophoræ, and five characteristic forms of ganglionic cells; *b.m.*, basal membrane, or septum, of the eye; *x*, a nerve-fibre terminating on a small ganglionic cell; *z*, and *y*, two methods of nerve endings upon the cell wall of the retinophoræ.

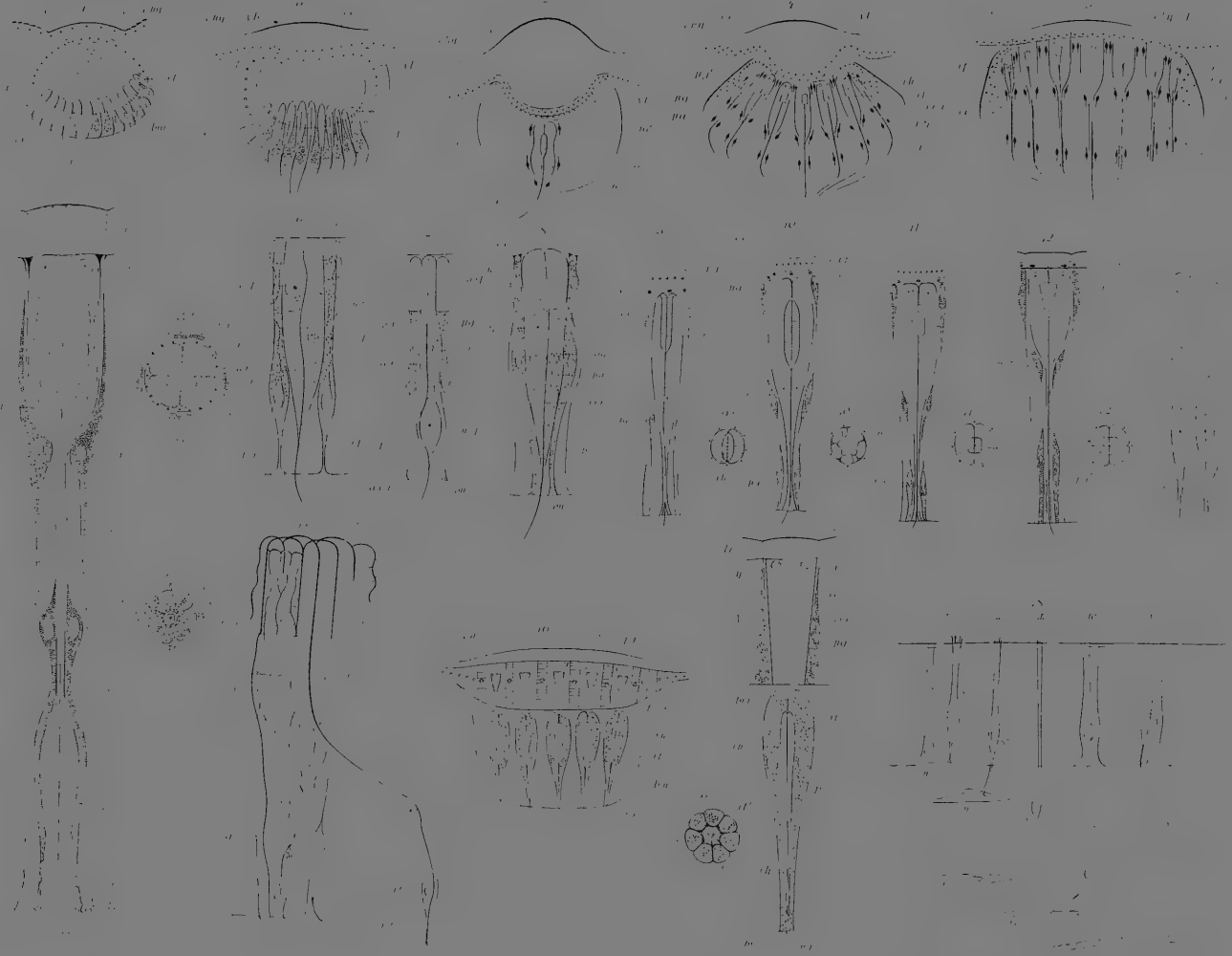
16. Diagram of compound eye, constructed according to Grenacher's views.

17. An ommatidium from a compound eye, constructed according to Grenacher's statements; he does not recognize the corneal hypodermis, and separates the eye into two layers at $b.m^1$; the dotted line, y, shows the position of the crystalline cone in certain cases where it appears to be absent.

17*a*, is a cross section of the retinulæ, showing the seven (or four) rods which they are supposed to secrete.

18. Diagram showing five stages in the transformation of a pair of epithelial cells; m^1 represents a myo-epithel cell connected with the sensory cell s^1. In II. and III., s^1 becomes a neuro-epithel cell, and m^1 has become a contractile cell. In IV., the neuro-epithel cell unites with a neighboring sense-cell by fine fibrillæ. In V., the outer end of the neuro-epithel cell has lost its sense-hairs, and is united to a neighboring sense-cell by numerous fibrillæ. Its outer end has become a true nerve-fibre, while its nucleated inner part develops new fibres uniting it with neighboring ganglionic cells. These five changes result in the transformation of the original epithel cell s^1, into the multipolar ganglionic one s^5.

The three cells s^6, s^5 and m^5 represent the three extremes in the modification of epithelial cells, at the same time, they represent the simplest combination of cells to form a mutually dependent sensory, nervous, and contractile system.



ON THE PHYLOGENETIC ARRANGEMENT OF THE SAUROPSIDA.

DR. G. BAUR,

YALE COLLEGE MUSEUM, NEW HAVEN, CONN.

Cope[1] has given the following classification of the Reptilia:—

A. Extremities not differentiated in form beyond proximal segment.

I. Os quadratum immovably articulated to squamosal, etc. Tubercular and capitular rib-articulations present and distinct.

1. *Ichthyopterygia.*

A.A. Elements of extremities differentiated.

II. Os quadratum immovably articulated, capitular and tubercular rib-articulations distinct. ARCHOSAURIA.

Pubis and ischium united, and with little or no obturater foramen; one posterior cranial arch; limbs ambulatory; a procoracoid. 2. *Theromorpha.*

Ischium and pubis distinct, the latter directed forwards, backwards, or downwards; two posterior cranial arches; limbs ambulatory; no procoracoid. 3. *Dinosauria* (+ *Crocodilia*).

Ischium and pubis united; two postcranial arches; anterior limbs volant. 4. *Ornithosauria.*

III. Os quadratum closely united to cranial arches; but one rib-articulation. SYNAPTOSAURIA.

Distinct hyposternal and postabdominal bones; ribs joining each two vertebræ, and generally forming a carapace; one posterior cranial arch. 5. *Testudinata.*

Hyposternal and postabdominal bones not distinct; two posterior cranial arches; ribs attached to one vertebra; a sternum; ? no procoracoid. 6. *Rhynchocephalia.*

[1] Cope, E. D. On the evolution of the vertebrata, progressive and retrogressive. Amer. Naturalist, March, 1885, pp. 245–246.

Hyposternal and postabdominal bones not distinct; two posterior cranial arches; ribs attached to one centrum; no sternum; a procoracoid. 7. *Sauropterygia.*

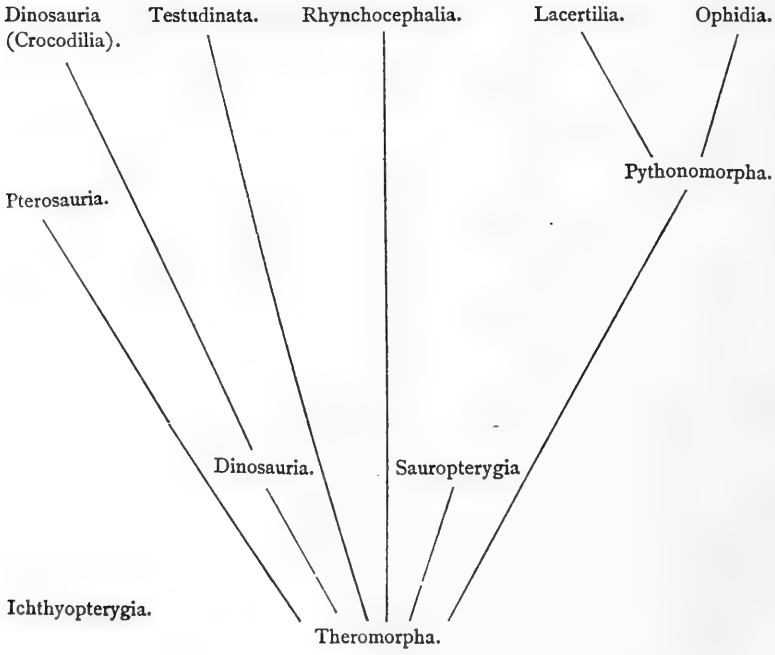
IV. Os quadratum attached only at the proximal extremity, and more or less immovable; ribs with one head. STREPTOSTYLICA.

Brain case membranous in front of proötic bone; trabecula not persistent. 8. *Lacertilia.*

Brain case with osseous walls anterior to proötic; a scapular arch and sternum. 9. *Pythonomorpha.*

Brain case with osseous walls anterior to proötic; no scapular arch nor sternum; trabecular grooves of sphenoid and presphenoid bones. 10. *Ophidia.*

The phylogeny of the Reptilia is expressed by Cope in the following way: —



Criticism of this Classification.

Ichthyopterygia.

The *Ichthyopterygia* are separated by Cope from the rest of the Reptilia because of the non-differentiation of the extremities in form beyond proximal segment.

That the Ichthyopterygia stand in the same relation to one or the other group of Reptiles as the Cetaceans do to the Ungulata and Carnivora seems to me unquestionable. Prof. Haeckel expressed this idea twenty years ago in his celebrated work: "Generelle Morphologie der Organismen." Vol. I., pp. 184–185. Berlin, 1866.

Of the Halisauria (= Enaliosauria = Ichthyopterygia + Sauropterygia), he says,—

"Ihre vielfachen Ähnlichkeiten mit den Fischen und insbesondere mit den Ganoiden haben zu der Annahme geführt, dass sie diesen näher als den übrigen Reptilien verwandt seien, und man hat selbst neuerdings versucht, die Ganoiden, Ganocephalen, Labyrinthodonten, Ichthyosaurier und Sauropterygier als fortlaufende Glieder einer einzigen Entwicklungsreihe darzustellen. *Indessen ist es viel wahrscheinlicher, dass diese Ähnlichkeiten mehr Anpassungs ähnlichkeiten sind, und dassdie Halisaurier sich zu den übrigen Reptilien verhalten, wie die Cetaceen zu den Säugethieren.*"

In 1881 Prof. C. Vogt[1] expressed the same opinion.

Quite lately I have brought forward new proofs for the correctness of this view.[2]

Therefore it is impossible to separate the Ichthyopterygia from the rest of the Reptiles; it would be the same as to separate the Cetaceans from the Mammals.

Weber[3] has shown, in a very important memoir, that the Cetaceans descended from a group of Mammals, probably ancestral to both the Ungulata and the Carnivora.

[1] Revue scientifique. 12. März, 1881; Kosmos, Vol. 9, 1881, pp. 318–319.

[2] Baur, G. Bemerkungen über Säuropterygia und Ichthyopterygia. Zool. Anz., No. 221. 1886.

[3] Weber, Max. Studien über Säugethiere. Ein Beitrag zur Frage nach dem Ursprung der Cetaceen. Jena, 1886.

The question now is, from which group of Reptiles descended the Ichthyopterygia?

The skull shows characters of the Rhynchocephalia, the oldest Crocodiles (Belodon) and the Dinosaurs; but it is still more generalized than in these groups. This is proved especially by two bones, — the opisthotic and the supratemporal.

The *opisthotic* is separate as in the Testudinata.[1]

Huxley[2] speaks of a flattened bone between the postorbital, postfrontal, and the squamosal; this bone (temporal, Cuvier — prosquamosal, Owen), according to Huxley, does not appear to have any precise homologue among other Reptilia.

I shall show, in another paper, that this peculiar "bone is nothing else than the supratemporal" of the Lacertilia, and the "squamosal" of the Stegocephali; that the "squamosal" of the Stegocephali is really the *supratemporal*, the "supratemporal" of the Stegocephali, the *squamosal*, of the Reptilia.

The Ichthyopterygia, therefore, are the only Reptiles, so far as now known, which have a supratemporal, like that of the old Stegocephali.

Another character common to the Rhynchocephalia, a few Lizards and Dinosauria, the oldest Crocodilia (Belodon), and Sauropterygia,[3] is the presence of the postorbital *and* the postfrontal in a separate condition.

The scapular arch of the Ichthyopterygia is Lacertilian or Rhynchocephalian.

The ribs are two-headed, like those of the Crocodilia, Dinosauria, etc. They are different from the ribs of all other known Reptiles, because they are never connected with the neurapophyses; they never leave the body of the vertebra (according to Owen's figures).

Abdominal ribs are developed, as in the Rhynchocephalia and Sauropterygia.

Therefore we have combined characters of the Rhynchocephalia, the oldest Crocodilia and Sauropterygia. To-day we do not know a group of Reptiles showing such characters;

[1] Cope, E. D. On the homologies of some of the cranial bones of Reptilia, and on the systematic arrangement of the class.
Proc. Amer. Assoc. Adv. Sc., Vol. XIX., p. 199. 1871.

[2] Huxley, T. H. A manual of the anatomy of vertebrated animals. London, 1871, p. 246.

[3] In *Simosaurus* these two bones have already united.

but that the Ichthyopterygia must have taken their origin from such a one I have no doubt.[1]

The Sauropterygia and Testudinata.

This group of Reptiles is classified by Cope with the *Synaptosauria*, which have but one rib articulation. This is not correct; the oldest Sauropterygia, the Lariosauridæ and Nothosauridæ, and even *Plesiosaurus*, have two-headed ribs in the cervicals and the former families even in the anterior dorsals. The posterior dorsals have the capitulum and tuberculum united, and therefore are morphologically two-headed.

It always seemed difficult to determine the systematic position of the Sauropterygia. Huxley considered this group allied to the Crocodilia, especially to the Teleosauridæ; Owen compared it with the Testudinata.

That they descended from land-living reptiles is certain.[2]

The Sauropterygia begin in the Triassic with the Lariosauridæ and Nothosauridæ. They are in no direct relation to the Ichthyopterygia. The skull and shoulder-girdle are entirely different in both.

The skull is very characteristic. It resembles the Rhynchocephalia and old Crocodilia (Belodon, Teleosaurus); the parietal foramen is present. The postorbital is free or united with the postfrontal. The whole shape (at least of some forms) resembles very much the skull of the Crocodilia; but one character shows at once the specialization of the Sauropterygian skull; it is the absence of the lower temporal arch, as in the Lacertilia; a quadratojugal seems never to be developed.

Another resemblance to the Rhynchocephalia consists in the structure of the abdominal ribs.

If we compare these elements in the Lariosauridæ and Nothosauridæ with those in Sphenodon we find exactly the same condition. In Lariosaurus and Sphenodon they are entirely identical, as the figures of Deecke prove.[3]

[1] The Baptanodontia (Sauranodontia), Marsh, are specialized forms of the Ichthyopterygia, like the Mystacoceti among the Cetacea, and the Pteranodontidæ among the Ornithosauria.

[2] Baur, G. Bemerkungen über Sauropterygia und Ichthyopterygia. Zool. Anz., No. 221, 1886.

[3] Deecke, W. Über Lariosaurus und einige andere Saurier der lombardischen Trias. Zeitschr. Deutsch. Geol. Ges. Bd. 38. Pl. III. Fig. 1.

There seem to be the larger number of connections with the Testudinata.

The cervical ribs of the Testudinata are entirely rudimentary. Hoffmann[1] has shown that they are developed in the embryos. In the adult Testudinata there are often well-developed diapophyses and parapophyses in the anterior cervicals. The former are connected with the neurapophyses, the latter with the body of the vertebra; both touch each other. Therefore we have a real para-diapophysis, and, consequently, if a rib should be developed, a rib with a capitulo-tuberculum. That the ancestors of the Testudinata had well-developed ribs on the cervicals is shown, not only by Hoffmann's researches, but also by the still developed para-diapophyses.

The pelvic arch of the Nothosauridæ is only comparable with that of the Testudinata. The shape of the pubis is very much alike in both. The obturator foramen is usually present in the Nothosauridæ, but is generally situated at the border of the bone. In the Testudinata the obturator foramen is generally wanting. Rudiments of it I find, however, in a specimen of *Eretmochelys imbricata*, and I do not doubt that a close examination of the pubic bones in the oldest Testudinata will show its rudimentary presence. The ischia are very much alike.

The femur of Nothosaurus, according to H. v. Meyer, is only comparable to that of the Testudinata.

The humerus of the Lariosauridæ and Nothosauridæ has the ectepicondylar foramen, like the Lacertilia, Rhynchocephalia, and Testudinata.

Other resemblances are to be found in the sacral and caudal vertebræ, in the condition of the chevrons, etc.

The sacral vertebræ of both the Sauropterygia and Testudinata have well-developed para- and diapophyses, to which the sacral rib is connected; the same is to be found in the anterior caudals; in the posterior caudals the para-diapophyses become rudimentary. In all the Sauropterygia the chevrons are attached only to the posterior part of the vertebræ, not between two vertebræ, as in the Rhynchocephalia, Crocodilia, etc.

The same has place in the Testudinata; if chevrons are de-

[1] Hoffmann, C. K. Über das Vorkommen von Halsrippen bei den Schildkröten. Beiträge zur verzleichenden Anatomie der Wirbelthiere, pp. 138-150. Leiden-Leipzig, 1879.

veloped, they are mainly connected with the posterior part of the vertebræ.

Another proof of the affinity of the Testudinata and Sauropterygia is given by Parker.[1]

He says: "There is one thing of great importance to be noted in the development of the Turtle, and that is the number of its body-segments at various stages, their rapid increase at first, and then the suppression or extinction of several afterwards.

"In embryos a little more than a quarter of an inch in length there are about 27 muscle-plates or somatomes.

"In embryos ranging from 6½ to 9 lines there are 51 of these divisions of the body visible externally.

"Now, in the adult I can only find 41 developed vertebræ, viz.: 8 cerv., 10 dorsals, 2 sacr., 21 caud., — 41 in all.

"But in the third and fourth stages there are at least 15 somatomes in the cervical region; in the dorso-lumbo-sacral, 12 (as in the adult), and 24 in the caudal, — 51 in all. Thus we miss in the adult 7 in the cervical and 3 in the caudal, — 10 in all.

"This free suppression of segments suggests a great secular modification by shortening of a form not unlike a *Plesiosaur*."

If it seems from the foregoing that the Testudinata are more or less related to the Sauropterygia, the question arises, which is the group of Reptiles ancestral to both? It is none of the known groups, and we can only admit that we do not know anything of the ancestors of the Testudinata and Sauropterygia; that it was a group allied to the Rhynchocephalia is probable. The Testudinata have an epipterygoid (columella), and it may be that the plastron of Testudinata was developed from or on abdominal ribs of a form like Sphenodon.

Rhynchocephalia, Lacertilia, Pythonomorpha, Ophidia.

Prof. Cope puts the Rhynchocephalia in one group, with the Testudinata and Sauropterygia. I do not find that natural. I find it very much more natural to combine the Rhynchocephalia with the Lacertilia, Pythonomorpha, and Ophidia.

The Rhynchocephalia are the most generalized of these;

[1] Parker, W. K. Report on the Development of the Green Turtle (Chelone viridis Schneid). The Voyage of H.M.S. Challenger. Zool., Vol. 1., p. 47.

they still have the complete lower cranial arch, lost in the other groups; they still have the intercentra, two Centralia in the carpus, and the proatlas. Their shoulder-girdle is entirely Lacertilian.

It is probable that the Homœosauria must be put together with the Rhynchocephalia; the shape of the jugal[1] shows that the lower arch was complete in *Homœosaurus*.

I do not doubt that *Homœosaurus* had intercentra between all vertebræ.

The Rhynchocephalia, together with the Protorosauria, to which they are allied, are certainly the most generalized group of all Reptiles, and come nearest, in many respects, to that order of Reptiles from which all the others took their origin. The embryology of *Sphenodon* (Hatteria) would be of the highest importance for the understanding of the phylogenesis of the Reptilia.

The "Simœdosauria." Dollo.

The "Simœdosauria," containing the cretaceous and eocene form, *Champsosaurus*, Cope, are considered by Dollo as a distinct order.[2]

I have to consider *Champsosaurus*, as Lydekker did, as a specialized member of the order Rhynchocephalia.

My principal proofs for that are, besides the common characters given by Dollo (l. c., p. 158): —

First. The loose condition[3] of the otic bones.[4]

Second. The condition of the rib-articulation.

Third. The articulation of the ribs in *Champsosaurus* is only comparable with that of *Sphenodon* and *Hyperodapedon*. The ribs of *Champsosaurus* are never placed *entirely* on the neurapophysis, as in the dorsals of Crocodilia, Dinosauria, Sauroptery-

[1] Ammon, L. V. Über Homœosaurus Maximiliani. Abhandlungen d. K. Bayr. Akad. d. Wiss. II. Cl. XV. Bd. II. Abth., München, 1885, p. 12.

[2] Dollo, L. Première note sur le Simœdosaurien d'Erquélinnes. Bull. Mus. Roy. d'Hist. Nat. Belg. Tome III. 1884.

[3] The loose condition of the otic bones, as described by Lemoine, is only found, in a similar way, in *Sphenodon*. In young specimens, and even in old ones, the sutures between the three bones are entirely distinct.

[4] Lemoine, L. Étude sur les caractères génériques du Simœdosaure. Reims, 1884.

gia, etc.; the same is to be found in *Sphenodon*. The differences between *Champsosaurus* and *Sphenodon*, given by Dollo (l. c., p. 159), are results of specialization of a Sphenodon-like form. *Champsosaurus* retains the neurocentral suture, which is lost in the adult *Sphenodon*.

Dinosauria — Crocodilia — Ornithosauria — Aves.

These four "orders" certainly form a natural group of the Sauropsida. The Dinosaurs are the oldest of these. It is not probable that *Protorosaurus* from the Permian is a Dinosaur, as Huxley[1] was inclined to suppose, and as Seeley[2] has quite lately suggested.

The Dinosauria form three well-distinguished groups.[3]

A. Carnivorous Dinosaurs,[4] *Harpagosauria*, Haeckel, 1866.
   I. *Goniopoda*, Cope, 1866 (Theropoda, Marsh, 1881).
B. Herbivorous Dinosaurs, *Therosauria*, Haeckel, 1866.
   II. *Orthopoda*, Cope, 1866.
      1. Ornithopoda, Marsh, 1881.
      2. Stegosauria, Marsh, 1877.
C. Crocodilian-like Dinosaurs, *Sauropoda*, Marsh, 1878.
   III. *Opisthocœlia*, Owen, 1859.[5]

It is possible that the Ornithosauria took their origin from true Dinosaurs; but at present we do not know such a group from which they could have originated. It may be that the Dinosaurs and Ornithosauria had a common ancestor.

[1] Huxley, T. H. On the classification of the Dinosauria, with observations on the Dinosauria of the Trias. Quart. Journ. Geol. Soc., Vol. 26, 1870, p. 37.

[2] Seeley, H. G. T. Philipps. Manual of geology, theoretical, and practical. Part 1. London, 1885, p. 515.

[3] I omit here the "Dinosauria" described by Owen in "Descriptive and illustrated catalogue of the fossil Reptilia of South Africa, in the collection of the British Museum, London, 1876." Owen creates a new group, *Tretospondylia: Tapinocephalus* with notochordal vertebræ. In the family *Serratidentia* he puts the genera *Pareiasaurus* and *Anthodon*. *Pareiasaurus* shows characters of the Sauropoda; *Anthodon* such of the Stegosauridæ. But from the present material the exact position of these forms cannot be determined.

[4] Baur, G. Der Tarsus der Voegel und Dinosaurier. Morph. Jahrb., Bd. VIII., 1882, p. 452.

[5] I retain the first name proposed by Owen, who put those "Saurians" among the Crocodilia. It is a fact that these forms show more characters common to the Crocodilia than to the *Orthopoda*.

The oldest *Crocodiles*, the Belodontidæ, and Aethosauridæ are very much allied to the Zanclodontidæ and the Sauropoda, and I feel quite sure that Crocodiles and Dinosaurs converge in the lower Triassic.

The Birds, and among those the Ratitæ with teeth, went off from the *Ornithopoda*,[1] but not from any known form.

Probably they started from some Ornithopoda-like form in the Triassic, because I do not doubt that some of the footprints in the Connecticut sandstone are made by true Birds.

The Orthopoda and Birds are the only Sauropsida in which the pubis is directed backwards;[2] a fact of great importance.

Archæopteryx is not on the direct line, but is a very much specialized member of a collateral branch.

The Theromorpha.

Prof. Cope includes the Theromorpha in the group consisting of the Dinosauria (Crocodilia) and Ornithosauria. I cannot agree with that proposition.

The Theromorpha form a natural group, like that containing the Crocodilia, Dinosauria, Ornithosauria, and Birds. They are limited to the Permian and Triassic.

This group is characterized by the absence of the postorbitosquamosal arch, the presence of the entepicondylar foramen, and the peculiar structure of the scapular arch.

The Pelycosauria, including Owen's Theriodontia, form the original stage, having the centra of the vertebræ notochordal and the intercentra present; the Anomodontia are very much specialized; the centra are not notochordal, the intercentra wanting, and the dentition in a rudimentary condition. Prof. Cope considers the Theromorpha as the ancestors of all other Reptilia with, possibly, the exception of the Ichthyopterygia. He also considers the Pelycosauria as the ancestors of the Mammalia. I tried to show in a lately published paper[3] that the Theromorpha are already too much specialized to be the ancestors of Mammals.

[1] Baur, G. Note on the Pelvis in Birds and Dinosaurs. Amer. Nat., Dec., 1884, p. 1274.

— Bemerkungen über das Becken der Vögel, und Dinosaurier. Morphol. Jahrb., Bd. 10, 1885, pp. 613–616.

[2] Baur, G. Zool. Anz., No. 216, 1886.

[3] Baur, G. Über die Kanaele im Humerus der Amnioten. Morph. Jahrb., Bd. XII., 1886, pp. 299–305.

The same I should like to say in regard to the Reptilia. I cannot see how the Testudinata, Lacertilia, Nothosauridæ, etc., with the *ectepicondylar* foramen in the humerus, could be developed from the Theromorpha with the *entepicondylar* foramen. It is true we know some Permian Reptiles which possess both the canals[1] in the same way as *Sphenodon;* but whether these forms are Theromorpha or Rhynchocephalia is a question.

I think it is very much more probable that the Theromorpha and Rhynchocephalia had a common reptilian ancestor below the Permian in the Carboniferous.

Cope[2] has described a fossil from the Carboniferous of Brazil, which he considers as Batrachian, with query. I have shown that it is probably a Reptile[3] allied to the Rhynchocephalia.

This fossil, which Professor Cope calls *Stereosternum tumidum*, has notochordal vertebræ, and the humerus with an epicondylar foramen; but the principal character of this animal consists in the presence of five distinct tarsal bones in the second row. This seems to me of very high importance. No Reptile, living or extinct, has more than four tarsal bones in the second row; the fourth and fifth digit is always supported by a single bone,—single even in the embryo. The Rynchocephalia, the Protorosauria, the Pelycosauria, all have only four tarsal bones in the second row.

This one character, if it is true, seems to be strong enough to allow the formation of a new order of Reptiles, which contains *Stereosternum;* for this order I propose the name *Proganosauria.*

But can we consider the Proganosauria as the ancestors of the other Reptiles? Possibly we may if we take the Proganosauria in a general sense. *Stereosterum* itself is certainly not on the direct line; it is a specialized member of the Proganosauria, like *Echidna*, a specialized member of the *Prototheria* (Monotremata). In some later time I hope to give more detailed communications on the philogeny of the single groups of Sauropsida.

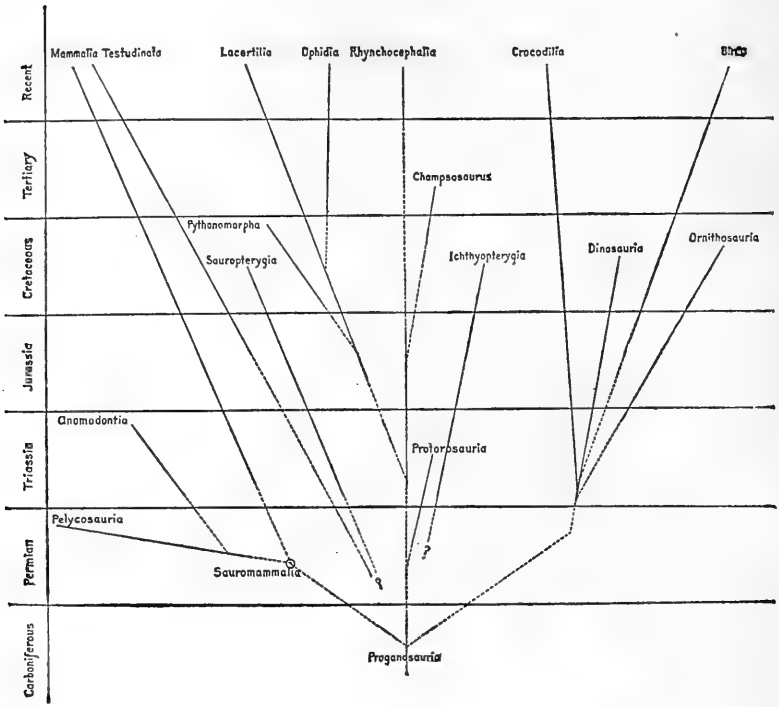
New Haven, Conn., Sept. 26, 1886.

[1] H. v. Meyer. Reptilien aus dem Kupfersandstein des West Uralischen Gouvernements Orenburg. Palæontographica, Bd. 15, Cassel, 1865–68, pp. 97–130.

[2] Cope, E. D. A Contribution to the Vertebrate Paleontology of Brazil. (Read before the American Phil. Soc., Apr. 17, 1885.) Pal. Bull., No. 40.

[3] Baur, G. Die zwei Centralia im Carpus von Sphenodon . . . Zool. Anz., No. 219. 1886.

The ideas brought forward in this paper may be expressed in the following diagram: —



A CONTRIBUTION TO THE HISTORY OF THE GERM-LAYERS IN CLEPSINE.

C. O. WHITMAN.

CONTENTS.

INTRODUCTORY.

THE origin and fate of the germ-layers in Clepsine have been the subject of investigation and critical discussion by a number of recent writers, who have reached conclusions so widely at variance that one might still say with Balfour, — "Our knowledge of the development of the Discophora is in a very unsatisfactory state." The origin of the mesenteron is very difficult to trace, and ignorance of its history has led to the greatest con-

fusion in the identification of the germ-layers, and to most contradictory interpretations of the strata composing the germ-bands. The association of neuroblastic with mesoblastic elements in the germ-bands, as maintained in my first paper on this subject, proved a serious stumbling-block to Balfour.

The germ-bands in a closely related group of annelids, the Oligochæta, were held to be purely mesoblastic, and my suggestion that they contained a neural stratum appeared to stand in plain contradiction with well-established views as to the origin of the nervous system. Moreover, Kowalevsky, whose brilliant success in extending the germ-layer theory to the invertebrates had rendered his authority preëminent, had stated, as a fact settled by his own observations, that the nervous system of Clepsine, like that of Lumbricus and other oligochætous annelids, was derived from the "upper layer," *i.e.*, the epidermal layer. This statement, although based upon an evidently hasty and unreliable examination of a few poorly preserved eggs of Clepsine, was corroborated by more extended studies on Rhynchelmis (Euaxes) and Lumbricus, and later, by the researches of Hatschek, Kleinenberg, and others. The more trustworthy statements of Metschnikoff, published in the same year with Kowalevsky's "Embryological Studies on Worms and Arthropods," escaped the attention of Balfour, and thus the testimony of numerous excellent observers as well as theoretical considerations appeared to stand in the way of accepting my conclusions. The contradictory results since reached by Bergh and Nusbaum have only made it still more desirable to reëxamine the subject with greater care and thoroughness.

Bergh's important researches on the development of the Gnathobdellidæ have led him to dispute the concurrent testimony of previous investigators on the origin of the epidermal layer in Clepsine; and thus we are left in a state of uncertainty regarding the origin and limitations of the germ-layers, not very far removed from that in which Hoffmann found himself, when, at the end of his second memoir on this subject, he frankly confessed his inability to distinguish "Keimblätter" in the Hirudinea.

Respecting the histological differentiation of the germ-band strata, there is still less unanimity of opinion. Even the latest writers, Bergh and Nusbaum, have failed to agree on the

derivation of the ventral nerve-cord, and my studies have led me to results which contradict the conclusions of both these authors. Although the origin and development of the nephridia and the sexual organs have received special attention in recent papers, these questions are still very far from being definitely settled. The precise origin of the nephridia forms just now a question of considerable theoretical importance, — an importance which will be found to be very much increased by the facts to be presented in this paper, and by the parallel results reached by Wilson in his study of Lumbricus. We have long been prepared to believe in the homology of the germ-bands of the Hirudinea and the Oligochæta; but such a complete parallel, both in the mode of origin and in the histological development, as is shown in these two papers, will certainly be a surprise to most embryologists. The observations have been made, independently, on representatives of two different groups of annelids, and they confirm each other in a manner too positive to leave any room for a reasonable doubt of their essential accuracy. Allowing that they are correct, it will be seen that they furnish what we have long stood in need of, — a satisfactory basis for the comparative study of the germ-layers in annelids, — and that they give us one more clue to the ancestral history of the vertebrates.

For the observations recorded in this paper, I had only a single batch of eggs of Clepsine parasitica (?) Say, and a few eggs of C. complanata obtained at Naples. This scanty material did not enable me to carry the investigation beyond the stage in which the concrescence of the germ-bands is nearly completed. I am now able, however, to account for the origin of all the germ-layers, and to give the earlier history of the germ-bands. I have been able to add something to our knowledge of the development of the head, and I hope soon to obtain material for a thorough study of this very important part of the subject. The methods employed have been described in the "American Naturalist," Nov., 1885, pp. 1134–1135; and a preliminary notice of the results obtained was published in the "Zoologischer Anzeiger," No. 218, 1886.

I. CLEAVAGE, AND THE EARLY ESTABLISHMENT OF BILATERALLY SYMMETRICAL RELATIONS.

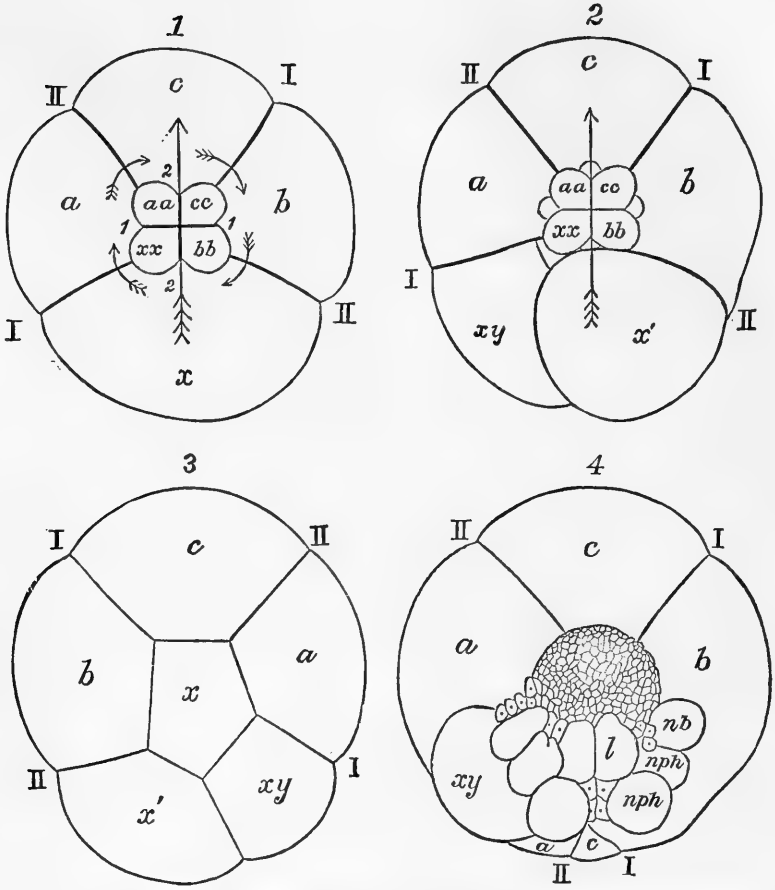
An accurate acquaintance with the principal events of cleavage is indispensable to a clear understanding of the derivation of the germ-layers; for these layers, and even the more important organs arising from them, can be traced directly to special blastomeres.

First Period, ending with the Eight-cell Stage.—A sketch of the cleavage, based upon results published in an earlier paper (No. 1, pp. 49–58, 75–76) is here introduced for the sake of clearness in the discussion and descriptions which are to follow.

The first two cleavage-planes are vertical, dividing the egg into four macromeres, three of which are relatively small, but nearly equal. The fourth, larger segment, which contains the remnants of the polar rings characteristic of this egg, occupies a *posterior* position with reference to the future embryo, while the *anterior* and the two *lateral* positions are held by the three smaller macromeres. The next step consists in the formation of four ectoblastic micromeres, which eventually present the figure of a quarterfoil at the animal pole. The posterior macromere (Diag. 1, *x*) first buds off the ectoblast *xx*, then the right macromere, *b*, gives rise to the second ectoblast, *bb*, and immediately afterward the third and fourth ectoblasts, *aa*, and *cc*, are produced simultaneously by the left macromere, *a*, and the anterior macromere *c*. We arrive thus at the eight-cell stage so common among worms and molluscs, represented by four macromeres surmounted by four micromeres that lie in cruciform order in the boundary-lines of the mother-cells.

The complete orientation of this stage embraces some relations of position yet to be noticed. The alternation of the micromeres with the macromeres is an arrangement brought about by a rotation of the whole quarterfoil, each segment having

(6.) Embryology of Clepsine. *Quart Journ. Mic. Sci.*, XVIII., 1878.



Semi-diagrammatic surface views of the egg of Clepsine in different stages of Cleavage.

Diag. 1. — The eight-cell stage, showing the relation of the embryonic axis to the first two cleavage-planes. The arrow, 2–2, shows the median plane of the embryo, and the four small arrows indicate the direction in which the four micromeres have rotated on the axis of the egg.

Diag. 2. — The posterior macromere (Diag. 1, *x*) has just divided into the neuro-nephroblast *x'*, and the two mesoblasts *x* and *xy*.

Diag. 3. — Same stage seen from the lower pole.

Diag. 4. — Stage in which all the teloblasts and the blastodisc have been formed.

I–I and 1–1. — First cleavage-plane; II–II and 2–2, second cleavage plane; *a*, left macromere; *c*, median and anterior macromere; *b*, right macromere; *x*, posterior macromere; *aa*, *cc*, *bb*, *xx*, micromeres derived from *a*, *c*, *b*, and *x'*, neuro-nephroblast; *xy*, left mesoblast; *x* (Diag. 3.), right mesoblast; *nb*, neuroblast; *nph*, nephroblast; *l*, lateral (median in this stage) teloblast.

moved through an arc of 45° from its place of origin. Had the segments maintained their original positions, it is evident that the arrangement would have been opposite, instead of alternate. The direction of rotation,[1] which is the same as that followed by the hands of a watch, is determined by the mode of origin of the segments. The segment *bb*, arising subsequently to the segment *xx*, pushes the latter to the left, and the positions thus established for the first two segments predetermine corresponding positions for the two last-formed segments (*aa* and *cc*). Such a point might at first sight appear too trivial for special notice, but its importance becomes apparent in view of a question, now attracting a good deal of attention, as to the relation of the first two vertical cleavage-planes to the median plane of the embryo. There is no danger of being too exact in regard to such relations, and the egg of Clepsine furnishes a case in which they can be determined with a certainty that precludes all controversy. As the micromeres represented, originally, the upper angles of the macromeres, it is evident that the planes of their apposed faces, marked by the boundary lines 1 and 2, must be regarded as parts of the cleavage-planes I and II, although, in consequence of the rotation of the quarterfoil, the former now form angles of about 45° with the latter. The boundary lines, 1 and 2, present the form of a cross, the arms of which, if extended, would bisect each of the macromeres. That limb (2) of the cross, which originally formed a part of the cleavage-line II, now marks the median plane of the future embryo. Here then is a case in which the median plane of the embryo is very clearly defined, and in which it coincides with neither of the first two cleavage-planes, but forms an angle of 45° with each of them.

The cleavages resulting in the eight-cell stage are comparable with the first two meridional cleavages and the first equatorial or horizontal cleavage in the eggs of amphibia and some fishes.

The further history of the quarterfoil of micromeres has not been worked out with sufficient thoroughness to say positively and precisely what part they play in forming the embryo; but it seems quite certain that they contribute to the formation of

[1] A similar rotation has been described by Hatschek (No. 35, p. 6) in Eupomatus.

the epidermal stratum of the ectoderm, and it is possible that they are directly employed in the formation of more important parts of the head. Smaller micromeres are gradually added round the primary quarterfoil, in the proliferation of which each of the macromeres appears to share. A disc of small cells is thus formed in which it is impossible to trace the genetic history of individual elements.

Second Period, ending with the Formation of Proliferating Blastomeres. — All regularity of cleavage ends with the eight-cell stage. Henceforth several distinct forms of cleavage will be carried on simultaneously, each restricted to special areas or blastomeres. Although there is scarcely anything in the external appearance of the eight-cell stage to indicate the relation of its parts to the future embryo, yet we know by what follows that an immense work has already been accomplished. All those fundamental conditions and relations implied in the terms anterior and posterior, right and left, dorsal and ventral, are now definitely established. The ground-plan of the future structure is there, and the segregation and distribution of the building material have advanced far toward completion.

The second period concludes the finishing strokes of cleavage, carrying us from the eight-cell stage to that in which the proliferation of the germ-bands begins. The prospective character of the work becomes more and more manifest, and architectural forecasts begin to reveal themselves. It is a period of preparation in which everything is ordered and appointed for the *formative* work with which the third or embryonic period begins. Proliferating blastomeres are created, grouped, and stationed according to the special kinds of work which they, as the artisans of the third period, are destined to accomplish. There are exactly *thirteen* of these blastomeres, symmetrically arranged in *three* primary groups. One group, consisting of *three entoblasts*, is represented by the macromeres, *a*, *b*, and *c*, or rather, will be represented by them at the end of this period, after they have ceased to contribute to the ectoblastic disc. The second and third groups, consisting respectively of *two mesoblasts* and *eight ectoblasts*, are yet to be developed from the large posterior macromere, *x*. The macromeres *a*, *b*, and *c* take no further part in the cleavage, if we except the budding off of ectoblastic micromeres at the animal pole, which does not sensibly diminish their size or alter

their general appearance. Throughout the embryonic period, until long after hatching, these huge segments preserve their individuality, only undergoing such slight changes in form and position as are induced by the development of the germ-bands and the epibolic expansion of the ectoderm. They contain most of the food-yolk, which is utilized in the long post-embryonic pseudo-larval period, and the elements which are to form the mesenteron.

The posterior macromere, x, on the other hand, undergoes sucessive cleavages, resulting in the production of *ten blastomeres*, or *teloblasts*, arranged in two bilaterally symmetrical groups, at the posterior edge of the blastodisc (Diag. 4).

The first cleavage-plane runs obliquely, beginning a little to the left of the upper angle and taking a slanting direction towards the right side, thus cutting off (x, Diag. 2) about one-third of the original macromere. Then follows a second cleavage, at right angles to the first, cutting the larger segment into nearly equal parts (x and xy, Diag. 3). The posterior macromere is now represented by three sub-equal segments: one of these, x', which may be called the *neuro-nephroblast* ("primary neuroblast," in my first paper), lies at the posterior edge of the blastodisc, more on the right than the left side. The second and third, representing the *mesoblasts*, are also asymmetrically placed, one (x) occupying a central position at the lower pole of the egg (Diag. 3), the other ($x\ y$) lying behind it, and to the left of the neuro-nephroblast (x'). Neither in the general appearance nor in the relative positions of the two mesoblasts is there anything indicative of their homotypical character. They appear to be vitelline spheres of the same nature as the three macromeres, a, b, and c, and have always been so regarded by earlier observers. In the course of this period a shifting of position among the cleavage-spheres takes place, which brings the mesoblasts into harmony with the bilateral symmetry of the egg. The three macromeres lengthen backward, slowly flowing over the mesoblasts and more or less completely enveloping them, so that one or both of them may entirely disappear from view, or, at least, become so obscure in outline as not to be easily recognized. In its backward elongation the anterior macromere c takes up a median ventral position between a and b, and usually carries the mesoblast x towards the hind end at the same time that it incloses it.

The right mesoblast x thus becomes imbedded mainly in c, but usually lies partly in b. Meanwhile, the mesoblast, xy, takes up an opposite position in the left macromere, a. I have never seen a single case in which bilateral symmetry was complete with respect to the mesoblasts; but the final position is always such that each proliferates exclusively for the germ-band of its own side. Their relative positions in transverse section are shown in Fig. 2, Pl. IV. In Fig. 3, representing a sagittal section of *C. complanata*, the right mesoblast, x, is placed much farther forward than is usually the case in *C. marginata*.

By successive vertical cleavages the neuro-nephroblast is split up into eight octoblasts, arranged symmetrically in two groups at the posterior edge of the blastodisc, as shown in Diag. 4. These cells have a superficial position at first, but the two median ones are very soon covered by the expanding blastodisc, and the rest are later overgrown by the same elements.

Towards the close of this period, when all arrangements for the formation of the embryo have been completed, free nuclei begin to appear in the surface of the three entoblasts, a, b, c. The origin and fate of these "entoblasts" have been traced with considerable care, and the results of my study on this point have confirmed the opinion that they give rise to the mesenteron.

II. ORIGIN OF THE MESENTERON.

1. *Historical and Comparative.*

Clepsine.—Grube (No. 2), and Leuckart and Rathke (No. 3), derived the entoderm by delamination from the blastoderm. But these authors, whose observations on this subject were made long before the introduction of the microtome, were not able to bring any direct evidence of such a mode of origin. It was simply the most rational conclusion open to them, considering the methods at their command, and what was then known

(2.) GRUBE, A. E. Untersuchungen über die Entwicklung der Clepsinen. *Königsberg.* 1884.

(3.) RATHKE, H., AND LEUCKART, R. Beiträge zur Entwicklungsgeschichte der Hirudineen. *Leipzig.* 1862.

respecting the genetic relations of the germ-layers. Even Hoffmann (No. 4, p. 45), working as late as 1877, with "Pikro-carmin gefärbten Querschnitten," arrives at the same conclusion, and expresses it in words that appear to be, in great part, a direct transcript of Leuckart's phraseology.

According to Robin (No. 5, pp. 297–298), the entoderm arises as a solid cord of cells in the axis of the mass represented by the three entoblasts, *a*, *b*, and *c*. A lumen first appears in the œsophageal portion of the axial cord, and is gradually extended backward, thus forming a digestive tube, with all the yolk lying external to it. Somewhat later (5–8 days after exclusion) the three entoblasts ("globes vitellins") undergo cleavage, breaking up into large cells, that form "*la couche moyenne de l'intestin et particulièrement la couche hepatique.*"

In my memoir on "the Embryology of Clepsine" I was unable to give a complete history of the origin of the mesenteron from the three entoblasts, but the facts there presented appeared to leave little room for doubt. Both my observations and conclusions have been contradicted by one of the latest writers on the subject. Soon after the publication of my paper came Hoffmann's (No. 6) second contribution to the embryology of the Hirudinea, in which he declares his opposition to my views in the following words: "Ich muss dieser Entstehungsweise des Darmepithels auf das bestimmteste wiedersprechen. Die 'dark spots in the opaque yolk,' welche nach Whitman in der Zeit, dass die Keimstränge sich ausbilden, so recht deutlich auftreten, sind, ich wiederhole es, nichts anderes als Protoplasma-Masse, welche sich aus dem Deutoplasma neu gebildet hat, sich abschnürt und so an der Bildung der Keimstränge sich betheiligt. *Bei ausgeschlüpften Embryonen findet man innerhalb des Dotters auch nicht einen Kern.* Wären sie vorhanden, so müssten sie an in Pikro-carmin gefärbten Schnitten sichtbar werden, denn die intensiv rothe Farbe der Kerne und des Proto-

(4.) Hoffmann, C. K. Zur Entwickelungsgeschichte der Clepsinen. *Niederländisches Archiv für Zoologie*, IV., H. 1, p. 31. 1887.

(5.) Robin, C. Mémoire sur le Développement Embryogénique des Hirudinées. *Paris.* 1875.

(6.) Hoffmann, C. K. Untersuchungen über den Bau und die Entwickelungsgeschichte der Hirudineen. *Haarlem.* 1880.

plasmas, wie die grüne der Dotterkörnchen ist zu charakteristisch, als dass man dieselbe übersehen könnte." (p. 53).

"Die Muskelfaserschicht und das Epithelium des Chylusdarmes entstehen beide aus ursprünglich von dem Keimstreifen herrührenden zelligen Elementen" (p. 51). The same origin is claimed for the intestine (Enddarm) and the œsophagus (Schlunddarm).

Metschnikoff (No. 7, p. 672) expresses no decided opinion on the origin of the mesenteron, but throws out the following suggestion. Speaking of the inner stratum (mesoderm) of the germ-bands, he says: "Man kann sehr leicht die Überzeugung gewinnen, dass das sich spaltende Blatt die äussere (*vielleicht auch die innere*) Wand des Mitteldarmes, den sog. Fettkörper, und die Segmentalorgane liefert."

The latest authority on this subject is Joseph Nusbaum (No. 8). In the introduction to his paper, Nusbaum states at some length Hoffmann's erroneous views concerning the nature of the "entoplasts," and then announces, as a discovery of his own, the fact that these entoplasts ("*îlots protoplasmiques*") represent the entoderm. Why so much attention should be lavished on Hoffmann's ideas, which had already been refuted by Bergh, and why the fact should be suppressed that the origin of the entoderm had been ascertained eight years before, I will leave Nusbaum to explain. To discover what has already been discovered, and refute what has already been refuted, is a double-headed offence, inexcusable if the result of ignorance, unpardonable if done deliberately. Nusbaum very narrowly escapes from this charge, for later on he feels constrained to drop the following saving remark: "Selon Whitman, l'épithélium intestinal se forme aux dépens de grands globes entodermiques, à la surface desquels se montrent les noyaux libres et ensuite une couche de cellules épithéliales qui limite de tous les côtés le vitellus nutritif. En un mot, d'après Whitman, l'entoderme secondaire se forme de l'entoderme primitif."

Nusbaum carefully refrains from any direct acknowledgment

(7.) METSCHNIKOFF, ELIAS. Beiträge zur Entwickelungesgeschichte einiger neideren Thiere. Vorläufige Mittheilung. *Mélanges Biologiques* VII., p. 671. 1871.

(8.) NUSBAUM, JOSEPH. Recherches sur l'organogénèse des Hirudinées (Clepsine complanata Sav.). *Archives slaves de Biologie, I. Fasc.* 2, pp. 310–340, *and Fasc.* 3, pp. 539–556. 1886. *Published separate.*

that his conclusions had been anticipated, and turns at once to the more agreeable task of contradicting Hoffmann's account. His own observations are then detailed as follows. Beginning with an embryo in which the somites are already distinctly marked from end to end, and the nephridia differentiated, he says: "Il se montre sur toute la surface externe du vitellus une couche protoplasmique granuleuse (*en*) avec des noyaux ovoides et arrondis; cette couche, développée aux dépens des éléments cellulaires de l'entoderme primitif, se transforme ensuite en épithélium intestinal. Hoffmann a observé aussi une semblable couche protoplasmique avec des noyaux ovoides, mais il l'a considérée comme un produit du mésoderme. Cependant j'ai vu plusieurs fois, sur des coupes très minces et parfaitement colorées, que cette couche est délimitée d'une manière très nette de la couche de cellules plates du feuillet viscéral du mésoderme, tandis que du côté interne la couche protoplasmique passe directement dans le vitellus; ainsi l'origine entodermique de cette couche ne peut être soumise à aucun doute" (p. 9).

This is all that Nusbaum has to tell us about the origin and history of the entoplasts. The whole alimentary tract is lined with cells of the same origin. "Ainsi le résultat final est que l'épithélium (plat) tapissant la cavité de la trompe représente un produit entodermique. Tout l'intestin postérieur représente de même un produit entodermique" (p. 11). The sequel will show that Nusbaum has added very little to what was already known on this subject.

Bergh (No. 9, pp. 259–260) confirms my statements in regard to the existence of peripheral nuclei in the three entoblasts, but rejects the hypothesis that they represent the entoderm, as incompatible with what is known about the origin of this layer in the Gnathobdellidæ.

Nephelis. — According to the observations of Kowalevsky (No. 10, p. 3), Bütschli (No. 11,), and Bergh (No. 9, p. 259), the entoderm in Nephelis arises immediately after the eight-cell

(9.) BERGH, R. S. Die Metamorphose von Aulastoma gulo. *Arbeiten a. d. zool.-zoot. Institut in Würzburg.* VII., H. 3, p. 231. 1885.

(10.) KOWALEVSKY, A. Embryologische Studien an Würmern und Arthropoden. *Mém. de l'Acad. Imp. de Sc. St. Pétersburg.* XVI., No. 12. 1871.

(11.) BÜTSCHLI, O. Entwicklungsgeschichtliche Beiträge. *Zeitschr. f. wiss. Zool.* XXIX., p. 239. 1877.

stage is reached, consisting at first of a few small cells lodged between the micromeres and the macromeres, and derived in all probability from the latter. Bergh is inclined to the belief that this mode of origin holds true of the Rhynchobdellidæ as well as the Gnathobdellidae. He finds in the egg of Clepsine certain cells beneath the blastodisc, in a position which corresponds perfectly to that of the first entoderm cells in Nephelis, and hence infers that they represent the entoderm of Clepsine, although he has not traced their development into this layer.

Notwithstanding the remarkable difference in the position of the "residual" yolk, which distinguishes the two types of leeches represented by Clepsine and Nephelis, it must be taken for granted, so long as no positive proof to the contrary has been produced, that the mesenteron arises in essentially the same manner in both cases. I have ascertained enough of the history of the cells in Clepsine, which Bergh identifies with the entoderm of Nephelis, to convince me that he is partly right on this point; but it is an error to suppose that they constitute the whole, or even the larger part, of the entoderm in Clepsine, and the observations of Kowalevsky and Bütschli fall very far short of establishing such a conclusion in the case of Nephelis. Kowalevsky gives no figures, and offers only the following brief sketch of the germ-layers, which he illustrates by referring to Rathke's plates:—

"Was die Scheidung der Blätter anbetrifft, so ist dieselbe schon auf der Fig. 13, Taf. I., bei Rathke zu entdecken; die oberen Zellen, *ff*, bilden das obere oder *sensorielle* Blatt, die zwischen denselben und den grossen Furchungskugeln liegenden — das *Darmdrüsenblatt* (Figs. 14 and 15), und die drei grossen Kugeln — das *mittlere* Blatt. Weiter umwachsen die Zellen des oberen Blattes die ganze Masse von allen Seiten; die Darmdrüsenblattzellen wachsen sehr schnell, verlieren ihr körniges Aussehen, werden zu grossen hellen Zellen und drängen dabei die grossen Zellen des mittleren Blattes nach hinten, welche durch Abschnürung zwei Zellenreihen bilden, die bekannten Keimstreifen der Nephelis sind." (No. 8, p. 3.)

Kowalevsky's interpretation of the three large segments — the homologues of the entoblasts in Clepsine — as mesoblasts, has been corrected by Bütschli, and his statement on the origin of the entoderm is as indefinite as it is brief.

So far as the published records show, Bütschli is the only one who has thus far undertaken to trace the origin of the entoderm in Nephelis with that precision and attention to details which are now required in such work. But there are some important gaps in his work which must be filled before the origin and relation of the germ-layers can be satisfactorily determined. The posterior macromere in Clepsine, as we have seen, is the sole source of the germ-bands, and its history is the key to subsequent development. Unfortunately our knowledge of this macromere in Nephelis is not complete enough to warrant the assertion that it plays the same rôle; but the facts, so far as they go, point in this direction. When we remember that the germ-bands of Nephelis have been traced by Bergh (No. 10) to ten terminal cells, which we have every reason to suppose are identical with the ten teloblasts of Clepsine, and further, that the eight-cell stage arises in the same manner in both cases, it seems incredible that there should be any radical differences in respect to the fate of the posterior macromere. If, however, this macromere in Nephelis is converted into the ten teloblasts of the germ-bands, — and there is nothing against, and everything for, such a supposition, — it is plain that Bütschli's observations on the origin of the germ-layers are very far from complete. It is simply incredible, in view of what happens in Clepsine, that the whole entoderm and mesoderm should arise in the manner described by him. If the deep cells discovered by Bütschli represent only the earliest and most anterior portion of the entoderm, then the chief difficulty in the way of reconciling the two types of development disappears. This appears to be the only mode of reconciliation open to us, if my observations on Clepsine are correct; and it is entirely permissible to hold this ground until some one has cleared up the history of the posterior macromere in Nephelis.

Robin (No. 5, p. 146) attempted to do this, but his methods of study were not equal to the task, and he fell into the error of supposing that the products of this macromere represented the dorsal moiety of the ectoderm. Bütschli has added but little on this important point; but a summary of his studies on the germ-layers will be needed in order to place the subject in a clear light.

Bütschli's account begins with the eight-cell stage, with what

I have called the second period in Clepsine. This period is initiated by two events, — (1) the cleavage of the posterior macromere, and (2) by the appearance of two small cells beneath the micromeres. Bütschli directs his attention almost wholly to the second of these events, and, with the exception of a few incidental remarks, completely ignores the first.

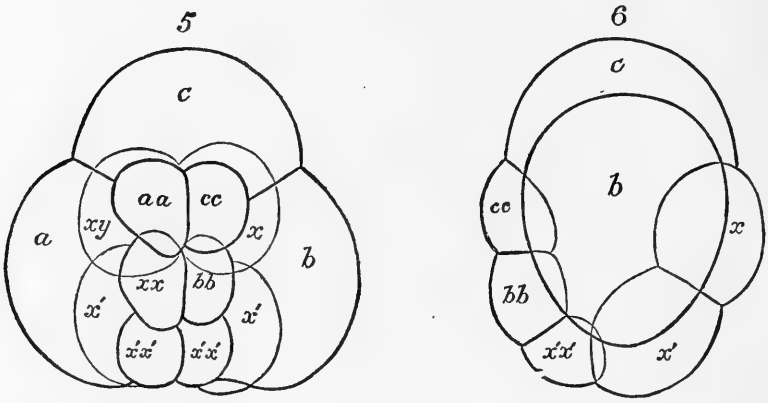
The posterior macromere first divides into two equal parts, one of which occupies nearly a central position on the lower face of the egg, while the other lies behind and slightly above, abutting against the posterior edge of the ectodermic quarter-foil. It will be remembered that similar relations are constant in the corresponding stage of Clepsine, with the single difference that the posterior of the two segments (the neuro-nephroblast) is usually only about one-half the size of the other. It is a fact of considerable importance to us that the second period is introduced in both cases in the same remarkable manner (*i.e.*, by the cleavage of one macromere), for we are thus assured that events are still moving on in parallel courses. I am quite confident that the two segments resulting from the division of the posterior macromere will be found to correspond to the *neuro-nephroblast* and the *primary mesoblast* of Clepsine, and I shall henceforth designate them by these names. One fact only, noticed by Bütschli, throws a little doubt on the identification of the neuro-nephroblast. This segment buds off two micromeres behind the four already formed at the animal pole, and the six micromeres of this stage are arranged in two longitudinal rows.[1] I have seen nothing in the egg of Clepsine comparable with the last two micromeres, but I have shown (No. 1, p. 54, x^4) that two ectoblastic micromeres arise in a little later stage, not directly from the neuro-nephroblast, but from the immediate products of this segment.

The neuro-nephroblast next divides by a sagittal cleavage into two equal parts, which are probably the equivalents of the cells marked x^2 (Fig. 31, Pl. XII.) in my first paper; and then follows the cleavage of the primary mesoblast into two equal parts, bilaterally disposed, and corresponding in position and origin to the two mesoblasts of Clepsine. According to Robin (No. 5, p. 150) the primary mesoblast divides before the neuro-nephroblast, in the same order as in Clepsine.

[1] Precisely like the "mesomeres" in Rhynchelmis.

Possibly the order in Nephelis is variable, in which case Bütschli's observations on this point would appear supplementary, rather than contradictory, to those of Robin.

Bütschli failed to trace the cleavage in detail beyond the sixteen-cell stage, in which (Fig. 5, Pl. XVIII.), as I have shown, every group of segments, and nearly every individual segment even, can be identified with those of the corresponding stage in Clepsine. The close correspondence in parts, relative size, position, and axial orientation, is shown in Diags. 5 and 6. The bulk of the egg is formed of the three macro-



Two views of the sixteen-cell stage of Nephelis. Three deep cells, representing the first entoderm cells, are not shown.

DIAG. 5. — Seen as a transparent object from the upper side. (After Bütschli.)

DIAG. 6. — Side view of the same stage. (Constructed after Bütschli and Robin.)

a, left macromere; *c*, median and anterior macromere; *b*, right macromere; *aa*, *cc*, *xx*, *bb*, micromeres derived from *a*, *c*, *b*, and *x*.

x'x' and *x'x'* micromeres derived from the neuro-nephroblast (*x'*), which is now represented by two cells, *x'* and *x'*.

xy, left mesoblast; *x*, right mesoblast.

meres, one (*c*) of which has an anterior median position, while the second and third (*a* and *b*) are laterally placed. The six micromeres are arranged in two bilaterally symmetrical rows, extending from the upper angle of the anterior macromere to the hind edge of the egg. The two mesoblasts (*x* and *xy*) are

symmetrically placed near the middle of the lower face of the egg. Behind and somewhat above them come the two daughter-cells of the neuro-nephroblast. The remaining three cells (not shown) lie beneath the surface, between the two anterior micromeres and the two mesoblasts, and represent the earliest portion of the entoderm. *The embryonic axis has precisely the same relations to the primary-cleavage planes as in Clepsine.*

A further confirmation of the opinion that all the essential details of the second period in the egg of Clepsine repeat themselves in the egg of Nephelis is found in the peculiar shifting of position among the cleavage-products. Bütschli (No. 11, p. 242) briefly alludes to the fact that the smaller cells become more or less completely imbedded in the macromeres, and a glance at his figures shows that the anterior macromere passes backward between the lateral macromeres, and, ultimately, takes a position at the hind end of the embryo. The same movement takes place in Clepsine, only it is not carried quite so far, leaving the macromere in a median ventral position, stretching from end to end.

Bütschli does not discuss the nature of the cells derived from the posterior macromere, and only alludes to them in one place (p. 242) as ectoderm cells. Abandoning the only clue that could guide him safely through subsequent phases, his statements become indefinite, if not obscure; and neither his descriptions nor his figures are free from confusion. Beyond the sixteen-cell stage he is not even able to say whether his figures represent the upper or lower side of the egg, or to state definitely which side corresponds to the ventral or dorsal aspect of the future embryo. Entoderm cells are first represented in red, then in blue, and each of the three germ-layers appears in turn in red. Such incongruity in diagrammatic representation can have but one explanation. Allowing that it was the best that could be done under the circumstances, it is obvious enough that Bütschli failed to leave the subject of the germ-layers in a satisfactory state, and that much remains to be done before the origin of these layers is as clear in Nephelis as in Clepsine.

The three deep cells, which are omitted in the diagrams, were discovered by Bütschli, and interpreted as the "Anlage des Entoderms." Their origin was not directly observed, but their

position was held to be sufficient evidence of derivation from the three macromeres. The number of these cells is very soon raised to six, but whether by renewed proliferation on the part of the macromeres, or by subdivision among themselves, we are not informed. A little later the still more numerous entoderm cells present the form of a solid axial cord, on each side of which is seen a row of three mesoderm cells. These two rows of cells are regarded as the basis of the germ-bands ("Keimstreifen"), but the important question of their origin is left undecided. The mesoderm cells increase rapidly, but not by proliferation on the part of the vitelline macromeres, as supposed by Kowalevsky. Bütschli expressly states that he has never seen these macromeres in process of division, until in a very late stage of the free embryonic life, when, as was first shown by Robin, they break up into a number of cells, which undergo resorption in the body-cavity (Bergh).

In the next stage represented by Bütschli, a narrow, slit-like lumen is seen between the entoderm cells, which is the incipient enteric cavity. The number of entoderm cells must be small, as only *eight* are shown in optical section (Fig. 9, Pl. XVIII.); and it must be noted as a still more remarkable fact that precisely the same number of cells appear in a much later stage (Fig. 12), after the formation of the œsophagus. They have increased immensely in size, while the three macromeres have become proportionately smaller. Bütschli thinks the entoderm cells enlarge at the expense of the fluid food-material contained in the egg-case; but his figures suggest that the growth is at the expense of the macromeres, and analogy would lead one to suppose that the embryo would exhaust its own stock of food-stuff before drawing upon external supplies. It is evident that these large entoderm cells have many changes to undergo before assuming the form of a lining epithelium; but what these changes are, and how the cells multiply, are questions left unanswered by Bütschli's observations. I find no mention of "free nuclei," and no indications of such bodies in his figures, unless the two supernumerary nuclei, shown in Fig. 12, may be so regarded. Such nuclei have been observed in the egg of Nephelis, according to Balfour (No. 12, pp. 3–9).

(12.) BALFOUR, F. M. A preliminary Account of the Development of the Elasmobranch Fishes. *Quart. Jour. Mic. Sc.* XXII., p. 323. 1874.

"Dr. Kleinenberg has followed a single egg through the whole course of its development, and concludes that the nuclei of Nephelis never become the nuclei of new cells." [1]

Assuming, then, that the ten terminal cells in Nephelis are the homologues of the teloblasts in Clepsine, and that, accordingly, the primitive mesoblastic cords discovered by Bütschli arise neither from the micromeres nor from the three entoblastic macromeres, *a*, *b*, *c*, but from a pair of cells (*x* and *xy*) derived from the posterior macromere, *xx*, the way is clear for comparing the entoderm cells. The chief differences are: (1) the absence of "free nuclei" in Nephelis, and (2) the contra-distinction in position of the three macromeres, which lie *within* the mesenteron in Clepsine, and *external* to it in the body cavity in Nephelis. The foundation of both differences is undoubtedly the relative abundance of food-yolk. The egg of Clepsine is much larger than that of Nephelis, and is completely filled with yolk-spherules, which are to serve as food during several weeks of larval life. The egg of Nephelis, on the other hand, has very little food-yolk, the larva depending upon the fluid albuminous substances contained in the egg-case. The abundance of yolk in the egg of Clepsine makes it necessary for the nuclei to seek a peripheral position, as in the case of so many arthropod eggs; and thus the yolk is left *within* the enteric epithelium. In the egg of Nephelis, the entoderm cells all escape from the macromeres at an early date, and henceforth they multiply by subdivision, and probably grow at the expense

[1] Balfour has here given Kleinenberg credit for what neither he nor any one else has ever yet accomplished. The method has not yet been discovered by which the egg of Nephelis can be kept alive for more than a few hours, after removal from the egg-case. Continuous observation on a single egg through all its stages of development is, therefore, a feat entirely beyond our present means. Kleinenberg's opinion as to the fate of certain nuclei must rest on evidence of a much less conclusive nature than supposed by Balfour. It is to be hoped that Kleinenberg will yet publish the results of his study on Nephelis, for we certainly stand in need of more light on this subject.

Bergh's brief remark on the origin of the mesenteron may be interpreted in favor of the existence of free nuclei. "Letzteres [Mitteldarmepithel] bildet sich nämlich aus dem primären Entoderm durch *fortdauernde Kernvermehrung;* erst nach dem Ausschlüpfen aus dem Kokon bildet es sich als eigentliches Epithel aus, *indem das Protoplasma sich um die Kerne herum in Zellen sondert.*" (No. 13, p. 294.)

(13.) Bergh, R. S. Ueber die Metamorphose von Nephelis. *Zeitschr. f. wiss. Zool.* XLI. H. 2. p. 284. 1884.

of the macromeres. The earliest entoderm cells are formed in precisely the same manner in Clepsine; but here a few cells are left to multiply in the macromeres, and these take the form of "entoplasts," and are to be regarded as the equivalents of the large mesenteric cells of Nephelis. The œsophagus of Nephelis is probably lined with cells derived from the first-formed entoderm cells, precisely as is the case in Clepsine. *That there is no fundamental distinction between the "entoplasts" of Clepsine and the entoderm cells of Nephelis is shown by the fact that we have both modes of formation in Clepsine, the one passing gradually and insensibly into the other.*

Bergh (No. 9, p. 260) explains the above-named difference in the position of the vitelline macromeres as the result of a retardation in the development of the mesenteron in Clepsine, and apparently regards the Nephelis type of development as the more primitive. The view presented above appears to me more satisfactory. I am not by any means ready to adopt the idea that the mode of development in Clepsine is to be regarded as a modified or derived form of that seen in Nephelis. It would be quite as rational to take just the opposite ground, and maintain that the egg of Nephelis has been derived from one that was heavily loaded with food-yolk. The mode of cleavage, and especially the persistence of the three macromeres, appears to support such a view.[1]

Rhynchelmis (Euaxes).— In spite of the many gaps in our knowledge of the development of Nephelis, it has been easy to find a close and interesting parallel between it and Clepsine in the early phases of the egg. This, to be sure, is no more than might have been expected in the case of two forms so closely allied, but it is more than could have been conceded without first showing how differences of opinion could be reconciled. If now we extend the comparison along the same lines to one of the Chætopods, *e. g.*, Rhynchelmis, we shall find the suggestions advanced in the foregoing pages corroborated in many points of fundamental importance.

[1] The power to elaborate and store nutritive yolk comes and goes with the need; but the mode of development induced by the presence of yolk appears to persist, to a greater or less extent, even after the yolk has been lost. Such has, in all probability, been the case with the mammalian egg, and there is reason to suspect that other alecithal eggs have had a similar history. Many anomalies of development may be accounted for in this way.

The cleavage in Rhynchelmis has been studied with considerable care by Kowalevsky (No. 10, p. 12), and by Vejdovsky (No. 14, p. 228). Some of the changes which the egg undergoes preparatory to cleavage, as described by Vejdovsky, are of great interest, on account of their manifest identity with certain remarkable polar phenomena which display themselves with great intensity in the egg of Clepsine. I refer to the polar rings of hyaline protoplasm, which concentrate at each pole in the form of a disc. The first two cleavage-planes divide the egg into four macromeres, three of which are nearly equal, and correspond to the anterior and the two lateral macromeres of Clepsine, while the fourth and largest one represents the posterior macromere, and contains most of the hyaline protoplasm of the polar discs, precisely as in the egg of Clepsine. Such a close correspondence in the primary steps of cleavage, resulting in the specialization of one of the four macromeres, affords the strongest possible evidence, short of verification by direct observation, that the subsequent history of this macromere will be essentially the same as that of the posterior macromere in Clepsine. Unfortunately, the observations on this point are too incomplete to be decisive; but two important facts may be noted which furnish, at least, a partial verification of the view here taken. First, the median plane of the embryo bears to the first two cleavage-planes relations which are analogous to, if not quite identical with, those described in Clepsine; and, secondly, a pair of mesoblasts arise from the posterior macromere. Remembering that only two teloblasts were hitherto recognized in Lumbricus, and that a careful study of the germ-bands from surface-preparations has led Wilson to the discovery of neuroblasts and nephroblasts, it is not venturing much to predict a similar discovery for Rhynchelmis. In the Hirudinea the mesoblasts lie beneath and usually in front of the remaining teloblasts, and their relations to the germ-bands are not easily ascertained without the aid of sections. Most of the remaining teloblasts (neuroblasts and nephroblasts) are very prominent even in the living egg. In the Oligochæta, on the contrary, the mesoblasts lie behind the other teloblasts, and are conspicuous in surface-views;

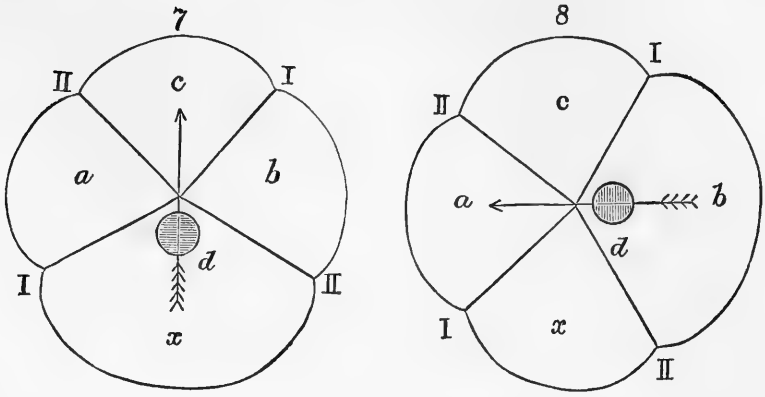
(**14.**) VEJDOVSKY, FR. Die Embryonalentwicklung von Rhynchelmis (Euaxes). Vorluäfige Bemerkungen.

Sitz.-Ber. d. k. böhm. Ges. d. Wiss. March, 1886.

while the neuroblasts and nephroblasts, lying in front of and differing but little in size from the cells which they produce, are discoverable only by careful microscopical examination. This accounts for the fact that they have hitherto been overlooked, and for the widespread belief that the mesoblasts are the sole proliferators of the germ-bands. The origin of the mesenteron, as described by Kowalevsky, is another evidence in favor of identifying the posterior macromere in Rhynchelmis with that of Clepsine.

Let us now consider the history of this macromere more in detail, in order to see how far observation supports the comparison with Clepsine. Vejdovsky has extended and corrected Kowalevsky's account of the cleavage in many particulars, and I shall therefore be guided by his statements.

The first cleavage-plane divides the egg into unequal parts, the larger of which receives the remnants of the polar discs of protoplasm, as in Clepsine. The second cleavage begins first on the larger segment, but passes to the small segment before the large one is completely divided, so that a three-cell stage does not really exist. All this seems at first sight to be in perfect accord with the first two cleavages in Clepsine. But there is one difference in regard to the second cleavage which threatens to upset the whole comparison. This cleavage does not divide the still visible remnant of the polar disc, but runs to the left of them, while in Clepsine it runs to the right. This difference introduces completely new axial relations, analogous to, but not identical with, those in Clepsine, as will be seen by comparing Diags. 7 and 8. It makes 90° difference in the direction of the embryonic axis, for *b* now becomes the posterior macromere, and is destined to play the same rôle as the macromere *x* in Clepsine. The embryonic axis now bisects *a* and *b* (Diag. 8), instead of *c* and *x*, as in Clepsine (Diag. 7). Thus arises a very serious difficulty in the way of identifying the macromeres. I see only two ways of meeting this point. 1. There is, of course, a possibility of error in observation, and the liability to error is all the greater, as Vejdovsky does not appear to have given any attention to the relations we are now considering. 2. If he is right in placing the second cleavage-plane to the left of the disc, it will still be possible to identify the macromeres, *provided the order of the first two cleavage-*



Diagrams of the four-cell stage of Clepsine and Rhynchelmis (Euaxes) to show the relations of the cleavage-planes to the median plane of the embryo.

DIAG. 7. — Clepsine. The axis of the embryo bisects two opposite macromeres (*c* and *x*).

DIAG. 8. — Rhynchelmis (constructed after Vejdovsky). The embryonic axis here bisects opposite macromeres, but stands at right angles to the position shown in DIAG. 7.

a, *c*, *b*, *x*, the four macromeres.

d, the polar disc of hyaline protoplasm, which marks the posterior macromere.

I – I, first cleavage-plane; II – II, second cleavage-plane.

planes in Rhynchelmis is the reverse of that in Clepsine. That is, if the first and second cleavage-planes in Rhynchelmis correspond respectively, to the second and first in Clepsine, there will be a complete correspondence of macromeres and axial relations in the two eggs. The appearances are so strongly in favor of the identity of the posterior macromeres, that either alternative appears to me more acceptable than the conclusion that there is a radical difference in axial relations that bear all the outward marks of being absolutely identical. I shall proceed on the assumption that the difficulty just considered arises from an error of observation, and that the relations between the median plane of the embryo and the cleavage-planes are precisely the same in both cases. Future observation must determine whether this position is well chosen.

The cleavage of the posterior macromere is described by Vejdovsky in the following words: —

" In den nachfolgenden Vorgängen spielt die hintere Makromere die wichtigste Rolle, in der, wie bemerkt, das Protoplasma

von beiden Polen sich concentrirte. Nachdem nämlich die ersten 4 Mikromeren ihre definitive und gleiche Grösse erlangt haben, knospet aus der hinteren Makromere eine grössere, aus Protoplasma bestehende Zelle, die, wie die vertikalen Längsschnitte zeigen, aus dem gemeinsamen Protoplasmanest ihren Ursprung hat und bezüglich der Grösse zwischen der des Mikromeren und Makromeren steht; somit werden wir sie als *Mesomere* bezeichnen.

"Dieselbe verdrängt die inzwischen vermehrten Mikromeren mehr nach vorne und bald darnach bildet sich in derselben Weise aus der hinteren Makromere, beziehungsweise aus deren Protoplasma die *zweite Mesomere*, die sich hinter der ersten stellt, und schliesslich entsteht die *dritte Mesomere*, welche in Bezug auf die Grösse und Gestalt den vorderen zwei vollständig gleich ist. Mikromeren sind bereits zahlreich zu beiden Seiten und nach vorne vorhanden. Die Längsschnitte durch dieses Stadium beweisen, dass *die hintere Makromere bereits des Protoplasma entbehrt, indem dasselbe zur Bildung der Mesomeren verwendet wurde.*

"Die vorderen zwei Mesomeren bleiben eine Zeit lang unverändert, während die dritte, hintere Mesomere sich mehr der Quere nach ausbreitet und schliesslich sich in zwei neue, gleich grosse Mesomeren theilt, die bald zu der ursprünglichen Grösse ihrer Mutterzelle heranwachsen. Bald darnach theilen sich in der Längsaxe auch die zwei vorderen Mesomeren, so dass *zwei Reihen von je drei gleich grossen Mesomeren entstehen*, die aus der Umgebung der inzwischen stark sich vermehrenden Mikromeren hervortreten. Die vorderen zwei Mesomeren theilen sich nun — immer in der Längsaxe — in zwei, dann in vier Zellen, die aber nicht wachsen, sondern durch weitere Theilung die Grösse der Mikromeren annehmen. An solchen Eiern treten nur die vier hinteren Mesomeren hervor. Dieselben Theilungsvorgänge wiederholen sich aber bald auch an dem zweiten Paare der jetzt vorderen Mesomeren und somit zerfallen dieselben in eine Anzahl der Mikromeren, während *das hinterste Paar der Mesomeren sowohl jetzt als auch später bei der späteren Furchung der Makromeren unverändert bleiben und als zwei weisse, stark gewölbte Kugeln dem hinteren Ende der Mikromeren aufsitzen.*" (No. 14, pp. 234–335).

It is evident from this description that Vejdovsky's "meso-

meres" correspond to the teloblasts of Clepsine. The two posterior mesomeres represent the mesoblasts, while the four anterior mesomeres probably represent the neuroblasts and the nephroblasts. Vejdovsky lets the anterior mesomeres divide up into micromeres, and fails to connect them with the nerve-cord and the nephridia. The discovery of a full set of teloblasts in Lumbricus makes it almost certain that such cells are concerned in the formation of the germ-bands of Rhynchelmis, and the mesomeres appear to be the only cells that could here be so identified. Allowing that the mesomeres admit of this interpretation, it is clear that the posterior macromere in Rhynchelmis fulfils the same ends as does the posterior macromere in Clepsine. The mesomeres differ from the teloblasts in number and in mode of origin, but agree with them in position, derivation, and purpose.

According to Vejdovsky (p. 235), the remnant of the posterior macromere, after the production of the three primary mesomeres, takes part with the other three macromeres in the formation of the mesenteron. I have not traced any entodermic elements to this macromere in Clepsine; but, in view of the fact that the mesoblasts persist for some time after they cease to contribute to the germ-bands, it would not be strange to find that their final products were entoplasts. Should this turn out to be the case, the origin of all the germ-layers would be as nearly the same in both eggs as one could well expect.

The origin of the mesenteron in Rhynchelmis has been well described and figured by Kowalevsky. A glance at his figures is sufficient to show that the mode of origin is essentially the same as in Clepsine. There are three primary entoblasts (four with the remnant of the posterior macromere), differing from those in Clepsine only in breaking up into a number of secondary entoblasts. The formation of entoplasts takes place in the same manner as in Clepsine, and the residual yolk is finally inclosed in the mesenteron. The whole process is so similar to what I have described in Clepsine that I have been surprised to find Hoffmann and Bergh, who must be familiar with the facts, disposed to reject my conclusions. It is hardly necessary to add that this mode of origin is very common among Arthropods; and that here, as in Rhynchelmis, the

similarity extends to the œsophageal portion of the alimentary canal.

It is an interesting fact that in those cases where the major portion of the mesenteron passes through well-marked stages of differentiation, beginning with one or more primary vitelline spheres, then, with or without further subdivision of these spheres, taking the form of free peripheral nuclei imbedded in protoplasm with no defined cell boundary-lines (entoplasts), and finally assuming the form of a distinct lining epithelium; there is always an anterior portion, which arises quite early, and which, from the first, consists of distinct cells, often scarcely distinguishable from the cells of the ectoderm and mesoderm. Numerous cases might be cited, but I must here limit the comparison to Rhynchelmis. On this point I may refer again to Vejdovsky. Speaking of the origin of the entoderm at the anterior end of the embryo, he makes the following remarks: —

"Es bildet sich hier eine Gruppe dicht neben und aneinander liegenden Hypoblastzellen, *die der Dotterkügelchen völlig entbehren und sich ebenso intensiv roth wie die Keimstreifzellen tingiren. Den ersten Anfang derartiger Hypoblastzellen kann man bereits in dem Stadium finden, als die Mesomeren sich einzustülpen*[1] *und die ersten Keimstreifzellen zu produciren beginnen. Aus den derart differencirten Hypoblastzellen bildet sich später die Epithelschicht des Anfangstheiles vom Mitteldarme — der Oesophagus.* (No. 14, p. 237).

Branchiobdella. — In a recent paper on the embryology of Branchiobdella, Salensky (No. 15, p. 1) has compared this form with Clepsine and Nephelis. The paper is a most welcome addition to our knowledge of this interesting parasite; but the points of chief interest here — the cleavage, axial relations, and origin of the germ-layers — are too imperfectly worked out to admit of a close comparison with Clepsine.

Salensky finds on one pole of the egg a clear spot, which

[1] The "Einstulpung" of the mesomeres, described by Vejdovsky, is comparable with the movements which result in imbedding the teloblasts of Clepsine in the vitelline spheres. I believe this imbedding process is best understood as a part of the invaginatory movement described in my former paper (No. 1, pp. 58–59). The mesoblasts lie at the hind end of the blastopore, *between* the entoblast and the ectoblast, and their products are carried inward between the two primary layers.

(**15.**) Salensky, W. Développement de Branchiobdella. *Arch. de Biol.* vi. *Fasc.* 1, p. 1. 1885.

corresponds in position to the archiamphiaster ("amphiaster de rebut"), and which is traversed by the first cleavage-groove. This pole is correctly identified with what I have called the "oral pole" in Clepsine. In view of these facts it is very remarkable that Salensky should regard the first cleavage-groove as equatorial. I fail to see a single fact which could be urged in support of such a view. The relation of this groove to the "clear spot" is conclusive evidence that it is meridian, precisely as it is in the Hirudinea and other annelids. If further proof be required, it may be found in the development of the eight-cell stage, which corresponds in every prominent feature with the same stage in Clepsine, Nephelis, and Rhynchlemis. This is a point of primary importance; for if the first cleavage were equatorial, the subsequent cleavages would be just as little comparable with those of Clepsine as the first, and the axial orientation would be radically different in the two cases. Precisely how the embryonic axis is related to the first two cleavage-planes is not clear; but, judging from Salensky's figures, it appears to be the same as that of the types before considered. There is a large macromere, from which the first micromere arises, and which divides, asymmetrically, prior to the division of the other three macromeres. It is this macromere that I would identify with the "posterior macromere" in Clepsine. According to Salensky (pp. 20–21) the two segments into which this macromere divides, participate equally in the formation of all the germ-layers. That is, they are not destined to play unlike parts, as they do in Clepsine, the one, representing a primary mesoblast, the other a neuro-nephroblast, but each alike gives rise to entodermic, mesodermic, and probably ectodermic elements. "Elles donnent naissance aux cellules *mésoentodermiques* et ne représentent point des ébauches spéciales ni du mésoderme, ni du système nerveux, comme c'est le cas chez Clepsine."

There appears to be the same confusion here as we found in Nephelis, in regard to the origin of the entoderm and mesoderm. The earlier "meso-entodermic" cells in Branchiobdella appear to correspond to the first deep cells which arise beneath the micromeres in Clepsine and Nephelis, and which, as I have shown, are purely entodermic. Salensky has found cells which are unmistakably the homologues of the teloblasts of Clepsine; but he has failed to trace their origin and to deter-

mine the special part which each plays in the formation of the embryo. Although he has thus left it impossible to distinguish the different kinds of teloblasts, we may safely assume that they represent mesoblasts, neuroblasts, and nephroblasts, and that they all originate from the large posterior macromere. Allowing that the teloblasts are here similar in origin and character to the teloblasts of Clepsine, Nephelis, and Lumbricus, it follows that the entoderm must arise, chiefly at least, from the other three macromeres. The chief difference, then, between Branchiobdella and Clepsine, in respect to the entoderm, would lie not in the *source*, but in the *mode* of origin. In the former the cleavage process is continued to the end, while in the latter it ceases with the formation of the primary macromeres, the work then being completed by the intermediation of entoplasts.

In regard to the extent to which the epithelium of the alimentary canal is of entodermic origin, Salensky remarks (p. 56): "Ce canal tout entier, à l'exception des parties insignifiantes avoisinant la bouche et l'anus, est exclusivement formé par l'entoderme." Speaking of the œsophagus, he says (p. 58): "Le processus de l'évolution de l'oesophage, chez Branchiobdella, démontre clairement que tout l'épithélium de cette partie nait *exclusivement* aux dépens de l'entoderme." The same will be shown to hold true in Clepsine.

Salensky (No. 15, p. 19) has misunderstood my statements with reference to the relation of the embryonic axis to the main axis of the egg. The cephalic lobe is very nearly centred on the upper pole of the egg in Clepsine, and the mouth arises at, or at least very near, this pole. It does not follow, however, that because the mouth is located at the oral pole, the posterior end of the embryo must be found at the opposite, or aboral pole. A glance at my figures will show that the two ends of the embryo are at first very near together, the caudal end itself lying just behind the area occupied by the four primary micromeres. The axis of the embryo may, therefore, be said to be at right angles to that of the egg, as it is in Branchiobdella and Nephelis.[1]

[1] I cannot agree with Salensky that the embryology of Branchiobdella establishes its title to be ranked among the Hirudinea. Both its development and adult structure appear to me to sustain the opinion, now held by most authorities, that it stands nearer the Oligochæta than the Hirudinea.

2. *Observations.*

The results of my study on Clepsine parasita are supplementary to those obtained on C. marginata (No. 1, pp. 57–58, 66–72) and C. complanata. For the sake of clearness as well as completeness, I have decided to give both the earlier and the later observations a place in the present paper. The existence of free nuclei in the surface of the three entoblasts, *a*, *b*, *c*, first pointed out in my paper on Clepsine, has been confirmed by Bergh and Nusbaum. The early history of these nuclei is given in the following citation: —

"About the time the germ-bands begin to form, a number of free nuclei appear in the surface of the entodermal blastomeres, *a*, *b*, *c*. These nuclei are very distinct in the egg of C. complanata, and it is remarkable that they have so long escaped observation. They appear like dark spots in the opaque yolk, just as the nuclei of the neuroblasts or of the blastodisc. They are oval, oblong, or biscuit-shaped, and measure .02 to .05 mm. At the time of appearance they number three to four in each blastomere, two or three of which occupy the position seen in the figure (No. 1, Fig. 37), while the others are near the lower pole. They are encircled by white rings, such as are generally seen around the nuclei of the neuroblasts. The substance of these rings is the same as that of the white borders of the rings and ring-discs.

"I have seen these nuclei pass through the successive forms of a dividing amphiaster. They multiply rapidly, and, in the stage of Fig. 38, are scattered over the whole outer surface of the blastomeres. In the following stages they can also be seen on the upper faces of *a*, *c*, and *b*, through the thin ectodermal layer. By the time the germ-bands are fully united they are very numerous, and much smaller than at first.

"Whence come these nuclei? In the stage of Fig. 35 they are not to be seen. A horizontal section of this stage (Fig. 80) shows that each blastomere possesses a single nucleus. The nucleoplasm has a somewhat stellate form. The rays vary in length, sometimes reaching to the irregular circular outline of the nucleus. The same condition has been described in Nephelis by Bütschli. Fig. 61 represents one of these nuclei in a little earlier phase. The nuclei now lie nearer the inner

than the outer faces. Fig. 83 represents a horizontal section of the stage of Fig. 37, which passes beneath the neuroblasts and the blastodisc. Here only two nuclei were hit, but these lie near the outer faces of the blastomeres. *The nuclei of the blastomeres then pass from their original central position to the periphery*, and can here be seen in the living egg." (No. 1, pp. 57–58.)

I was unable to trace these nuclei directly through all their later stages of multiplication, but various facts led me to conclude that they gave rise to the mesenteron. Between the last stage in which I could recognize these nuclei and that in which the mesenteron became distinct, a number of stages intervened in which I was unable to demonstrate their existence. This failure, due to imperfections in methods of preparation, was a source of doubt, in spite of the many indications which made it appear almost certain that they represented the mesenteron. I satisfied myself that the entoderm could not have its origin in elements derived from the germ-bands, for all these elements were plainly turned to other uses. Besides, the earliest appearance of the entoderm cells, — their loose and irregular order *in the periphery of the yolk*, — pointed to their origin from the free superficial nuclei of earlier stages. After describing the earliest appearance of the mesenteron (p. 66), I stated my conclusion in the following words: —

"These superficial nuclei go on multiplying by division during the whole period of the epiboly. Finally they are seen as mere white dots scattered over the entire surface of the yolk. Six to seven days after exclusion the entoderm cells make their appearance as *clear cells* with small nuclei, in the periphery of the yolk already cut up into compartments by the septa. What hypothesis is more probable than that these cells originate from the free nuclei? My sections have convinced me that *these entoderm cells arise in the surface of the yolk, and that they do not originate in the products of the blastodisc*" (p. 67).

The condition of the mesenteron represented in Figs. 93 and 95 of my former paper, is very similar to that shown in Nusbaum's Figs. 13 and 14, Pl. II.

With regard to the origin of the free nuclei from the primary nuclei of the entoblasts, I have nothing to add to the statements above cited. The observations which follow begin with

an early stage of the embryo, before the germ-bands have reached the equator of the egg, and the illustrations for the earlier phases described (Figs. 1–5) are drawn from eggs of C. complanata, obtained at Naples.

Figure 1 represents a surface view of the egg, with the germ-bands in an equatorial position, before they have united at the cephalic end. The white patches (*enp*) seen in the yolk, below the germ-bands, are *nucleated* masses of finely granular protoplasm, to which I have given the name *entoplasts*. The existence of such bodies was entirely overlooked by Hoffmann in his first paper; but in his second paper he describes them as "*Protoplasmaflecke*," and devotes considerable space to refuting my interpretation of them. Nusbaum (No. 8, p. 2) carefully translates Hoffmann's description, as if it were something original, using "*îlots protoplasmiques*" as the equivalent of Hoffmann's term.

Sections of the egg (Figs. 2–5) show that many of the entoplasts have not yet reached a peripheral position. In a sagittal section of the head (Fig. 4) we see scattered entoplasts (*enp*) in the yolk, and external to them some large entoderm cells (*en*). Some of these cells are only faintly or imperfectly circumscribed by boundary lines, representing transitional phases between the entoplast and the clearly defined entoderm cell. The differentiation of the entoplasts is going on more rapidly in this region than elsewhere, and it is here that they first assume an epithelial character. Another sagittal section of the same egg, nearer the median plane, is seen in Fig. 3. Here the same large entoderm cells are seen beneath the head (*cl*), and a few neighboring entoplasts, not yet delimited, against the yolk. This one section shows fourteen entoplasts, only three of which could have been seen from the surface. The rest lie beneath the cephalic lobe (*cl*) and around the mesoblast *x*, which is here nearer the anterior than the posterior end of the egg. In the transverse section (Fig. 2) fewer entoplasts are seen, — four in *a*, three in *c*, and one in *b*. The protoplasm surrounding the two nuclei at the upper angle of *c* is continuous with the ectoblast, which raises the question whether contributions to the latter have continued up to so late a stage. I am not able to decide the question, but I am inclined to think that the macromeres cease to proliferate ectoblastic elements

when the formation of free nuclei begins. Near the upper angle of *xy*, between *xy* and *a*, is seen a well-defined entoderm cell. It is rare to find the entoplasts assuming the cell form at such a depth. Fig. 5 (a horizontal section in the plane of the arrow 5–5, Fig. 3) shows entoplasts at different depths, one of which has become defined as a cell (*en*), while another near by shows faint indications of its future outline.

Passing now to the stage in which the germ-bands have united for about one-half their length, we find all the entoplasts in the periphery of the yolk, and a marked advancement already made in the development of the cephalic end. Beneath the stomodæal thickening (Fig. 20, Pl. VI.) is seen a mass of large clear cells (*en*), as yet presenting no definite form and giving no indication of their future histological character. They are easily identified with the entoderm cells beneath the cephalic lobe in Figs. 3 and 4.

In a little later stage (Fig. 21, *en*), when the germ-bands are nearly closed, these cells are smaller, and appear to be taking a more definite shape, as they become more sharply delimited from mesodermic (*m*) and neural (*sup*, *oe*, *g*) elements. We now distinguish an anterior axial portion, forming a solid pad beneath the stomodæum, and a posterior portion (*sgl*), consisting of larger cells, stretching towards the dorsal and ventral sides. Those of the dorsal side are more deeply stained than those of the ventral side. In several instances I have seen a column of these clear cells extending through the centre of the stomodæum, as shown in Fig. 21; and this leads me to believe that the canal of the proboscis is lined throughout with cells of entodermal origin. The condition shown in Fig. 28 fully bears out such a view. Along the ventral side of the yolk (Fig. 22) there is a peripheral layer of coarsely granular protoplasm ("conche protoplasmique granuleuse" of Nusbaum), feebly stained, in which may be seen free nuclei (*enp*). Similar nuclei are found on the dorsal side, but there they are less numerous, and the peripheral layer of granular, uncolored protoplasm is not present.

By the time the germ-bands are completely closed, and the embryo is ready to leave the egg membrane (Fig. 28), the entoderm of the œsophageal region has differentiated into small, axially placed cells, of a distinctly epithelial character, and

larger cells, destined to form the several pairs of massive cell-groups, known as the salivary glands (*sgl*), which are later found on the dorsal and ventral sides of the pharynx, with canals leading into the extreme hind end of the proboscis. The epithelial portion extends through the proboscis, now distinctly marked off from the rest of the stomodæum, and reaches backward between the dorsal and ventral masses of glandular cells. There is still no lumen recognizable in any portion of the œsophagus, but the entodermal epithelium is so well differentiated in color and general appearance, and agrees so perfectly with the conditions seen later in the posterior portions of the alimentary canal, that there is not the least difficulty in distinguishing it from the other embryonic tissues associated with it.

On the dorsal side of the yolk the entoderm is still represented by entoplasts (*enp*), but on the ventral side, by an extremely thin layer of epithelial cells (*en*), which would be easily overlooked, except for the strongly stained oval nuclei. In my study of C. marginata I failed to recognize this very obscure layer, and thus overlooked a stage of development which connects the entoplasts with the epithelium derived from them. The same condition is seen along the middle region of the ventral side, as shown in Fig. 26, *en*, but the layer vanishes a little farther on, behind which point we find scattered entoplasts (Fig. 27, *enp*). The gradual transition from this flattened epithelium into columnar epithelium is well shown in Fig. 25, which represents the dorsal half of one of the anterior cæca in C. marginata, nine days after hatching.

Recapitulation. — The history of the mesenteron may now be recapitulated.

1. The earlier entoderm cells arise beneath the cephalic lobe, and are probably budded off from the entoblasts, *a*, *b*, *c*, as distinct cells, precisely as in Nephelis. But to these earlier and regularly formed cells are soon added others, which appear first as entoplasts, so that it is impossible to draw any line of distinction based on the mode of origin.

2. The larger portion of the mesenteron, embracing the whole alimentary tract, with the exception of a small, anterior (œsophageal) portion, passes through the following stages of development. The first stage is represented by the *three large macromeres*, or *entoblasts* (*a*, *b*, *c*); the second by *entoplasts*

(each represented by a nucleated mass of protoplasm without cell-boundary); the third by an exceedingly thin layer of *flattened epithelium;* and the fourth by a *columnar epithelium.*

3. The development of the mesenteron begins at the anterior end, and progresses towards the posterior end, but more rapidly along the ventral than the dorsal side.

4. The phases of development are essentially the same as in Rhynchelmis, the chief difference being that, in the latter, the three primary entoblasts *a*, *b*, *c*, split up into secondary entoblasts before the entoplastic phase appears.

5. The history of the mesenteron in Nephelis is very imperfectly known, but there is nothing in the observations thus far published which appears to be irreconcilable with the results obtained in Clepsine. The development in Clepsine is more complicated, owing to the larger amount of food-yolk. It is doubtful whether the entoplastic phase is represented in Nephelis.

6. It is possible that the residual mesoblasts (the remnants left after the completion of the germ-bands) contribute to the formation of the mesenteron. Such a termination of their history has not been ascertained, but is suggested by the fate of the posterior macromere in Rhynchelmis.

7. The proboscis — the homologue of the muscular pharynx of the Gnathobdellidæ — is lined with cells of entodermal origin. The rest of the proboscis, together with the proboscidial or pharyngeal pocket, is derived from the stomodæal thickening of the ectoderm.

8. A knowledge of the history of the teloblasts clears up many obscurities in regard to the origin and relations of the germ-layers, particularly the entoderm and mesoderm. If the precise origin of the teloblasts be known, that of the entoderm may be inferred, and *vice versâ*.

III. THE ECTODERM AND ITS PRODUCTS.

1. *The Ectoderm.*

The origin of the first four ectodermic cells (micromeres) has been described under the head of cleavage and axial relations. By the addition of numerous other micromeres, arising, mainly, from the anterior and the lateral macromeres, a sort of blastodisc is gradually formed, centered at the upper pole of the egg. This blastodisc is not wholly ectodermic, for a few of its deeper cells, as we have seen, represent the earlier entoderm cells, as was first suggested by Bergh. The superficial, ectodermic portion of the blastodisc gives rise to the epidermal layer and its derivatives, the stomodæum, sense-organs, etc.

The ectoderm includes, in addition to the superficial portion of the blastodisc, all the teloblasts, except the two larger and and deeper ones, which represent mesoblasts. The grounds for regarding the eight smaller teloblasts as part of the ectoderm are the following: 1. They have at the outset a superficial position at the hind edge of the blastodisc. 2. Two of them give rise to the ventral nerve-cord. 3. In Lumbricus (*vide* Wilson) they lie in, and plainly form a part of, the general ectoderm.

2. *The Ventral Nerve-chain.*

In a preliminary paper (No. 16) I have already stated that the nerve-chain of Clepsine first appears in the form of two simple, unsegmented rows of cells; and, further, that each row is the product of a single cell, the neuroblast. At the time this fact was announced nothing of the kind was known in any other animal; and Nusbaum, the latest authority on Clepsine, had just arrived at an entirely different conclusion, and one altogether more in harmony with traditional views. A similar discovery has since been made by Wilson in Lumbricus, and, fortunately, the evidences in both cases can now be presented side by side. The subject is one which has received a good deal of attention, and given rise to considerable discussion. Before reviewing the opinions of other writers, or giving my

(**16.**) WHITMAN, C. O. The Germ-Layers of Clepsine. *Zool. Anz.* No. 218. 1886.

own observations, it will be well to consider briefly some points respecting the germ-bands.

Use of the Term Germ-bands. — It is important to settle at the outset precisely what is to be understood by the term "germ-bands." Keimstreifen, the German equivalent, is usually restricted to the strata derived from the teloblasts, the epidermal layer being excluded. It is, furthermore, generally believed that the germ-bands of the annelids embrace only mesoblastic elements. Although appearances may often favor such a restricted use of the term, we cannot so limit it in all cases. The idea that the germ-bands are purely mesoblastic has already led to much confusion. In the Hirudinea it is perfectly certain that ectoblastic elements must be included, and hence the matter is not in the least simplified by excluding the epidermal stratum. In Lumbricus, where, according to Wilson, the neuroblasts and nephroblasts are at first ordinary ectoblastic cells, and where, after sinking beneath the surface, they remain imbedded in the epidermal layer, it is obvious that this layer cannot well be held to be distinct from the elements of the germ-bands. According to Bergh (No. 9) the "definitive epidermis" in the Gnathobdellidæ arises from what I shall call the *neuronephric* stratum of the germ-bands. I cannot, therefore, follow Kowalevsky, Bütschli, Hatschek, Balfour, Goette, and numerous other writers in the use of "mesoblastic bands" as the equivalent of germ-bands, nor can I accept the alternative offered by Bergh (No. 9, p. 285), which denies the homology of the germ-bands. The moment we undertake to exclude ectodermic elements, the basis for homology is sacrificed, and the door is open to endless confusion. I shall, therefore, include in the term germ-bands both the "mesoblastic bands" and the superjacent ectodermic strata. It must be remembered, also, that the term, as here used, has reference only to the body of the embryo.

Germ-bands of the Head. — The relations of the cephalic lobe to the germ-bands have not yet been made clear in Clepsine. In Aulostoma, Bergh (No. 9) finds in the head two distinct germ-bands, which arise independently of each other and of the germ-bands of the body. The head-bands ("Kopfkeime") contain epidermal, neural, and muscular elements, and are regarded as homodynamous with the trunk-bands ("Rumpf-

keime"). Leuckart (No. 17, p. 706) described the head-bands in Hirudo as "Zwei einfache seitliche Anschwellungen, die rechts und links vor der Mundöffnung gelegen sind und durch eine ziemlich lange Commissur sowohl unter sich, als auch mit den jetzt hornförmig ausgezogenen Vorderenden der Unterschlundganglienmasse zusammenhängen." Semper (No. 18, p. 215) was the first to point out two distinct head-bands ("Sinnesplatten," "Kopfkeimstreifen") in the Hirudinea. "Bei Clepsine, wie bei Nephelis, der Schlundring und das dorsale Schlundganglion entsteht gerade so wie bei Hirudo, durch Verwachsen zweier Sinnesplatten." He insists, however, that these sense-plates (p. 247) are "echte Kopfkeimstreifen, von deren Bildungszellmasse nur ein Theil zum Nervensystem wird, während ein anderer Theil sich in die übrigen Organe des Kopfes, vor Allem in die mit dem Schlunde sich verbindenden Organe umwandelt."

As above remarked, I am not prepared to discuss the question as to the existence of two independent germ-bands in the cephalic lobe. I have stated (No. 1) my conviction that this lobe is formed at the expense of the first four micromeres. Should there prove to be two head-bands, as maintained by Semper and Bergh, it would be an interesting problem to determine whether they hold the same relation to the first four micromeres as the germ-bands of the trunk to the teloblasts. If such a relation could be demonstrated, the homodynamy of head and trunk would be placed in a very instructive light. The entire embryo, with exception of the mesenteron and epidermis, would then be built up in fundamentally the same manner, at the expense of terminal blastomeres, ten teloblasts, and four micromeres, or acroblasts. The theoretical difficulty presented by independent rudiments for the head and trunk could then be disposed of. The temporary separation of the rudiments might be regarded as an accident of their present mode of origin rather than as an expression of their primitive relations. Their real and essential unity is discoverable in the history of the originating blastomeres. It will be remembered that the posterior macromere produces not only the mesoblasts, neuroblasts,

(**17.**) LEUCKART, R. Die Menschlichen Parasiten. I. 1863.

(**18.**) SEMPER, C. Die Verwandtschaftsbeziehungen der gegliederten Thiere. *Arb. a. d. Zool.-Zoot. Inst. in Würzburg.* III. 1876.

and nephroblasts, but also one of the four primary micromeres. The teloblasts stand thus in the direct line of descent with the acroblasts, and are at first in close contact with them. The full significance of the teloblasts and their original relations can only be made clear by comparison with the larval forms of other annelids. Farther on I shall indicate briefly some points in this comparison.

Germ-bands of the Trunk. — Each germ-band consists of three distinct layers: (1) A thin epidermal layer, (2) a neuro-nephric layer, and (3) a mesoblastic layer. The character and relative positions of these layers may be seen in Figs. 2, 6, and 7, Pl. IV. The epidermal layer (*ep*) consists of flattened cells, more deeply stained with osmic acid than the underlying strata. The neuro-nephric layer is represented by four longitudinal rows of cubical or oval cells (*nc*, *nph*, and *m'*), as is best seen in surface views (Fig. 8, Pl. V.). The mesoblastic layer (*m*) consists of large, rounded, or polygonal cells, two or more deep, filling the space between the neuro-nephric layer and the yolk.

Origin of the Ventral Nerve-Chain. — As my observations on the origin of the nerve-chain contradict those of Kowalevsky and Nusbaum, and as they do not confirm the anticipations of such clear-sighted embryologists as Balfour, I can hardly do justice to the subject without dealing briefly with its historical side.

(*a*) *Historical and Critical.* — Filippi (No. 19, p. 23), the earliest writer on the embryology of Clepsine, tells us that it is impossible to trace the origin of individual organs, owing to the small size of the embryos.

Grube (No. 2, p. 35) derived the nerve-cord from the germ-bands ("Bauchwülsten"), the defective technique of the times not enabling him to reach more definite results.

In the posthumous work of Rathke, edited and revised by Leuckart, the nerve-chain is said to be formed from the median part of the germ-bands ("Bauchplatten"). This result, obtained long before the introduction of the microtome, comes much nearer the truth than the statements of Kowalevsky or Nusbaum. Without the aid of sections, the inner stratum of the

(19.) FILIPPI, F. DE. Sopra l'Anatomia e lo Sviluppo delle Clepsine. *Pavia*, 1839.

germ-bands could not be distinguished from the neuro-nephric stratum, and this is the principal failure in Rathke and Leuckart's description. They recognized four rows of cells, each terminated by a teloblast, but were mistaken in supposing that these rows alone made up the entire thickness of the bands. Their conclusion is stated in the following words (No. 3, p. 94): "Wie bei Nephelis, so scheidet sich auch bei den Clepsinen ein jedes dieser Täfelchen [metamere] in zwei neben einander liegende Hälften, von denen die eine, *die der Medianlinie des Körpers anliegt, zu einem Theile des Bauchmarkes wird*, während sich die andere in ein plattes und dünnes Bündel quer verlaufender Muskelfasern entwickelt."

In Leuckart's celebrated work on Human Parasites (No. 17, pp. 702–703), the longitudinal commissures and the lateral ganglia of the nerve-chain are described (in Hirudo) as arising independently of each other, the ganglia being formed from the median portions of the bands, while the commissures are derived from a "helle Furche" (presumably ectodermic) which separates the bands. "Der Primitivstreifen besteht aus zwei Hälften, die trotz ihrer dichten Anlagerung in der Mittellinie durch einen schmalen Zwischenraum getrennt sind. Bei durchfallendem Lichte erkennt man hier eine *helle Furche*, die ziemlich bald, von den sich rasch entwickelnden Längsfasern, ein etwas streifiges Aussehen annimmt."

The origin of the ganglia from the median parts of the germbands, subsequent to the division into metameres, and of the commissures from the median groove, is then described as follows: "Die Entwicklung der Ganglien geht von dem Innenrande der einzelnen Felder [metameres] aus und geschieht dadurch, *dass dieser zapfenförmig in die Längsfurche zwischen den beiden Hälften des Primitivstreifens hineinwächst, sich an den hier, wie erwähnt, schon früher vorhandenen Längsfaserstrang anlegt und schliesslich von der übrigen Zellenmasse des Feldes abtrennt.*"

Metschnikoff (No. 7, pp. 671–673) was the first to recognize three distinct strata in the germ-bands, and also the first to determine the origin of the nerve-chain from the neuro-nephric stratum. He was wrong, however, in supposing that this whole stratum is converted into the nerve-cord. His brief description runs thus: "Bei dem ersten Erscheinen der beiden Keimstreifen bestanden dieselben bereits aus drei Keimblättern. Das oberste

Blatt erschien in Form eines dünnen Häutchens, welches den ganzen Embryo von allen Seiten umgab. Die beiden anderen Keimblätter beschränkten sich blos auf die Keimstreifen. Das eine von diesen Blättern, dasjenige nämlich, welches unmittelbar unter dem obersten Häutchen lag, bestand aus einer Reihe grosser Zellen, welche in vier Reihen in jedem Keimstreifen geordnet waren. Das untere, dicht dem Dotter anliegende Blatt erschien in Form eines dicken, aus kleinen Zellen bestehenden Wulstes. Bei weiterer Entwicklung, zur Zeit wann sich die beiden Keimstreifen in ein Ganzes verschmolzen haben, erfahren die Keimblätter wichtige Umänderungen wobei übrigens das oberste dünne Blatt nur eine untergeordnete Rolle spielt. Dieses behält seine ursprünglichen Eigenschaften und erweist sich bald als die Epidermis des Embryo. Das zweite Blatt, welches nunmehr aus kleineren Zellen zusammengesetzt wird, bildet dann das centrale Nervensystem."

Speaking of the germ-layers of Clepsine from a comparative stand-point, Metschnikoff remarks, — "Der Hauptunterschied bei Clepsine besteht darin, dass sich das epidermoidale Blatt sehr früh von der Nervenanlage absondert. . . . Die beiden ersten Keimblätter von Clepsineembryonen werden somit dem oberen Blatte des Skorpions und anderer Articulaten entsprechen."

Independently of Metschnikoff's paper, which had escaped my attention, I came to precisely the same conclusion (No. 1); and a little later Hoffmann (No. 6, p. 42) repeated my observations on this point, without adding to or correcting them. Other writers, with a single exception, have failed to get as near the truth as this, and their observations have tended to confusion rather than enlightenment.

Bergh is the only one among the more recent writers who has made any advancement on the observations of Metschnikoff, Rathke, and Leuckart. In the course of his extensive papers on the metamorphosis of the Gnathobdellidæ, and in several reviews, he has stated very briefly the results of studies yet to be published, as I am led to infer, on the origin of the nerve-chain. According to Bergh, the original epidermal layer is lost, and the "definitive epidermis" is then formed from the lateral portions of what I have called the neuro-nephric stratum, while the nerve-cord is formed from the median portion of the same stratum. Although "median portion" is still an indefinite

quantity, it is evident that Bergh has made a closer approximation to precision than any of his predecessors. Grube is the least definite of all; Robin (No. 5, pp. 199, 344) regarded the entire germ-bands as the central nervous system. Rathke and Leuckart traced its origin to the median portion of the bands; Metschnikoff limited it to a single stratum, and Bergh to the median portion of the same stratum.

From the following comments by Bergh it will be seen that he thought it impossible to trace the nerve-cord to special neuroblasts in Aulostoma, and a little more than impossible (!) in the case of Clepsine: —

"Whitman hat die zehn Zellen am Hinterende der Rumpfkeime gefunden, welche bei Clepsine durch ganz besondere Grösse ausgezeichnet sind; die acht derselben nennt er Neuroblasten, die zwei dagegen Mesoblasten, indem er annimmt, dass aus den ersteren nur Nervensystem, aus den anderen nur Mesoderm entstehe. Vergeblich sucht man in der genannten Arbeit ebenso wie in der Natur selbst irgend einen Beweis für diese Behauptung, die eben nur eine solche ist. Es ist bei Aulostoma *vollkommen unmöglich*, die Descendenten jeder einzelnen der erwähnten grösseren Zellen für sich zu verfolgen, und *bei Clepsine wird die Sache noch viel schwieriger*, indem die Rumpfkeime hier stark gekrümmt sind. Richtig ist es aber, wenn Whitman *ganz im Allgemeinen* die Bauchkette aus den Rumpfkeimen herleitet." (No. 9, p. 259).

In Bergh's Fig. 23*a*, Pl. XV., all the strata of the germ-bands are clearly defined; but if we compare this Fig. with Fig. 24*a* and *b*, the entire neuro-nephric stratum appears to be employed in the formation of the "definitive epidermis." On page 263 the nerve-cord is said to arise *beneath* the "Anlage der definitiven Rumpfepidermis." This statement, taken in connection with the illustrations, of Pl. XV., would lead one to suppose that the nerve-chain had its origin in the inner (mesoblastic) layer of the germ-bands. Scarcely more definite are his descriptions in the case of Nephelis (No. 13, p. 295, Pl. XIX.). All the strata are clearly represented in the figures, but the neuro-nephric stratum is marked *ep* ("definitive epidermis"), and there is not the slightest indication of any distinction between neural and epidermal elements. The origin of the nervous system from this layer is conceded in a foot-note (p. 295), in

which my interpretation of the teloblasts is briefly noticed.[1] The same fact is more definitely stated in later papers (No. 20, p. 4, and No. 21, p. 408). Bergh's interpretation of the neuro-nephric stratum, as a neuro-epidermal layer, will be considered after my own observations have been presented.

For the latest contribution on the subject we are indebted to Joseph Nusbaum (Nos. 8, 22, and 23). Nusbaum has given a detailed account of the origin of the nerve-cord from the epidermal layer, thus confirming the conclusion reached by Kowalevsky and sustaining the position taken by Balfour (No. 12). Nusbaum is more fortunate in his company than in his observations, and this is almost the only reason that compels me to burden the reader with a review of his statements. His elaborate account of the mode of origin of the nerve-system from the epidermal layer, in view of the fact that this layer has absolutely nothing whatever to do with its formation, is, to say the least, a most singular production, and one for which it would seem, at first sight, very difficult to find any very satisfactory explanation. But it undoubtedly has an explanation, and I am under the disagreeable necessity of pointing it out. Nusbaum's observations, not only on the nerve-system, but on nearly every point that he has discussed, show a most evident lack of thoroughness. He has neglected to acquaint himself with the more important facts in regard to the origin and structure of the germ-bands, and the consequence is that he has misinterpreted and blundered at nearly every step. He has just as little knowledge of the anatomy as of the embryology of Clepsine; for he starts out under the persuasion that there is only one pair of testicular sacs, (No. 22, p. 614). And yet these sacs are so large and conspicuous

[1] In justice to Bergh it should be stated that his remarks on the origin of the nerve-cord are intended only to serve as a preliminary account.

(**20.**) BERGH, R. S. Über die Deutung der allgemeinen Anlagen am Ei der Clepsinen. *Zool. Anz.* No. 216. 1886.

(**21.**) Id. — Die Entwicklungsgeschichte der Anneliden. *Kosmos.* II. p. 401 1886.

(**8.**) NUSBAUM, JOSEPH. Recherches sur l'Organogénèse des Hirudinées (Clepsine complanata Sav.).
Arch. Slaves de Biologie. I. fasc. 2, pp. 320–340; fasc. pp. 539–556. 1886.

(**22.**) Id. — Zur Entwicklungsgeschichte der Hirudineen (Clepsine).
Zool. Anz. VII., No. 181, p. 609. Nov., 1884.

(**23.**) Id. — Zur Entwicklungsgeschichte der Geschlechtsorgane der Hirudineen.
Zool. Anz. VIII., No. 191, p. 181. Mar., 1885.

that they can easily be seen through the body-wall with the naked eye. What a spectacle is presented when one undertakes to instruct us about the genesis of organs he has never seen! In his second contribution (No. 23, p. 182) he discovers his error, but charges it to Moquin-Tandon. Has Nusbaum never heard of Leuckart, Leydig, or Claus, that he should go back to an authority of half a century ago to find out how many testiculi Clepsine has? But this is a trivial error in comparison with inaccuracies in observation and reading, such as we shall find in his description of the origin of the nerve-chain.

Nusbaum (No. 8, p. 21) first attempts to explain why others have been less successful than himself in tracing the derivation of the nervous system. He discovers — so he affirms — that the egg-membrane is composed of two layers, the *inner* of which is provided with pores. In the course of development this porous layer is penetrated with vitelline granules, and, sometimes, with protoplasmic particles. "*D'où il résulte que cette couche peut être prise assez facilement pour l'ectoderme. Alors il doit sembler que le système nerveux se forme aux dépens du feuillet moyen*, de sa couche la plus externe, aboutissant à l'ectoderme." The invention of such a blunder is as preposterous as its commission is impossible. Furthermore, such a condition of the egg-membrane as Nusbaum has represented in Fig. 32, Pl. III. is either artificial or altogether imaginary. Had Nusbaum begun his observations on the germ-bands at an early stage of their development, it would probably never have occurred to him to suspect his predecessors of such a stupid blunder. In these early stages the egg-membrane is not in contact with the germ-bands, and I fail to see how such a strange condition of the membrane could arise.

In a brief historical review, the opinion which I formerly held (No. 1) on the origin of the nervous system is cited as something "strange enough," and as opposed to the ideas of Metschnikoff and Hoffmann. I have already made it clear that this opinion coincided with that of Metschnikoff and Hoffmann. Bergh's conclusions on this point are not even mentioned.

Nusbaum has described the development of the nervous system in his preliminary article with quite as much detail as in his final paper, and in nearly the same words. I shall, therefore, give here the original description, which runs as follows: —

"Während der Embryo noch eine ovale Form besitzt und nach der Rückenseite hin stark gebogen ist, finden wir *das dünne, einschichtige Ectoderm in dem vorderen und mittleren Theile des Embryo in der Mitte der Bauchseite verdickt;* es entsteht hier eine Schicht grösserer, zuerst runder, später in kubische übergehender Zellen. *Dies ist die Anlage des Bauchnervenstranges, die eine continuirliche Schicht mit dem Ectoderm bildet, und dicht unter der dicken derben, zweischichtigen Chorionmembran zu liegen kommt. Die Zellen dieser einschichtigen Nervensystemanlage beginnen sich in der Richtung nach innen hin zu vermehren, und verursachen somit die Verdickung derselben.* Ganz unabhängig vom Bauchnervenstrange entsteht eine ähnliche ectodermale Verdickung auf dem Kopfende des Embryo, die Anlage des Gehirnganglion bildend. . . . Die Trennung des Nervensystems vom Ectoderm findet auf folgende Weise Statt. *Von beiden Seiten der Bauchnervensystemanlage bildet sich je eine dünne ectodermale Falte nach aussen hin; die beiden Falten, sehr dicht dem Chorion anliegend, wenden sich in der Richtung nach der Mittellinie der Bauchseite des Embryo, um hier später an einander zu stossen. Es bildet sich also eine Art breiter Nervenrinne, von der Seite des Chorion geöffnet; sie ist aber fast vollständig flach und seicht, so dass ihr Boden, d. h. die zuerst gebildete Nervensystemanlage, dem Chorion nahe anliegt. Nach der Aneinanderstossung der obengenannten Falten, bildet sich eine continuirliche Ectodermschicht und der Bauchnervenstrang stellt eine Art platten und breiten Rohrs mit einem excentrischen, engen und spaltförmigen Lumen, dessen innere Wand dick ist und den eigentlichen Bauchnervenstrang vorstellt, während die äussere, dem Ectoderm zugekehrte, von einer einzigen Schicht platter Zellen gebildet ist.* Diese Spalte sieht man noch eine Zeit lang nachher; auf späteren Stadien verschwindet sie spurlos. Es geht aus diesem Entwicklungsmodus hervor, dass derselbe eine nur wenig modificirte Art der Nervensystembildung der Branchiobdella darstellt, wo sich, nach Herrn Prof. Salensky, eine grosse und tiefe Nervenrinne bildet, die sich in einem Nervenrohr schliesst" (No. 22, pp. 610–611).

How beautifully all this chimes with the idea that a neural canal, comparable with that of the vertebrates, should be expected in the annelids! How much detail in describing some-

thing so eminently satisfactory in theory, but without a particle of foundation in fact!

Among the figures given by Nusbaum (No. 8), I find only one (Fig. 33, Pl. III.) in which there is a median ventral thickening of the epidermal layer that might be mistaken for the basis of the nerve-chain. I have seen the same thing, and my first thought about it was that it represented a neural thickening. I shall show that this thickening is a glandular organ, and that the nerve-chain arises beneath it, and never has any connection with it. Nusbaum gives no figures in which such a thickening is seen at any point far behind the anterior ends of the germ-bands. In his Fig. 34, representing a transverse section of some part of the trunk of the embryo, there is not the slightest evidence of an epidermal thickening. On the contrary, the nerve-chain is here sharply marked off from the epidermis, although in contact with it. How does it happen that the nerve-chain is here farther advanced in development than at the anterior end? We ought, of course, to find the development less and less advanced as we go from the head towards the hind end. The broad neural groove ("breiter Nervenrinne") shown in his Fig. 34 is purely ideal. Both Bergh and Hoffmann agree with me in affirming that the epidermal layer is here continuous, and not interrupted, as represented by Nusbaum. The epidermis completely covers the neuro-nephric stratum at a very early stage, and even advances more rapidly than the deeper portions of the germ-bands, meeting in the median ventral line, and forming a continuous layer before their junction, as shown in Fig. 7, Pl. IV. Both the "neural groove" and the "thin ectodermal folds" are inventions, pure and simple, — products of a fertile imagination, which pays more respect to the supposed requirements of some fascinating theory than to the needs of thorough and accurate observation.

Nusbaum goes completely astray in his account of the second layer (neuro-nephric) of the germ-bands, as will be seen from the following (No. 22, p. 613): "Die acht grossen Zellen, von Whitman 'Neuroblasten' genannt, die am Hinterende des Embryo früh auftreten und als Producte des primitiven Entoderms aufzufassen sind, erleiden folgende Veränderungen. Sie unterliegen einer energischen Theilung und vermehren sich fort und fort in der Richtung von hinten nach vorn. Auf einem frühen Stadium

wo das Nervensystem vom Ectoderm sich noch nicht abgetrennt hat, findet man auf einem Querschnitt durch den hintersten Theil des Embryo zwei Reihen dieser Zellen, zu vier an jeder Seite der Bauchfläche liegend; in dem etwas mehr vorderen Theile des Embryo sind nur deren zwei jederseits, und noch näher zum Vorderende beobachtet man jederseits blos eine einzige solche Zelle im Mesoderm, nahe der Bauchseite des Embryo liegend. Die Vermehrung dieser Zellen geht bis zum vordersten Theile des Embryo vor sich, so dass zuletzt in jedem Somite des Embryo jederseits je eine einzige Zelle vorkommt. . . . Diese grossen, in jedem Leibessegmente hervortretenden Zellen beobachtete auch Whitman, und nannte sie 'Segmentzellen.' Da er sich aber die Entwicklung des Nervensystems nicht richtig vorstellte und es von den 'Neuroblasten,' d. i. den acht grossen, oben erwähnten hinteren Zellen herleitete, so bemerkte er keinen genetischen Zusammenhang zwischen letzteren und den Segmentzellen. Whitman vermuthete aber nicht unrichtig dass die Segmentzellen vielleicht an der Bildung der Geschlechtsorgane (Testiculi) Theil nehmen." (Compare, No. 8, p. 17.)

The elements of the two inner and principal strata of the germ-bands are here confounded. Nusbaum appears to be entirely ignorant of the existence of the two large mesoblasts and their relation to the germ-bands. The sexual cells ("segment cells"), which are derived from the mesoblasts, and which form a part of the third (inner) layer of the bands, are identified with the cells of the second (neuro-nephric) layer. At the hind end of the embryo, as Nusbaum affirms, are found *four* rows of these sexual cells on the ventral side of each band; farther forward only *two* rows are present; and at the anterior end there is only *one* row. Four rows reduced to one! and no explanation offered. It is quite true that there are four rows of cells in the neuro-nephric stratum of each band; but the number of these cells seen in transverse section does not diminish, but increases from behind forward.

It remains to notice the opinions of a few writers who, although they have made less extended studies on the development of the Hirudinea, or none at all, are nevertheless regarded as important authorities on the subject. The conclusion reached by Kowalevsky has had greater weight with many authors than

it is really entitled to. That it is not based upon sufficiently thorough observations is evident enough from Kowalevsky's own words. "Die Embryonen der Hirudineen," says Kowalevsky (No. 10, pp. 1–2), "erwiesen sich aber zu Querschnitten nicht ganz passend, und *es gelang mir nur mit grösster Mühe, einige feine Querschnitte anzufertigen, auf welchen ich die Scheidung in Keimblätter und die Bildung des Nervensystems aus dem oberen Blatte sehen könnte, aber nicht den Keimstreifen,* aus dem sich lediglich die Muskeln entwickeln."

In regard to Clepsine, Kowalevsky remarks (p. 3): "Da ich aber zur Zeit der Entwickelung derselben gerade mit den Accipensern beschäftigt war, so bewahrte ich *nur mehrere Stadien in schwacher Chromsäure auf; an Querschnitten derselben könnte ich mich später nur von der Abstammung des Nervensystems vom oberen Blatte überzeugen.*"

Kowalevsky's conclusion as to the origin of the nerve-system rests, then, as he himself acknowledges, on the study of a few eggs so imperfectly preserved and prepared that it was impossible to understand the structure of the germ-bands. How Kowalevsky convinced himself, with such material as this, that the nerve-chain arises from the epidermal layer, must be left entirely to conjecture; for not a word of explanation is offered, nor a single figure given.

Semper (No. 18), in his well-known work on "*Die Verwandtschaftsbeziehungen der gegliederten Thiere,*" reviews the statements of Rathke, Leuckart, Kowalevsky, and Metschnikoff on the origin of the nerve-system in the Hirudinea, and endeavors to reconcile them with his own observations on Nephelis, Nais, Chaetogaster, etc. "Nach eigenen Untersuchungen," says Semper, "kann ich für Nephelis die Angaben Rathke's bestätigen, dass die Ganglien entstehen durch Sonderung der medialen Parthien des Keimstreifens; *eine mittlere, von diesem unabhängige Ectodermverdickung tritt bei dieser Gattung so wenig, wie bei Chaetogaster ein*" (p. 246).

Semper, however, maintains that the nerve-chain has a double origin, its median portion being derived directly from the ectoderm, and the lateral portions (ganglia) from the mesodermic layer of the germ-bands. This view enables him to explain the contradictory results of different writers. Metschnikoff's statements regarding Clepsine are commented on as follows (p. 178):

"Metschnikoff's inneres Blatt des Keimstreifens allein ist das Mesoderm, dessen Betheiligung am Aufbau des Bauchmarks er nicht gekannt hat; sein äusseres Blatt des Keimstreifens gehört dem Ectoderm an, und er hat hier ganz richtig dessen Theilnahme an der Bildung des Nervensystems erkannt, dagegen seine erste Entstehung aus dem ectoderm nicht beobachtet."

According to Semper, Metschnikoff and Kowalevsky are wrong only in supposing that the *whole* of the nerve-chain arises from the ectoderm, while Rathke was equally wrong in deriving it wholly from the mesoderm. Semper (p. 295) extends the idea of a double origin of the central nervous system to all segmented animals. The median part ("centrale Ganglionzellenstrang"), which is supposed to arise as an unsegmented cord, from a thickening of the ectoderm, is regarded as homologous with the spinal cord of vertebrates; the lateral ganglia, arising in the manner described by Rathke from the already segmented mesoderm, are homologized with the spinal ganglia of vertebrates.[1]

Hatschek's remarks (Nos. 24, 25) on the origin of the nervous system in the Hirudinea are of an incidental character, and are of interest only on account of the general scheme set up for the annelids. The ventral nerve-chain, according to Hatschek, arises from three rudiments, one median and two lateral. The median rudiment is a longitudinal infolding of the ectoderm along the ventral line, and is marked with a groove like the medullary groove of vertebrates. The lateral rudiments are prolongations of a pre-oral, neural plate ("Scheitelplatte"), which had its origin in an unpaired thickening of the ectoderm.

The "neural groove," which forms the most seductive feature of Hatschek's scheme, has nothing whatever to do with the formation of the ventral nerve-chain, as has been clearly shown by Kleinenberg. In concluding his excellent review of Hatschek's observations, Kleinenberg (No. 26, p. 123,) states the case thus:

[1] In a foot-note (p. 179) Semper admits that the spinal ganglia may originate in the neural plate, as maintained by Balfour; but, in this case, he would still maintain their homology with the lateral ganglia of annelids.

(**24.**) HATSCHEK, B. Beiträge zur Entwickelungsgeschichte und Morphologie der Anneliden. *Sitzb. Akad. Wiss. in Wien.* LXXIV. 1876.

(**25.**) Studien über Entwicklungsgeschichte der Anneliden. *Abeiten a. a. zool. Inst. zu Wien.* I. 3. 1878.

"In ihren wesentlichen Grundlagen halte ich aber meine Ansichten von der Entstehung des Bauchmarkes, wie sie 1878 für Lumbricus und 1881 für Lopadorhynchus entwickelt wurden, aufrecht, da sie durch fortgesetzte Beobachtungen an anderen Anneliden nicht bloss bestätigt sondern auch weiter ausgebildet werden konnten. *Überall entsteht der Bauchstrang unabhängig vom Kopfganglion aus zwei seitlichen Anlagen, ohne Betheiligung einer soliden oder röhrenförmigen medianen Einstülpung des Ektoderms.*"

It is remarkable that so careful an investigator as Kleinenberg should have entirely overlooked the relation of these "*seitlichen Anlagen*" to the terminal neuroblasts.

The "neural canal" of Branchiobdella, which Salensky (No. 15) homologizes with the medullary canal of vertebrates, has also been effectually disposed of by Kleinenberg (No. 26, p. 127). It has just as little morphological significance as the "neural groove" which Nusbaum describes in Clepsine. Salensky discovered eight teloblasts in Branchiobdella, but missed their special relations and significance.

Kleinenberg's studies on Nephelis (No. 26, p. 129), which were begun some time ago, and not carried to completion, failed to give him the clue to the precise origin of the nerve-chain and its relation to the epidermal layer. The loss of the original epidermis, as stated by Rathke, and more fully described by Bergh, was also observed by Kleinenberg.

Goette (No. 27, p. 91), adheres to the belief that the germ-bands are wholly mesoblastic, and discredits the testimony of those who derive from them the ventral chain, which, as every one will now admit, is ectodermic in origin.[1]

(**26.**) KLEINENBERG, N. Die Entstehung des Annelids aus der Larve von Lopadorhynchus.
Zeitschr. f. wiss. Zool. XLIV. 1 and 2. 1886.

(**27.**) GOETTE. A. Abhandl. z. Entwicklungsgesch. der Thiere. Heft. 2. Vergleichender Theil. 1884.

[1] So strong is Goette's faith on this point that he feels inspired to prepare for his heterodox brethren a very edifying homily in the form of a lengthy foot-note, warning them of the unpleasant consequences of an "einseitige Beurtheilung," and admonishing them of the saving efficacy of the "vergleichende Methode." The counter homily needs no quill from the wing of the angel Gabriel. When facts conflict with theory we know on which side the error lies; and we have little respect for a (not *the*) "comparative method," which begins by denying well-authenticated facts.

Balfour based his account of the formation of the germ-layers in Clepsine on the observations presented in my earlier paper (No. 1), and offered one or two critical remarks that deserve notice. Referring to my statements on the origin of the nerve-chain from the neuroblasts, he says (No. 12, pp. 288–9): "Such a mode of origin for a ventral ganglionic chain is, so far as I know, without a parallel in the whole animal kingdom. . . . Till more evidence is brought forward by Whitman or some other observer in support of the view that the so-called neuroblasts have any share in forming the nervous system, they must, in my opinion, be regarded as probably forming, in conjunction with the mesoblasts, two simple mesoblastic bands. Kowalevsky has, moreover, briefly stated that he has satisfied himself that the nervous system in Clepsine originates from the epiblast, — a statement which certainly could not be brought into harmony with Whitman's account."

In reply to these objections the following considerations have been offered (No. 28, p. 392): —

"With reference to Clepsine, Kowalevsky remarks: 'I preserved only several stages in weak chromic acid, and from sections of these I could only convince myself later of the origin of the nervous system from the upper layer.' This is all he has said on this point; and I will now show that, if we do not go behind the verbal statement itself, it does not even require to be brought into harmony with my account, since it is precisely what I have claimed. The four rows of neuroblasts in each germ-band lie, at the outset, at the surface, and must therefore be considered a part of the epiblast, although a specialized part. It is simply a precocious differentiation of the edge of the epiblast, by which epidermal and neural elements become distinctly marked at an unusually early stage. In the course of the epibolic growth of the ectoderm the epidermal portion progresses somewhat more rapidly towards the lower pole than the germ-bands, and thus sweeps over the neural portion. But it seems to me plainly a matter of little importance whether the neural portion loses its surface position during the epiboly, or immediately after the conclusion of the concrescence of the

(28.) WHITMAN, C. O. A Rare Form of the Blastoderm of the Chick, and its Bearing on the Question of the Formation of the Vertebrate Embryo. *Quart. Journ. Mic. Sc., XXIII.* 1883.

germ-bands; and I confess that I do not see wherein this view requires 'any special support.' At the time Balfour penned the above criticism he evidently was not aware that my observations on the origin of the nervous system in Clepsine were but little more than a corroboration of those of an eminent Russian embryologist."

(*b*) *Observations on the Origin of the Nerve-chain.*—The proof that the entire ventral nerve-chain arises as two simple longitudinal rows of cells, and that each row is produced by the continued proliferation of a single cell, — the neuroblast, — is to be obtained by the study of surface-preparations in connection with sections made in the planes of the three axes. Sections show that the fourfold striated appearance of the germ-bands is due to the presence of four rows of cells beneath the epidermal stratum of each band; and surface-preparations enable us to trace these rows forward into the special organs developed from them. I have been helped towards precise results by the differential action of the preservative fluids employed, and by natural distinctions between the neural and the nephric cell-rows. These distinctions are less conspicuous in the species hitherto studied in Europe than in the American species (C. parasita), and hence have been overlooked. The nephridial rows are more granular and stain more deeply with osmic acid than the neural and lateral rows. A glance at Plates IV. and V. will show how extremely useful such distinctions have been in the analysis of the neuro-nephric stratum.

Having already briefly indicated the different layers of the germ-bands, it remains to consider more in detail the elements composing the neuro-nephric stratum. As was made clear in my former paper (No. 1), there are exactly four rows of cells in this stratum in each germ-band. The outlines of these rows can easily be seen in germ-bands hardened in situ (Fig. 1, Pl. IV.), but better in surface-preparations which have been freed from the yolk (Fig. 8, Pl. V.). They are most distinctly marked at the hind ends of the bands, but can be traced forward nearly to the cephalic lobe in many preparations. The reason for their becoming less and less sharply marked as we pass from the hind end forwards, lies in the fact that development is more and more advanced in this direction. Behind, the rows are simple (*i.e.*, each consists of a line of single cells), and

often separate from one another for a short distance in front of the teloblasts. Farther forward, each row becomes double, then triple or quadruple, and at the same time its boundary lines become less clearly defined, as shown in Figs. 8–11. Fig. 7 is a transverse section of the stage seen in Fig. 8, at a point where the bands are still separated by a narrow interval. The rows of cells are here simple, each showing a single cell in section. In another section, taken just in front of this, where the bands have already closed, three median cells (Fig. 6, *nc*) are seen in place of the two shown in Fig. 7. It is possible that there has been a duplication of cells in one of the median rows, but it is more probable that the section has passed through two more or less wedged-shaped cells belonging to the same row, but inversely placed, as shown in the left neural row of Fig. 11. Fig. 11 represents a horizontal (frontal) section near the posterior end of an embryo in which the germ-bands are fully closed. All the rows are here simple, but towards the middle of the same embryo (Fig. 10) we find the neural row doubled on one side and tripled on the other. A little in front of the middle a still more advanced condition is found (Fig. 9); for here not only have the neural cells become smaller by division, but there is a plain differentiation into median (Long. commissures + median ganglia) and lateral (lateral ganglia) portions.

A more complete and instructive picture may be obtained by mounting the embryo entire, after stripping it from the yolk. In such a preparation (Fig. 8), passing from the hind end forwards, we meet with successively higher stages in the development of the nerve-chain, beginning with two simple rows of cells (*nc*) and ending with well-defined ganglia. The neural rows are so clearly delimited against the darker, nephridial rows, and the steps in development follow in such a perfect ascending series, that every doubt about the conversion of these rows into the nerve-chain is removed.

In Figs. 15–19, representing transverse sections from the posterior end of an embryo just hatched, the neural rows have united into a median flattened cord (*nc*) which shows a differentiation into median (*lc*) and lateral portions, as in Fig. 9. In these sections, selected from a series beginning in the middle of one somite and extending to the middle of the next in front, the contrast in color between the neural and nephridial elements

is well-marked. The contrast between the nephridial (*nph*) and the lateral (*m'*) cells is equally strong, so that in this advanced stage of development it is still easy to find all the derivatives of the neuro-nephric stratum, and to connect them with the primary cell-rows shown in Fig. 6.

3. *The Larval Gland-Cells.*

Passing now to the anterior end of the same embryo we find the nerve-cord presenting the same general form, with the median and lateral parts more clearly defined (Fig. 23). The median part does not yet show the double commissures. In this section, taken in the region marked *gl* in Fig. 8, we meet with a very interesting larval organ, consisting of numerous large gland-cells, each with its own duct leading to the exterior. These massive gland-cells lie between the sub-œsophageal ganglia and the epidermis, and extend over an area of greater breadth than the ganglia. These glands arise as a pair of thickenings of the epidermal layer immediately behind the cephalic lobe, and appear as rounded prominences in quite an early stage of the germ-bands. (Fig. 1). The epidermal thickening is always clearly distinct from the neural cells, as may be seen to best advantage in longitudinal sections (Figs. 20 and 21). It is undoubtedly this thickening which Nusbaum has figured and described as the basis of the nerve-chain. Only the deeper cells of the thickening are destined to become gland-cells, and these appear to sink gradually beneath the surface, the ducts forming in situ rather than by subsequent outgrowths. A median sagittal section of this stage (Fig. 28) often shows only a few gland-cells compared to the number met with in sections passing about midway between the median ventral line and the side of the embryo (Fig. 29), showing that they still form two more or less distinct groups.

Nusbaum (No. 8, p. 28) has described a "provisional dorsal organ" entirely different from anything I have seen. "Chez l'embryon, dont le système nerveux est déjà complètement séparé de l'ectoderme, j'ai remarqué, au milieu de la paroi dorsale du corps, dans la troisième partie antérieure de sa longueur, une couche de cellules hautes, cylindriques, de l'ectoderme. . . . Les cellules ectodermiques, qui forment cette proéminence, emettent ensuite des fils externes, minces, très

longs. . . . Le rôle physiologique de ces fils consiste, comme il me semble, dans la fixation réciproque de jeunes individus, tournés ordinairement l'un vers l'autre par leurs faces dorsales et fixés à la paroi ventrale de la mère par leurs ventouses antérieures. Cet organe dorsal n'existe cependant pas longtemps; il disparaît sans laisser de traces avant la séparation des jeunes individus de la paroi du corps maternel."

Nusbaum and Hoffmann (No. 4, p. 45) have both fallen into the same error of supposing that the larvæ attach themselves to the ventral side of the parent by the oral sucker. The young are further, according to Nusbaum, fixed to one another by means of glutinous threads formed by the "dorsal organ."

While studying the embryology of C. marginata and complanata, I noticed that larvæ hatched from eggs that were taken away from the parent and kept in a watch-glass, soon became attached to one another in pairs. The point of attachment, however, was not dorsal, but ventral, just behind the part destined to form the oral sucker. The attachment, as I have since learned, is effected through an adhesive secretion of the larval glands above described. When the leech is allowed to remain over the eggs until they hatch, the larvæ become fixed to its underside, not by the still undeveloped oral sucker, but by the secretion of the post-oral, ventral glands. I have never noticed the "reciprocal attachment" by means of a "dorsal organ;" but, without further examination, I would not venture to dispute Nusbaum's statement. But the description and figures given certainly awaken the suspicion that the "dorsal organ" is a pathological formation. The larval glands which I have described serve only the temporary purpose of fixing the young to the parent leech at a time when neither sucker is sufficiently developed to perform this office. As soon as the posterior sucker becomes serviceable, it is used as an organ of attachment, and the larval glands disappear; at least, I have not been able to connect them with any organ in the adult leech.

The larval organs of adhesion occupy a position which corresponds to that of the Ganoid suctorial disc. The means of fixation in the young fish (Amia), at least in the youngest larvæ, is an adhesive secretion. My attention was first called to the secretion in Amia by Dr. Patten.

4. *Primary Sense-organs of the Lip.*

At the time of hatching, long before the eyes and their serial homologues, the segmental sense-organs, appear, two pairs of sense-bulbs are found, symmetrically placed on the surface that is to form the margin of the lip. These organs arise as bulb-like thickenings of the epidermis. Figs. 12 and 13 represent successive sections through the anterior pair of bulbs (*sb'*), in the direction shown by the arrows 12–12 and 13–13 in Fig. 29. The fifth section behind that seen in Fig. 13 hits the posterior pair of bulbs (*sb''*), passing above the super-œsophageal ganglia, as indicated by the arrow 14–14 in Fig. 29. These sense-bulbs are nearer together than those of the anterior pair, and they are a little depressed, as if there were a slight infolding. The scanty material at my disposal did not permit me to trace the history of these organs farther. I have since given some time to the study of the development of the eyes and sense-organs of the lip, in much later stages, and I have found that all the sense-organs of Clepsine arise in the same manner as these two pairs of bulbs. As the entire œsophageal nerve-collar is already formed, there is absolutely no ground for supposing that these bulbs are rudiments of the nervous system. The basis for the super-œsophageal ganglia (*sup. œ. g.*) is present as a distinct body of cells in the stage of Fig. 20, long before the appearance of the primary sense-bulbs. Even as early as the stage of Fig. 3, I find between the epidermal layer of the cephalic lobe and the primary entoderm cells a layer of cells which I regard as the basis of both the neural and the mesodermic elements of the head. The precise origin of this layer I have not thus far determined.

5. *The Nephridia.*

In describing the origin of the nerve-chain I have called attention to the nephridial rows of cells, two of which are found in each germ-band, lying between the median (neural) and the lateral rows (Figs. 2, 6, 7, 8, 9, 11, *nph*), and forming one stratum with them. These relations are shown in Diag. 9.

In C. parasita the nephridial rows are remarkably distinct, owing to the contrast in color between them and the rows by which they are bounded. This contrast, due to the coarse

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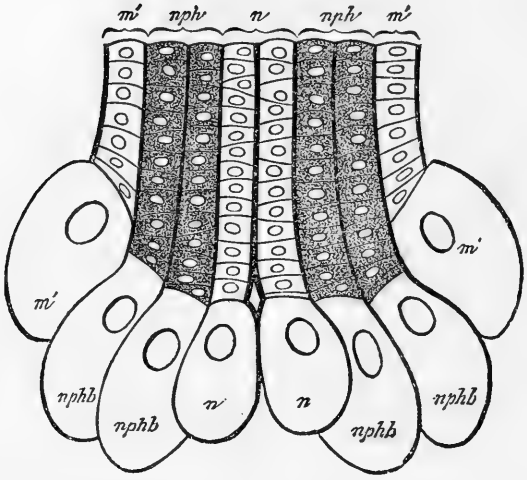

Diag. 9. — *A diagrammatic surface-view of the neuro-nephric stratum at the posterior end of the nearly completed germ-bands.*

n, neural rows; *nb*, neuroblasts; *nph*, nephridial rows; *nphb*, nephroblasts; *m'*, lateral rows.

granules of the cells, is strengthened by the action of osmic acid, and thus becomes a most important aid in determining the fate of the cell-rows.

Tracing the nephridial rows forward in a surface-preparation (Fig. 8, Pl. V.), we find each represented behind by a line of single cells; towards the middle, they become double or triple, while still maintaining the same diameter; near the beginning of the anterior third, the two rows blend; and here outlines appear, at first shadowy, then more distinct as we advance, cutting the rows into quadrangular plates with rounded angles. The formation of the nephric plates progresses from the cephalic end backwards, keeping exact pace with the metameric division of the embryo. Thus the basis is laid for a pair of nephridia in each somite, although only sixteen pairs are retained in the adult. The details of the process by which these plates are converted into the nephridial organs I have not attempted to follow. It is a point, however, which is worthy of a most careful study.

The series of sections shown in Figs. 15–19, beginning near the middle of a posterior somite, and running forwards to the middle of the next in advance, shows that the cells of the nephric plates multiply in depth as well as breadth. Fig. 18 represents a section on the boundary-line of two somites, in which not a single nephridial cell could be found. It is in this region that we meet with a pair of large cells (*sex*) lodged in the mesoderm. A single pair of these cells occurs in each somite, and their position in the walls of the septa suggests that they may be the mother-cells of the testicular organs. Nusbaum claims to have traced the development of these cells into the sexual organs; but he has evidently confounded the cells of the neuro-nephric stratum with the sexual cells.

Returning to the nephridia, the points of chief interest in their development appear to be the following: —

1. Derivation from the ectoderm.

2. Earliest appearance in the form of simple, longitudinal cell-strings.

3. Each nephridial cell-string is a product of a single terminal cell, — the nephroblast.

As soon as I became aware of the precise origin of the nephridia, I began to question the validity of the opinion that the nephroblasts were ectoblastic. It was almost universally believed that the nephridia take their origin in mesoblastic elements. In view of this, I did not venture to discuss the question in my preliminary paper (No. 16).

Wilson's paper on Lumbricus settles the point, leaving little room for a reasonable doubt as to the ectoblastic nature of all the teloblasts concerned in the production of the neuro-nephric stratum. The establishment of this fact, taken in connection with recent discoveries pointing to the ectoblastic origin of the vertebrate segmental ducts, paves the way to a better understanding of the phylogenetic derivation of these organs.

In this connection Bergh's discovery that the larval nephridia of the Gnathobdellidæ arise as lateral outgrowths from the germ-bands is especially important. Adding this to the discovery of distinct nephridial cell-strings, we have a remarkably perfect picture of the more important steps in the development of the pronephros of Petromyzon.

6. *The "Pharyngeal Clefts."*

In Fig. 1, Pl. IV., are shown two remarkable grooves just in front of the thickened anterior ends of the germ-bands, marking the line of junction with the cephalic lobe. These groove-like formations are remarkably distinct in C. complanata, and are found in every species of Clepsine that I have examined. I have before described them as "pharyngeal clefts" (No. 1, pp. 60–61), but I now have considerable doubt as to the correctness of this interpretation. They were not so well marked in C. parasita, and I have not thus far obtained the material necessary for a detailed study of them.

Salensky (No. 15, p. 25) describes in Branchiobdella what he calls a "bifurcation de la gouttière médullaire," which corresponds nearly in position and appearance with the "pharyngeal clefts" of Clepsine.

IV. SPECIAL AND GENERAL QUESTIONS.

1. *Larval Nephridia.*

No larval nephridia have thus far been discovered in the Rhynchobdellidæ, and the relations of these organs to the permanent nephridia in the Gnathobdellidæ have not yet been made clear. Bergh was not aware of the existence of distinct nephric cell-strings, and hence his descriptions and figures do not settle the place of origin of the larval nephridia with the precision that could be desired. It would seem from Bergh's statements that these organs are derived from the two "outer strings," *i.e.*, the lateral row (*m'*), and the adjacent nephridial row in my figures. "An den äusseren Strängen sind als schräg nach aussen und hinten gerichtete Zweige die Anlagen der Urnieren, deren Aulastoma vier Paare besitzt, sichtbar. . . . Die vier Urnierenpaare entstehen somit als seitliche Sprossen von zwei Längssträngen, welche letztere sich später mit den erwähnten inneren Strängen vereinigen" (No. 29, p. 91). As the nature and fate of these "longitudinal strings" remained

(29.) BERGH, R. S. Thatsachen aus der Entwicklungsgeschichte der Blutegel. *Zool. Anz.* VII. No. 160, p. 90. Feb. 18, 1884.

unknown, Bergh (No. 30, p. 115) was led to believe that "die Segmentalorgane typisch segmental, *ohne die geringste Verbindung untereinander entstehen.*" In regard to the relations of the larval to the permanent nephridia, Bergh says: "Bei den Blutegeln tritt mit grosser Klarheit hervor, dass *Urnieren und Segmentalorgane durchaus nichts miteinander zu thun haben.* Letztere legen sich nämlich (in den Rumpfkeimen) erst an nachdem die Urnieren sich schon lange von diesen abgelöst haben. Ebensowenig kann von einem ursprünglichen Zusammenhang zwischen den einzelnen Anlagen der Segmentalorgane die Rede sein (No. 30, p. 113).

If we examine Bergh's Fig. 5, Pl. XII. (No. 9), in the light of what we now know about the origin of the nephridia, we see at once that the provisional nephridia arise from one or both of the cell-rows, which must be identified with the nephridial rows of Clepsine. In this figure, the fourth nephridium ("Urniere") of the left side is represented by a single cell, which still retains its original position in the outer nephridial row. It is thus highly probable, if not quite certain, that both the larval and the permanent organs do arise from the same basis, — the nephridial rows. Allowing this to be the case the relations of the two sets of organs would be very clear. One thing is perfectly certain: it can no longer be said with Bergh and Vejdovsky (No. 31, p. 123), that the permanent nephridia arise from disconnected bases or rudiments.

The view first advanced by Hatschek (No. 25), in spite of the theoretical objections raised by Balfour (No. 32, pp. 565–6) and the lack of confirmation on the part of other writers, is, after all, the one most easily reconciled with the results presented in this paper. In my opinion it not only accords better with known facts, but presents a more rational basis for explaining the morphogenetic relations of these organs, than the theory of disconnected rudiments. I refer, of course, not to the details of the conditions described in Polygordius, but to general features; such as, the derivation of the whole excretory system of the head and trunk from a common basis, and the formation of the trunk

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(30.) Bergh, R. S. Die Exkretionsorgane der Würmer. *Kosmos.* II. p. 97. 1885.

(31.) Vejdovsky, F. System und Morphologie der Oligochaeten. Prag, 1884.

nephridia by metameric division of a pair of continuous longitudinal rudiments. There can be no doubt about the homology of the nephridial rows in Clepsine and Lumbricus with the longitudinal "cell-strings" of Criodrilus; and the ciliated posterior ducts, which appear to develop as sprouts from the "head-kidneys" in Polygordius, are only more highly developed forms of the simple nephridial rows I have described.

Nephroblasts were not discovered in Criodrilus, but we may now be almost certain that they are present. We may be equally confident, I think, that nephroblasts, or equivalents, are present in Polygordius. Until they are discovered and their exact relations to the "head-kidneys" made out, it will be difficult to decide the question as to the strict identity of the larval nephridia in the Gnathobdellidæ with the cephalic nephridia of marine annelids, such as Polygordius, Echiurus, Eupomatus (Serpula), etc.

Balfour (No. 32, p. 567) was very decided in the opinion that "the provisional excretory organs of the leeches cannot be identified with the anterior provisional organs of Polygordius and Echiurus." The question now stands in a somewhat different light. Vejdovsky (No. 31, pp. 121–122) has discovered larval excretory organs in Rhynchelmis, Aeolosoma, Nais, and Chætogaster, and thinks it probable that they occur in the early embryonic stages of all the Oligochæta. Bergh has traced the development of such organs in the leeches; and his observations, in connection with mine, make it almost certain that both the provisional and the permanent organs arise from the same cell-cords. Wilson has made the important discovery of nephric cell-cords in Lumbricus; and this, in connection with the wide-spread occurrence of teloblasts, leaves little room to doubt that such cell-cords are common to all annelids. The case is made still stronger by the earlier observations of Hatschek on Polygordius, Echiurus, and Criodrilus, and by E. Meyer's discovery (No. 33, p. 677) of a longitudinal canal connecting the permanent nephridia of Terebella (Lanice) conchilega. The general occurrence of these larval organs, their relatively early origin

(32.) BALFOUR, F. M. Comparative Embryology. II. 1881.

(33.) LANG, ARNOLD. Die Polycladen. *Fauna und Flora des Golfes von Neapel. Monographie* XI. 1884.

from, or in connection with, the nephric cell-cords, the general uniformity in their position, their non-metameric character, their atrophy and replacement by the permanent, metameric nephridia, appear to indicate that they all belong to one and the same system of organs. So far I am in accord with Bergh (No. 9, pp. 269–272, and No. 30, p. 116); but I am not of his opinion that this conclusion makes it impossible to homologize the larval with the permanent organs.

2. *Significance of Nephric Cell-Cords.*

The important bearing of the discovery of nephric cell-cords on the question of the derivation of the vertebrate nephric system has been ably presented by Wilson. Without entering into the discussion of this side of the question, I may say that I fully concur in his general views on this subject. There is one point only to which I will briefly call attention. If both the provisional and the permanent nephridia arise from the same cell-cords, how are we to know, from the occurrence of such cords, which system, if either, has been retained in the vertebrates? We may, as it seems to me, be quite certain about the homology of the nephric cell-cords, and yet be quite unable to decide whether one, both, or neither of the two nephridial systems seen in the annelids is represented in the vertebrates. We are not even certain that the larval nephridia represent the same system throughout the annelids. The mode of origin of the larval organs in leeches, as lateral-buds, reminds one of the formation of the pronephros in Petromyzon; but the outgrowths[1] from the "segmental duct" have a metameric arrangement. In the formation of the permanent nephridia of leeches we have the metameric arrangement without the lateral outgrowths, the entire cell-cords being cut up into consecutive cell-plates. The fundamental importance of homologous nephric cell-cords is not, however, lessened by any such difficulties in identification as are here presented.

[1] Scott (Morph. Jahrb. VII.) states that the pronephric funnels arise as outgrowths from the segmental duct, while Shipley (Quart. Jour. Mic. Sc. XXVII., Jan., 1887, p. 344) represents them as arising from a groove in the parietal peritoneum. As this groove (which is continuous with the lumen of the segmental duct) closes up, it leaves four or five openings which persist as the openings of the ciliated funnels.

3. *Questions Relating to the Nephridia.*

A few questions of a general nature remain to be considered. What morphological element (or elements) represents the primitive nephric basis? Is the non-metameric (larval) system to be regarded as the main stock from which the metameric (permanent) system arises by a process of budding, as held by Hatschek? Or are the relations of the two systems better expressed when both are represented as buds from a common stock? In either case what is the primitive form of the stock itself? Is it a pair of simple cell-cords, or a pair of single cells? What was the original function of these organs?

The answers to these questions will vary according to the views we entertain on the origin and significance of the metamere and its genetic relations to the head. This problem logically takes precedence of the others; but we are not yet in a position to solve it, and a presentation of the leading theories could not well be brought within the limits of this paper. Besides, such work is rendered unnecessary by the excellent review given by Fraipont (No. 34, pp. 102–125) in the latest of the Naples Monographs. Bergh has recently given a comprehensive and critical review of all that is known in the comparative morphology of the excretory organs of the Vermes (No. 30 and No. 21, p. 417). I shall therefore limit myself here to a few suggestions, which appear to be warranted by the facts presented in this paper, when considered in the light of what was previously known on the same subject.

Original Function. — Of the two functions now served by the nephridia, which is primary and which secondary? Bergh (No. 30, p. 120) holds that "*die segmentierte Leibeshöhle der Anneliden den Höhlen der Geschlechtsfollikel der Plattwürmer und Nemertinen homolog ist; jede Hälfte einer Segmenthöhle mit dem sie begrenzenden Epithel entspricht einem Geschlechtsfollikel.* Um diesen Vergleich durchzuführen, muss man sich vor allem das Verschwinden des Parenchyms bei den Anneliden vergegenwärtigen. Dabei legen sich die Wände benachbarter Follikel (Mesodermsegmente) aneinander und in dieser Weise

(35.) FRAIPONT, JULIEN. Polygordius. *Fauna u. Flora des Golfes von Neapel. Monographie* XIV. 1887.

entstehen einerseits die Mesenterien, anderseits die Dissepimente."

In harmony herewith, it is inferred that the permanent nephridia served primarily as ducts for the escape of the sexual products, the excretory function having developed later. This view may appear plausible enough so long as we assume with Bergh that the larval and the permanent nephridia represent two unrelated systems of organs, having no connection with each other either ontogenetically or phylogenetically. But, let their homogeny be conceded, and there will be no escape from the conclusion that the functional relations of the permanent nephridia to the sexual organs, wherever such relations exist, have been acquired secondarily. The question then becomes simplified; for we have only to determine the primitive function of the provisional nephridia. As these organs never function as sexual ducts we have no reason to suppose that they have ever served any other purpose than that of excretory organs. As the permanent nephridia arose later, either directly from the larval organs, or, at least, from the same basis; as they exhibit the same general structural features; and as their appearance is followed by the atrophy of the larval system, there is every reason to believe that they assumed the work of the organs which they superseded. It is easy to understand how such organs could be pressed into the service of the sexual organs, and how their original function might be suppressed as the result of adaptation to this new work. The conversion of sexual ducts into excretory ones, presenting the typical structure of the primordial excretory organs, could not, on the other hand, be so readily explained.

Original Basis. — The question of original basis, like that of original function, must be considered in the light of what is known about the development of the larval nephridia. In the leeches these organs appear to arise from single cells, which develop, by division, into simple cell-cords. This simple mode of development is repeated in the ontogeny of the metameric nephridia, as seen in the formation of nephridial cell-rows from terminal nephroblasts. Although the nephric cell-plates, into which the primary cell-cords are metamerically divided, consist of numerous cells, it is probable that each plate represents a simple (or double) string of cells, with its

coils so closely packed that the linear arrangement of the cells is obscured. I think this is a fair inference from the appearance of the nephric plate. (*Vide* No. 1, Fig. 92, Pl. XV.)

The larval nephridia of other annelids, so far as known, consist at most of only a few cells; and in some cases, *e.g.*, Eupomatus, the duct of the fully developed organ is formed within a single elongated cell, stretching from the œsophagus back to the mesoblast of the same side. A number of spherical cells are found around the anterior end of this elongated cell, and these are regarded by Hatschek (No. 35, p. 143) as belonging to the excretory organ. *The entire organ arises from two cells*, one of which forms the duct, while the other splits up into the spherical "end-cells" (p. 134). Whether the two cells arise by successive divisions of the mesoblast, or by division of a primary nephroblast, we are not informed by Hatschek. Both cells are regarded as mesoblastic, but this interpretation would be perfectly consistent with the second mode of origin.

Hatschek finds a pair of primary mesoblasts ("Urmesodermzellen"). Each of these divides into two unequal parts, a large "pole-cell" and a small "daughter-cell." The "pole-cells" evidently correspond to the two "mesoblasts" of Clepsine; and the "daughter-cells" appear to me to represent nephroblasts. But, if Hatschek is right in regard to the origin of these cells, there is one difficulty in the way of identifying the "daughter-cells" with the nephroblasts; for the former are mesoblastic, while the latter are ectoblastic. If, however, we examine the facts a little more closely, the objection appears less formidable than at first sight. In Clepsine we have seen one cell give rise by division to the mesoblasts, the nephroblasts, and the neuroblasts. The first division separates the cell into a "primary mesoblast" and a "neuro-nephroblast." The point of fundamental importance for our comparison is the *twin origin* of these cells. If we call one cell mesoblastic and the other ectoblastic, that is a matter of interpretation, which may be justified by appearances in the one case, and contradicted by them in the other. The fact remains, that the genetic relation between mesoblast and nephroblast is equally close in both

(35.) HATSCHEK, B. Entwicklung der Trochophora von Eupomatus uncinatus Philippi (Serpula uncinata). *Arbeit. a. d. zool. Inst. z. Wien. VI.* H. 1., p. 121. 1885.

cases; and no artificial lines of distinction, such as we are accustomed to draw between the germ-layers, can lessen its significance. When our definitions of the germ-layers fail us we must appeal to *the precise genealogy of the cells*. To deny the existence of a mesoderm is of no avail; for, with two primary layers, — ectoderm and entoderm, — we are just as far from being able to settle the question of morphological identity. When, as in the case under consideration, we find an organ arising sometimes from the ectoderm, and at other times from the mesoderm, we have to admit that there is no fixed and impassable boundary-line between these two layers; and that its association with this or that germ-layer is not an infallible guide to its morphological identity.

The following view offers a fair explanation of the point in question: Both the mesoblasts and the nephroblasts arose primarily from a common ectodermic basis. The genetic relations of the two cells have remained essentially the same; but the time of their differentiation as distinct cells varies. If the division takes place within the ectoderm, then each makes its exit from the original seat separately and independently of the other; if, on the other hand, the division is delayed until after the separation from the ectoderm is accomplished, then the nephroblast appears to arise from the same source as the mesoblastic bands, and thus to form a part of these bands. The differences noted between Eupomatus and Clepsine may be reconciled in this way. The conflicting accounts given of the origin of the vertebrate "segmental duct" admit of a similar explanation.

There are, then, some very positive indications that the larval nephridium consisted, originally, of a single cell; and the general occurrence of nephroblasts, as the basis of both systems of organs, is in favor of this view.[1]

4. *The Origin of the Epidermis.*

Bergh has shown that it is necessary to distinguish between the larval and the definitive epidermis of the Gnathobdellidæ.

[1] I am reminded of the opinion long ago expressed by Leuckart (No. 17, pp. 698–699), that the teloblasts of Clepsine ("Colossale Zellen") represent "*Urnieren*." Leuckart supposed, however, that they were provided with ducts, and that they were functionally active.

The larval epidermis arises in precisely the same manner as the epidermis of Clepsine; it is lost during larval life, and replaced by the "definitive" epidermis, which is an entirely separate and independent formation from the neuro-nephric stratum, having absolutely no direct genetic relations with the original epidermis. The origin of the definitive epidermis, as described by Bergh, has no parallel in other animals, and it is clearly impossible to reconcile it with my observations on the fate of the neuro-nephric stratum. The mode of reconciliation suggested by Bergh (No. 20, p. 6), according to which the epidermis of Clepsine is the homologue, not of the larval, but of the definitive epidermis of the Gnathobdellidæ, must be set aside as entirely incompatible with the facts presented in this paper.

There can be no doubt about the accuracy of Bergh's observations on the loss of the larval epidermis; but his theory of the origin of the definitive epidermis I am not able to accept on the evidence adduced. I have shown that three of the four rows of cells constituting the neuro-nephric stratum of each band are employed in the formation of the nerve-chain and the nephridia. I have not been able to satisfy myself fully as to the fate of the fourth or lateral row; but I have followed it far enough to ascertain that it has nothing whatever to do with the formation of the epidermis. The epidermis is perfectly distinct at every stage from the neuro-nephric stratum, and I cannot discover the shadow of a reason for thinking that it ever receives any contributions from this stratum.

In this connection I must mention one fact which links the teloblasts with the epidermis. I have shown that the neuroblast is the twin cell of the median nephroblast. The mother-cell (No. 1, Pl. XII., Fig. 35, x^3), before dividing into these two cells, produces a small median cell (x^4), which, together with its homotype of the opposite side, is converted directly into true epidermal cells. This pair of epidermal cells (x^4) is a constant and striking feature of the stage referred to. This is the nearest point of connection between the epidermic layer and the neuro-nephric stratum. But I venture to say that no one acquainted with the development of Clepsine would risk the suggestion that the whole epidermis is derived from this median pair of cells.

Kleinenberg (No. 26, p. 129) confirms Bergh's statement as to the loss of the original epidermis in Nephelis, but raises objec-

tions to his idea of the origin of the definitive epidermis. Kleinenberg adds (p. 130), that in all Polychæta, whose development is known to him, the epidermis of the larva is replaced by the definitive, outer epithelium of the annelid; but this takes place through a process of transformation, which has its point of departure in the larval epidermis itself, and only in a few cases are the parts of the old ectoderm actually thrown off.

Is it not possible that the permanent epidermis in Aulostoma and Nephelis has its origin in the larval epidermis? Such an origin would accord perfectly with what takes place in other annelids, and remove the apparent discrepancies in development between the Rhynchobdellidæ and the Gnathobdellidæ. Bergh's figures appear to lend no support to the suggestion; but important points in the history of this neuro-nephric stratum have escaped his attention, and a reëxamination is required in order to settle them. If it turns out that this stratum gives origin to the nephridia and nerve-chain, as in Clepsine, the conformity in development between the two classes of leeches will be settled beyond a doubt.

5. *Significance of the Teloblasts.*

The teloblasts form one of the most remarkable features of annelid development. They represent specialized centres of proliferation, with most marvellous powers of assimilation and reproduction. Their occurrence in worms, molluscs, and vertebrates (only mesoblastic teloblasts have thus far been discovered outside the annelids), in larval as well as in fœtal types of development, makes it sufficiently evident that they are not to be regarded as an accidental phenomenon without morphological significance.

The embryos of all bilateral animals, from the worms up to the vertebrates, lengthen by cell-proliferation at the posterior end. The question arises, Is this proliferating power invariably localized in special cells or groups of cells? It is generally believed that the posterior end of the embryo represents a mass of indifferent, non-specialized elements. It is supposed that here histological differentiation has its vanishing point; that here the germ-layers blend in a common basis.

The case of Lumbricus (*vide* Wilson's paper) shows us that

the teloblasts may differ so little in size from the cells which they produce that their terminal position is about the only means of distinguishing them. This accounts for their having been overlooked by such embryologists as Kowalevsky, Kleinenberg, and Hatschek. In the Hirudinea we see the different kinds of teloblasts of each band represented either by one or two cells. Wilson informs me that in one species of Lumbricus he finds only one nephroblast on each side; in another species, two, as in Clepsine.

We do not know to what extent this variation in number may be carried; but it adds another difficulty of recognition, which might easily become insuperable. Instead of only one or two teloblasts of a given kind, there may be many, all taking equal shares in a common work, or correlative parts of a complex work. Some such condition may be supposed to exist in the higher bilateral animals.

We already have sufficient grounds for regarding the teloblasts as an archaic feature of development. Obviously they do not represent primitive organs, but the undeveloped, embryological bases of such organs. They constitute the trunk-bud, and are thus the primary seat of all the truly metameric elements of the animal. Primarily they represented, as we have reason to suppose, the bases of non-metameric organs, in which the regenerative power was, or became, preëminent.

6. *The Fœtal and the Larval Type of Development.*

The relations of the fœtal and the larval types of development have never been made clear by those who hold that the latter represents, approximately, the ancestral line of development. Some have maintained that the phylogenetic history of the annelid is retraced in larval metamorphoses; while others have denied any such morphogenetic significance to the larva, claiming that it is a secondary form reached through adaptive changes which have been called forth by its pelagic mode of life.

Balfour has given us a broad and comprehensive discussion of the nature, origin, and affinities of larval forms, and has considered, in a general way, the nature and extent of the secondary changes likely to occur in the fœtal or the larval state. According to Balfour (No. 32, p. 299), "the relative chances

of the ancestral history being preserved in the fœtus or the larva may be summed up in the following way: There is a greater chance of the ancestral history being *lost* in forms which develop in the egg; and of its being *masked* in those which are hatched as larvæ."

Balfour's phylogenetic conclusions were based on a comparison of the various larval forms with one another. No attempt was made to identify larval with fœtal features of development, and to verify in this way deductions based on the occurrence of similar larvæ in different groups. It cannot be denied, however, that in this direction lies a crucial test of our theories respecting larval forms. As long as it remains impossible to find a parallel in fundamental features between the fœtus and the larva, so long will it be impossible to decide how much is ancestral and how much adaptive in the larva.

In spite of volumes devoted to the discussion of the subject the larva of Polygordius still remains a morphological puzzle. After an extended, critical analysis of the leading theories relating to this larva, Fraipont closes his magnificent monograph on Polygordius with the following confession: "It is not yet possible, in the present state of our knowledge, to determine what is the morphological significance of the larva of Polygordius, the Trochophora (Trochosphere) of the annelids."

In the history of the teloblasts we find a satisfactory basis for the direct comparison of the fœtal with the larval course of development. What, then, is the fœtal Trochosphere? and of what importance is it to our theoretical conceptions of the annelid embryo? Does it throw any light on the structure of the ancestral Trochosphere, — the Trochozoon? and does it assist us to a better understanding of the nature and extent of the abbreviations and modifications represented in direct development?

For the purpose I have in view Clepsine furnishes an excellent example of the direct type of annelid development, while Polygordius affords a well-known example of the larval type. These forms may, therefore, serve as points of departure for the few suggestions to be offered here. The development of the Trochosphere of Polygordius is very imperfectly known, but the gap is now bridged by Hatschek's studies on Eupomatus (No. 35).

We have seen that radial symmetry, so far as outward appearances go, is preserved in the egg of Clepsine until the eight-cell stage is reached; and that bilateral symmetry attains its fullest expression through the cleavage of the posterior macromere, which ends in the establishment of ten teloblasts. In Eupomatus we reach the same important stage of development by the time the blastopore has been reduced, by closure advancing from behind forward, to a small pore, — the future mouth. The radial Gastrula has passed into the bilateral, embryonic stage of the larva. The teloblasts are represented by a pair of mesoblasts (" pole-cells ") and a pair of nephroblasts. The neuroblasts are not distinguishable, which may be explained on the supposition that they still lie in the ectoderm, and present no conspicuous differential characters. A præ-oral band of cilia (prototroch, Kleinenberg), occupying an equatorial position with respect to the primary axis, is already present, and the apex of the præoral lobe (Scheitelfeld) bears a few long cilia. With the exception of the ring and the tuft of cilia, the organs of the larva are as yet undeveloped, and exist only in the form of more or less definite rudiments. The *embryonic Trochosphere*, as we may call this stage, is represented in Hatschek's Figs. 25 and 26, Pl. XI.

In Clepsine, as I have said, the formation of the teloblasts, as the closing act of cleavage, brings us to the stage which corresponds most nearly to the embryonic Trochosphere. For the sake of distinction this stage (Diag. 4) may be named the *fœtal Trochosphere*.

In both forms we meet with the same fundamental features, and the differences are precisely such as general principles would lead us to anticipate. Ciliated organs of locomotion, specially adapted to the needs of a roving, larval life, but without functional importance for the fœtus, are not developed in Clepsine. As the fœtal Trochosphere is supplied with a large stock of food-material, ready for absorption without the aid of a digestive system, there is no necessity for an early development of the mouth and gastric cavity, such as must exist in the case of the embryonic Trochosphere; and, accordingly, we find the larval development far in advance of the fœtal in these particulars. In respect to the trunk-bud (teloblasts), the case is reversed; for in the larval embryo the differentiation of the bud

is incomplete, and its development is retarded in the interest of the Trochosphere proper; while in the fœtal form, the trochospheral development is abbreviated for the sake of a more perfect bud with accelerated development.

Among the more important differences remaining to be noticed are those which have been brought about under the influence of the food-yolk. The process of gastrulation, the form of the blastopore and its relations to the mouth, have been very profoundly modified in this way. The trunk-bud of the fœtal Trochosphere has been carried far from its original, post-oral position; and, as the result of this displacement, we see the halves (germ-bands) of the trunk, which develop side by side as a unit in the larva, formed separately, and carried over the massive sphere of yolk in such a manner as to meet along the median ventral line. This whole process of circumcrescence and concrescence has arisen secondarily, in adaptation to fœtal conditions that do not exist in the larval form. The blastopore, if we include the space traversed in the closure of the germ-bands, has been stretched out of all proportion to its original dimensions, so that it no longer represents the primitive Gastrula-mouth, but merely a secondary prolongation of it backwards along the whole ventral line of the body. In the embryonic Trochosphere we find the blastopore already closed before the trunk-bud begins to develop; hence the line of closure ("Gastrula-raphe") is limited to the ventral line of the Trochosphere. As the metameric body-region is not yet developed, it is evident that the posterior limit of the primitive blastopore falls within the non-metameric region, from which the head-segment of the adult animal is formed. This region is represented in Clepsine by the cephalic lobe, and the primitive blastopore is not, strictly speaking, represented at all. The most that can be said is that its position is sometimes indicated by a linear depression (No. 1, p. 55), extending from the mouth to the hind edge of the cephalic lobe. The line of junction of the germ-bands becomes continuous with the post-oral groove; but the two things have nothing further in common, and they are as distinct in meaning as in mode of origin. What is usually called the blastopore is therefore a secondary opening resulting from the mechanical separation of the halves of the trunk-bud, and its closure is simply a restoration of original conditions.

This closure advances from before, backwards, following the direction of the bud-development. The closure of the primitive blastopore, on the contrary, progresses in the opposite direction, and represents, not a restoration, but a reconstruction. In the embryonic Trochosphere the anterior remnant of the blastopore persists as the mouth, while in the fœtal Trochosphere, where the primitive blastopore never comes to development, the mouth appears to form as a secondary perforation.

Grant that teloblasts exist in both the larval and the fœtal form, and that the conception of them as a trunk-bud is correct, and I see no escape from the above view of the blastopore. The larval form has the primitive blastopore with typical relations to the mouth and to the non-metameric portion of the animal; the fœtal form has lost the primitive blastopore, and acquired a secondary one, which may be regarded as a posterior extension of the original opening along an entirely new region — the metameric trunk-region.

The primitive Gastrula stage is passed long before the establishment of the first metamere; the secondary Gastrula is the primitive one extended, and so retarded in development that the process of gastrulation is prolonged through the whole formative period of the embryo.

The occurrence of the Trochosphere, its origin from a typical invaginate Gastrula, the persistent relations of the blastopore to the mouth, and the presence of a teloblastic trunk-bud, all appear to me to support the views developed in the foregoing comparison.

It is conceivable that the Gastrula from which the Trochosphere arises represents not a primitive, but a derived form, which has been much reduced in extent through a retarded development of the trunk. The objections to this view are numerous, and so obvious that they need not be enumerated.

The comparison above made between the fœtal and the larval Trochosphere has important bearings on the interpretation of the blastopore in higher forms, on the concrescence theory of the formation of the vertebrate embryo, and on some recent theories of the origin of metameric segmentation, — bearings which cannot be considered *in extenso* within the limits of the present paper. I may say, however, that I see no reason for abandoning the so-called theory of concrescent growth. In all

cases where separate germ-bands are formed concrescence must be conceded. The formation of the vertebrate embryo may be easily regarded as a modification of the same process, and a more rational view has not yet been propounded, so far as I am able to judge. Concrescence seems, indeed, to be the very essence of Sedgwick's theory (No. 36) of the origin of the mouth and anus, and yet he rejects this view as applied to vertebrates.

It will be obvious to the reader of the foregoing pages that I regard as untenable those theories according to which the somites of segmented animals are derived from gut-pouches. It is not the archenteron, nor yet the mesenteron, in which metamerism first exhibits itself.

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EXPLANATION OF PLATE IV.

Reference Letters.

a, left macromere.
b, right macromere.
c, anterior and median macromere.
cl, cephalic lobe.
ec, ectoderm.
en, entoderm.
enp, entoplast.
ep, epidermal layer.
m, mesoderm.
m', lateral cell-row.
nb, teloblasts of the neuronephric stratum.
nc, nerve-cord, neural cell-rows, neuroblast.
nph, nephridial rows.
p, posterior end of cephalic lobe.
x, right mesoblast.
xy, left mesoblast.
y, yolk.

FIG. 1. *C. complanata* from Naples. Surface view of germ-bands in an equatorial position. The white blotches in the yolk are entoplasts (*enp.*) × 120.

FIG. 2. *C. complanata* (Naples). Transverse section near the middle of the egg, in a little earlier stage. The contrast in color between the superficial and the deeper cells of the ectoderm has been made too great by the lithographer. × 120.

FIG. 3. *C. complanata* (Naples). Median sagittal section of the same stage. × 120.

FIG. 4. Cephalic lobe and underlying yolk, from the same series of sections, but a little to one side of the median plane. × 280.

FIG. 5. An obliquely horizontal section of the same stage, at the level of the arrow 5–5 in Fig. 3. × 120.

FIG. 6 *C. parasita* (Cambridge, Mass.). Transverse section of the embryo, when the germ-bands are a little more than two-thirds closed. Section is just in front of the unclosed portion of the bands. × 280.

FIG. 7. From the same series, just behind the point of closure.

EXPLANATION OF PLATE V.

Reference Letters.

c, coelom.
en, entoderm.
ep, epidermis.
g, lateral ganglia.
gl, larval glands.
lc, longitudinal commissures.
m, mesoderm.
m', lateral cell-row.
nc, nerve-cord — neural rows.
nph, nephridial rows — nephridia.
oe.c, œsophageal collar.
s, septum.
sb,' anterior pair of sense-bulbs.
sb,'' posterior pair of sense-bulbs.
sex, sexual cells.
sp, somatopleure.
spp, splanchnopleure.
st, mouth.
st.d, stomodæum.
y, yolk.

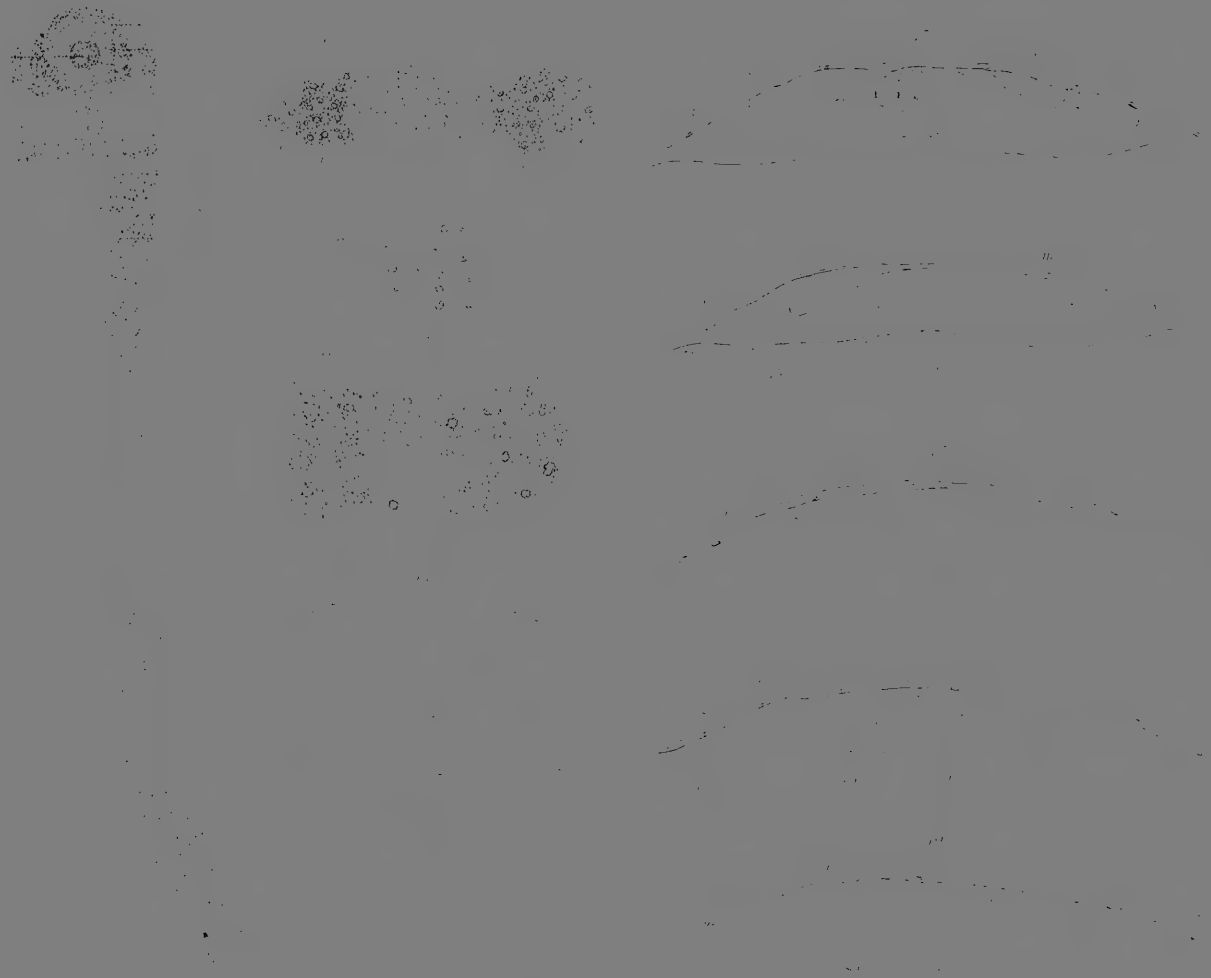
FIG. 8. *C. parasita.* Surface view of the embryo when the germ-bands are about two-thirds closed.

FIGS. 9–11. Three portions of a horizontal (frontal) section of the embryo, after the complete closure of the germ-bands, showing different stages in the development of the neuronephric stratum. Fig. 9, near the end of the first third; Fig. 10, near the beginning of the last third; and Fig. 11, very near the hind end. × 280.

FIGS. 12–14. From a series of transverse sections of the head at time of hatching. Fig. 12 hits the dorsal edge of the stomodæal ingrowth, and grazes the anterior pair of sense-bulbs (*sb''*); Fig. 13 is the next section above, taking in a strip of the œsophageal collar and the centre of the sense-bulbs; Fig. 14 is the fifth section (= .0075^{mm}) above Fig. 13, and hits the posterior pair of sense-bulbs (*sb''*); *y* = the line of the yolk. The position of these sections is shown by the arrows in Fig. 29. × 280.

FIGS. 15–19. Selected from a series of transverse sections through the hind end of the embryo at time of hatching, showing an early stage in the development of the nerve-chain and the nephridia. The sections run from near the middle of one somite to the middle of the next in front. Fig. 15 gives the first section; Fig. 16, the second; Fig. 17, the fourth; Fig. 18, the fifth; and Fig. 19, the seventh.

FIG. 18 is taken on the boundary line of the two somites. × 280.



EXPLANATION OF PLATE VI.

Reference Letters.

c, cœlom.
d, ducts of larval gland-cells.
en, entoderm.
enp, entoplasts.
ep, epidermis.
g, ganglia.
gl, larval gland-cells.
m, mesoderm.
nc, nerve-cord.
nl, neurilem.
p, proboscis.
s, septa.
sb', anterior pair of sense-bulbs.
sgl, salivary glands.
sp, somatopleure.
spp, splanchnopleure.
st.d, stomodæum.
sub.œ.g, subœsophageal ganglia.
sup.œ.g, superœsophageal ganglia.
tr, transverse nerve-fibres.
y, yolk.

FIG. 20. *C. parasita.* Median sagittal section of an embryo in which the germ-bands are one-half closed. × 280.

FIG. 21. Similar section of a stage in which the germ-bands are nearly closed. × 280.

FIG. 22. Middle region of ventral side (from the same section), showing entoplasts in the periphery of the yolk. × 280.

FIG. 23. Transverse section through the region of the larval gland-cells at time of hatching. × 280.

FIG. 24. Near the middle of the ventral side. From a sagittal section following that seen in Fig. 28.

FIG. 25. *C. marginata.* Nine days old. Longitudinal section of one of the anterior gastric diverticula. The dorsal half alone is shown. × 280.

FIG. 26. *C. parasita.* Same series as Fig. 28. Middle region of ventral side, showing the septa and an early stage of the entoderm. × 465.

FIG. 27. From the same section, nearer the hind end. × 465.

FIG. 28. Median sagittal section at time of hatching. × 280.

FIG. 29. Sixth section from that shown in Fig. 28. × 280.

THE GERM-BANDS OF LUMBRICUS.

EDMUND B. WILSON.

THE foundation of our knowledge of the germ-layers of the Chætopoda was laid by the classical memoir of Kowalevsky on the embryology of *Lumbricus rubellus* and *L. agricola* (1871), and the subsequent researches of Kleinenberg, Hatschek, and other embryologists seemed sufficient for a clear understanding of all the essential phenomena of development. An examination of the development of *Lumbricus olidus*, Hoffmeister (= *L. fœtidus*, Dug.) has nevertheless shown that some of the most important and characteristic phenomena have hitherto escaped attention; and I am led to publish this preliminary article partly on account of the light that my results throw on the interesting discoveries of Whitman and others in the development of the Hirudinea, partly on account of their close bearings on recent studies of the excretory system in the Vertebrata. In the present communication I shall describe only the general structure and mode of growth of the germ-bands, reserving for a future paper an account of the early embryonic stages and a detailed description of the development of organs. (See postscript p. 121.)

I. GENERAL ACCOUNT.

As in *L. rubellus* (*t.* Kowalevsky) and in *L.* (*trapezoides*) *communis* (*t.* Kleinenberg) the germ-bands end behind in a pair of large "mesoblasts" at the expense of which the bands increase in length throughout the whole course of development. At the outset these mesoblasts appear to be the sole source whence new cells are added to the germ-bands. But as development proceeds six other large cells are set aside as builders of the bands. For the sake of brevity I shall designate the eight large cells (the mesoblasts included) as *teloblasts* — a term suggested to me by Professor Whitman. The arrangement of the teloblasts is symmetrical with respect to the ventral

median line, as shown in Figs. 1 and 2. At the extreme posterior end lie the two mesoblasts (*M*) in contact with each other at the median line. Two others (*N*) are placed on either side the median line at a considerable distance anterior to the mesoblasts. A third pair (*Np*) lie, one on either side, a short distance behind and lateral to the second pair, and a fourth pair (*X*) are found just outside the last.

Each of the eight teloblasts gives rise to a row of cells, at first single, that extends forwards between the ectoblast and entoblast. The rows proceeding from the mesoblasts soon widen into a pair of broad mesoblastic plates that form the greater part of the germ-bands and ultimately give rise to the dissepiments, muscles, vessels, and, as I believe, to the setigerous glands. The six remaining rows lie between the mesoblastic bands and the ectoblast, but are intimately related with the ectoblast. The two inner rows (r^1, Figs. 1, 2, 6), arising from the anterior pair of teloblasts, give rise to the corresponding halves of the ventral nerve-cord, and the large cells are, therefore, *neuroblasts*, precisely as in *Clepsine*. The adjoining rows (r^2, Figs. 1, 2, 7) furnish the basis of the nephridia, and the third pair of teloblasts (*Np*, Fig. 1) are, therefore, *nephroblasts* (of which Whitman describes *two* pairs in *Clepsine*). The fourth or outer row (r^3) apparently gives rise to a solid band of cells (*x*, Fig. 6) that lies between the nephridia and the outer setigerous glands, and may be followed to the anterior end of the body. I have not yet succeeded in determining the ultimate fate of this structure. The arrangement just described persists unaltered for a considerable period, and in its main features may be recognized in embryos eight or ten millimetres long, and nearly ready to hatch. As development proceeds, however, the six anterior teloblasts gradually lose their prominence, and finally can no longer be distinguished, and at the same time the corresponding cell-rows become more than one cell wide throughout their whole length. The mesoblasts persist until a very late stage.

It is evident from the foregoing account that the germ-bands of *Lumbricus*, in one species at least, are closely similar to those of *Clepsine*. As in the Hirudinea generally, each germ-band may be said to consist of three strata of cells, viz.: (1) the ectoblast, (2) the three rows of cells produced by the three

anterior teloblasts, and (3) the mesoblastic band, which lies in contact with the entoblast. The second of these layers is, however, intimately related with the ectoblast, and, as will appear farther on, is derived from it.

II. The Nerve-cord.

The neuroblasts fit closely into the ectoblast (*N.*, Fig. 5), and *in some cases unquestionably extend to the outer surface*, as may be seen with especial clearness when they are rounded and swollen at the time of division. The position of the amphiaster visible at this time demonstrates the fact that cells are added to the neural row at its posterior end by division of the neuroblast; and my preparations indicate that all the neural cells have a like origin. Although the neural rows are closely applied to the ectoblast, they are always separated from it by a distinct line (Figs. 3, 7), and they assume a clear red color with borax-carmine, while the ectoblast cells are stained brownish red. In late stages, after disappearance of the neuroblasts, the neural rows, now several cells wide, still appear to increase in length by division of the cells at their posterior ends, and not by proliferation of the ectoblast (Fig. 7).

The ventral nerve-cord is formed by the gradual concrescence of the neural rows in the median line (Fig. 6). There is no indication that the subjacent ectoblast takes part in the formation of the cord, and, so far as *L. olidus* is concerned, Hatschek's account of the matter is certainly incorrect.[1] No invagination from the exterior takes place; no medullary canal is formed, and the continuity of the ectoblast across the median line is never broken. Unless the development of *L. rubellus* differs very widely from that of *L. olidus*, Hatschek has mistaken the narrow angular interval between the converging halves of the cord for an evidence of invagination.

III. The Nephridia.

The foregoing account of the neuroblasts and the neural rows will apply, *mutatis mutandis*, to the nephroblasts and the nephridial rows (compare Figs. 4 and 5). Anteriorly these rows are always clearly defined at the sides by delicate bundles

[1] Sitzungsb. d. k. Akad. Wien. lxxiv. 1876.

of longitudial muscles, developed out of mesoblastic cells that pass down from the overlying mesoblastic band. *The nephridia arise as paired metameric outgrowths from the nephridial rows,* there being a single pair in each somite just behind and in contact with the rudiment of the dissepiment. Each nephridium appears to consist at first of a single cell, though it is difficult to determine this with certainty. However this may be, it soon assumes the form of a U-shaped cord of cells, that projects into the cœlom and becomes invested with flattened mesoblastic cells. One limb of the U remains attached to the body-wall, and ultimately forms the distal part of the nephridium; the other limb becomes free from the body-wall, but remains attached to the dissepiment. Opposite to this point of attachment, on the anterior face of the dissepiment, is a single large *mesoblastic* cell, which gives rise to the ciliated funnel. The subsequent development of the nephridium consists in, (1) the elongation and convolution of the loop; (2) the perforation of the central cord of cells from end to end, by a delicate canal, and (3) the conversion of the mesoblastic covering into tne outer layers of the organ.

IV. The outer row of Cells.

I am still in doubt as to the fate of the outer cell-row (r^3, Figs. 1, 2). The teloblast from which it arises is precisely like the nephroblast, and the cell-row itself differs from the nephridial row only in being, as a rule, somewhat narrower. Passing forwards, the row becomes less distinct, and is for a time very hard to follow, whether in surface-views or in sections. In early stages it sometimes fuses in front with the nephridial row, but in some specimens it seems to be distinct. In later stages it appears to be continuous with the band of cells (x, Fig. 6) described on p. 114. In embryos nearly ready to hatch this band can no longer be distinguished, and I have been unable to establish any connection between it and any adult structure.

V. The Mesoblast.

The mesoblastic bands arise as single rows of cells at the latero-posterior angle of the mesoblasts, curve around their outer sides so as nearly to meet in the median line, then bend

rather abruptly outwards, and run forwards, soon becoming broad bands that pass between the entoblast and the remaining six cell-rows (compare Figs. 1, 3, 4, 5, and 6). Posteriorly the mesoblastic bands consist of a single layer, which splits farther forwards into two layers (somatic and splanchnic) the cells of which dovetail together with great regularity (Fig. 7). Still farther forward the cells arrange themselves in groups, so as to give rise to metameric cavities separated by rudiments of the dissepiments, as shown in Fig. 3.

The mesoblastic bands give rise to all the muscles and vessels of the body, as well as to the ciliated funnels and outer investment of the nephridia, but the origin of the setigerous glands is not so easy to determine. They arise at the same time with the nephridia, the outer series lying just outside the outer cell-row (Fig. 6), while the inner series is situated close to the row of nephridia. The setigerous gland is at first very similar to the rudiment of a nephridium, consisting of a single (?) large central cell (which ultimately gives origin to the setæ) surrounded by a mesoblastic investment. It is extremely difficult to determine certainly how the central cells arise, but I believe them to be of mesoblastic origin on account of their behavior with reagents, and because, in transparent surface-views, the outer series of setigerous glands can be traced backwards until it fades away in the mesoblast that lies outside the outer cell-row.

VI. Growth and Concrescence of the Germ-bands. Origin of the Teloblasts.

Cleavage of the ovum is unequal, and in its general features is similar to that of *L. communis* (*trapezoides*) as described by Kleinenberg. The gastrula is formed by embolic invagination, and at the time of infolding is saucer-shaped, the blastopore occupying the entire ventral aspect. Closure of the blastopore proceeds from behind forwards, and the anterior part, after a stomodæal ingrowth of ectoblast, persists as the mouth. The mesoblasts are differentiated at a very early stage, and some time before the invagination, are pushed into the cleavage-cavity, where they lie side by side at the posterior end. Each gives rise to a row of (mesoblastic) cells that extends forwards near the lip of the wide blastopore, and forms the rudiment of a germ-

band. As the blastopore narrows, the two cell-rows extend forwards and upwards, and finally meet each other in the median line above the mouth. The middle part of each row is at the same time carried downwards, so as to lie at the side of the body, somewhat towards the ventral aspect. Each row has, therefore, a double curvature (the embryo now being nearly spherical), passing downwards and forwards from the "mesoblast," and then forwards and upwards to meet its fellow above the mouth. As growth progresses the rows (now widened into the mesoblastic bands) grow towards each other, and finally unite just behind the mouth. From the initial point of union concrescence proceeds regularly backwards throughout the whole period of embryonic life, and is completed about the time of hatching. During this process the bands constantly increase in width until they join each other along the whole dorsal median line, and thus completely surround the alimentary canal.

The six anterior teloblasts, viz., the neuroblasts, nephroblasts and the "lateral teloblasts" (*X*), can first be distinguished with certainty in spherical embryos towards the end of invagination. At this period they have the same arrangement as in later stages, but *lie in the ectoblast, extending to the surface of the body.* Each gives rise to a row of cells that can be traced forwards a short distance, and then is lost amongst the surrounding cells. In later stages these teloblasts are gradually crowded below the surface by adjoining ectoblast cells, though they always remain embedded in the ectoblast, and sometimes reach the surface in stages as late as that shown in Fig. 3. There is no evidence that they are originally formed below the ectoblast, and are afterwards pushed out to the surface. The only interpretation that I can put upon these observations is, that *not only the neuroblasts, but also the nephroblasts and "lateral teloblasts," are modified ectoblastic cells.*

VII. Summary. Comparisons.

It is not my intention to give at present a full discussion of the observations outlined in the preceding pages, but it seems desirable to call attention to their bearing on certain morphological discussions relating to the origin and homologies of

the excretory system. As we have seen, this system first appears as a continuous longitudinal cord of cells ("the nephridial row") lying in the somatopleure, and my observations on this point are in accord with those of Whitman on *Clepsine*,[1] of Hatschek on *Criodrilus*,[2] and of Edouard Meyer on *Polymnia nebulosa*.[3] Although this cord never acquires a lumen in *Lumbricus* there can be no doubt from Meyer's observations and my own that it is homologous with the longitudinal excretory canal of *Polygordius*, *Lanice*, and *Polymnia*, which is likewise solid at first (Meyer), and in *Polymnia* consists of a single cell-row. Several morphologists have compared this canal directly with the segmental duct of vertebrates; but the homology has thus far remained an open question on account of the lack of decisive embryological evidence. This evidence, I venture to believe, is afforded by my observations on the origin of the nephridia in *Lumbricus*, taken in connection with recent studies on the segmental duct. It is impossible to doubt that the nephroblasts of *Lumbricus*, and, therefore, the nephridial rows and the nephridia (excepting the funnels), are derivatives of the *outer germ-layer*, and, in view of this conclusion, the likeness between the development of the nephridial row and that of the segmental duct, as described in the recent papers of Spee,[4] Flemming,[5] and van Wijhe,[6] is very significant. In the rabbit (Hensen, Flemming), guinea-pig (Spee), and in *Raja* (van Wijhe) the segmental duct arises as a solid cord of cells that is split off from the outer layer and grows at its hinder end by the proliferation of a limited area of the ectoblast. This area is continually carried backwards as the embryo elongates, and, barring certain unimportant differences in the number and arrangement of the cells, Flemming's figures of cross-sections, near the growing end of the segmental duct, agree closely with my own sections through the hinder part of the nephridial row in *Lumbricus*. An essentially similar account of the origin of the

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1 Zoologischer Anzeiger, No. 218, 1886.

2 Studien über Entw. d. Anneliden. Arb. ans. d. zool. Inst. Wien, I., 1878.

3 Communicated by Lang, Fauna und Flora d. Golfes von Neapl; XI. Die Polycladen, p. 678.

4 Arch. f. Anat. und Phys., 1884.

5 Arch. f. Anat. und Phys., 1886.

6 Zoologischer Anzeiger, No. 236, 1886.

segmental duct in Amphibia (*Rana*) and Reptilia (*Lacerta*) is given in a brief note just published by Perenyi.[1]

Now, in some cases at any rate, the segmental tubules of the vertebrate pronephros are formed wholly or in part as outgrowths of the segmental duct, and thus agree precisely in mode of origin with the nephridia of *Lumbricus*. As far, therefore, as exact likeness in the development of special parts can be taken to indicate homology, *the "nephridial row" of Lumbricus must be regarded as homologous with the segmental duct, and the series of nephridia as homologous with the vertebrate pronephros.*[2]

It must, however, still remain an open question how far the comparison between the nephridia of annelides and those of vertebrates can be carried out until it is ascertained how far these structures are serially homologous with one another. Van Wijhe's positive statements are strongly confirmatory of the ordinary view that the mesonephric tubules are of mesoblastic origin, and only secondarily become joined to the ectoblastic segmental duct, so that they would seem not to be serially homologous with the pronephric tubules. We have seen, however, that the annelidan nephridia consist of a proximal mesoblastic portion (funnel) and a distal ectoblastic portion. If we suppose these two portions to have existed in all the nephridial tubules of the common ancestral type, and to have subsequently varied in importance in different regions of the body, one or the other portion increasing at the expense of the other, the difficulty of understanding the serial homologies disappears.

Turning now to more special questions, we find a likeness between the germ-bands of *Lumbricus* and those of *Clepsine* so great as to indicate a very near relationship between the Oligochæta and the Hirudinea. This resemblance can hardly be adaptive merely, since *Lumbricus* and *Clepsine* differ very widely in respect to the mode of gastrulation, and there is nothing in the conditions of larval life to suggest an explanation of the likeness in the germ-bands. Probably no one will question the homology of the neuroblasts in the two animals. The four nephroblasts of

[1] Zoologischer Anzeiger, No. 243, 1887.

[2] The validity of the comparison is not affected by the absence of special nephoblasts in the vertebrates, for these cells have arisen in *Lumbricus* and *Clepsine* simply through extreme concentration of development, and represent the entire proliferating group in the vertebrates.

Clepsine have apparently become reduced to two in *Lumbricus* (see postscript), and the "lateral teloblasts" of the two animals appear to be homologous. It is possible, however, that the cells so-called in *Lumbricus* are homologous with the outer nephroblasts in *Clepsine*, the outer rows having disappeared.

The development of the six anterior teloblasts in *Lumbricus* seems to give the key to an explanation of the phylogenetic origin of their homologues in *Clepsine*. It is easy to understand the origin of these cells in *Lumbricus* from the ectoblast, through greater and greater concentration of development at the posterior ends of the germ-bands until first a small group and ultimately single cells, or pairs of cells, became differentiated into neuroblasts, or nephroblasts. In *Lumbricus* these cells are at first ordinary ectoblast cells, which afterwards sink below the surface, though still remaining imbedded in the ectoblast. In *Clepsine*, by acceleration of development, they are covered by the ectoblast at a very early stage, but it is a significant fact that they arise from a blastomere (see Whitman's well-known paper) the remaining portion of which breaks up into ordinary ectoblastic cells.

BRYN MAWR, PA., Feb., 1887.

Postscript, July, 1887. — Since the foregoing article was sent to the printer I have examined the development of *L. communis* (= *trapezoides*), and of *L. agricola*. The former is the species studied by Kleinenberg, and the latter has been examined especially by Kowalevsky. Both agree closely with *L. olidus* in the structure and development of the germ-bands, and the teloblasts are especially well seen in *L. communis*.

L. agricola agrees very closely with *L. olidus*, but *L. communis* differs from both these species in one very interesting respect, namely, that there are five instead of four teloblasts in each germ-band. The mesoblasts and neuroblast are precisely as in the other species, but there are two nephroblasts in addition to the so-called lateral teloblast, which is situated a little farther forwards. *L. communis* therefore agrees in every respect with *Clepsine*.

A careful comparison of these embryos with those of the other two species has convinced me that the cell I have called the "lateral teloblast" in *L. olidus* (X.), is, in reality, a second nephroblast, and that the lateral teloblast of *L. communis* and *Clepsine* has disappeared in *L. olidus* and *L. agricola*, as suggested on p. 121. I have not always been able to find the lateral teloblast in *L. communis*, but the corresponding cell-row is always present. This row is likewise present in the single embryo of *L. agricola* of the proper stage that I have been able to examine, though the lateral teloblast is absent. This embryo (which has been identified beyond question) has a pair of very large primary mesoblasts, although Kowalevsky states that he was unable to find them in embryos of *L. agricola*.

[METHODS. — The best results have been obtained by treatment of the embryos with Perenyi's fluid (10–15 minutes) and subsequent staining with borax-carmine, alum-carmine, or picro-carmine. Very clear temporary preparations of early stages may be

made by fixing with Perenyi's fluid, staining with the ordinary dilute iodine solution used in the study of vegetal tissues, and clearing, first, in dilute, afterwards in strong glycerine. The color fades in a few hours, when the specimens may be stained with the carmine-solutions and sectioned or mounted whole in glycerine or balsam. Perenyi's fluid is the only fixing fluid, so far as my experience goes, that causes absolutely no shrinkage. In studying any but the very early stages, it is necessary to remove the hardened mass of albumen from the alimentary canal. This operation (which requires some practice) may best be performed under glycerine with a pair of fine pointed scalpels, the dorsal wall of the body being cut away.]

EXPLANATION OF PLATE.

I have made many actual sections that clearly demonstrate the relations shown in Figs. 3, 4, and 5, but it is preferable to figure optical sections, since they show the structure with equal clearness, and better preserve the natural relations of the parts. For making actual sections it is usually necessary to remove the contents of the mesenteron, a process which often mutilates the entoblast.

c. Large ciliated cells of the ectoblast, extending along the ventral median line.
d. Dissepiments.
ec. Ectoblast.
en. Entoblast.
lm. Longitudinal muscle-fibres.
m. mouth.
M. Primary "mesoblast."
mes. Mesoblastic cells.
N. Neuroblast.
n. Neural row.
Np. Nephroblast.
np. Nephridia.
o.g. Oral gland.
pg. Supra-œsophageal ganglion.
r^1. Neural row.
r^2. Nephridial row.
r^3. "Outer row."
si. Inner series of setigerous glands.
so. Outer series of setigerous glands.
vn. Ventral nerve-cord.
X. "Lateral teloblast."
x. Anterior continuation of the "outer row."

FIG. 1. Diagrammatic view of the hinder part of the germ-bands of an oval embryo, seen from the ventral aspect, showing the eight teloblasts, the mesoblastic, neural, nephridial, and "outer" cell-rows, and part of the ectoblast.

FIG. 2. Camera drawing of part of the ventral view of an embryo in the same stage as the last, showing the six anterior teloblasts, the neural, nephridial, and outer cell-rows and part of the ectoblast. At *mes.* is shown a row of mesoblastic cells that give rise to longitudinal muscle-fibres.

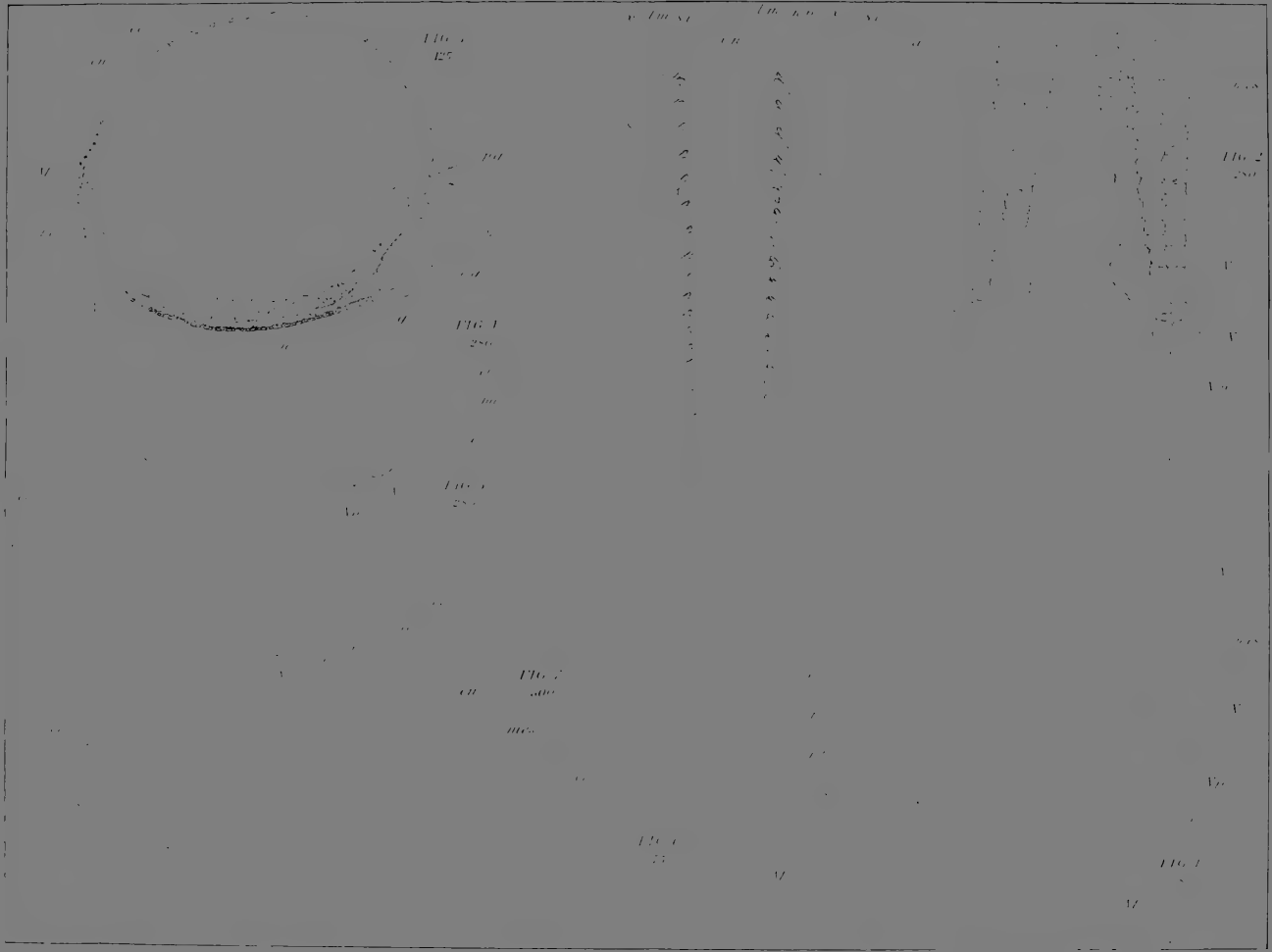
FIG. 3. Optical longitudinal section through an embryo somewhat older than that from which Fig. 2 is taken. Camera.

FIG. 4. Optical transverse section through a slightly older embryo, showing the nephroblasts and outer teloblasts, with the overlying mesoblastic bands. Camera.

FIG. 5. Optical transverse section in front of the last, showing the neuroblasts, nephridial rows, and outer rows. The precise relations of these structures to the ectoblast and mesoblast are shown. Camera.

FIG. 6. Diagrammatic view, from the ventral aspect, of the germ-bands of an embryo about the middle of larval life, after disappearance of the six anterior teloblasts. To show the relation of the eight cell-rows to the adult structures which appear in the anterior part of the figure. The figure is made diagrammatic for the sake of clearness, but is a fair representation of the actual appearance of the preparation.

FIG. 7. Constructed from two consecutive longitudinal sections through the posterior part of an embryo somewhat younger than the last. It shows the entoblast and ectoblast, one of the mesoblasts, and the beginning of the mesoblastic and neural rows. The neuroblast has disappeared. Camera.



STUDIES ON THE EYES OF ARTHROPODS.

WILLIAM PATTEN, PH.D.

1. Development of the Eyes of Vespa, with Observations on the Ocelli of some Insects.

In a former paper (27) I gave an account of some observations on the structure of the compound eyes of Arthropods. Those observations, which were made almost entirely upon adult eyes, differed widely from those of recent writers on this subject. I desired to confirm, by embryological data, my observations on the continuity of the so-called rhabdom with the crystalline-cone cells, and on the nature of the *corneagen*.[1] It was also important to determine whether there was any similarity between the development of the compound eyes and the ocelli. Moreover, if the opinion expressed in my former paper, that the primitive Arthropod eye was an invaginated and closed vesicle, be tenable, then it is necessary to show that the simplest Arthropod ocelli, which have heretofore been regarded as simple cup-like pits, are in reality closed vesicles. It is also necessary, in order to maintain my views on the origin of the com-

[1] The term "corneal hypodermis" employed in the paper referred to above is unsatisfactory on account of its length. In this paper I shall substitute *corneagen* for corneal hypodermis.

Postscript. — Dr. Mark's memoir on "Simple Eyes in Arthropods," was received as this paper was going to the printer. He suggests "lentigen" to designate the layer of cells called by me corneagen. There are some considerations which make the word "lentigen" preferable to "corneagen;" but it seems to me they do not outweigh the objections which might be urged against it. It is important that the term be applicable to the layer of cells in question throughout the Arthropods at least. But there are numerous instances in which this layer is undoubtedly present, although no lenses are formed by it: *Branchipus*, *Phronima*, and *Gammarus*, etc. On the other hand in *Peripatus* a lens is present, although it is not secreted by that layer of cells which, on morphological grounds, should be called lentigen, provided Dr. Mark's nomenclature were adopted.

pound eye, and the position and structure of the ommatidia, to show that the ommateum develops from the inner wall of the primitive optic vesicle.

The recent observations of Carrière (20, 21), Locy (28), Reichenbach (24), Claus (22), and Kingsley (23), on the development of the simple and the compound Arthropod eye differ widely; and it seems, at first sight, hardly possible to reconcile them either with one another or with my interpretation of the structure of the compound eye. I am convinced, however, that this difficulty arises from a difference in interpretation rather than in observation. The compound eye and optic ganglion of *Vespa* develop so slowly, and the successive stages are so clearly defined, that it is not difficult to follow the process through all its changes. The observations described in this paper, it seems to me, render it easy to harmonize the conflicting interpretations of facts observed by Reichenbach and Kingsley, while at the same time they furnish a confirmation of my views upon the origin and structure of the compound eye.

This paper contains observations on the compound eyes of *Vespa*, *Blatta* and *Phryganids*, and upon the ocelli of *Dytiscus*, *Hydrophilus* and *Phalangium*. Only the more important facts, and those of theoretical significance, are mentioned here, since a more detailed account will be published later.

VESPA.

In unhatched embryos whose mouth-parts have just appeared, the ventral plate extends to the anterior extremity of the egg, over the apex of which the head-end is bent at nearly right angles, so that the mouth lies at the tip of the egg, while the cephalic lobes are directed posteriorly and dorsally. The formation of the brain is initiated by a thickening of the distal and ventral edges of the diverging lobes. A gentle depression soon appears in the middle of the lobes, while at the same time their distal and ventral edges break away from the ectoderm, which then begins to grow over them (Figs. 1 and 2, *y*). Just below the free edge of the ectoderm, the cells of the cephalic lobes are contracted at their outer

ends, as though forcibly pinched in by the advancing ectoderm.

The cephalic lobes, before they are completely shut off from the exterior, are composed of distinct groups of spindle-shaped cells, *which strongly resemble certain kinds of sense-organs.* These groups of cells, whatever their significance may be, divide rapidly, and subsequently form quite regularly arranged balls of cells, which give the brain, when cut in certain directions, a segmented appearance somewhat like that of the ventral cord. The number and fate of these nerve-balls I have not been able to determine with certainty. There are at least six for each lobe; three of them undoubtedly form the optic ganglia; it seems probable that the fourth develops into the antennary lobe, and the remaining two into the mushroom-shaped bodies on the dorsal side of the brain. The thin layer of ectoderm continues to advance over the cephalic lobe until it has enclosed all but an oblong space, whose long axis is parallel with that of the lobe, on the median part of its ventral edge. This still uncovered part is deeply depressed, and is composed of three cords of cells (Fig. 2, *op.g.*$^{1-3}$). The middle cord is widest at the proximal end of the lobe, and gradually narrows toward the opposite extremity, where the other two cords are widest. I shall speak of these three cords which ultimately develop into the optic ganglion, as the *inner*, *middle* and *outer* walls of the ganglionic fold.

The superficial ectoderm finally bridges the ganglionic fold and coalesces with the undifferentiated ectoderm on the ventral edges of the cephalic lobes. It is worthy of remark that instead of the brain being formed in the usual way from the inner of two layers arising from the splitting of the cephalic lobes, the cephalic lobes themselves are depressed and finally covered by an advancing sheet of ectoderm, the edge of which was formerly continuous with the dorsal and distal edges of the cephalic lobes (Figs. 1–3).

The ectoderm, at the point where it is continuous with the ventral edge of the cephalic lobes, and therefore in close proximity to the ganglionic fold, becomes slightly thickened to form the foundation of the compound eye (Fig. 1, *E*).

Before the advancing sheet of ectoderm has covered the ganglionic fold the latter is continuous with the thickened patch of

ectoderm which gives rise to the compound eye (Figs. 1 and 2, *op. g*[1]). The ganglionic fold finally breaks away from the optic thickening, the free edge of which immediately unites with the advancing sheet of ectoderm to form a continuous layer over the brain (Figs. 2 and 3).

The optic thickening, therefore, does not lie in the cephalic lobes, properly speaking, or those parts which give rise to the brain, but just on one side of them.

While the ganglionic fold is still uncovered, *the optic nerve* appears as a cord of cells arising from the optic thickening and uniting it with the middle wall of the ganglionic fold (Figs. 1–3, *op.n.*).

The inclosing of the ganglionic fold, the formation of the optic nerve, and the slight depression in the optic thickening, take place before the embryo hatches.

In larvæ about 3 mm. long, the optic cavity (Fig. 4, *E*) has assumed a very characteristic shape, in that the invagination is deepest on its ventral edge, and gradually grows shallower towards the opposite side. The optic nerve is attached to the ventral and deepest part of the invagination (Fig. 4, *op.n.*).

In the 5 mm. larvæ, the optic invagination is very deep and may be recognized as such without difficulty (Fig. 5, *E*). The optic nerve is still attached only to the deeper part of the invagination. The ganglionic fold is, in this stage, completely filled by the middle wall (*op.g.*[2]), which has pushed the outer wall (*op.g.*[1]), now transformed into a layer of columnar cells, just below the floor of the optic cup.

The brain-sheath (*br.sh.*) forms a thin but distinct layer of cells between them.

The cells of the middle wall multiply rapidly in a remarkable manner. One of the cells divides into two pear-shaped ones, united at the base by slender stalks, but free at the nucleolated apices. One of these cells divides again in the same manner. This process is repeated many times, until a long and nearly straight fibre is produced with pear-shaped cells arranged upon it like leaves upon a growing shoot. These clusters of pear-shaped cells, of which the whole optic ganglion is composed, are unipolar ganglion-cells. The main stalks to which the cells are attached, are probably composed of a bundle of fibrillæ, each fibrilla being the prolongation of the stalk of a single

ganglion-cell. Even at the time when the pupa is ready to come out of its cell, the ganglion-cells of the middle wall, which develops into the enormous optic ganglion of the adult (epiopticon of Hickson), have exactly the same arrangement as that just described, only the main stalks and cells are more numerous and more closely packed (Figs. 5, 6,7, *op. g.*^{2}). The arrangement of the unipolar ganglion-cells upon long stalks, and their method of division, are so evident, both in sections and in macerated specimens, that there can be no doubt concerning the matter.

Beneath the ganglion-cell layer there is a mass of nerve-fibres which finally develop into the *medulla* of the optic ganglion, or the *outer medulla* (Fig. 7, *o.md.*).

The ganglion-cell layer of the middle wall is directly continuous with the infolded edge of the outer wall (Figs. 5 and 6) which appears to be a proliferating point, supplying the optic ganglion with new elements (Fig. 5).

The inner edge of the inner wall (*op. g.*^{3}) becomes thickened and bent to form a ridge which appears like a papilla in cross-sections. The inner wall, at the base of the papilla, is perforated by a bundle of nerve-fibres arising from the base of the middle wall. This bundle of fibres (*n.f.*^{2}) is crossed by a layer of fibres (*n.f.*^{1}) arising from the inner face of the ridge, and extending along the under surface of the inner wall, and finally passing to the optic nerve, after mingling with the fibres at the outer edge of the medullary mass.

In the 10 mm. larvæ (Fig. 6), the middle wall, which we shall now speak of as the optic ganglion, has greatly increased in size, and is strongly arched. The outer medulla is composed of two distinct sets of crossing fibres, while the outer wall (*op. g.*^{1}) is reduced to a convex, single layer of cells, from the upper edge of which arises a small tuft of ganglion-cells, which are subsequently added to the optic ganglion.

The brain-sheath, (*br.sh.*) has become a stratum of widely separated, columnar cells between two distinct membranes. On the dorsal side of the brain it runs close under the ommateum as a very thin layer, and, bending inwards, terminates at the lower edge of the outer wall (Fig. 6). On the ventral side it is very thick below the outer medulla, but is rapidly reduced to a thin membrane, with an occasional nucleus, beneath the optic

nerve, at the distal end of which it terminates, just beneath the lower edge of the ommateum.

Cross-sections through the middle of the inner wall show the remnants of the ridge, or fold, on its outer edge, and the bundle of nerve-fibres from the outer medulla, which penetrate the wall just below the ridge (Fig. 6). A considerable space has been formed between the outer wall and the inner surface of the outer medulla. In median cross-sections this space opens outward; on either side of the median line, however, it is closed. The cavity is nearly V-shaped, with the opening at the apex of the V, on the front side of the brain.

There is a thick layer of densely packed nerve-fibres continuous with the medullary substance of the brain, and running under the inner wall to mingle with the fibres of the outer medulla (Fig. 6, *n.f.*[2]). These fibres develop into the inner medulla (Fig. 7, *i.md.*).

The cells of the inner and outer walls are characterized, up to a late period, by their deeply stained and turbid protoplasm. They are closely packed in a single layer, and stand out in strong contrast with the graceful outlines of the beautifully arranged and delicately stained ganglion-cells of the middle wall. Toward the inner edge of the middle wall there are some very large, either pear-shaped or conical, ganglion-cells, containing deeply-stained granules surrounding one or two distally placed nuclei. These cells are unipolar ganglion-cells, although it is possible that some of the conical ones are tripolar, for I have occasionally seen indications of two prolongations of the broad distal ends of such cells. It is certain that some fibres, in the 10 mm. stage at least, connect the peripheral layer of the optic ganglion with the adjacent neurilemma.

During this stage the nucleated, heretofore irregularly arranged nerve-fibres connecting the outer medulla with the ommateum undergo a remarkable change, in that they now issue from the apex of the medulla as perfectly straight, radiating fibres, which run against the neurilemma and are then deflected toward the lower edge of the ommateum as a confused mass of wavy fibres, which finally pass from there upwards between the ommateum and the neurilemma, distributing on the way small bundles of nerves to each of the minute om-

matidia which have now begun to form in the ommateum (Fig. 6).

A distinct row of unipolar ganglion-cells appears about half way between the apex of the outer medulla and the brain-sheath. Each cell is attached to a fibre by a minute stalk, just as the ganglion-cells in the cortical layer of the optic ganglion are attached to the main stalks. On either side of this row of cells are others, irregularly arranged. Toward the apex of the outer medulla the cells are more numerous, and they soon form there a second layer of cells (Fig. 7, *rt.g.*^{2}). This cone of remarkably straight fibres (fan-shaped in section), with their attached ganglion-cells, is the rudiment of the retinal ganglion. The outer row of cells is the foundation of the *ganglion-cell layer*, and the second row, of the *nerve-spindle layer* of the retinal ganglion.

Beneath the medulla of the optic ganglion is a mass of unipolar ganglion-cells exactly like those on the opposite side (Figs. 6 and 7, *inf.gl*). The stalks of these cells contribute their fibrous prolongations to the formation of the outer medulla.

The optic invagination, which, in the 5 mm. stage was a deep, narrow pit, is now, when seen from the surface, a crescent-shaped and rather shallow depression. The ventral lip of the depression (*vf*) is rounded and composed of nearly columnar cells. The dorsal lip (*df*), which is formed later than the other, is sharp-edged and composed of flattened, overlying cells which cling closely to the cuticula over the invagination. *The floor of the optic invagination forms the ommateum; its cells are therefore upright.* The nuclei of the ommateum are at first arranged in a single row; but finally, the cells become so numerous that their nuclei are forced to arrange themselves in three or four superimposed layers, although the ommateum is still composed of but a single layer of cells.

In the 10 mm. larvæ the nuclei of the retinophoræ, or crystalline cone-cells, form a distinct row just beneath the outer surface of the ommateum.

In surface views of the isolated ommateum, and in macerated specimens, *the retinophoræ are seen to be arranged in pairs, with their swollen outer ends extending inwards as slender stalks which rest upon the basal membrane.* Around each pair of *retinophoræ* is a spindle-shaped group of cells,— the retinula cells. Between the groups of cells formed by the retinulæ and retino-

phoræ are rather large, round nuclei belonging to other pigment-cells of the ommatidia. Although there are, at first, but two retinophoræ for each ommatidium, this number is increased to four just before the first formation of pigment in the eye, by the appearance of two very small cells just below the nuclei of the two retinophoræ already present. These two cells continue to grow until all four are equal in size and configuration, forming the cup-like swelling of the outer ends of the retinophoræ, which contains eventually the crystalline cone, but which is now almost filled by the four nuclei.

Pigment first appears in the outer ends of the retinula cells, as a pair of dark spots on either side of the retinophoræ, just below the neck of the calyx. Each dark spot belongs to a single retinula cell. The other retinula cells subsequently develop pigment in the same way. But for a long time the pigment remains as two distinct semicircular areas with the neck of the calyx in the middle. These two patches of pigment finally unite, so that the base of the calyx is completely enclosed in a circle of pigment.

Pigment is next deposited in the inner ends of the retinula cells. Finally, pigment appears diffusely distributed in the cells whose expanded outer ends surround the calyx.

The following facts are worthy of special notice: — 1. *The crystalline-cone cells, or any of the eventually pigmented cells surrounding them, do not form a layer of cells distinct from and superimposed on the retinulæ; on the contrary, the crystalline-cone cells, the retinulæ, and the other pigmented cells are derived from, and remain, a single layer of cells.* 2. *The rhabdom is not a product of the retinulæ; it is merely the inward prolongation, or stalk, of the crystalline cone-cells.* 3. *The layer of cells from which the ommateum arises is the inner wall of an optic vesicle formed by an invagination of the ectoderm, consequently the ommateal cells are upright.* 4. *The retinophoræ which, in the adult, are grouped in fours, in the youngest stages are arranged in twos, thus repeating the permanent condition of the retinophoræ found in the ocelli of most Insects, and in the simpler compound eyes of Crustacea.* 5. *The pigment first appears as paired patches around the paired retinophoræ and is retained until after the retinophoræ have increased to four. This transitory condition of the ommatidial cells in the compound eye probably corresponds*

with the permanently paired arrangement of the pigment patches and retinophoræ of the ocelli.

The ommateum, in the 10 mm. stage, is divided into two nearly equal parts by a shallow, longitudinal depression of the surface, below which are a number of round, irregularly-arranged nuclei (Fig. 6, *a*). The absence of cell-boundaries and the irregular arrangement of the nuclei in this part of the ommateum are in sharp contrast with the regularly arranged ommatidia in the adjacent parts. At the beginning of the pupal stages this median cord of nuclei has disappeared, and its place is occupied by well-formed ommatidia.

In the 8 mm. stage, of which no drawings are given, *the ommateum is supplied with two nerve-branches, one going to the dorsal and the other to the ventral part of the ommateum.*

In the pupæ the optic ganglion which during the preceding stage was an oblong and nearly flat mass of ganglionic cells, by the continued growth of its peripheral portions, becomes an enormous, almost spherical, ganglion. Its medulla is now a thick, nearly hemispherical shell, with its convex face directed toward the ommateum (Fig. 7, *omd*).

The change in the shape of the ganglion has been caused by the growth of its inner edges (*gc*[1]) toward the brain, while, at the same time, the anterior and posterior boundaries of the inner edge grow in opposite directions completely around the medulla and finally unite with each other on its ventral side.

In median sections through both optic ganglia, one therefore sees beneath the outer and inner medullæ, a wedge-shaped mass of cells (Fig. 7, *inf. ge.*), which, at first sight, appears to form a distinct ganglion. Such, however, is not the case; it is merely a ventral continuation of the anterior face of the optic ganglion. The "Keilförmiges" ganglion of Berger (7), and the "couronne ganglionnaire," and "ganglion en coin" of Viallanes (15), are merely continuous parts of the cortical layer of the optic ganglion, apparently bounded by the fibres connecting the optic, with the retinal ganglion.

The fan-shaped row of nerve-fibres, with its two rows of ganglion-cells, has now developed into a convex layer easily recognized as the *retinal ganglion* (Fig. 7, *rt.g.*[1] and *rt.g.*[2]). The coarse, straight fibres of the preceding stage have enlarged between the cells of the inner layer, into refractive, spindle-shaped thick-

enings which I shall call *nerve-spindles;* the part of the retinal ganglion in which they are formed, I have called the *nerve-spindle layer.* Surrounding the nerve-spindles are unipolar ganglion-cells which appear to be attached to the spindles by very short fibres. In the outer layer of the retinal ganglion the spindles are reduced to fibres, to which are attached clusters of unipolar ganglion-cells. These clusters of cells attached to the main fibre are similar to the much longer ganglion cell-stalks forming the cortical layer of the optic ganglion.

After passing that part of the brain-sheath lying against the distal surface of the retinal ganglion the main fibres unite in large bundles to form the nerve-bundle layer.

Beneath the nerve-spindle layer is what appears to be a distinct membrane, but careful examination shows that it is a layer of nerve-fibres running at right angles to the spindles. There are also a few scattered ganglion-cells beneath the nerve-spindle layer.

The nerve-bundle layer contains, in the pupal stage, large, round cells filled with deeply-stained yolk-like globules (Fig. 7, *ft.c*).

During the pupal stage the optic invagination closes. The actual closure I have not observed. At the latest period seen before the closure the anterior and posterior folds covered more than half of the eye. On the edges of the eye the cells composing the two limbs of the folds had already assumed the shape and arrangement characteristic of the outer and middle layer of the eye in the next stage. In the earliest pupal stage after the closure the folds had disappeared, *while above the ommateum was a double layer of cells exactly like those seen on the periphery of the eye in the preceding stage.* It is therefore evident that these two layers are derived from the coalesced limbs of the dorsal and ventral folds.

At the beginning of the pupal stage, then, the eye consists of three layers, the inner one being the ommateum, which is in practically the same condition that it was before the closing of the flattened invagination; the middle layer is composed of cells containing large, round nuclei arranged at regular intervals over the retinophoræ; the third layer is composed of flattened cells with quite small nuclei; it is retained with very slight modifications as the corneagen of the adult. The cells of the middle

layer become sickle-shaped and arrange themselves in pairs, a single cell on either side of a calyx. These cells grow inward as far as the neck of the calyx, where they terminate in a rounded swelling containing a large nucleus. Their inner ends soon become deeply pigmented, and appear to form a part of each ommatidium. On surface views of the eye at this time we can distinguish the small, faint nuclei of the corneagen and the sharp outlines of the calyx, on each side of which is a large semicircular cell derived from the middle layer. Surrounding the sickle-shaped cells are the ends of a circle of 18 more cells; in some cases I could only count 16 or 17. Each ommatidium, therefore, including the four retinophoræ, but not the two middle layer-cells, is composed of 22 cells.

The inner wall of the ganglion-fold, towards the close of the pupal stage, breaks up into short strings of unipolar ganglion-cells, similar to those of the optic ganglion, which become continuous with the layer of ganglion-cells covering the brain.

The inner wall decreases in size until, at the close of the pupal stage, it is no longer visible.

The fibres of the nerve-bundle layer are not single fibres, although they appear to be at first sight. They are composed of a bundle of fibrillæ which can only be recognized as such at certain places. One of these fibres may be followed with certainty from the basal membrane through the nerve-bundle layer and the neurilemma, to the retinal ganglion, where it is reënforced by the fibrillæ arising from the ganglion-cells of the outer layer; immediately afterwards it expands into a nerve-spindle of the inner layer. The nerve-spindle is not, so far as I can observe, a "neurospongium," such as described by Hickson (16), but merely a point where its constituent fibrillæ become swollen and refractive, and less compactly arranged. From the retinal ganglion the fibre, which can no longer be resolved into its elements, is continued to the convex surface of the outer medulla, where it turns at right angles into the fibrous mass of the medulla, just below the outer surface of which it expands again into a short nerve-spindle, and is then continued straight onwards to the opposite surface. On tangential section of the outer surface of the medulla the nerve-spindles are seen in cross-sections as dark points arranged with perfect regularity, and probably corre-

spond in number with the nerve-spindles of the retinal ganglion and with the ommatidia.

The outer medulla is composed of concentric layers of fibres, those of adjacent layers being at right angles with each other. These layers are crossed at right angles by those fibres just described, and by the inward prolongation of the stalks of those ganglion-cells which form the cortical layer of the optic ganglion.

In every case where it is possible to distinguish anything with certainty the outer medulla can be resolved into single fibres running in different directions in adjacent layers, but not uniting with one another by anastomosing fibrillæ. Nothing like the confused net-work of anastomosing fibrillæ, or "neurospongium," described by Hickson in *Musca*, has been seen in *Vespa*, except indeed, in poorly preserved specimens, where the medullary substance has coagulated into a homogeneous and finely granular mass which has some such appearance as Hickson describes. The same remarks apply to the nerve-spindles of the retinal ganglion. I have seen the hazy appearance of the nerve-spindles when macerated and preserved in glycerine; but believe that it is due to a partial coagulation of the fibrillæ composing them, for fresh specimens teased in weak chromic acid show no such appearance. Hickson proposes a new name for the widely present "granular matrix" in the optic, and other ganglia of the body; for he says, p. 219, although "very commonly met with in the animal kingdom," it has not, as far as he is aware, received any separate name. The very fine "granular matrix" he has reference to is the celebrated "Punktsubstanz" of Leydig (2.) It has also been variously designated by later authors, who have treated this or kindred subjects.

Dujardin (1), according to Berger, first described this substance in the mushroom bodies of the Bee as the "*granuläre substanz*," and the cell-covering of the same as the "*substance corticale pulpeuse.*" Subsequently, Leydig (2) demonstrated the cellular nature of the latter substance, and showed that the former, which he named the "*Punktsubstanz*," was present in the ganglia of the ventral nerve-cord as well. Dietl (4) called the same substance "*Marksubstanz*," and Berger(6), in his pioneer work on the brain and optic ganglia of Arthropods, used the same term, and called that part in the epiopticon of

Hickson the outer "*Marklager*," and that in the opticon, the inner "*Marklager*." Viallanes (14) called the same substance, according to its position, the "*masse medullaire externe*," "*interne*," or "*terminale*." It does not seem advisable to adopt the term "neurospongium," for the existence of any such structure has not been demonstrated, and if it had been, the supply of terms already in use would be quite ample for its designation.

Hickson is inclined to criticise (p. 228) Leydig's successors, because they overlooked or misunderstood the nature of the cells composing the "Punktsubstanz," which, he says, was described by Leydig as a nerve-cell sheath surrounding the other parts of the ganglia. Leydig did not describe the "Punktsubstanz" as a sheath of nerve-cells, but, on the contrary, as a central mass of net-like and interwoven nerve-fibres.

Hickson takes some pains to confirm this supposed observation of Leydig, by showing that the *Punktsubstanz* in the silkworm moth is composed of nucleated cells. He probably had in mind the "*graue granläre Rindensubstanz*" which Leydig showed to be composed of cells, or what he called "*ganglionkugeln*."

The same author finds a variety of ganglion-cells in the optic tract, among them some "apolar ones." He also affirms that ganglion-cells are seldom found in the optic tract of Insects, although he says some are occasionally present. This is probably due to the fact that, according to his definition, a ganglion-cell has considerable cell protoplasm, and a nerve-cell comparatively little. It is probable that there is a very similar difference between the "nerve-fibrils and fibrillæ" (p. 227), which, according to Hickson, form distinct constituents of the optic tract.

According to Berger (p. 40), the layers of cells called a "retina" are to be regarded as such because they "stets innig mit dem Licht percipirenden Apparat, den Sehstäben, verbunden bleiben, während die übrigen Bestandtheile des Augenganglions von demselben durch Nerven abgetrennt sein können." In the majority of cases, however, the so-called "retina" of Berger is separated from the ommateum by nerve-bundles more clearly than it is from the optic ganglion. Moreover, the "retina" is separated from the ommateum by the brain-sheath, which encloses the brain and all the parts of the optic ganglion and "retina" in one sac; showing that the "retina" is far more

closely connected with the brain than it is with the ommateum.

Then, again, if the retinal ganglion of Arthropods is to be regarded as a part of the ommateum, both structures should arise from the same cell-layer. This, however, is not the case; for I have shown that the retinal ganglion of *Vespa* arises as a secondary specialization of the nerve-fibres and ganglion-cells arising from the optic ganglion. In my opinion there is not sufficient evidence for supposing with Hickson, that all the optic ganglia, as well as the retinulæ, form one complete retina, for we have seen that the optic ganglion develops as a part distinguishable from, but continuous with, the brain; while the layer to which the retinulæ belong is an entirely independent formation. The retinal ganglion cannot be regarded, I believe, as distinct, functionally or morphologically, from the rest of the optic ganglion. The similarity in structure of the two parts points towards this conclusion. I regard the shape of the optic ganglia, and the, at first sight, marvellously intricate arrangement of nerve-fibres in the medulla, as the resultant of two tendencies: the one is to increase the number of ganglionic cells, necessitating an extension of the cortical layer; the other is to arrange the ganglionic cells, and the fibres arising from them, in the least possible space. As soon as the cortical layer of the optic ganglion has increased in extent so as to form a nearly spherical mass, with the nerve-fibres in the centre, it appears to have reached a limit to its advantageous growth in that manner. The further increase of ganglion-cells is obtained by the formation of a new centre of growth, — the retinal ganglion. This process might go on until several ganglia had been formed.

Although I have not as yet arrived at any conclusion regarding the arrangement of the nerve-fibres in the medulla, it seems probable that the successive layers of fibres running at right angles to each other have been formed by alternating growths of the ganglion-cells and nerve-fibres on different sides of the optic ganglion. If we had a mass of fibres shaped like an hour-glass, and composed of layers of spirally-wound fibres, those in alternating layers being at any point nearly at right angles with each other, we should have some such arrangement as that in the medullæ. A median longitudinal sec-

tion of the glass would show a decussation of fibres at the neck. — and also at either end, provided the fibres were continued onwards, — like that between the optic and retinal ganglion and that between the inner and outer medulla.

But there is another factor which renders the arrangement of the fibres still more complicated. There are, besides those fibres which run to the eye, others which leave the medulla in the opposite direction, and go to the brain, perhaps to the eye on the opposite side of the head.

I have made some observations which render it probable to my mind that each stalk of ganglion-cells in the optic ganglion divides at the surface of the medulla into two branches, going in opposite directions. It is possible that of the two sets of fibres thus formed, one, after its passage through the medulla, goes to the eye, and the other to the brain, or to the eye on the opposite side of the head.

On pp. 226–7, Leydig (2), in speaking of the origin of the nerves from various ganglionic centres, says: "Gegen diese centrale Punktsubstanz richten sich die Stiele der Ganglionkugeln, um ihre fibrilläre Materie dort beizumengen und aus diesen centralen Herden von Punktmasse geht erst die einfach streifige Substanz der peripherischen Nerven hervor."

Dietl (4) supports Leydig's observation, and asserts that fibres never pass directly from the ganglion-cells to the peripheral nerves; the latter receive their fibres only from the net-like "Marksubstanz." Claus (10), however, declares (p. 46) that this view is untenable, since he has observed in *Phronima* which is specially adapted to the study of this point, that fibres arising from the cortical ganglion-cells pass diagonally through the Punktsubstanz, without coming into any closer relation to the latter substance, directly into the nerves arising from the double ganglia of the ventral cord. My observations, as far as the optic ganglion is concerned, agree with those of Leydig and Dietl, for nerve-fibres running through the retinal ganglion to the eye do not arise from the cortical ganglion-cells, but from the depths of the medulla.

There is apparently a wide discrepancy between Reichenbach's (24) recent observations on *Astacus* and my own on the development of the eye and optic ganglion of *Vespa*. But closer inspection will show, I think, that this difference is rather

one of interpretation than of observation. The "Augenfalte" of Reichenbach is undoubtedly what I have called the ganglionic fold. Compare his Figs. 148–152, Pl. XI., with Figs. 3 and 4 of this paper. The resemblance of Reichenbach's figures to my unpublished drawings is more evident. The crystalline-cone layer of Reichenbach is what I have described as the optic thickening. The slight depression in the middle of this layer would correspond to the optic invagination in the Wasp, only in the latter it is much deeper. The absence, in *Astacus*, of the dorsal and ventral folds which enclose the eye of *Vespa* is easily explained by a comparison with what I have observed in *Blatta* and *Phryganids*. There, the optic invagination is a barely recognizable depression; the dorsal and ventral lips of the optic cavity, by whose union the corneagen and middle layer are formed, are represented by a very obscure ingrowth of flattened cells over the outer surface of the nearly flat optic thickening. A very similar process probably takes place in *Astacus*, perhaps even more obscurely than in Blatta or in the Phryganids. If this be so, it would account for the failure of Reichenbach to observe the formation of the real optic invagination.

Moreover, we can hardly doubt, after Kingsley's (23) and my own observations, that the ommateum is a single layer of cells, and it is therefore quite certain that the crystalline cone-layer of Reichenbach represents the whole ommateum, and corresponds, in its early stages, to the optic thickening of *Vespa*.

If what I have just said be true, then it must of necessity follow, that the outer wall of Reichenbach's "Augenfalte," cannot, as he believes, develop into the layer of retinulæ and rhabdoms. We may also note the fact in this connection that in *Crangon*, according to Kingsley's observations, and in *Vespa*, *Blatta* and *Phryganids*, according to my own, pigment first appears in the cells immediately surrounding the rhabdoms, while in *Astacus*, the supposed rhabdom layer, even up to a very late period, has developed no pigment, while at the points where, according to my supposition, the rhabdom should be, that is, in the crystalline cone-layer of Reichenbach, there is an early deposition of pigment.

On p. 92, Reichenbach, in discussing the fate of the outer wall of the "Augenfalte," says, "*Es unterliegt wohl keinem*

Zweifel, das wir in diesen langen, stabförmigen, abgeflachten Gebilden die Rhabdome vor uns haben, während die peripheren Schichten der Aussenwand die eigentlichen Retinulazellen enthalten." On p. 96, however, he admits, "Es könnte nämlich das von mir als Rhabdomschicht gedeutete Stratum eine Nervenfaserschicht sein." It is thus evident that Reichenbach had no very conclusive evidence for regarding the outer wall of the "Augenfalte" as the retinula and rhabdom layer; the "rhabdoms," are possibly nerve-fibres, while the fact that the supposed retinula cells are arranged in groups, which apparently correspond to the crystalline-cone cells, is of no importance, as evidence on this point, since the cells of the optic ganglion in *Vespa* show a very similar arrangement. This leads us finally to the conclusion, in favor of which still other facts might be adduced, that the outer wall of the "Augenfalte" does not develop, as Reichenbach believes, into the layer of retinula cells and rhabdoms; as already indicated, the "Augenfalte" undoubtedly corresponds to what I have called the ganglionic fold, no part of which has anything to do with the formation of the ommateum; the middle wall of this fold, however, develops into the optic ganglion. Reichenbach's description of the fate of the Augenfalte is not quite clear to me; but, on comparing his Fig. 224, with Fig. 6 of this paper, it seems probable that the retinula layer represents the cortical layer of the optic ganglion. He himself notices (p. 87,) the resemblance of the cells in the Augenfalte to ganglion cells. The layer of rhabdoms, which he admits may be a layer of nerve-fibres, would then correspond to the medulla of the optic ganglion, together with its nerve-spindles, which in Astacus may resemble rhabdoms. It would not be the first time that nerve-spindles — *e.g.*, those of the retinal ganglion — had been mistaken for rods. His inner wall (*I.w.*) would then correspond to the same named structure in the optic ganglion of *Vespa*, and the outer wall to a layer of ganglion-cells (*G.K.*), which he represents in Fig. 224, but, so far as I can discover, he does not mention in the text whence it came.

In order to show, beyond a doubt, what parts of the optic and ganglionic invaginations develop either into the retinulæ, rhabdoms, or optic ganglion, of the adult, it is necessary to carry on the observations up to a stage in which the resemblance of the parts in

question to those of the adult is so evident as to preclude all doubt as to their identity. Unfortunately, Reichenbach regarded the observations of these developmental stages, as "*nicht so brennend notwendig*, da sie uns nur über die weiteren Verschmelzungsprocesse den Sehzellen einerseits mit dem Ganglion opticum anderseits Aufschluss geben kann." (p. 92.)

Claus, in his paper on the development of Branchipus, labors under the same disadvantage as did Reichenbach in supposing, according to older observation, that the crystalline cone-cells and retinulæ formed two distinct layers. It is improbable that the first stages in the development of the eyes of *Branchipus* should differ fundamentally from those of other Arthropods, and, therefore, there is room to doubt Claus' observation, that the retinulæ arose from the mass of proliferating cells which gave rise to the optic ganglion. Claus represents this as taking place in the *metanauplius stage*, where it is probable that the retinulæ had been already formed some time, for they are covered by a corneagen, which is not developed in *Vespa*, *Blatta*, and *Phryganids*, until *after* the formation of ommatidia. Moreover, the presence of a retinal ganglion in the earliest stage figured by Claus, likewise indicates that that stage was too far advanced to show the first steps in the development of the ommatidia and optic ganglion.

Kingsley's (23) observations on the development of the compound eye of *Crangon*, are, in some points, difficult to reconcile with those of Reichenbach and my own. Kingsley describes an invagination of the epiderm to form the optic cavity. The epiderm overlying the invaginated pouch, becomes the corneagen, the outer wall of the pouch, the "retina," and the inner wall, the optic ganglion.

It is probable that Kingsley's "optic invagination," Reichenbach's "Augenfalte," and my ganglionic fold, are one and the same structure. If we accept the interpretation of the first two writers, we are led into several serious difficulties, since we must suppose that the "retinal" cells are inverted, a supposition which cannot be made to harmonize with any observations as yet made on the structure of the compound eye. I would suggest, therefore, that in *Crangon*, the "optic invagination" develops into the optic ganglion, and that the ommateum and corneagen were formed in a stage overlooked by Kingsley,

and in an obscure way, similar to that seen in *Blatta* and *Phryganids*.

I have made some observations on the development of the compound eyes in *Blatta* and *Phryganids*, which will be of interest here, since the closing-in of the ommateum is quite different from that in *Vespa*. The process in these two forms is, very likely, the more prevalent one; and, as I have already suggested, a similar process probably takes place in *Astacus* and *Crangon*.

There is a thickening of the hypodermis on one side of the infolding of the optic ganglion. The cells on the periphery of the thickening become flattened, and their nuclei are gradually pushed over the outer ends of the ommateal cells to form a very thin, but continuous layer over them, but without the formation of any fold, and with only a barely noticeable depression of the thickening. It is remarkable that the slight depression is not deepest in the middle of the thickening, but on its posterior ventral edge, as in *Vespa*.

The formation in this way of a double layer of cells is undoubtedly a modification of the process seen in *Vespa*. In my paper on the "Eyes of Molluscs and Arthropods" (27) I gave reasons for supposing that the primitive Arthropod ocellus was a closed sac, the whole eye consisting of three layers. I further suggested that the compound eye was a modified ocellus, and consisted of two layers, the middle one, the outer wall of the optic vesicle, having disappeared. The development of the eyes of *Vespa*, *Blatta*, and *Phryganids*, shows that surmise to be very nearly correct; for the compound eye of Vespa is at one period a closed but flattened vesicle, covered by a layer of hypodermis, in other words, it consists of three layers similar to to those we supposed to exist in a primitive ocellus.

The *ocelli* of *Vespa* appear at the close of the larval period as *four* deep pits whose walls consist of a single layer of cells. The two anterior pits are situated close together, and soon fuse to form a single depression which develops into the anterior unpaired ocellus.

Just after the cessation of larval activity the lips of the pits probably close; for, at the beginning of the pupal stage, the cavities have disappeared, and we have three depressed, oval thickenings, composed of two layers of cells. I have, unfortu-

nately, not been able to procure the right stages for observing the rapid changes which take place just after the formation of the deep and open pits. Whether the two layers are formed by a delamination of the floor of the optic cups or by their closure, I cannot say. On theoretical grounds a delamination of the walls of an open pit does not seem probable, yet, in the youngest stages of the double-layered condition observed, the two layers *appeared* as though they had been formed by delamination, rather than by folding. It is possible, however, that this appearance was due to the fact that those stages had been treated only with alcohol, and, consequently, were not well preserved. Be this as it may, the fact I desire to emphasize here, is that *the three ocelli arise from four single-layered pits, the median ocellus being formed from a coalescence of the two ventral ones.*

The double nature of the median ocellus is also shown by the fact that, even in the latest stages, the root of the nerve is double, while that of the other two is single.

My observations, therefore, differ considerably from those of Carrière (21) on the Hymenoptera, since he describes the first stages as disc-like thickenings, which soon split into two layers. On the periphery of the double layer thus formed, is a "schräg nach unten gerichtete Einstülpung in welche die beiden Schichten in ihrer normalen Lage eingehen." This description corresponds with what I have seen in young pupæ of Vespa. But we have seen that the first stages of the ocelli were not to be found in pupæ, but in the oldest larvæ. Therefore, it is probable that what Carrière regarded as the "anlage" of the ocelli was nothing but the already closed optic cups of the larvæ.

ACILIUS AND OTHER FORMS.

In my paper on the Eyes of Molluscs and Arthropods I expressed the opinion that the ancestral Arthropod eye was three-layered, composed of the two walls of an optic cup, and an overlying layer of epidermis. In order to maintain this supposition it is important to show that the eyes of *Dytiscus* and related forms, described by Grenacher as open cups, and regarded by him and his followers as the simplest type of Arthro-

pod eyes, are in reality closed vesicles, and composed primarily of three layers of cells.

With this object in view I have examined the ocelli in the larvæ of *Hydrophilus*, *Dytiscus*, and *Acilius*, and find the facts such as my supposition would lead one to expect. From Grenacher's description, there would be little room to doubt his assertion that the ocelli of these Coleopterous larvæ are composed of a single layer of cells. Graber (Arch f. mik. Anat. xvii., Bd. Hft. 1, p. 67), however, hinted that Grenacher was in error on that point, and then asserted, in a very short foot-note, that he had subsequently found a "membrana limitans" in the ocelli of Dytiscus. Sograff (Zool. Anz. No. 18, 1879) remarks that the eyes of *Lithobidæ* and *Scolopendridæ* are exactly like those of Coleopterous larvæ (Acilius), and Spiders, although, in this preliminary notice, we can find no evidence in confirmation of his statement. There are a number of very important differences in the structure of the ocelli of these three groups. It is not clear in what points he regarded them as similar.

In the larvæ of *Acilius* there are six pairs of ocelli. Longitudinal vertical sections of the head show that the two dorsal pairs are very deep, and resemble the two-layered ocelli of certain spiders. The space between the lens and retina is completely filled by a layer of very long cells, *corneagen*, whose deep, nucleated ends are somewhat swollen and bent away from the centre of the eye. The axial cells of this layer are the longest, and their nucleated ends terminate about half way between the centre of the retina and its periphery (Fig. 8). The abaxial cells are shorter and thinner on the periphery of the eye. It thus happens that there are no nuclei of the corneagen just above the centre of the retina, while there is a distinct layer of them over its periphery, as well as on the walls of the inner half of the eye.

The periphery of the corneagen contains a thin layer of very large, dark globules, many of which contain a still darker corpuscle. This layer of pigment-like bodies extends from the edge of the lens to the retina.

The floor of the eye is formed by a layer of upright retinal cells, each provided with a double rod. In the middle of the retina is a deep furrow, filled with two rows of very large

rods, separated by a band of deep black pigment. The rods on either side of the median furrow are short, and gradually decrease in length towards the periphery of the retina.

The peripheral cells of the retina are rodless, and appear to be continuous with a layer of small flattened nuclei outside of those belonging to the corneal hypodermis, and extending outwards as far as the edge of the lens. The pigment must be removed in order to see this layer of nuclei. The outer part of the eye is surrounded by a delicate membrane.

The ocelli are formed in the embryos of *Hydrophilus* by invaginations of the ectoderm, directed diagonally inwards. In the just-hatched larvæ these pits are apparently still open, although the rods are well developed. The ocellus is composed of three distinct layers of cells (Fig. 10), of which the thick inner layer, the retina, is directly continuous on the dorsal side (*ab*) with the hypodermis. In the middle of the retina is a deep furrow, in which lies a single (?) row of rods. On the ventral side the edge of the retina is rounded, and, as can be easily seen, is not directly continuous, as on the opposite side, with the hypodermis. It appears to be continuous, however, with a thin layer of cells (*vb*) between it and the overlying corneagen. In the centre of the eyes this layer (*vb*) is thickened, and contains a cluster of deeply-stained nuclei. The limits of the layer are not very clear; but, from observations made on other material, I can hardly doubt that it is continuous, as I have indicated in the semi-diagrammatic drawing, at *z*, with the thick and very distinct corneagen which is continuous, on its ventral side, with the unmodified hypodermis.

It is thus evident, if my observations are correct, that the eye, although practically a closed vesicle, is not so in reality, as is shown by the absence of nuclei at the point *z*, and the continuity of the three layers.

In the older larvæ of *Hydrophilus* and *Dytiscus*, from 5 to 8 mm. long (Fig. 8), the retina contains much dark pigment. In the centre of the eye is a core of nuclei (*vb*) which probably belong to the outer wall of the optic vesicle. Between them and the lens is a distinct layer of cells.

I have taken special care to see that my sections, both of the larvæ and embryos, passed through the axis of the eye, and not to be misled by the deceptive appearances of sections which

were not cut in that plane. I think, therefore, that the fact is satisfactorily established that there are ocelli in the larvæ of Insects which are very similar to what, in my former paper, I regarded as the ancestral eye of Arthropods.

In that paper I also had occasion to refer to the lateral eyes of *Scorpions*, which, for various reasons, I regarded, in opposition to Lankester's observations, as double-layered. Prof. Lankester (26) mentions this point in his criticism of my paper and maintains that there is not the "*slightest doubt*" that the lateral eyes are single-layered, "there being no folding in of the edges of the depression so as to form a vesicle, and consequently no duplication or triplication of the layers." This evidence is far from being conclusive; for it is well known that there are few, if any, instances of admittedly two-layered ocelli in which there is any evidence of *infolding*. It has been recently shown that the compound eye is supplied with a layer of cells corresponding to the vitreous layer of the ocelli, yet this layer has been overlooked by the most careful students of this subject. Claus could not find the corneagen in the compound eyes of other Arthropods than the Edriopthalmidae, although, from theoretical reasons, he confidently expected to find such a layer. The lateral eyes of Scorpions are more difficult to study than the compound eyes; and yet, in spite of these facts, Prof. Lankester asserts that there is not the "slightest doubt" that the vitreous body is absent in these eyes, although nothing whatever is known of the development, which alone can furnish conclusive evidence in favor of his assertion.

In the paper referred to I gave reasons for not accepting as conclusive Lankester's observations on the lateral eyes of Scorpions. This proceeding of mine has been severely criticised by Prof. Lankester. It seems my objections did not outweigh my audacity in presuming to doubt the accuracy of his observations. It is reassuring, however, to think that the weight of Prof. Lankester's indignation may be divided in the future, since Prof. Mark,[1] on essentially the same ground offered by myself, has also presumed to question the decisiveness of the evidence.

[1] Simple Eyes in Arthropods. Bulletin of the Mus. Comp. Zool. Cambridge, Vol. xiii, No. 3. Prof. Mark's paper was received as this article was going to the printer.

Now that the ocelli of Dytiscus and Hydrophilus, which for so long have been regarded as open cups, are seen to be in reality composed of three layers of cells, it is all the more necessary to supply a satisfactory confirmation of Lankester's observations on the lateral eyes of Limulus and Scorpions.

In the larvæ of *Hydrophilus*, *Dytiscus*, and *Acilius*, there is a remarkable organ on the dorsal side of the posterior dorsal ocellus (Fig. 8), formed of two layers of cells; *the thin outer layer, judging from embryological data, is probably continuous with the corneagen, and the thick inner one with the retina of the adjacent ocellus.* The inner layer of the organ in question is composed of long, spindle-shaped cells drawn out to single nerve-fibres at the inner end, and terminating at the opposite extremity in double rods (*rd*) similar to those in the ocelli of Vespa. In the oldest larvæ there is an irregular layer of pigment around the base of the rods. Although there is no lens-like thickening of the cuticula over this organ the disposition of the pigment and the structure of the cells point to the conclusion that it is some form of ocellus.

In *Dytiscus* this organ is pigmented only in the oldest larvæ; in *Acilius* the pigment appears much earlier. In just-hatched larvæ of Hydrophilus the organ is a thickened patch of cells (Fig. 10 *ab*), apparently continuous with the retinal layer of the eye on its dorsal side, which at that point is practically open. The outer cell layer of this organ is probably formed later than the inner by the dorsal growth of the corneagen. In the older larvæ the two structures are apparently perfectly distinct (Fig. 8), although touching each other. It is thus evident that the organ we have been describing is nothing more than a dorsal extension of the ocellus to which it is attached.

This double ocellus, in its youngest stages, resembles the compound eye of *Vespa* in its early stages.

In other drawings, not published here for lack of space, the resemblance is greater than in those in the plate, which were selected to illustrate other points. At present it seems to me probable that the compound eye of Arthropods has developed from a single ocellus, which increased in area by a process similar to that observed in the ocelli of Dytiscus and Acilius. The ventral pit of the compound eye in Vespa, with its bundle of

nerve-fibres, would correspond to the primitive ocellus with its optic nerve, while the dorsal half of the compound eye would correspond to the dorsal extension of the ocellus.

Just as in the early stages of *Acilius*, whose ocelli we shall regard as typical, the ventral half of the double ocellus, or the primitive ocellus, is alone supplied with a special nerve, so in the beginning, the ventral half of the compound eye in *Vespa* is likewise the only part supplied with a special bundle of nerves. The comparison may be carried farther, for in the next stages of both organs, each half has a special nerve-branch. Still later, the two parts of the ocellus become practically independent, being separated by a layer of indifferent cells. The same occurs in the compound eye, which, as I have shown, is divided into a dorsal and ventral half by a median cord of indifferent cells. Again, the dorsal half of the ocellus, being a mere extension of the original ocellus, must be regarded as a later formation. The correspondence of the dorsal half of the ocellus with the similarly situated part of the compound eye is shown by the fact that the dorsal half of the latter is the last to develop ommatidia. Still another point of resemblance between the compound eye of *Vespa* and the double ocellus of *Acilius* is to be seen in the development of the dorsal and ventral folds which enclose the eye. We have shown that the ocellus in Hydrophilus arises as a pouch of epidermal cells pushed ventrally beneath the epidermis. No fold is formed on the dorsal side of the ocellus (Fig. 10, *ab*). Its closure is effected by the growth of a ventral fold dorsalwards until it unites with the dorsal edge of the optic cup. But it seems that the complete closure of the optic cup does not take place, if at all, until some time after hatching. Meantime, however, the dorsal edge of the retina has travelled dorsally (Fig. 10, *ab*), but is finally overtaken by the ventral fold (Fig. 10, *z*), which is growing in the same direction, and perhaps completely fuses with it, although it is possible that a complete fusion never takes place, the subsequent extension of the ocellus being effected by the dorsal growth of the ventral fold and by the dorsal edge of the retina.

The inclosing and growth of the compound eye is effected in a similar manner. The development of the eye is initiated by the formation, in a thickened patch of ectoderm, of a *ventrally* directed pouch. The pouch is consequently covered on its

ventral side with a *dorsally directed fold* (Figs. 4 and 5), while on the opposite side the inner wall of the pouch gradually passes into the thickened ectoderm of that side without the formation of a fold. The dorsal fold, which develops much later than the ventral one, is quite different from it in structure and general appearance, and may be regarded as a secondary formation. The compound eye grows, like the double ocellus, toward the dorsal side by the formation of ommatidia on its dorsal edge.

If the compound eye of Arthropods arose from such an ocellus with its dorsal extension as I have described in *Acilius*, *Dytiscus* and *Hydrophilus*, we ought to find traces of a similar structure in the larvæ of other Insects. In the larvæ of *Chironimus*, I have seen on the dorsal side, and a little in front of the ocelli, an oblong patch of hypodermic cells which has a strong superficial resemblance to the dorsal outgrowth of the ocellus in Dytiscus, the only important difference being that, in the former case, the colorless patch of cells is apparently completely separated from the ocelli.

In young *Corethra* larvæ there is a similarly thickened patch of cells on the dorsal side of the compound eye. In this case it is probable that the larval compound eye is a modification of an ocellus, while that of the adult represents the larval compound eye, plus the dorsal patch of colorless cells, which, in the adult, have developed ommatidia like those in the rest of the eye.

In *Neophalax* there is a thickening on the posterior dorsal side of the larval compound eye, similar to that in *Corethra*.

In the *Hydrachnida* the apparently single ocellus is in reality double, or at least has two distinct lenses imbedded in a common mass of pigment; on the dorsal side of each double ocellus is a rosette-shaped organ which I believe to be homologous with the dorsal extension of the ocellus in *Acilius*. In *Hydrachna* the organ in question is rosette-shaped and, in surface views, appears to be composed of a circle of wedge-shaped cells with peripheral nuclei; in the centre is a distinct granular core. The inner ends of the large cells are prolonged into nerve-fibres, which unite to form a bundle of nerves. The outer ends are capped with a hyaline layer of rods which resemble those of the dorsal extension in Acilius. Neither the rosette-shaped organs of *Hydrachna*, nor the similar structures in *Chironimus*,

Neophalax, or *Corethra* have been examined by means of sections. The superficial resemblance of the organs, however, both in position and structure, with the dorsal extension of Acilius is such that we may confidently expect sections to show a similar agreement in their minute anatomy.

There is another set of organs that at one time I felt inclined to include under this head; they are the organs described by Weismann, Leydig, and Claus, as the *frontal Sinnesorgane* of the Phyllopod Crustacea. The double cells and rod-like inclosures of these organs, as described by Claus, are suggestive of the double rods and cells in the dorsal extension of *Acilius* and *Dysticus*. The fact that the nerve supplying these organs, in some Daphniden, is connected with the three-fold ocellus is probably of no significance and of secondary importance, as Claus states; for the undoubtedly homologous organs, in *Simocephalus*, and in *Branchipus*, are supplied by independent nerves arising directly from the brain. The chief difficulty in comparing these organs with those in *Acilius* is their wide separation from the ocelli from which they are supposed to originate, and the fact that the insertion of the nerves supplying them is some distance from that of the nerve supplying the eye to which these peculiar organs presumably belong. This separation, however, may be secondary, and perhaps more apparent than real, as is the case with *Acilius* in its later larval stages. In an adult *Limenetis*, Grobben (2) has placed the frontal Sinnesorgan just in front of and close to the compound eyes, a condition that corresponds very nearly with that seen in larval Insects. At present, however, it seems to me that we can only indicate some points of resemblance between the frontal organ of Crustacea and the dorsal extension of the ocellus of Acilius. It is very possible that embryological studies directed especially to this point may furnish us with evidence of a closer relation between these problematical organs in Phyllopods and the dorsal extension of the ocelli in Insects.

If the compound Arthropod eye is in reality formed of two parts, at one period in the development supplied with separate nerves, but having a common optic ganglion, then it is possible that we may find, and indeed we should expect to find, among all the variations in structure of the Arthopod eye, that in some species the two parts had retained their embryonic character,

and remained separated. This supposition is rendered all the more probable since there are indications that the primitive ocellus and its dorsal extension, from which, in my opinion, the compound eye arose, are also in some cases (Hydrachna) completely separated. In fact we do find a separation of the compound eye into its primitive parts in the adult *Phronima*, in which the dorsal and ventral eyes are apparently distinct and are supplied with separate retinal ganglia and optic nerves, although there is but a single optic ganglion. In Insects there are numerous instances of this kind. In *Gyrinnus natator* there are two pairs of compound eyes which are supplied, according to Carrière (20) with separate nerves and retinal ganglia, the middle ganglion being paired, but united, while the inner is unpaired. Carrière considers that the two pairs of eyes arose from the division of a primitive pair. This statement, however, if I am correct, may need modification, since the facts I have presented render it probable that *double compound eyes have not arisen by the division of a primitive homogeneous eye, but by the separation of two originally different parts.* In most cases these parts are alike, and so closely united as to form one eye, the difference being discernible only in the youngest stages.

Another instance of double eyes, a description of which we owe to Carrière, (19) is found in *Bibio* and *Cloë diptera*. In these cases, however, only the males have double eyes, while in Gyrinnus both sexes are thus provided. In Bibio and Cloë, the accessory eye, as Carrière calls it, arises at the beginning of the pupal stage as a thickening of the epithelial cells, very similar to the primitive thickening for the compound eye, on the *dorsal* edge of the "Gattungsauge," from the upper, inner edge of which it appears to take its origin.

Although Carrière distinctly states that there is no transition of the ommatidia of the lower eye into those of the developing upper one, I feel inclined to doubt the complete accuracy of this statement when the condition described in Acilius and Vespa is borne in mind; for, in the former case, there is, at first sight, no connection between the dorsal extension and the ocellus in the older larvæ, although this connection is apparent in the embryos. Perhaps Carrière did not see the very first stages of the accessory eye, since he states that it appeared at the beginning of the pupal stage, while the dorsal half of the compound

eye in Vespa, which I regard as the homologue of the accessory eye in Bibio and Cloë, is visible at a much earlier period.

In the larvæ of the *Libellulidæ*, we have an indication, similar to that in Vespa, of the dual nature of the compound eye in the fact that the ventral part of the eye is separated by a furrow from the dorsal part, which is flatter, and contains ommatidia much less developed than those in the ventral part. Still other evidence of this nature is to be found in the stalked eyes of *Euphausia*, which I have found to be divided into two distinct parts by a thick layer of black pigment. In one part the corneal facets and ommatidia are much larger than those in the remaining part, while there are several striking differences in the structure of the ommatidia.

A consideration of these facts has led me to the supposition that *the dorsal and ventral eyes of Phronima and Gyrinnus, and those of the males of Bibio and Cloë as well as the dorsal and ventral parts of the eyes in Libellulidæ and Euphausia are homologous with the dorsal and ventral halves of the larval compound eyes of Vespa. The parts of the compound eyes of Vespa, and in all probability of most other Insects, are in turn homologous with the posterior upper ocelli of Acilius and their dorsal extensions. In such cases as those seen in the larvæ of Corethra and Phryganids, the ocellus has already become a compound eye, while its dorsal extension does not attain that perfection until the imago stage has been reached.*

In Coleopterous larvæ with six pairs of ocelli I have never seen more than a single pair with dorsal extensions. Although the position of these double ocelli appears to vary somewhat in different genera, I see no reason to doubt that they are, in all these cases, modifications of the same pair of ocelli.

In my paper on the "Eyes of Molluscs and Arthropods," I stated that the arrangement of the retinal cells in the ocelli of *Spiders* closely resembles the arrangement of cells in the ommateum of the compound eye. Since then I have examined the ocelli of *Phalangium*, and find the resemblance even closer than I anticipated.

The *ocelli* of *Phalangium* consist of three layers of cells, a thick, sublenticular one, the corneagen, beneath which is a delicate, cuticular-like membrane, overlying a very thin, middle layer of

cells, the *outer wall of the optic vesicle*. Beneath the centre of the lens this layer is seldom visible; it is thickest on the periphery of the ommateum, where it contains a single layer of oval and faintly-stained nuclei. It was only on very successful sections that this layer could be observed at all.

The inner layer, or ommateum, consists of well-developed ommatidia, each one composed of at least nine cells. The centre of each ommatidium is occupied by *three retinophoræ*, each one bearing a flattened rod, broad at its outer extremity, and running to a point at the opposite end. The axial faces of the three rods unite to form a cone-shaped body, with the base directed outwards, and giving either a T-shaped or triradiate figure in cross-sections. The ends of the three arms of the figure are indented, while in the centre of the figure is a triradiate mark, which indicates the boundaries of the rods. The nuclei of the retinophoræ are probably situated over the outer end of the rods, although this is a point which I have not determined with certainty.

The retinophoræ are surrounded by six pigmented cells arranged in two circles. The outer circle is formed by slender cells, one in each angle of the trifluted cone. Their hardly distinguishable nuclei are situated about opposite the middle of the cone, while their inner ends terminate in long hyaline *bacilli*, similar to those of the outer pigmented cells in *Pinaeus*. The cells of the inner circle are much larger, and contain large oval nuclei, situated below the inner ends of the rods. At first sight they appear to be the only nuclei present in the ommateum. The thickened edges of the outer ends of these deeply pigmented cells are folded over the flutings of the cone. In isolating these cells, the median part of their outer ends is so thin that it often ruptures. Such cells then appear to be forked. In cross-sections there appear, at first sight, to be nine pigmented bands, surrounding the outer end of the cone. There are, however, only six, since the two bands, one on either side of the fluting, belong to a single cell.

The three rods formed by the retinophoræ terminate inwardly in three slender fibres, very much like the slender inward prolongation often formed by crystalline-cones in the compound eye. This fibrous prolongation of the rods is enclosed in a delicate tube, formed by the coalescence of the inner ends of the

retinophoræ. This *tube*, or *style*, for it is formed in exactly the same way as the style in the compound eye, is continuous at its inner end, with an axial nerve-fibre which passes through the centre of the tube into the centre of the cone. It then divides into several longitudinal branches, passing between the axial faces of the rods. On the surface of the cone, in each angle, are two longitudinal nerve-fibres. Each one of the rods has very fine cross-striations, undoubtedly caused by the presence of extraordinarily fine nerve-fibrillæ uniting the axial nerves with the superficial ones, thus forming a system of cross-nerve fibrillæ, or a *retinidium*, similar to that described by me in *Pecten*.

MILWAUKEE, April 11.

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EXPLANATION OF PLATE.

a. Raphe of indifferent cells in the ommateum.
ab. Dorsal extension of retina.
b. Ganglionic(?) cells in the retina.
br. Brain.
br.sh. Brain-sheath.
c.f. Layer of cross fibres beneath the retinal ganglion.
c.hy. Corneagen.
d.f. Dorsal fold of comp. eye.
E. Optic thickening.
ft.c. Fat cells.
g.c.^{1} Large unipolar ganglionic cells at inner edge of optic ganglion.
g.c.br. Ganglionic cells of the brain.
hy. Hypodermis.
i.ch. Inner chiasma.
i.md. Inner medulla.
inf.g.l. Inferior ganglionic cells.
n. Nerve of the dorsal extension.
n.b.l. Nerve-bundle layer.
n.f.^{1} and *n.f.*^{2} Decussating nerve-fibres.
o.ch. Outer chiasma.
o.md. Outer medulla.
op.g.^{1} Outer wall of ganglionic fold.
op.g.^{2} Middle wall of ganglionic fold.
op.g.^{3} Inner wall of ganglionic fold.
op.n. Optic nerve of Vespa.
op.n.^{1} Optic nerve of Acilius.
rd. Rods.
rd.^{1} Large median rods.
rd.^{11} Rods of the dorsal extension.
rt.f. Retinophoræ.
rt.g. Retinal ganglion.
rt.g.^{1} Cortical layer of retinal ganglion.
rt.g.^{2} Nerve-spindle layer of retinal ganglion.
v.b. Outer wall of optic vesicle.
v.f. Ventral fold of compound eye.
x. Connection of optic nerve with the middle wall of ganglionic fold.
xy. Nuclei imbedded in pigment.
y. Edge of the sheet of ectoderm that is growing over the brain.
z. Dorsal edge of corneagen, when it is continuous with the middle layer, *d.f*.

Figs. 1 to 6 have been selected from a number of drawings in my possession, representing the development of the compound eye and optic ganglion of *Vespa*. They have been chosen and reproduced as simply as possible, in order to illustrate the salient features in the development of the parts under consideration.

Figs. 1 to 6 represent cross-sections of the heads of embryonic and larval Wasps.

Fig. 1. Section of the cephalic lobes of an embryo, whose appendages have just appeared. At *y* is the sheet of ectoderm advancing towards the optic thickening *E*, with the edge of which it finally unites.

Fig. 2. Section of a later stage in which the ectodermic layer (*y*) has covered in all the brain except that part which develops into the optic ganglion (*op.g.*$^{1\,2\,3}$). The neurilemma (*br.sh*.) is forming from a layer of cells split off from the brain.

Fig. 3. Section of an embryo just ready to hatch. The sheet of ectoderm (*y*, of Figs. 1 and 2) has united with the edge of the optic thickening *E*.

Fig. 4. Section from a 3 mm. larva, showing the first stage of the invagination in the optic thickening (*E*). The brain-sheath (*br.sh*.) completely covers the optic ganglion and extends along the optic nerve to the eye.

Fig. 5. Section of a 5 mm. larva. There is an ectodermic fold only over the ventral edge of the invagination. The middle wall of the ganglionic fold (*op.g.*^{2}) has greatly increased in size.

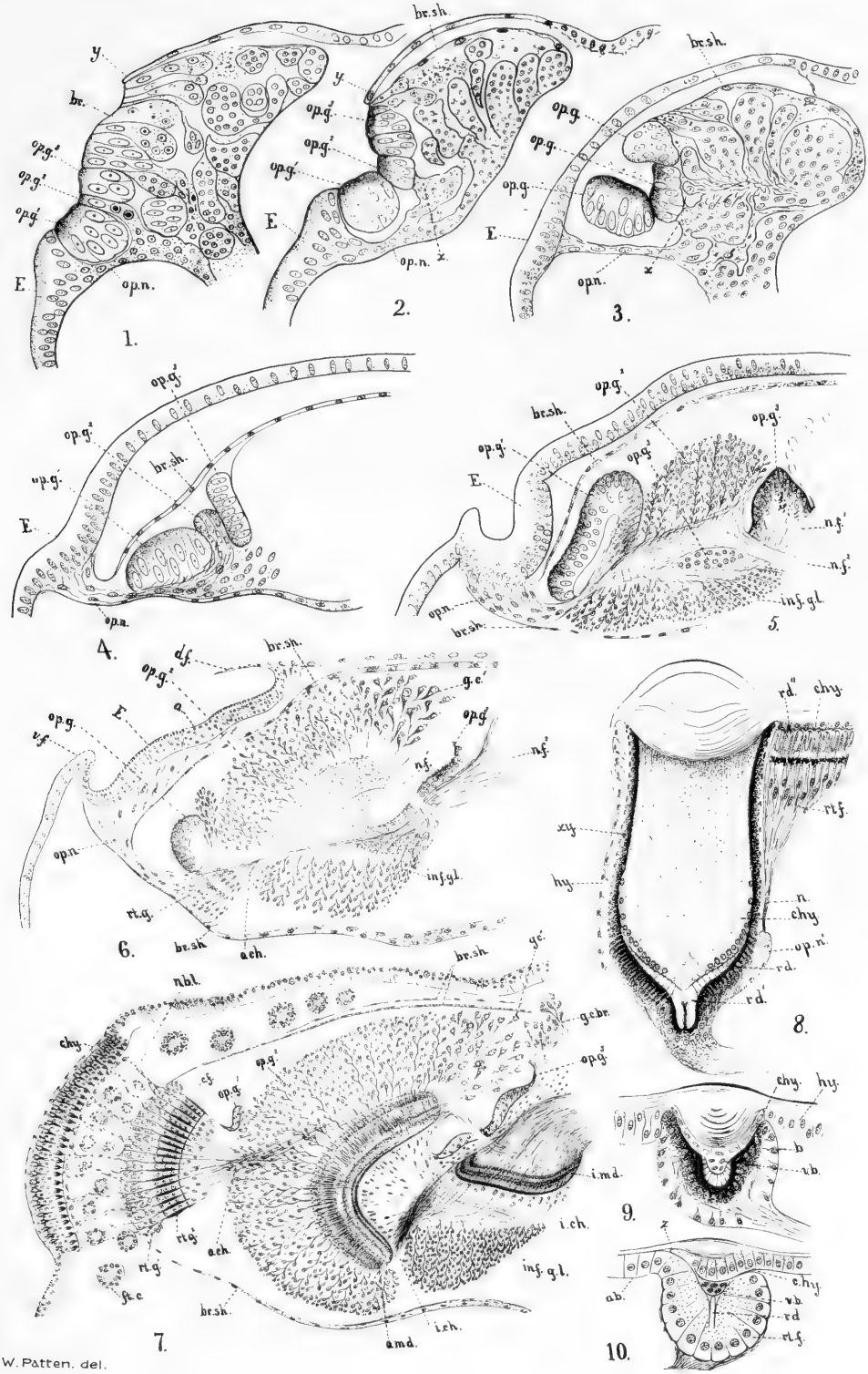
FIG. 6. Section of a 10 mm. larva. The middle wall of the ganglionic fold (*op.g*^{2}.) now resembles the optic ganglion of the adult. The floor of the optic invagination has developed into the ommateum, divided into a dorsal and ventral half by a raphe of irregularly arranged nuclei (*a*). The ommateum is bounded by a dorsal and ventral fold of the ectoderm (*d.f.* and *v.f.*), which, in the succeeding stage, unite with each other to form a double layer of cells over the ommateum. The retinal ganglion (*rt.g.*) appears in this stage as a cone of straight nerve-fibres interspersed with nuclei.

FIG. 7. Section through the head of a young pupa. The optic ganglion (*op.g*^{2}.) has nearly completed its development. The outer wall of the ganglionic fold (*op.g*^{1}.) has almost disappeared, while the inner wall is breaking up into unipolar ganglionic cells continuous with those forming the cortical layer of the brain. The mass of nerve-fibres (*n.f.*^{2}, of Fig. 6) has developed into the inner medulla, *i.md.*

FIG. 8. Cross-section through the head of *Acilius*, showing the posterior dorsal ocellus with its dorsal extension. It is important to notice the extension of the nuclei of the corneal hypodermis *over* the peripheral parts of the retina.

FIG. 9. Section through the eye of a young *Hydrophilus* larva, showing the corneal hypodermis (*c.hy.*), and the cluster of nuclei (*v.b.*) which probably belong to the outer wall of the optic vesicle.

FIG. 10. Section, semi-diagrammatic, through the ocellus of a just-hatched *Hydrophilus* larva. The ocellus contains but a single row of rods, and is still open at *z*, although practically closed. The hypodermis at *ab.*, on the dorsal side of the ocellus, thickens, while it is still continuous with the retina, and gives rise to the "dorsal extension" which becomes double-layered by the subsequent growth of the edge of the corneagen over it.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OÖKINESIS.

C. O. WHITMAN.

THE period of maturation and fecundation of the egg is pre-eminently one of kinetic phenomena. We have here two complex series of events, which together form the prelude to development. Though overlapping, and blending at the point where development begins, they must, nevertheless, be regarded as distinct, inasmuch as maturation, at least in cases of parthenogenesis, may be completed quite independently of fecundation.

The phenomena of maturation embrace the closing chapter in the history of the germinal vesicle, and such concomitant changes in the vitelline protoplasm as prepare the egg for the reception of the spermatic element. The phenomena of fecundation[1] embrace the history of the pronuclei, and those attendant changes in the protoplasm which form the concluding steps in the premorphological organization of the egg. The first series culminates in the production of polar globules; the second, in the formation of the cleavage-nucleus by the union of the pronuclei. In each series we recognize two factors; namely, *nuclei* and *cell-protoplasm*.

To what extent these factors act independently, and how far

[1] The distinction proposed by E. Van Beneden (*Arch. de Biol.*, IV., p. 283) between the *copulation* of the sexual cells and *fecundation* is here adopted.

they influence or react upon each other, are questions still awaiting decisive answers. The natural boundary line between the two, at first clearly defined, begins to fade almost simultaneously with the earliest appearance of kinetic changes, becomes rapidly effaced as the energy of display increases, and is resumed only at regularly recurring epochs, when outward manifestations of activity cease. Under such conditions, with karyoplasm passing uninterruptedly into cytoplasm, it is certainly very difficult, and perhaps quite impossible with the micrographical means at our command, to determine precisely the part played by each.

The majority of writers are inclined to seek the *primum mobile* in the nucleus, and to make the nucleus responsible for the kinetic phenomena displayed in the cytoplasm. Attempts have been made to settle this question experimentally, through the artificial division of infusoria; but thus far no one has undertaken a critical analysis of the phenomena of maturation and impregnation, with a view to finding test cases. It is this side of the subject which I propose to consider in the present paper.

It may be noticed, first of all, that the phenomena in question are not all of an active nature. Some are plainly induced by outside influences, or are simply secondary effects resulting from altered internal conditions. The rapid clearing up of a pelagic fish egg the moment it comes in contact with water, owing to the dissolving of its opaque granules, is an example of this kind. All changes in the relative position of the constituent elements of the egg that result from differences of specific gravity may be referred to the same category. We have, then, to distinguish between active and passive changes, and the latter can be set aside as unimportant to the inquiry. The former may be conveniently divided into two classes, one of which I shall designate *cytokinetic*, in distinction from the other, which is now generally called, after Schleicher's example, *karyokinetic*.[1]

[1] The term *karyokinesis* has been objected to by Flemming (Zellsubstanze, Kern und Zelltheilung, p. 376) as neither describing the form nor indicating the nature of nuclear metamorphoses. *Karyomitosis*, or simply *mitosis*, is the substitute proposed by Flemming. Priority and general usage are in favor of karyokinesis; besides, this term commends itself, in my opinion, as the simpler and more comprehensive, and as expressing better the essence of the phenomena. Its leading idea is *motion*, but motion viewed as an exponent of forces residing in, or acting upon, the nucleus. It

The whole series of movements and form-changes, progressive and regressive, through which the nucleus passes in the process of division, together with all the kinetic changes displayed in the germinal vesicle and pronuclei, are karyokinetic phenomena.

The phenomena which may be regarded as oökinetic, or cytokinetic, display themselves in the vitelline protoplasm and in the cytoplasm of cells in general. *They are diversiform in the extreme, rarely presenting regular form-series, and thus stand in marked contrast with nuclear metamorphoses, which everywhere, both in plant and animal cells, exhibit a most remarkable uniformity.* This irregularity makes it quite impossible, in the present state of our knowledge, to formulate, or express in general terms, the phenomena embraced under this head.

I. Movements of the Germinal Vesicle and Pronuclei.

The unique character of many of these cytokinetic displays appears to me incompatible with the idea that they are the direct effect of nuclear influence. Any changes in the protoplasm, induced and sustained at the expense of changes taking place in the nucleus, should be as regular and uniform as the karyokinetic processes themselves. On any hypothesis that refuses to admit that the cytoplasm is endowed with subtle powers of its own, and capable of automatic as well as responsive action, how can we account for the characteristic difference between telolecithal and centrolecithal eggs? By what power are the passive yolk-elements restrained from taking the position which they would assume under the influence of gravitation alone? What force drives the germinal vesicle from the centre

regards the nucleus as a *seat of energy, which displays itself in phenomena of motion.*

Mitosis is at best only a synecdochial expression, in which a part is put for the whole. Allowing that the form-changes of the chromatic loops can be thus characterized, it is evident that the movements of the achromatic elements are entirely ignored. But, even in this limited sense, the word is not free from objection. Flemming defines it as "thread-metamorphoses;" but Carnoy (*La Cellule*, III., p. 319) points out that, etymologically interpreted, it signifies "reduction to thread." Now, it is during kinesis, or mitosis, as Carnoy justly remarks, that the chromatin of the nucleus loses its thread-like form, breaking up into loops or rods, and resuming its filoid aspect only after the division is completed.

to the periphery of the egg during the period of maturation? As this centrifugal movement may be upward, downward, or to one side, even in the same class of eggs (*e.g.*, Teleostei), it cannot be said to be controlled by gravitation, nor can it be purely automatic.

How are we to explain that remarkable centripetal movement of the pronuclei which always forms the concluding step in the arrangements preparatory to development? As is well known, these bodies are invariably formed at or near the surface of the egg, sometimes near the same pole, and at other times at opposite poles. Any attraction assumed to exist between them would only be competent to account for their coming together, but would afford no explanation of their centripetal movement. The direct influence of gravitation can no more account for this than for the centrifugal movement of the germinal vesicle. We are driven to the conclusion that the phenomenon is due to the *interaction of nuclear and cytoplasmic forces*. It is not admissible to assume that either factor is passive, but rather that each acts and reacts upon the other. It is by virtue of this subtle interaction (Wechselwirkung) that the pronuclei ultimately assume a position of equilibrium with respect to the active constituents of the oöplasm.

But will the attraction which we must assume to exist between the oöplasm and the pronuclei account for all that we know about the behavior of the latter? Or are we under the necessity of assuming still another attraction acting between the pronuclei themselves? The majority of writers would answer unhesitatingly the first question in the negative, and the second in the affirmative. While I fully concur in this opinion, it seems to me that the reasons generally assigned for it require examination.

The formation of asters in connection with the pronuclei, and the fact that these bodies approach each other and eventually unite at the centre of the egg, or at some point which, though not the geometric centre, yet represents the virtual centre of their sphere of action, are commonly regarded as proof that they attract each other. If we analyze these facts, we shall find that they afford very little evidence in support of this view.

The astral displays demonstrate action of some unknown kind between the oöplasm and the pronuclei, but they give no posi-

tive indication of any action whatever between the two pronuclei. Now this action, which we may call *centripetal attraction* for want of a more definite term, is, as before stated, all that is required to account for the centripetal movement of the pronuclei. But would this movement result in bringing the pronuclei together? Evidently it would, if the attraction was the same for each pronucleus.

Wherein, then, lies the evidence of attraction between the pronuclei? In order to elucidate this question let us consider how such a force would manifest itself in the movements of these bodies. We should have two forces, one tending to bring the pronuclei together in a direction marked by the chord joining them, the other tending to draw them along their respective radii to the centre of the egg. The place of meeting and the nature of the lines described by the pronuclei will depend on a variety of circumstances. Prominent among these are the *place* and *time* of origin. The probability that neither of these forces acts uniformly throughout, and the possibility that the attraction between the pronuclei may act only within shorter distances than those by which these bodies are often separated, will have to be taken into account.

To begin with, let us suppose that the pronuclei are contemporaneous in origin and located at opposite poles. A typical illustration of such conditions is furnished in the nematode egg. The component forces would here act in the same direction and drive the pronuclei straight to the point of meeting (*c*). The possibility of meeting at the centre would depend upon the obvious conditions, — (1) that they start at the same moment and move with equal velocity, or (2) that any difference in one of these respects is neutralized by a counter difference in the other.

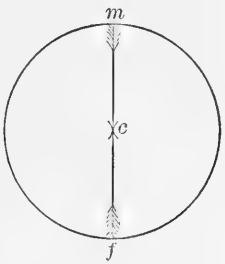

Fig. 1.

If the starting-points (*m* and *f*, Fig. 2) were near the same pole, the other conditions remaining the same, the place of meeting (*x*) would be eccentric, and the nature of the paths described would, of course, depend on the relation sustained between the component forces. If this relation is uniform, the pronuclei will move in *straight* lines to the

point of meeting (x'), and then along the radius in which this point lies to the centre (c). If, on the other hand, it varies, it may do so in many ways, three of which may be noticed here.

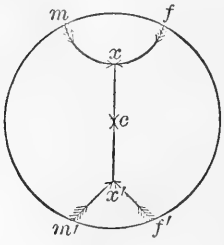

Fig. 2.

Allowing, what seems most probable, that the centripetal attraction steadily diminishes, the zero-point being reached at the centre, we may assume (1) that the nuclear attraction remains constant; or (2) that it increases as the distance between the pronuclei diminishes; or (3) that, acting only at relatively short distances, it does not come into play until the centripetal migration is partially completed. In the first two cases the paths of the pronuclei would be represented by curves, with the concave sides towards the pole, with the difference only that the curves would be stronger in the second case than in the first. In the third case the paths would continue straight until nuclear attraction began to act, and then curve towards the pole until the point of junction was reached. In all three cases the course after meeting would be centripetal along the radius in which the point x (Fig. 2) lies. Essentially the same thing would happen in a telolecithal egg, except that the point c, representing the centre of equilibrium rather than the geometric centre, would be nearer the active pole.

In the movements thus far considered there are only two peculiarities, the occurrence of which could be regarded as conclusive evidence of nuclear attraction; namely, the *curved* paths of the pronuclei (Fig. 2), and their meeting *before* reaching the centre. The first of these peculiarities is remarkably well shown in the amphibian egg (1, 2, 3, 4), where the path of the male pronucleus is plainly marked by a streak of pigment concave towards the dark pole; and the second has been repeatedly

1. OSCAR HERTWIG. Beiträge zur Kenntniss der Bildung, Befruchtung und Theilung des thierischen Eies. *Morph. Jahrb.*, III., 1877.

2. CHARLES VAN BAMBEKE. Recherches sur l'Embryologie des Batraciens. *Bull. de l'Acad. roy. de Belgique*, LXI., 1876.

3. Id. — Sur les trous vitellins que présentent les œufs fécondés des Amphibiens. *Bull. de l'Acad.*, etc., 2e sér., t. XXX., 1870.

4. WILHELM ROUX. Beiträge zur Entwickelungsmechanik des Embryo. *Arch. f. mik. Anat.*, XIX., II. 2, Pl. x., 1887.

observed by Hertwig (5), Fol (6), Selenka (p. 87), Mark (8), and others.

For the sake of simplicity, we have proceeded thus far on the assumption that the pronuclei are contemporaneous in origin, and that they begin to migrate simultaneously. But the conditions will agree more nearly with those generally occurring in nature, if we represent them as varying considerably both in the time of origin and the time of starting. The slight advantage in respect to starting-point, which the female pronucleus has over the male pronucleus, and the velocity of movement, can be left out of account in considering the influences that affect the course and direction of migration.

The time of starting is the chief source of the variations which we have now to consider. The difference in this respect is carried to the extreme in those cases where one of the pronuclei reaches its destination before the other is ready to begin its march. In such cases the earlier pronucleus, starting from any point (as f or f') in the periphery, would be carried, by centripetal attraction alone, in a straight line to the centre (c). From this point it would then advance along another radius to meet the later pronucleus at some point (as x); and, after meeting, the united pronuclei would move back to c. The centrifugal movement of the early pronucleus from c to x would be slower than the centripetal movement of the later pronucleus from m to x, since the former movement would represent the *difference*, and the latter the *sum* of the same two forces. Precisely similar cases of centrifugal movement have been observed, and they furnish another decisive proof of nuclear attraction.

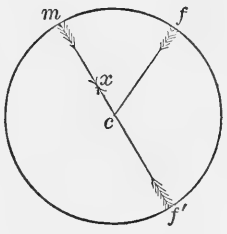

Fig. 3.

A capital illustration is furnished in the egg of *Toxopneustes lividus*. After saying that the male pronucleus leaves the

5. Oscar Hertwig. Beiträge z. Kenntniss d. Bildung, Befruchtung u. Theilung d. thier. Eies. *Morphol. Jahrb.*, I., 1876, p. 381.

6. Hermann Fol. Recherches sur la Fécondation et le Commencement de l'Hénogénie, 1879, p. 259.

7. Emil Selenka. Befruchtung des Eies von Toxopneustes variegatus. Leipzig, 1878.

8. Edward L. Mark. Maturation, Fecundation, and Segmentation of Limax campestris. *Bull. Mus. Comp. Zool*, VI., 1881, p. 222.

periphery of the egg with "*wahrnehmbarer Geschwindigkeit*," travelling in the direction of the female pronucleus ("Eikern"), Hertwig (5, p. 380) remarks as follows: "Während dieser so beachtenswerthe Vorgang sich abspielt, verharrt der Eikern nicht in Unthätigkeit; vielmehr setzt sich derselbe gleichfalls in Bewegung, sobald als die Radienfigure [male pronucleus] von der Oberfläche sich entfernt, und rückt näher nach der Eimitte zu. Doch ist seine Bewegung *langsam* und kann leicht übersehen werden, wenn man nicht ein Object gewählt hat, in welchem der Eikern recht peripher gelagert ist. Dass aber eine Bewegung stattfindet, davon habe ich mich ganz sicher überzeugt, indem ich die Lageveränderung des Kerns mit dem Mikrometer controlirte.

"Das resultat dieser Vorgänge ist, dass beide Körper endlich sich treffen entweder in der Eimitte oder wenigstens in der Nähe derselben. *In letzterem Falle verändern dann dieselben noch nachträglich zusammen allmälig ihre Lage, bis sie das Eicentrum einnehmen.*"

With reference to the same point, Fol (6, p. 259) says: "L'attraction se manifeste bien plus vivement sur le pronucléus mâle que sur l'autre noyau, puisque ce dernier ne commence à se mouvoir et à se déformer que lorsque le noyau mâle arrive presque à le toucher."

According to both Hertwig and Fol it is the *male* pronucleus that moves with the greater velocity. But if the explanation I have offered is correct, it depends upon the *time of formation*, which pronucleus will make the longer journey, and which will move the more rapidly. If the male pronucleus reaches the centre of the egg before the female pronucleus is formed, the former will travel the longer distance, but the latter, when it begins to move, will advance with the greater velocity.

A full confirmation of this view is furnished by the observations of Van Beneden (9) on *Ascaris megalocephala;* and it is all the more satisfactory as this investigator evidently had not directed his attention to the points under discussion. First of all we have the important fact (confirmed by Zacharias, 10) that

9. ED. VAN BENEDEN. Recherches sur la maturation de l'œuf et la fécondation. *Arch. de Biol.*, IV., 1883.

10. OTTO ZACHARIAS. Neue Untersuchungen, etc. *Arch. f. mik. Anat.*, XXX., H. 1, p. 149, 1887.

the male pronucleus reaches the centre of the egg before the female pronucleus is formed, as shown in the following statement: "Le spermatozöide, modifié comme je l'ai exposé plus haut, occupe pendant toute la période qui se termine par l'expulsion du second globule polaire et la libération de la seconde couche périvitelline *le centre géométrique* de l'œuf" (p. 489). Morever, Van Beneden states that, in order to meet the male pronucleus, the female pronucleus traverses the greater distance, and hence, by inference, moves the more rapidly: "Pour se rejoindre à son congénère le pronucléus femelle, qui prend toujours naissance au voisinage du pôle supérieur de l'œuf, parcourt un chemin beaucoup plus long que le pronucléus mâle; celui-ci se déplace relativement peu" (p. 524).

In saying that the female pronucleus makes a longer journey than the male pronucleus, the author evidently wholly ignores the distance previously traversed by the latter in reaching its central position.[1]

We find, then, three facts, which can be said to furnish indisputable evidence of attraction between the pronuclei. These are: —

1. The *curved* path of the male pronucleus in the amphibian egg.

2. The *meeting* of the pronuclei *before* reaching the centre of equilibrium.

3. The *centrifugal* movement of the *earlier* pronucleus to meet the later formed pronucleus.

That this attraction acts only at comparatively short distances, and not at all distances which may separate the places of origin of the pronuclei, is demonstrated by the following very interesting observation of Fol's (6, p. 106): "A mesure que l'aster mâle s'avance dans le vitellus, ses rayons deviennent toujours plus longs et plus accentués; sa liaison avec son point d'origine à la surface du vitellus se perd. *Sa direction, d'abord*

[1] It will probably occur to any one tolerably familiar with Van Beneden's paper, that he holds that the pronuclei are contemporaneous in origin, while the validity of my explanation rests upon a contrary assumption. The contradiction is, however, only apparent, not real; for it is one of words rather than facts. The difference is accounted for by the fact that Van Beneden maintains that the spermatozoon undergoes a sort of maturation after penetrating the ovum, and that the changes through which it becomes a proper pronucleus take place only *after* it has reached the centre, simultaneously with the formation of the female pronucleus.

centripète, change, lorsque le pronucléus femelle n'occupe pas le centre de l'œuf, pour se rapprocher de ce dernier noyau. Enfin le pronucléus femelle, jusqu'alors immobile se met en mouvement au moment où il est atteint par les rayons de l'aster mâle, et la réunion des deux noyaux s'opère promptement."

The same fact is brought out most clearly by the researches of Wilhelm Roux (4) on the frog's ovum. Roux's observations are all the more conclusive, as he has made a special study of the movements of the pronuclei, with a view to determining the precise axial relations of the paths described. The spermatic body takes at first a centripetal direction, penetrating to a depth of .29–.35 *mm.*, and thus describing the "*penetration-path.*" By a more or less abrupt curve the path then becomes directed towards the female pronucleus, — becomes nucleopetal, — and is thenceforth called the "*copulation-path.*" The angle formed by the first and second parts of the course varies from 90° to 180°, according to the distance of the point of penetration from the pole. The first part of the course is accomplished through a simple "movement of penetration;" the second, under the influence of nuclear attraction. Roux does not undertake to assign any reason for the change from a centripetal to a nucleopetal direction, but alludes to the possibility that the nucleopetal course sets in when the transformation of the spermatozoon into the male pronucleus becomes complete.

Centripetal attraction, as we have seen, may, under certain conditions, act entirely alone upon one or both pronuclei; but the attraction between the pronuclei must act, under all normal conditions at least, concomitantly with centripetal attraction. I am aware that the language often used in describing the movements of the pronuclei is not in full accord with the last statement. Both Hertwig and Fol seem to be very strongly impressed with the idea that the male pronucleus must, under all circumstances, move more rapidly than the female pronucleus; and both declare that the path taken by the pronuclei, as they advance to meet each other, is represented by a single straight line, instead of two straight or two curved lines meeting at an angle, as here maintained. In one of his latest papers Hertwig (11, p. 281) expresses himself as follows: "Wie nun

11. OSCAR HERTWIG. Das Problem der Befruchtung und der Isotropie des Eies. *Jenaische Zeitschrift*, XVIII., 1885.

früher der Samenfaden das Ei aufgesucht hat, so wandert jetzt der Spermakern *in gerader Richtung dem Eikern entgegen*, welcher sich gleichfalls, wenn auch *viel langsamer*, in Bewegung setzt." Fol (6, p. 259) puts it thus: "Ainsi lorsque le noyau femelle se trouve dans une position excentrique et que le noyau mâle prend naissance près de ce dernier, il marche directement à sa rencontre *suivant une corde de cercle* au lieu de se rendre d'abord au centre du vitellus."

These descriptions would be strictly accurate if our two forces acted consecutively and not concomitantly; *i.e.*, if nuclear attraction acted alone before the meeting of the pronuclei, and centripetal attraction alone after this event. It is possible — and this observation of Fol makes it highly probable — that the attraction between the pronuclei is much stronger than the centripetal attraction. We know of no fact that forbids this supposition, and, if this point be conceded, the difficulty of reconciling the observation with our views practically disappears.

That what we have called centripetal attraction is a reciprocal action between oöplasm and pronuclei is a conclusion supported by still another interesting fact. In the centrifugal march of the germinal vesicle and in the maintenance of a peripheral position by the archiamphiaster during the production of the polar globules, there is satisfactory evidence, as Fol has already pointed out, of a *repellent action*. In those cases where the spermatozoon penetrates the ovum before the elimination of polar globules (Teleostei, Nematoidea, Hirudinea, etc.), we have attraction and repulsion exhibited at one and the same time, and the oöplasm is the *common factor* in both actions. It is thus made evident, first, that the body attracted and the body repelled cannot be identical in molecular constitution; and second, that the two modes of action are due to the unlike physico-chemical relations which these bodies respectively sustain towards the oöplasm.

Allowing that both the attraction and the repulsion represent reciprocal action, we are brought face to face with the question of the relative importance of the two factors engaged in each case. Do the points just noticed throw any light on this question? I believe they furnish at least one very important evidence in support of the opinion that the nucleus takes the initiative in action.

Up to a certain time we see the germinal vesicle held in place by centripetal attraction; then, owing to unknown changes either in itself or in the oöplasm, or in both, repellent action sets in, and it begins to move centrifugally. Now, if it can be shown that the conditions of centripetal attraction remain unchanged, so far as the oöplasm is concerned, the changes which induce repellent action must evidently be located in the germinal vesicle. That no changes take place in the oöplasm which can interrupt the action of centripetal attraction is shown by the deportment of the male pronucleus at the very time when the expulsion of polar globules is in progress, and by the fact that this attraction acts alike[1] on both pronuclei, irrespective of the time, place, or order of their origin. All the essential conditions of centripetal attraction in the oöplasm may then be said to be unaffected by the processes of maturation and fecundation. The primary cause of centrifugal movement must, therefore, lie in the germinal vesicle itself. To ascertain the nature of the causal changes is a task of the future.

Various causes have been assigned for the centrifugal movement of the germinal vesicle. In the meroblastic eggs of vertebrates this movement is connected with the formation of the *latebra* (Purkinje); and in the holoblastic eggs of the Amphibia, with the origin of the "*figure claviforme*" (Bambeke). The fact that this movement in the hen's egg follows so closely upon the appearance of the white yolk spherules, might raise suspicion of a passive displacement, the germinal vesicle being pushed upward by the formation of the yolk elements beneath it. This view, advanced by Van Beneden (12, p. 206), is com-

[1] It cannot perhaps be said that in all cases this force acts with *equal intensity* on both pronuclei. In *Toxopneustes variegatus*, for instance, Selenka ("Befruchtung des Eies, etc.," Leipzig, 1878) states that the "Eikern" (female pronucleus) always takes an eccentric position (p. 4), and there remains until after the formation of the male pronucleus. The male pronucleus, on the contrary, makes no delay in its centripetal movement, and, having gained the centre, awaits there the approach of the female pronucleus, which does not appear to move until reached by the astral rays of the former (pp. 7–8). It would appear from Selenka's description that the Eikern is drawn from its eccentric position by nuclear attraction alone. In exceptional cases the spermatozoon penetrates the egg in the immediate vicinity of the Eikern, and then the two pronuclei unite and move slowly towards the centre (p. 8). Selenka makes no mention of a centrifugal movement on the part of the male pronucleus.

12. ED. VAN BENEDEN. Recherches sur la composition et la signification de l'œuf. *Mem. cour. d. l'Acad. roy. des Sciences de Belg.*, XXXIV., 1870.

pletely disproved by the mode of origin of the latebra (13, p. 67). Oellacher's (14, pp. 18, 24) theory of the expulsion of the germinal vesicle by contractions of the vitellus has been disposed of by more recent observations.

The idea of passive displacement has recently been amplified to a general theory by Ryder (15, pp. 95–101). When we reflect that this movement is common to all types of eggs, that in the majority of cases it takes place after the egg has attained its full size, and after the formation of the food-yolk is completed, we find it difficult to accept this mode of explanation, and quite impossible to concede that the "*law of displacement*" is to be found in this direction. The holoblastic eggs mentioned by Ryder (p. 101), not to mention numerous others, furnish evidence quite fatal to his theory. Take the single example of the frog's egg, where the germinal vesicle maintains a nearly central position until a short time before deposit, taking up its centrifugal march after the food-yolk is all present. If the germinal vesicle is simply crowded to the surface by food-yolk, how can the penetration of the yolk by the pronuclei be accounted for? Equally fatal to the theory is the persistence of the latebra and the cord of protoplasm connecting it with the cicatricula.

II. Receptivity of the Ovum for Spermatozoa.

The idea seems to have been widely accepted that the accessibility of the ovum to the spermatozoa is regulated by external, mechanical means, rather than by internal, physiological conditions. It is well known that the period of fecundation is, generally speaking, relatively a short one; but the distinction between *receptivity* and *accessibility* is very generally ignored.

If centripetal attraction is persistent, as maintained in the foregoing chapter, why should the ovum enjoy only a transitory receptivity? Can the duration of this period be brought into

13. Wilh. Waldeyer. Eierstock und Ei. Leipzig, 1870.

14. J. Oellacher. Beiträge zur Geschichte des Keimbläschens im Wirbelthiereie. *Arch. f. mik. Anat.*, VIII., 1872.

15. John A. Ryder. "Embryography of Osseous Fishes." *Annual Report of the Commissioner of Fish and Fisheries for 1882.*

definite relation with any of the internal changes before considered? If the view presented in the preceding pages be tenable, the period of receptivity may be said to date, not from the expulsion of the polar globules, but *from the moment the conditions of centripetal attraction are reversed in the germinal vesicle.* A period of non-saturation begins with the centrifugal movement of the germinal vesicle, and terminates with the penetration of the spermatic body. As soon as all the elements of saturation are present, external manifestations of centripetal attraction cease, and there remains only the work of internal equilibration, which ends with the centripetal march of the pronuclei.

From this standpoint, it is idle to talk about mechanical contrivances for preventing the admission of supernumerary spermatozoa, as if the receptivity of the ovum were not self-regulating. The idea that the micropyle may be closed against spermatozoa by a polar globule, as held by Hoffmann (16, p. 68), is inadmissible, as will be shown by observations soon to be published. Allowing Hoffmann's observation to be correct, what grounds have we for supposing that spermatozoa could not pass directly through a polar globule lodged in the micropyle? Has not the penetration of polar globules by spermatozoa been repeatedly observed? And is it at all probable that a polar globule would prove more renitent within the micropyle than elsewhere?

Equally untenable is the suggestion of Calberla (17, p. 458), that the tail of the spermatozoon is left in the micropylar canal for the purpose of blocking the way to other spermatozoa.[1]

In the Echinoderm egg, according to Fol (6, p. 94), it is the rapid formation of an impenetrable vitelline membrane, at the moment when the spermatozoon comes in contact with the vitellus, which makes it impossible for other spermatozoa to enter. There is no evidence that superfetation would follow if such a membrane were not formed; hence its formation would not necessarily be connected with any such function as Fol has ascribed to it.

16. C. K. HOFFMANN. Zur Ontogenie der Knochenfische. Amsterdam, 1881.

17. E. CALBERLA. Der Befruchtungsvorgang beim Ei von Petromyzon Planeri. *Zeitschr. f. wiss. Zool.*, XXX., 1877.

[1] Kupffer and Benecke affirm, positively, that the tail is not left in the micropyle.

Besides, Hertwig (18, p. 173) has shown quite conclusively that this membrane is already present *before* any spermatozoon reaches the vitellus, and concludes that it is not a mechanism for limiting the number of spermatozoa admitted. Hertwig remarks (p. 173), that the existence of membraneless eggs is against the idea advanced by Fol, and adds, — "*It seems to me, therefore, that it is the egg-plasma itself which alone, during unimpaired vitality, can prevent the entrance of more than one spermatozoon. At all events, this phenomenon finds its analogue in the copulation of the lowest unicellular plants and animals, where one also sees only two cells uniting in the sexual act.*"

Selenka (19, p. 4) places himself on the side of Fol.

Kupffer and Benecke (20, p. 21) have advanced the idea that the spermatozoon is drawn into the vitellus by an attractive influence emanating from an egg-nucleus. Mutual attraction between the vitellus and this nucleus carries the latter from its place of origin near the formative pole, towards the centre of the egg; and as the attraction between the nucleus and the spermatozoa diminishes as the distance increases, only the foremost spermatozoon may be supposed to keep within its influence, all the rest being left behind. As this view is plainly incompatible with what is now known about the movements of the pronuclei, it requires no further notice.

We are not infrequently told that, when the spermatozoon enters the egg before maturation is complete, it remains unchanged and inactive in the periphery of the egg until the polar globules are ejected. It is evident, however, that observations on this point have not been sufficiently close and searching in many cases, as often happens when observations are made at random, and in ignorance of their theoretical bearings. The time of appearance of the male aster, in some cases, would raise a suspicion that centripetal attraction manifests itself more strongly after the elimination of the polar globules than before; but I have failed to find a single well-ascertained fact in support

18. O. Hertwig. Beiträge z. Kenntniss d. Bildung, Befruchtung u. Theilung d. thier. Eies. Dritter Theil. I. Abschnit. *Morph. Jahrb.*, IV., 1878.

19. Emil Selenka. "Befruchtung des Eies von Toxopneustes variegatus." Leipzig, 1878.

20. Kupffer and Benecke. Der Vorgang der Befruchtung am Eie der Neunaugen. Königsberg, 1878.

of the opinion that, under perfectly normal conditions, the spermatic body remains inactive and stationary at the surface of the egg until the polar globules are excluded.[1]

In the case of the teleostean egg, I have positive proof, not only that the spermatozoon enters the egg *before* any polar globule is formed, but that the pronucleus derived from it attains a central position before the female pronucleus begins to move.

In the Hirudinea, Bütschli (21, p. 5) and Hertwig (1, p. 30) failed to find the male aster before the appearance of the first polar globule, and my earlier efforts were equally unsuccessful. I have since succeeded in finding a distinct male aster in the fresh-laid egg of Clepsine, *i.e.*, from thirty to forty minutes before the first polar globule arises. The aster at this time has already penetrated to a depth of one-quarter or even one-half the radius of the egg, and is soon after found at, or near, the centre. I am unable to say positively whether it advances somewhat from this position to meet the female pronucleus, but the appearances indicate a slight movement of this kind. The phenomena of fecundation in the Hirudinea, so far as at present known, accord with the conclusions already set forth.

With reference to *Ascaris megalocephala*, Oscar Hertwig (11, p. 282) makes the following somewhat surprising remark: "Hier bleiben die grossen Samenkörper, welche die Gestalt einer Spitzkugel haben, *längere Zeit nach ihren Eindringen ganz unverändert in ihrer ursprünglichen Gestalt in der Eirinde liegen.*" The investigations of Van Beneden (9) and Nussbaum (22), cited in support of this statement, unfortunately con-

[1] Fol (No. 24, pp. 106–107) states that in those cases where the egg is fecundated before the formation of the polar globules is completed, the male pronucleus remains at the edge of the vitellus in the condition of a small, immobile, and hardly visible spot until the moment when the elimination of those globules is accomplished. Both pronuclei then arise simultaneously, and each takes its own independent course towards the centre. *The place of meeting, however, is between the centre and the formative pole, because the male pronucleus advances more rapidly.* It is to be remembered that, under normal conditions, the female pronucleus is formed before fecundation; and, further, that the male pronucleus, in the cases referred to, started from, or near, the nutritive pole.

21. O. BÜTSCHLI. Studien über die ersten Entwicklungsvorgänge der Eizelle, etc. Frankfurt a. M., 1876.

22. MORITZ NUSSBAUM. "Ueber die Veränderungen der Geschlechtsproducte bis zur Eifurchung." *Arch. f. mik. Anat.*, 1884.

tradict it in both points. On pages 392–394, Van Beneden formally enumerates the changes exhibited in the protoplasmic substance of the spermatozoon the moment it comes in contact with the egg; and on page 395, he distinctly states that the nucleus undergoes, at the same time, "une modification très apparente." Nussbaum's statements are a little less explicit; but, so far as they go, confirm those of Van Beneden. But it is only after the expulsion of the second polar globule, according to Van Beneden (p. 355), that the spermatozoon undergoes "*those modifications which announce the imminence of fecundation*," and this statement may possibly have misled Hertwig. A citation, previously made (p. 235), from Van Beneden shows that Hertwig was also mistaken in supposing that the spermatozoon remained stationary for a considerable time at the surface of the egg.

Zacharias (10) fully confirms Van Beneden's statements in regard to the centripetal movement of the spermatic body, and gives, besides, a detailed description of the changes which take place in it before the formation of the second polar globule. In unfertilized eggs the first polar globule is formed, but not the second. This fact shows how erroneous is the idea that the spermatozoon remains passive until after the extrusion of the polar globules.

III. The Pole of Impregnation.

The copulation of the sexual cells is attended with very interesting oökinetic phenomena. A remarkable example has been described by Fol (6, pp. 91, 249), in the egg of *Asterias glacialis*. The protoplasm rises up at one point in the form of a cone, which continues to elongate until it meets the spermatozoon on its way through the mucous envelope ("oölemma"). The height of the cone depends on the rapidity with which the spermatozoon advances. If it progresses slowly, the cone may attain a height equal to half the thickness of the oölemma. As soon as contact is established, the cone begins to shorten, but rarely disappears entirely. Its summit, terminating in the remnant of the tail of the spermatozoon, usually remains above the surface of the egg, and soon becomes the point of departure for a new cone, — the "cone of exudation," — which is supposed to arise by expulsion of the vitellus.

The point of chief interest here is the fact that attraction between the oöplasm and the spermatozoon can manifest itself *at a distance*. Difficult as it is to explain the mechanism of such action, the fact itself is so conclusively established that we are compelled to accept it. At first sight, the fact appears to stand entirely alone; but there is something very closely analogous in the attraction between the pronuclei. They certainly attract each other at very considerable distances; but it may be a question whether they act directly on each other, or through the medium of the oöplasm which bridges the distance between them. That the action of the pronuclei upon the oöplasm — whether on the hypothesis of currents or that of polar attraction — cannot account for the behavior of these bodies toward each other is conclusively shown by the fact that *supernumerary male pronuclei do not unite, although they do develop astral radiations*. If the influences which manifest themselves in these astral lines are not competent to account for all the movements of the pronuclei, how can we escape the conclusion that the pronuclei act directly on each other?

The necessity of recognizing two distinct kinds of attraction is thus made very clear. On the one hand, we have the direct action of one nuclear body upon another nuclear body, which we have called *nuclear attraction;* and, on the other, the action of nuclear bodies on the oöplasm, which manifests itself in astral lines, and to which we have given the name *centripetal attraction*. The cone of attraction in Asterias may be regarded as a manifestation of centripetal attraction under exceptional conditions; for, although the cone moves toward the spermatozoon, this fact does not exclude the idea of reciprocal attraction. Although, from the nature of things, we do not expect to see the egg move, as a whole, towards the spermatozoon, there are, at least, very strong grounds for believing that it attracts at the same time that it is attracted, and that its attractive influence is always felt before actual contact takes place.

I am fully aware that most writers hold that the sexual products are brought together, not by attractive influences, but by the impelling action of the tail of the spermatic body. Fol is so strongly impressed with this belief that he examines all other hypotheses before accepting that of attraction at a dis-

tance. We believe that such attraction is exerted, not only by the spermatozoon, but also by the egg, and that the part it plays in bringing together the sexual cells is no less important than that taken by the tail of the spermatozoon. It is highly probable, also, that *this attraction is polar, and that the place of penetration is a predetermined point or region.*

On this question, however, we have conflicting testimony, which has been considered at some length by Van Beneden (9, pp. 371–376). Fol and Hertwig concur in the opinion that the spermatozoon may penetrate at any point; while Selenka (7, p. 6) holds that, *as a rule, it enters a preformed protuberance of the vitellus* ("Dotterhügel"), but adds that in about a dozen cases out of a hundred, it may effect an entrance at any other point. Van Beneden suggests that this "Dotterhügel" of *Toxopneustes variegatus* (Selenka), and of *T. lividus* (Flemming, 23), corresponds to the "bouchon d'imprégnation" in *Ascaris;* and this view appears to be well taken. It is supported by one very important consideration, not mentioned by Van Beneden. I refer to the evidence of a micropyle, or micropylar region, which may be drawn from the observations of Hertwig, Fol, and Selenka. Hertwig (18, p. 173) has demonstrated clearly that the vitelline membrane is present before the copulation of the sexual products, and Fol (6, p. 94) has obtained equally positive evidence of a small opening ("micropyle d'occasion") located in a crater-like inflection of this membrane. Fol did not, however, recognize any such crater before the spermatozoon entered the vitellus, but he did find the "cone of attraction" before this event, and observed that *the cone remained in continuity with the vitellus "à travers la membrane,"* after the penetration of the spermatozoon. Allowing, then, that both the cone and the membrane exist prior to fecundation, the same must be conceded for the micropyle. Only one way of avoiding this conclusion occurs to me. The cone might be regarded as a portion of the membrane which is thrown into continuity with the vitellus secondarily, as the result of the penetration of the male element. There is an obvious objection, however, to considering the "cone of attraction" as a part of the vitelline membrane. On this very im-

23. W. Flemming. Beiträge z. Kenntniss d. Zelle u. ihrer Lebenserscheinungen. *Arch. f. mik. Anat.*, XX., 1882.

portant point Fol's investigations leave us in the lurch; for, while he describes the cone (p. 91) as arising from the cortical layer of hyaline protoplasm (which layer represents the vitelline membrane *ab initio* Hertwig, *in posterum* Fol) he finds himself unable, when he comes to a final discussion of the matter (pp. 250–251), to decide whether the cone forms a part of this layer or a part of the vitellus proper. As there is no doubt expressed about the continuity of the cone with the vitellus *after* the penetration of the spermatozoon, it may be safely inferred that the continuity exists from the outset.

If Fol's theory of the origin of the vitelline membrane were correct, — it is difficult to accept it in the face of Hertwig's observations, which accord so much better with our general knowledge of related phenomena, — the origin of the cone from the cortical layer could easily be reconciled with its continuity with the vitellus, since this entire layer is supposed to be an integral portion of the egg protoplasm up to the moment of impregnation. The delimitation and separation from the vitellus, resulting from impregnation, would take place all around the cone, leaving the cone still continuous with the vitellus. This view finds its strongest support in the fact that when several spermatozoa enter the same egg at different points, as may happen in pathological cases, a cone of attraction is developed at each of these points (p. 119).

Although any point of the egg may give rise to one of these cones, it is still probable that, in all normal cases, the single cone arises from a differentiated place, which corresponds to the Dotterhügel of Toxopneustes. When the cone is first seen, it has the form of a low, "nipple-like prominence" (p. 91), like the "Dotterhügel" in the egg of the sea-urchin.

Flemming states that this prominence is quite difficult to find in *T. lividus*, and that it must be carefully searched for by rolling the egg slowly, and examining attentively every point of the surface. It is probable that Fol overlooked this prominence until, at the penetration of the spermatozoon, its exact position was marked and easily brought into the field of vision.

The "Dotterhügel" of Selenka, the "Höckerchen" of Flemming, the "Protoplasmabrücke" of Hertwig (18, p. 173), and the "cône d'attraction" of Fol are, in all probability, identical in origin and function; and Selenka's observations on the

behavior of the spermatozoa towards the "Dotterhügel" go to show that it is really a "bouchon d'imprégnation." Selenka's interpretation of this as a protuberance caused by the extrusion of the polar globules is entirely unsupported by analogy in other eggs; and, besides, it offers no explanation of the decided preference shown by the spermatozoa for entering the egg at this point. This *preference* must mean that the attraction between the egg and the spermatozoon is strongest at the "Dotterhügel." Selenka accounts for it by supposing that the gelatinous envelope of the egg is more easily pierced in the immediate vicinity of the Dotterhügel than elsewhere.

The fact that spermatozoa generally penetrate the envelope *vis-a-vis* the "Dotterhügel" may be quite as readily explained on the hypothesis of attraction; and this view is supported by analogy, as we shall presently see, and by one of Selenka's own observations. In case the spermatozoon passes through the mucous envelope at some point more or less remote from the "Dotterhügel," it does not continue to advance straight through the vitelline membrane ("Rindenschicht"), but "*swims about over the membrane for from one-half to several minutes, until, by its whip-like movement, it accidentally strikes its head against the Dotterhügel*" (p. 6). It then bores its way through the membrane into the vitellus.

The careful investigations of Zacharias have led him to conclude that there is no predetermined point of impregnation in the egg of Ascaris. According to this author, the spermatozoon penetrates at any point of the surface of the egg, and Van Beneden's statements as to the existence of a micropylar orifice are represented as entirely incorrect. "It is very remarkable, however," says Zacharias (10, p. 143), "that in the great majority of cases only a single spermatozoon copulates with the egg of Ascaris. *If no predetermined point of impregnation is present, it is wholly inexplicable that only one of the many hundred spermatozoa which surround the egg in the upper part of the uterus should become attached to it.*" The idea that the receptivity of the egg is self-regulating does not seem to have occurred to Zacharias.

A very striking and convincing proof of attraction at a distance, and at the same time a confirmation of the interpretation above given to Selenka's observations, is found in the fecunda-

tion of the Lamprey egg, as described by A. Müller (24) and by Kupffer and Benecke (20). The micropylar area is represented by a watchglass-shaped segment of the egg membrane, and is surmounted by a prominent hyaline dome, which here replaces the mucous envelope ("Schleimhülle").

The first point which is of special interest in the description is the fact that *the spermatozoa which come in contact with the mucous envelope do not try to penetrate it, while those which reach the dome* (Müller's "Flocke") *immediately take a direction radial to the "watch-glass."* The whole dome becomes so thickly beset with spermatozoa that it presents the "picture of a beard." A. Müller likened the appearance to that of iron filings arranged in a feathery tuft around the end of a magnet, and the comparison is fully indorsed by Kupffer and Benecke. The whole account impresses one very strongly with the fact that attraction is felt *before* contact with the vitellus, and that its influence is strongest at, possibly confined to, the micropylar area.

With reference to this point, Kupffer and Benecke remark: "The micropyle is, therefore, not an open passage, as it would appear to be from the statements and figures of Calberla, but merely a more permeable place. But it remains a mystery how this point is always hit by a zoosperm, unless one is permitted to assume that *the interaction between egg and zoosperms is more energetic in that radius which passes through the micropyle than in any of the other radii*" (p. 15).

The attraction manifests itself not only in the behavior of the spermatozoa, but also in a *contraction* of the egg, which shows itself in the ring-like space left between the rim of the "watch-glass" and the vitellus. A single spermatozoon, after it has penetrated the dome and placed itself in a position radial to the micropylar surface, is sufficient to induce the immediate retraction of the vitellus; but the intensity of this action increases with the number of spermatozoa. Kupffer and Benecke justly infer, therefore, "*dass die Zurückziehung des Dotters nicht auf einer Contactwirkung, sondern auf einer Fernwirkung der radiär geordneten Zoospermien beruht*" (p. 11).

The second point of interest is the fact that *all undulations*

24. AUG. MÜLLER. Beobachtungen ü. d. Befruchtungserscheinungen im Ei d. Neunaugen. *Verhandl. d. Königsberger phys.-öconomischen Gesellschaft*, 1864.

of the tail cease as soon as the head of the spermatozoon penetrates fully into the egg membrane. Its further progress, then, is due not to any automatic movements, but solely to centripetal attraction. As Kupffer and Benecke put it, "Es wird angezogen."

This attraction is felt even before the undulatory movement ceases, as shown by amœboid changes in the head. "Eine Welle erhebt sich am hintern Ende des Kopfes und läuft an demselben bis an die Spitze hin ab, dort sich kuglig zusammenballend, weicht dann wieder zurück, um in erneuetem Anlaufe abermals vorzudringen. Dieses Spiel kann sich minutenlang wiederholen. Ist der Kopf bis in die Nähe der inneren Oberfläche der Eihaut gelangt, so sendet derselbe häufig einen feinen Faden pseudopodienartig vor, der den Rest der Strecke durchsetzt" (pp. 13–14).

A third fact of great significance is the *elongation of the head of the spermatozoon during its passage through the perivitelline space.* The nearer it comes to the vitellus the more elongated it becomes, the increase in length amounting in the end to about one-third. These remarkable changes show the attractive influence of the egg quite as clearly as Fol's "cone of attraction" demonstrates such action for the spermatozoon.

Taken all in all, Kupffer and Benecke's paper is by far the most important contribution to the evidences of attraction at a distance that has yet appeared, and whoever doubts such action will do well to read carefully their work.

The explanation which they offer for the phenomena is not one that I can accept, in so far as it refers the attractive influence exerted upon the spermatozoon, not to the vitelline protoplasm, but to a centripetally moving egg-nucleus. The idea that this egg-nucleus owes its origin to an impulse given to the vitellus by the spermatozoa of course requires no refutation. The method of accounting for the centripetal movement of the egg-nucleus accords fully with the views presented in the foregoing pages. The reason assigned for the failure of more than one spermatozoon to effect an entrance is entirely untenable, as before pointed out (p. 241).

The "Leitband des Samens," described by Calberla (17, p. 458), in the egg of Petromyzon, is regarded by that author (p. 485) as the functional equivalent of the "cone of attraction" in Asterias, and by Van Beneden (9, p. 373) it is likened

to the "bouchon d'imprégnation" in Ascaris. Kupffer and Benecke deny that the "Leitband" has the functional importance attributed to it by Calberla, and hence they prefer to call it an "Axenstrang."[1] Allowing that it serves to guide the spermatozoon into the vitellus, as asserted by Calberla, it is difficult to see how it furnishes any evidence of attraction at a distance. It is simply one of many protoplasmic threads, left by the *contraction of the vitellus*, not a cone of attraction *rising from the vitellus* to meet the spermatozoon. Neither the observations of Calberla nor those of Kupffer and Benecke permit us to identify the "Leitband" with the "bouchon" in Ascaris. This "bouchon"[2] arises before impregnation and quite independently of any spermatic influences, while the Leitband arises merely as a secondary result of the action of the spermatozoa. In view of the fact brought out by Kupffer and Benecke, that the spermatozoa may penetrate at any point of the micropylar area ("watch-glass"), and follow any one of the protoplasmic filaments formed in this region, or even pass between them in order to reach the vitellus, we would suggest that the "bouchon"—if such a structure is present in the Lamprey egg—is represented by the discoid thickening (A. Müller's "Deckel des Urbläschens") of the thin protoplasmic mantle. This disc occupies the active pole, and in extent corresponds closely with the micropylar area. (Cf. Kupffer and Benecke's Fig. 7.)

Nothing has yet been described in the teleostean egg that could with certainty be said to function as a "cone of attraction." The observations which I have made on pelagic fish eggs in conjunction with Mr. Agassiz are soon to be published elsewhere, and I will not here anticipate the results further than to say that a careful study of surface-preparations and sections has revealed not the slightest trace of such a cone. I may say, however, that conclusive evidence has been found that the area of impregnation is, as might have been predicted from the existence of a definitely localized micropyle, a limited one, with boundary lines encircling the germinal pole. It is per-

[1] The Axenstrang is not constant in Petronyzon fluviatilis (Kupffer and Benecke, p. 18).

[2] There is no such structure according to Zacharias.

fectly certain that these eggs have a polar area, comparable with the disc of impregnation in Petromyzon.

In many teleostean eggs a well-defined germinal disc is present before impregnation takes place, and in such cases the micropyle is usually described as occupying the centre of the disc. Such a position would indicate, as van Beneden (9, p. 376) has pointed out, that the spermatozoon penetrates at a predetermined point. It is probable, however, that the micropyle is a little eccentric, and that its polar distance is variable within certain narrow limits, — within an area which may be called the *pole of impregnation.*

Prof. Kupffer (25) has discovered in the egg of the trout (thirty m. or more after fertilization) small discs distributed over the surface of the blastodisc, which he compares with the "disque polaire" described by Van Beneden in the egg of Ascaris.

In surface views and under a low magnifying power, these "Polscheiben," a dozen or more in number, appear as light flecks. "Diese Scheiben bestehen aus *einer senkrecht gestrichelten hellen Substanz, die als Fortsetzung der dünnen Dotterhaut erscheint, gegen die Mitte zu an Dicke zunimmt und hier von einem Pfropfe der Keimsubstanz durchbohrt wird,* über dessen Oberfläche sich die Dotterhaut nicht fortzusetzen scheint. An der Basis des Propfes sieht man jederseits zwei kleine Hohlräume, die Durchschnitte eines die Basis ringsum umziehenden Kanals" (p. 6).

Kupffer holds that the germinal protoplasm ("Pfropfe") filling the central perforation of one of these "Polscheiben" fulfils the function of the "*bouchon d'impregnation*" of the Ascaris egg; but he has failed to verify this by observation.

The occurrence of several polar discs is taken as an evidence that more than one spermatozoon is required to fertilize the egg. But such evidence can have very little weight so long as the polar discs themselves remain entirely problematical. Kupffer defends his interpretation on the ground that the discs are similar in structure to the polar disc of Ascaris. But Zacharias has given very strong reasons for believing that the "disque polaire" and its central "bouchon d'impregnation" are artificial

25. C. KUPFFER. Die Befruchtung des Forellenies. *Bayerische Fischerei-Zeitung,* 1886.

productions, and hence the argument from analogy falls to the ground. When Kupffer or any other investigator succeeds in showing, under perfectly normal conditions, that more than one spermatozoon is concerned in the formation of the male pronucleus, we shall be ready to concede that a case of polyspermatic fecundation has been established. Decisive evidence can be obtained in no other way than by tracing the history of the male pronucleus, and it is precisely in this direction that Kupffer's observations are most incomplete.

Kupffer claims also "Copulationshügel" and polyspermatic fecundation for the amphibian egg and the lamprey egg. He has seen the hügel penetrated by spermatozoa, but has failed to determine their fate, and thus left it entirely uncertain whether more than one of them is concerned in the act of fecundation. Through the investigations of Bambeke, O. Hertwig, Roux, and Born, the history of the male pronucleus in the amphibian egg has been very completely ascertained. Thus far no positive evidence has been produced to show that the male pronucleus is the product of several spermatozoa. The indications are plainly in favor of monospermatic fecundation. If several spermatozoa took part in fecundation we should expect to find a corresponding number of pigment paths. Referring to this point, Roux (4, p. 173) remarks: "Van Bembeke fand wiederholt mehrere solcher Pigmentstrassen im Ei, *während ich in etwa 100 geschnittenen Eiern dies blos einmal beobachtet und dies Ei stammte vom Ende der Laichperiode, wo Abnormitäten sehr häufig sind. Meine Untersuchungen bestätigen also die Angaben von O. Hertwig und Born, dass normaler Weise blos ein Samenkörper in das Ei eindringt.*"

With reference to the place of penetration, O. Hertwig (1, p. 82) makes the following statement: "Bei Rana temporaria erfolgt der Eintritt des befruchtenden Spermatozoon in den Dotter stets *am schwarzen Pol zur Seite des schleierförmig ausgebreiteten Excretkörpers* auf der vom Eikern abgewandten Eihälfte." Roux admits that the spermatozoon generally enters the egg near the upper pole, but denies that this is invariably the case (4, p. 174).

NOTES ON THE DEVELOPMENT OF PETROMYZON.

W. B. SCOTT,

College of New Jersey, Princeton.

THE present paper is a continuation of an article which appeared in the "Morphologisches Jahrbuch" for 1881, and, like that article, is for the most part founded upon study of the great series of sections prepared by the late Dr. Ernst Calberla, of Heidelberg, which was placed in my hands by Professor Gegenbaur. This paper has been nearly ready for publication for several years past, but its completion has been long delayed by circumstances which need not be enumerated here.

Since the appearance of my first article several errors have been detected in it, some by other observers and some by myself; but as these have all been commented upon by Shipley, it is needless to dwell upon them further than to mention them. Thus my account of the formation of the ventral mesoblast by splitting from the yolk-hypoblast, of the development of the pronephros from diverticula of the segmental duct, of the formation of the anus and neurenteric canal, are incorrect. With regard to the epithelial layer of the central nervous system, a word of explanation may not be out of place. The existence of this layer in the first rudiment of the nervous axis has been affirmed by Calberla and myself, and denied by Balfour and Shipley. At Balfour's request I carefully reëxamined all of Calberla's preparations which bear upon this point, and was, at the time, fully convinced of the accuracy of Calberla's statements. I have not had an opportunity of again examining the question since the appearance of Shipley's paper; and, in view of his positive statements, my confidence in my former account is somewhat shaken, especially as sections prepared by the more recent methods are so much clearer than those prepared by the methods in use in Calberla's time. Nevertheless, the evidence appeared so perfectly satisfactory, when I last had the

opportunity of investigating it, that I am as yet not convinced of the accuracy of Shipley's views.

I am under great obligations for assistance and supplies of needed material to many persons. First and foremost to Professor Gegenbaur, who placed Calberla's collections at my disposal, and who constantly exhibited the greatest interest and kindness while I was in his laboratory. Professors Balfour, Benecke, Wiedersheim, and Gage have sent me material of much value, and Mr. Shipley has most kindly sent me an extensive series of his beautiful preparations, which have proved of the utmost service. To all these gentlemen I wish here to express my very cordial thanks.

I. THE CENTRAL NERVOUS SYSTEM.

In order to understand the alterations in the brain and in the position of the sense-organs, it will be necessary to take into account the changes which take place in the character and shape of the mouth, as they have a profound effect upon the position of the surrounding organs. The mouth in an embryo of seventeen days (Fig. 1, Pl. VIII) is a deep depression of the epiblast, very large internally, but with a somewhat contracted opening. The upper lip is short, and, seen in longitudinal section, has a rounded anterior margin, and does not descend to the level of the lower lip. By the eighteenth day (Fig. 2) the cranial flexure has increased, and, indeed, attained its maximum; the nasal pit opens directly downwards; the upper lip has become longer, and now extends somewhat below the level of the lower lip; it has also extended antero-posteriorly, deepening the nasal involution, and becoming somewhat triangular in sagittal section. At this stage the mouth is altogether ventral in position, and when the head of the entire embryo is viewed from below, the resemblance of the mouth to that of a Selachian in shape and position is very striking. Nothing could be more different from the suctorial disk of the adult lamprey, or even from the mouth of the larva, than the shark-like mouth of the embryo at this stage. Shortly after hatching (Fig. 3) a remarkable change takes place: the cranial flexure begins to correct itself by an upward rotation of the fore and mid-brain, about a tranverse axis passing through the

mid-brain, so that the *lamina terminalis* which formerly faced directly downwards, now faces obliquely forwards and somewhat downwards (Fig. 3). The upper lip has greatly increased in size, especially in the vertical direction, the posterior edge growing out into a long process. This growth keeps pace with the correction of the cranial flexure, so that the upper lip still extends down to the level of the lower, and the mouth is still ventral in position. Later, however, the upper lip itself begins to rotate about a transverse axis; the point marked UL in the drawings, which in Figs. 2 and 3 points backwards, in Fig. 12 (Ammocœtes of 12 mm.) points directly forwards, having thus moved through an arc of 180°. At the same time the lip increases in vertical thickness, and thus completely encloses the olfactory epithelium, which was at first exposed on the surface, but now comes to lie deep within the head. Further, this rotation and method of growth of the upper lip transfers the opening of the nasal pit from the ventral to the dorsal surface of the head. Several intermediate stages of this transformation between those shown in Figs. 3 and 12 might be given, but they are doubtless sufficient.

1. *The Brain.* — With the exception of the olfactory lobes, the brain of the larva at the time of hatching contains all the parts which are to be observed in the adult brain, though the structure and proportions of the various parts are very different from those found in the later stages of larvæ, and still more so from those of the adult. The brain, as a whole, is exceedingly small compared with that of fishes or Amphibia at a corresponding stage of general development, and this reduction is especially marked in the fore and mid-brains, which may fairly be called minute. The exceedingly small size of these parts is no doubt due to the retarded development of the sense-organs connected with them, as both the olfactory organ and the eye, especially the latter, remain in a very backward and incomplete state during the whole of larval life. The larva lives, as is well known, buried in sand or mud, and the organs of higher sense are nearly functionless; this condition must necessarily have a very considerable effect upon the growth of the brain. Wiedersheim (41) has called attention to the very large size of the hind-brain in the Ammocœte. This is quite true of the hind-brain, as compared with the other divisions of the encephalon,

but in relation to the size of the animal, even the hind-brain is seen to be exceedingly small, very much smaller than in the Selachii or Amphibia, for example.

In the embryo of *Petromyzon* nearly ready for hatching, or in the newly hatched larva, the cranial flexure is comparatively slight. At its maximum it scarcely, if at all, exceeds a right angle, and, as we have already seen, the flexure is gradually corrected. In the higher vertebrates, the correction of the cranial flexure is, as Balfour has shown, only apparent, and is brought about by foldings in the opposite direction, the effect of which is to apparently straighten the axis of the brain, but in reality to make it more sinuous than before. Something of this may also be seen in *Petromyzon*, where the dorsal fold which marks the limits of the mid and hind-brains becomes gradually deepened as the correction proceeds, and the cerebral hemispheres are folded slightly upwards and backwards. But these processes have little effect, and in addition to them there is a real correction of the flexure brought about by an upward rotation of the fore and mid-brains about a transverse axis. The correction, though real, is not altogether complete; as may be seen in Fig. 13, Pl. IX, there is still a slight bend in the axis of the brain.

(*a*) *The Fore-Brain.* — At the time of hatching the fore-brain consists of the thalamencephalon and the hemispheres, the olfactory lobes not having yet become differentiated from the latter. The hemispheres, as I showed in my former paper, arise as an unpaired and solid rudiment, which later divides into two portions; these remain solid for a considerable period, nor have I been able to observe any trace of the lateral ventricles earlier than in larvæ of 14 mm. in length. In young larvæ the hemispheres are very minute, and they enlarge but slowly, as their small size is characteristic of full-grown Ammocœtes, and even of the adult. The olfactory lobes are not formed, as Shipley (37) has supposed possible, from special ganglionic detachments of the olfactory and pituitary epithelium; but by enlargement and constriction of the anterior portion of the hemispheres. Shortly after their formation a small lumen appears in each one, which communicates by a common chamber with the lateral ventricles of the hemispheres (Fig. 16, Pl. IX). At first the lobes are smaller than the hem-

ispheres, but in the adult they are considerably larger, a growth which is probably to be referred to the extraordinary size and complexity of the olfactory apparatus in the adult. Both the olfactory lobes and the hemispheres are at first solid masses of nerve-cells, closely packed together and without any appearance of fibres. After the formation of the ventricles, a lining layer of epithelial cells forms around them, and considerable bundles of fibres make their appearance. In larvæ of 40 to 50 mm. in length, the minute structure of these bodies is very much as in the adult, consisting of nerve-cells imbedded in fibrous bundles. Towards the centre the nerve-cells are most closely crowded, gradually separating towards the circumference, which is formed by a thin layer of white matter.

The relative position of the *lamina terminalis* changes with advancing age. At first it is in close contact with the nasal epithelium, and thus remains for a considerable period (Figs. 2 and 3, Pl. VIII; Fig. 11, Pl. IX), the hemispheres extending but little in advance of it. The formation of the olfactory lobes is accompanied by a depression of the lamina (Fig. 16), and as the lobes increase in size, the lamina is carried further and further away from the nasal capsule, and the depression becomes more marked. I cannot state at what period the anterior commissure between the hemispheres first makes its appearance, but it is very distinctly marked in larvæ of medium size (60 mm. and upwards).

The development of the *thalamencephalon* presents several points of considerable interest. At the time of hatching this region has walls of considerable thickness, with much thinner roof and floor; in the median line, both dorsal and ventral, there is but a single layer of cells; the ventricle is quite widely open, and especially expanded above the middle of its height. In later stages the lower part of the ventricle becomes much narrower and is reduced to a mere slit, while the upper portion increases in width, and, as a whole, the thalamencephalon becomes very much higher and narrower. The roof of the ventricle thins out, and is pressed down by the overlying epiphysis and its accompanying structures, while the thalami increase in height and thickness and gradually overtop the cerebral hemispheres. Fibres appear in this region much later than in the mid and hind-brains, so that in young larvæ the thalamen-

cephalon consists entirely of cells (Fig. 11, Pl. IX). In large larvæ, however, the optic thalami consist almost entirely of fibres, though some layers of cells surround the ventricle, and scattered cells may be seen among the fibres. These fibres first appear as two bundles, one on each side, about midway in the height of the thalami, but interrupted by the cells both above and below (Fig. 8, Pl. VIII). The fibres gradually encroach upon the cells until the latter are reduced to very small proportions.

The *infundibulum* appears very early (Fig. 2, Pl. VIII) as a diverticulum of the floor of the thalamencephalon, immediately behind the optic chiasma. It is of large size from the first, especially in the antero-posterior direction, but rapidly increases in size. As Ahlborn (1) has remarked, it remains single in larvæ of 22 mm. in length, but in Ammocœtes of 26 to 30 mm. the division into lobus and saccus is to be found. The *saccus infundibuli* has very thin walls, and its cavity is much expanded below and somewhat contracted above, so that in transverse section the saccus is seen to project much beyond the sides of the thalamencephalon. This condition is reached in larvæ of 26 mm., and perhaps earlier. At a similar stage the *lobus infundibuli* extends backwards until it comes to underlie the mid-brain, and in some transverse sections appears to form a part of that region. Immediately in front of the infundibulum there appears very early a constriction in the floor of the brain (Fig. 4, Pl. VIII) which encloses a small cavity, the *recessus chiasmaticus*, formed by the union of the hollow bases of the optic tracts. In the adult the optic tracts and the recessus project very prominently from the floor of the brain (see Ahlborn (1), Figs. 33–35, Pl. XV), but during larval life all the structures connected with the eye remain very small, and the recessus retains essentially the same characters as those shown in Fig. 4, except that transverse fibres begin to appear in its walls, and thus form the optic chiasma.

The discoveries of Spencer (39) have latterly drawn great attention to the *pineal gland*, which Rabl-Rückhard, from a study of the Teleosts (28), and Ahlborn (3), from his observations on the lamprey, had already conjectured to be a rudimentary eye. Its history in *Petromyzon* is of great interest. It first arises, as was shown in my former paper, in embryos of about the seven-

teenth day as a small median diverticulum of the roof of the fore-brain, and until after the time of hatching the communication between the cavity of the diverticulum and the third ventricle remains open. This communication is, however, shut off shortly after hatching, though a narrow prolongation of the ventricle long persists as a recessus pinealis. At first the pineal rudiment is composed of a layer of columnar epithelial cells in no way differing from the surrounding cells in the roof of the brain. In a short time, however, the cells forming the lower wall of the diverticulum become perceptibly thicker, a result which is produced not by division, but by vertical elongation of the cells and gradual assumption of a more or less rod-like form. The epiphysis rapidly increases in size, and grows especially forwards, so that the distal vesicle comes to overlie and even extend beyond the ganglia habenulæ, whereas, at its first formation, it lies altogether behind the region of these bodies. At this stage, and for some time afterwards, the vesicle is much compressed by the surrounding structures, so that its cavity is nearly obliterated and its structure very much obscured. When viewed with low powers, the body appears to consist of a flattened, lenticular, and solid mass of rounded cells (Figs. 5, 6, and 9, Pl. VIII; 12, Pl. IX); but a closer examination of more favorable sections reveals a small cavity with a thin roof of much flattened cells and a thickened floor of columnar cells. It is to be observed that the portion of the vesicle which gives rise to the retina-like structure is the inner wall, that is to say, the wall turned toward the brain, while in the paired eyes the retina arises in the side of the vesicle which is turned away from the brain and nearest to the skin. Thus not only is the position of the retinal elements with reference to the nerve fibres different in the pineal and the paired eyes, but the portion of the primitive vesicle which becomes the retina is also different. Or, if the retinal side be considered homologous in the two sets of organs, the vesicle has changed its position with reference to the brain and the skin. Beard (7 and 8) states that the epiphysis is pigmented in some specimens of the adult and in the very young larvæ. Though not wishing in any way to throw doubt upon this positive statement of fact, I am unable to confirm it, as none of my sections of Ammocœtes show any trace of pigmentation. The presence of pigment is, of course, an

important element in the view to be taken of the homologies of the epiphysis. That portion of the pineal stalk which lies beneath the distant vesicle remains, throughout larval life at least, cellular, and retains its lumen. It may be seen as a small thin-walled canal lying between the pineal vesicle and the left ganglion habenulæ. The proximal portion of the stalk becomes fibrous, and loses its lumen. The stalk is short in the larvæ, but elongates very considerably at the time of metamorphosis.

The epiphysis is at first entirely symmetrical and median in position, but afterwards, as has been pointed out by Ahlborn, it assumes a strikingly asymmetrical position, moving to the left of the median line and coming into special relations with the left ganglion habenulæ. I have no observations which show the mode of origin of the second epiphysial vesicle, which, according to Ahlborn, is already present in Ammocœtes of 22 mm., nor have I been able to detect anything which suggests the formation of a lens at any stage in the development.

In a larva of 26 mm., a series of transverse sections reveals the following condition of this structure. A section passing through the forward portion of the hemispheres shows the anterior edge of the primary pineal vesicle, which is median in position and symmetrical with reference to the hemispheres. In the following section both vesicles of the epiphysis are shown, and here they are distinctly to the left of the median line. In structure the two vesicles are very similar, but the cells of the lower are smaller and less distinctly rod-like than those of the upper. In this section the right ganglion habenulæ is seen, but the left is not yet reached. A third section (omitting intermediate ones) exhibits the hollow pineal stalk resting upon the left ganglion habenulæ; while farther back the stalk becomes solid and passes into the roof of the recessus pinealis. This is the condition in which the organ remains during larval life, and the only important change which takes place at the time of metamorphosis is the great increase in the length of the pineal stalk, by which the vesicle is carried nearly as far forwards as the lamina terminalis, and entirely in advance of the right ganglion habenulæ, and the stalk lies in close contact with the optic thalami, which project above the cerebral hemispheres.

These remarkable changes in the character and relations of the pineal body in *Petromyzon* would certainly seem to indicate that it has acquired some secondary function of importance. Of its primitive structure as an eye there still remain, as already stated, recognizable traces in the distinctly retina-like appearance of the upper vesicle, and, according to Beard, in the pigmentation, although apparently the lens is not even indicated at any time. But any visual powers the organ may once have possessed are now obviously lost, though it may perhaps be sensitive to light, as is suggested by the fact that the epiphysis is visible through the wall of the head. Nevertheless the lamprey does not exhibit a simple degeneration of the organ, such as occurs in most of the higher vertebrates, but rather a transformation of it. The formation of the second epiphysial vesicle and the intimate connection found with the left ganglion habenulæ are not known to occur in any other type, and certainly suggest a transformation, and not a mere loss of function. What this secondary and acquired function may be, if any such exists, it would, in the present state of our knowledge, be idle to conjecture. It is, however, important to emphasize the fact that in *Petromyzon*, the pineal gland, in some of its stages at least, is more distinctly like an optical organ than in any known vertebrate outside of the lacertilian type. From this it would seem to follow that the pineal eye is an organ which was originally functional in the lower vertebrates, even if we admit Dohrn's hypothesis that the *Cyclostomata* are the degenerate descendants of fishes which were of a comparatively highly organized type.

The first formation of the *Ganglia Habenulæ* is thus described by Shipley (37): "This superior commissure is at first covered with but a few ganglion cells, but these afterwards increase until two bodies are formed, the Ganglia Habenulæ. The left one is very small, but the right is a structure of considerable size, projecting downwards, and backwards, and reducing the lumen of the fore-brain to a Y-shaped slit. These bodies have been fully described by Ahlborn in the adult; it is interesting to note that the curious asymmetry they possess is present from their first appearance." The commissure here referred to passes transversely through the roof of the thalamencephalon immediately in front of the epiphysial rudiment. Ahlborn has

described it as the commissura tenuissima; Osborn (27) has found it in several of the Amphibia, and named it the Superior Commissure. It also seems to occur in the Selachians, as shown by Balfour. My own observations on the formation of the ganglia habenulæ agree entirely with those of Shipley. The asymmetrical development of those bodies is even more decidedly marked at the period of their origin than in the adult, and is carried so far that a deceptive appearance of symmetry is produced, for the left ganglion is so small that unless one's attention is particularly directed to it, it may easily be overlooked, and in slightly oblique sections the right ganglion occupies so much of the roof that it appears like a median unpaired structure. The history of these two bodies is very different, and they must be described separately. The right one, as already stated, is much the larger from its first appearance; it depends from the roof of the third ventricle, occupying most of the breadth of that roof, above the level of which it does not at all project (Fig. 10, Pl. VIII). At this stage its broadest part is the base of attachment, gradually tapering to the free end, forming in transverse section a triangle. In young larvæ the point of attachment becomes considerably constricted and the free portion thus exceeds the base in transverse diameter; at the same time the point of attachment is shifted more towards the right side. In many sections the body appears to lie isolated within the ventricle, owing to the constriction of the neck. In later stages the changes in this body are apparently confined to an increase in size, which occurs rapidly, and especially to a marked increase vertically, so that the body begins to project conspicuously above the roof of the ventricle, and thus forces the pineal stalk over towards the left side. Even the point of attachment of the stalk is to the left of the median line; but this cannot be regarded as a mere mechanical displacement, but rather as a result of the more rapid and extensive growth of the right-side in this region, the left side lagging behind; what was formerly the median line thus coming to be at the left side. I have seen nothing which suggests an actual shifting in the point of attachment of the pineal stalk.

The left ganglion habenulæ is at first very minute, consisting of a few ganglion cells on the lower side of the superior com-

missure (Fig. 8, Pl. VIII. This drawing is erroneous in so far that the commissure is not shown, and a histological difference is indicated between the right and left ganglion, which does not exist). For some time the left ganglion persists in this condition without essential change. Its later increase in size is at first in the antero-posterior direction, accompanying the epiphysis in its forward growth, but not extending quite so far forwards as the pineal vesicle. In larvæ, between the lengths of 12 and 25 mm., a great change takes place. As Ahlborn has shown, the left ganglion habenulæ has by this time become distinctly divided into two parts, of which the anterior is in close contact with the lower vesicle of the epiphysis (see his Fig. 43). In the adult, these two portions become quite widely separated, the anterior one accompanying the growth of the pineal vesicle, and partially coalescing with the lower division of that vesicle; a fibrous tract connects the two divisions, and, at first sight, appears to be a portion of the pineal stalk. These changes apparently take place at the time of metamorphosis, as I have not detected them even in advanced Ammocœtes. The posterior division of the left ganglion retains its relative position in the adult; it is comparatively small, and does not, like the right, project above the level of the brain and form a conspicuous object from without.

The Pituitary Body may be conveniently described in connection with this region of the brain. In my former paper I stated that the hypophysis was derived from the nasal involution. Götte has very justly claimed this discovery as his own (16), and complained that I did not acknowledge the priority due to him. This was done for two reasons: (1.) The subject was barely touched upon in my first paper, which dealt only with the embryonic as distinguished from the larval development, while the complete history of the pituitary body, including a discussion of Götte's statements and theoretical views, was reserved for my second paper, which was at that time nearly ready, and was expected to appear immediately. (2.) From the letter which Götte very courteously wrote me, and the sketches which accompanied that letter, I inferred that he had mistaken the process of development of the body in question, the original statement in the "Entwickl. d. Unke" being very brief, and not very clear. But in this, as appears from what he

has since written (16), I was mistaken. This statement is inserted here simply because I am anxious to avoid any appearance of injustice to Götte's work. The misunderstanding has arisen solely from the very long and entirely unforeseen interruption in the publication of my observations.

Dohrn (12) has objected that my account of the origin of the pituitary body is incorrect, inasmuch as that body arises from an independent involution, and not from the nasal invagination. But, as will be seen from his figures, he has differed from me more with regard to names than to facts. I never meant to assert that the hypophysis was derived from the *olfactory epithelium*, using the word "Nasengrube" to include the rudiments of all the structures connected with the olfactory organ.

Götte's original account of the development of the hypophysis in the Cyclostomata is as follows (Unke, pp. 318, 319): "Da die Entwickelung des Hirnanhangs vom medianen Schlussstücke der Sinnesplatte, also, einer sehr wichtigen Embryonalanlage ausgeht, von dem ganzen Fortsatze aber die vordere Hälfte, nämlich der obliterirende Kanal vollständig verkümmert und schwindet, so liegt es nahe in diesem ganzen Vorgange einen Rückbildungsprocess zu vermuthen. Da ferner bei den Batrachiern die beiden Anlagen der Geruchsorgane median — und abwärts mit der trichterförmigen Anlage des Hirnanhanges zusammenhangen (vgl. den nächsten Abschnitt), so kann man sich zur Hypothese veranlasst fühlen, dass die vollkräftige Entwickelung der Hypophysisanlage unter Einbeziehung der beiden Geruchsplatten den unpaaren Nasenrachengang der Cyklostomen bilde, welcher ja nachweislich als ein von vorn ausgehender Blindsack erst nachträglich, d. h. gerade so wie die Nasengruben der Batrachier in die Mundhöhle durchbricht. Die Anwesenheit eines Hirnanhanges bei den Cyklostomen wäre kein Grund gegen jene Annahme, *denn derselbe entsteht eben nicht aus der ganzen Anlage, sondern nur aus deren Endabschnitte;* [the italics are mine], und was die verschiedene Lage der äusseren Oeffnung des unpaaren Nasenrachenganges und der Hypohysisanlage betrifft, so erinnerere ich an die Unterschiede der Naseneingänge bei den amphirrinen Selachiern und Delphinen. Daher glaube ich, dass wenn man zunächst die Batrachier zum Ausgangspunkte wählt, die Hypothese von einer Homologie ihrer

dreitheilige vorderen Sinnesplatte (Anlage des Hirnanhangs und der Geruchsplatten) mit dem unpaaren Nasenrachengang nicht ohne weiteres von der Hand zu weisen wäre."

The earliest stage that I have observed in the development of the pituitary body is in an embryo of seventeen days (Hy. Fig. 1, Pl. VIII), where a slight ingrowth of epiblastic cells is seen immediately in advance of the upper lip, the nasal epithelium not having as yet become differentiated from the general epiblast. Dohrn's Fig. 1 represents a stage which I have not succeeded in finding, but I altogether agree with his statement that the hypophysis is formed quite independently of the olfactory epithelium. In the following stage (18 days, Fig. 2), a considerable change is observable; the olfactory epithelium is now well developed and passes posteriorly into a canal, which is a common involution for the nasal canal and the pituitary body. This involution is now much deeper than in the former stage, it having lengthened *pari passu* with the growth of the fore-brain; but of still greater moment in producing this effect is the lengthening of the upper lip, already described, which deepens the canal by extending its inferior wall. The end of the involution has in this stage reached the infundibulum, with which it is in close contact. In larvæ which have just come out of the egg (4.8 mm.) the involution is very much longer (Fig. 4), its lower end still remaining in contact with the infundibulum. This increase in length has been entirely conditioned by the growth of the upper lip and its commencing rotation, together with the correction of the cranial flexure, which now begins to be apparent. As can be seen in Fig. 4, these changes in the lip have very much contracted the entrance to the nasal pit (compare Fig. 2). The prolongation of cells to the infundibulum is shown in transverse sections to be a canal with a very small lumen and thicker dorsal than ventral walls. The involution is of nearly uniform size throughout, though gradually tapering as we pass backwards. In later stages the further increase in size of the lip, its rotation so as to point directly forwards, together with the growth of the hemispheres and olfactory lobes, produce a corresponding increase in the length of the canal, and the formation of the hypophysis proper begins by a transformation of the mass of cells which underlie the infundibulum. In this

region the lumen of the canal disappears and the solid, proliferating mass of cells broadens into a lens-shaped body, which embraces the free end of the infundibulum, the upper surface being concave and the lower convex. The portion of the canal immediately in front of the pituitary rudiment thins out, and apparently develops fibres which still retain a connection with the posterior end of the nasal canal proper. This connection persists for a considerable period, and, so far as I have been able to observe, it does not appear to be severed at all during larval life. In the larva of 53 mm. in length (Fig. 17, Pl. IX), the hypophysis consists of a broad mass of closely crowded epithelium cells, which, as yet, show no division into follicles. It is no longer in contact with the roof of the mouth, as was the case in the earlier stages (Fig. 4, Pl. VIII), but separated from it by a thick layer of connective tissue developed from the mesoblast. At a later stage this connective tissue apparently sends trabeculæ into the substance of the pituitary body and break it up into a series of solid follicles, as has been described by W. Müller for the adult (25). At the time of metamorphosis, as is well known, the nasal canal becomes greatly enlarged, both in length and breadth, extending downwards and backwards between the brain and the pharynx. The hypophysis (Fig. 21, Pl. IX) now lies imbedded in the upper wall of the canal, and in contact with the infundibulum.

The morphological signification of the pituitary body is a question of great difficulty, as to which the most diverse opinions have been held. The study of its development in *Petromyzon* would seem to bring us at least one step nearer to the solution of the problem. In most vertebrates this body is derived from a diverticulum of the stomodæum, agreeing with the process in *Petromyzon* only in so far as it is of epiblastic origin. The mode of formation in the lamprey is apparently so divergent from that seen in the higher vertebrates, that they seem to have very little in common, and on that ground Balfour declined to accept my account. However, when examined in the light of comparative embryology, much of this apparent divergence disappears, and with the possible exception of the Teleosts, the mode of formation is seen to be fundamentally the same throughout the vertebrate series.

The old hypothesis that the conario-hypophysial tract repre-

sents the invertebrate mouth and gullet is now entirely abandoned, especially since Spencer has conclusively shown the nature of the pineal gland. Götte, as we have already seen, believes that the hypophysis of the higher vertebrates is the representative of the nasal canal (Nasenrachengang) of the Cyclostomata, a view which is mainly supported by the fact that in *Bombinator* a connection between the pituitary involution and the nasal pits occurs. But, as appears from Götte's figures and descriptions, this connection is only by slight superficial folds which also connect the hypophysis with the mouth. In other Amphibia there is no such connection; in the frog and newt, according to Miss Johnson and Miss Sheldon (19), the stomodæum arises as a solid ingrowth of the deeper layer of the epiblast, of which the posterior portion fuses with the archenteron, while the free anterior portion gives rise to the pituitary body. Dr. Orr, who is working in this laboratory upon the development of *Amblystoma*, kindly allows me to make use of the results which he has obtained. Here a solid epiblastic cord grows in from the front of the head and reaches the infundibulum before the formation of the mouth, and there is no connection at any time of the pituitary involution with either the olfactory pits or the mouth. There is no good reason for considering the nasal canal of the Cyclostomata as essentially different from the canals of other vertebrates. In the Cyclostomata, it is true, this canal is median and unpaired; but, as we shall see later, the single olfactory organ of this group has almost certainly been produced by a coalescence of paired organs, and, if this is true, the canals would also coalesce in the same manner. Otherwise the only peculiarity in the nasal canal of the lamprey is in its great size; this implies nothing new, but rather the extension of parts already present. This extension, as J. Müller showed long ago, has been conditioned by the unusual situation of the nasal opening. There is no more reason for regarding the entire canal of the Cyclostomata as belonging to the hypophysis than in the case of the higher vertebrates for regarding the entire stomodæum as belonging to that body.

Dohrn (12) has independently reached Götte's conclusion as to the nature of the canal in *Petromyzon*, and further regards this canal as produced by the coalescence in the median line

of a pair of pre-oral gill-clefts. "Die Hypophysis von Petromyzon ist aber beträchtlich länger, denn wie schon angedeutet, *ist die ganze von den Autoren als Nasengang, blinder Nasensack oder Spritzsack beschriebene Bildung nichts anderes als die vergrösserte Hypophysis.* . . . Ganz anders aber liegt das Ding bei der Hypothese die in der zweiten meiner 'Studien, etc.,' dargelegt ward. Dieser Hypothese zufolge handelt es sich bei der Hypophysis um den letzten Rest einer ursprünglich selbständigen, *vor* dem Munde befindlichen Kiemenspalte. Einer solchen Deutung fügt sich die dauernd erhaltene Structur der Hypophysis in ihren Beziehungen zu den Blutgefässen welche sie in grosser Zahl durchziehen und sie auf einer Stufe mit anderen abgeschnürten Kiemensäcken bringen, die wir als Drüsen unbekannter Function, sogennante Blutgefässdrüsen, an einer grossen Zahl von Vertebraten kennen. Eine solche Deutung wird auch unterstützt durch den Ursprung diesen Blutgefässe aus den gegen den Kopf gerichten Verlängerungen der Kiemenarterien, der Carotiden, und einer solchen Deutung fügt sich nicht nur auf das Bequemste der hier dargestellte thatsächliche Entwicklungsgang der Hypophysis ein, es scheint vielmehr, dass die bei den Petromyzonten beobachtete Function des Nasenganges und Nasensackes, Wasser einzuziehen und wieder auszustossen in directer Linie aus der Function einer Kiemenspalte abzuleiten ist. . . . Bei der Annahme der Hypothese, dass die Hypophysis eine vor dem Munde gelegene, natürlich wie alle übrigen ursprünglich paarig angelegte Kiemenspalte gewesen, gewinnt man auch ein Verständniss für den Umstand, dass sie bei Petromyzon in den Bereich der Nase, bei allen übrigen Vertebraten aber in die Mundhöhle gerathen ist. Als Kiemenspalte gehörte sie bei den gemeinsamen Vorfahren der Petromyzonten und der anderen Vertebraten weder zur Nase noch zum Munde; vielmehr war sie ein selbständiges, beiden, Nase wie Mund, gleichgeartetes, homodynames Gebilde. Erst als aus den auf sie folgenden Kiemenspalten durch Durchbruch der medianen Scheidewand eine einzige grosse Oeffnung, der gegenwärtige Mund, ward, erst da verlor sie ihre Selbtändigkeit und ward bei den nun erfolgenden tieferen Einbuchtung der Körperwandung, welche den ectodermalen Theil des jetzigen Mundes lieferte und bei der Ausbildung einer besonderen Oberlippe bei der einen Gruppe

weiter gegen den Grund der sogenannten Mundbucht geschoben, wo sie dann auch allmählich, wie so viele andere ursprüngliche Einstülpungen der Haut oder Ausstülpungen des Darmes abgeschnürt ward, um durch Wachsthums-Verschiedenheiten gänzlich dislocirt zu werden und in Beziehungen zu treten, die ihr ursprünglich fremd waren, — bei der anderen Gruppe aber wurde sie mitsammt der Nase auf den Rücken über und vor das Gehirn geschoben, büsste aber nicht ihre selbständige Mündung ein."

The chief fact upon which Dohrn relies in support of this hypothesis is derived from his observations on the Teleosts (11), that the hypophysis is formed from a pair of hypoblastic diverticula toward the infundibulum. But, aside from the very great obscurity in the process and uncertainty as to the origin of the cells in question, which Dohrn very candidly admits, the Teleosts form such an exceedingly specialized group, and their developmental history, as at present interpreted, deviates in so many important respects from that of other vertebrates, both higher and lower, that morphological generalizations should be made from their structure only with the greatest caution. In the present case the facts as stated by Dohrn are opposed by the process observed in other groups, where the hypophysis is of unmistakably epiblastic origin. Even the paired condition of the pituitary rudiment in the Teleosts, upon which Dohrn lays such stress, is by no means clear from his figures. It would more accurately be described as a continuous mass of cells somewhat thinned in the median line on account of the close approximation of the infundibulum to the fore-gut.

In *Petromyzon* Dohrn agrees with Götte in regarding the entire nasal canal as belonging to the hypophysis, though without any cogent reason for such a view. I can see no better ground for accepting this hypothesis than in the case of the higher vertebrates for regarding the entire stomodæum as a part of the hypophysis. One assumption is quite as reasonable as the other. Dohrn's view that the hypophysis represents a pair of coalesced gill-clefts, I regard as altogether untenable, if the facts of embryology are to be allowed any weight. A median epiblastic involution from the surface of the head, with no special nerve supply, no remnants of skeletal or muscular structures, and no arterial arch at any stage of its development, has very little in

common with paired hypoblastic diverticula from the alimentary canal provided with segmental nerves and arterial arches, with cartilages and muscles. In the case of the aborted first pair of gill-clefts in the lamprey, which I first identified, and which, as Dohrn has shown, give rise to the ciliated grooves, all the accessory structures of gill-clefts are present at one time or another in the course of development. But it may be replied, that, admitting the facts in the case of the Teleosts to be as Dohrn believes them, and as Ryder (30) is also inclined to believe, this group may have retained the primitive mode of formation of the hypophysis, while other vertebrates have acquired a secondary mode, just as the pineal eye best shows its primitive character in the comparatively highly organized Lacertilia. Now, the two cases are not at all parallel: the pineal eye has been retained in one group, and simply degenerated in the other — a degeneration which might easily occur independently in many groups. With regard to the hypophysis, the question may thus be stated: Is it probable that the primary mode of development should be retained in one highly specialized type, while an entirely new method should be independently assumed by all other groups, higher and lower? Here there is no case of simple degeneracy, but of a transformation occurring independently in widely separated groups, and following essentially the same method in all.

My own view of the relations of the pituitary body in *Petromyzon* has been explained in another place (35), and need only be referred to here. I believe the primary mode of development to be that exhibited by *Amblystomæ* and *Bombinator*, namely, a median epiblastic involution from the surface of the head to the infundibulum, which has either no connection, or a later or secondary connection, with either the mouth or the olfactory organs. In the Selachians, a connection between the stomodæum and the pituitary rudiment is necessarily produced by the immense size of the fore-brain and the great extent of the cranial flexure. In *Petromyzon*, the coalescence of the olfactory organs in the median line of course involves a coincidence of the nasal with the pituitary involution, which is thus seen to be secondary in the same sense as the connection with the stomodæum in the higher groups is secondary. If, as Balfour has maintained, the origin of the involution from

the stomodæum be primitive, the connection between it and the olfactory organ in the Cyclostomata is altogether unintelligible.

With regard to the homologies of the pituitary body with organs of invertebrates, the most probable suggestion seems to be the one made by Balfour (4), Julin (20), and, latterly, by Miss Sheldon (36) comparing it with the ciliated pit of the Ascidians. Hubrecht's view (17) of homology with the proboscis of Nemerteans may, perhaps, have some truth in it, but the evidence is as yet very insufficient. At all events, I think we may safely regard the hypophysis as an organ derived from invertebrate ancestors, and as originally forming a cul-de-sac, which opened upon the surface of the head and was in close contact with the nervous axis.

(*b*) *The Mid-Brain.* — As Shipley has remarked, the line of demarcation between the fore and mid-brains is for some time obscure; but it may, nevertheless, be unquestionably made out even before the time of hatching. In horizontal sections (Fig. 23, Pl. X) there is a widening of the ventricle, produced by a thinning of the walls at the plane where the fore-brain passes into the mid-brain. At a later stage, when the bands of nerve fibres make their appearance on the sides of the spinal cord, hind and mid-brains, it is seen that they stop at the anterior end of the latter, and do not form a definite envelope for the fore-brain (Fig. 24). This condition persists for a considerable period after hatching (Fig. 11, Pl. IX); but now there is an external constriction which shows the limits of the two regions very clearly, and in larvæ of 15 mm. length and upwards the relations are perfectly plain (Fig. 15, Pl. IX). The separation between the mid and hind-brains is much more obviously indicated from the first. Several observers, especially Shipley, have noticed that in advanced embryos the roof of the entire brain is in the median line only a single cell thick, and that at this stage a dorsal fold of some depth occurs at the junction of the mid and hind-brains. With the gradual correction of the cranial flexure, already mentioned, this dorsal fold deepens and becomes more conspicuous.

There has never been any difference of opinion as to the dorsal limits of the mid-brain; but Ahlborn's account of the structure in the adult calls for some examination. According to this observer, the mid-brain cannot be regarded as having

any floor at all, the hind-brain coming up to the constriction immediately behind the infundibulum, and the third nerve arising from the anterior part of the hind-brain. (See Ahlborn's Fig. 2, Pl. XIII, Zeitschr. f. wissensch. Zool. Bd. XXXIX.) Now, without discussing the considerations which have led Ahlborn to adopt this view, as they are more or less of an *à priori* nature, it will suffice to say that his interpretation does not at all correspond to the condition found in embryos and young larvæ, as in them a vertical constriction runs down the sides of the brain, from the roof, where the dorsal fold, already mentioned, occurs, to the floor, enclosing a ventral section of the mid-brain of very considerable length. As to the third nerve, there can be no doubt that in the Selachians this nerve arises from the mid-brain, and it would require very strong evidence to prove that in *Petromyzon* it has a different origin. The ciliary ganglion and its root, whether or not that be the third nerve, undoubtedly arises from the embryonic mid-brain, and sections of embryos demonstrate in the clearest manner that the floor of the hind-brain does not extend to the infundibulum.

If Ahlborn has been led to his view by the fact that the notochord extends as far forward as the infundibulum, it should be remembered that this extension is a secondary matter. Primarily, of course, the notochord does not extend beyond the hypoblast, from which it has been derived, but after its separation it grows considerably in advance of the hypoblast. But the anterior end of the hypoblast is in very nearly the same vertical plane as the division between the mid and hind-brains (Figs. 2, 3, and 6, Pl. VIII). Primarily, at least, the mid-brain is, as Ahlborn calls it, præchordal, though the notochord does eventually extend beneath it for nearly its entire length.

The mid-brain undergoes comparatively slight changes in the course of larval development. At the time of hatching, its ventricle is large proportionally, and somewhat expanded above. The roof is thin, and two considerable bands of fibres occupy the sides. These fibrous bands increase rapidly at the expense of the cells, which become reduced to a thin layer surrounding the cavity. The ventricle narrows very much except dorsally, where the roof expands and becomes exceedingly thin, and, receiving a large number of blood-vessels, projects in complex

folds into the cavity, thus forming the choroid plexus of the mid-brain. This is not shown in any of the figures, but does not differ except as to size from the plexus of the hind-brain (Figs. 13 and 14, Pl. VIII). Special protuberances to form the optic lobes do not appear till late in larval life; obviously a retardation, due to the rudimentary condition of the eye during the larval state, which, we have already seen, has a profound effect upon the development of the brain.

(*c*) *The Hind-Brain.* — This region of the encephalon has likewise a very simple course of development. It is from the beginning very much larger than either of the anterior regions of the brain, though proportionally short when compared with the length of the entire animal. In early stages it but slightly exceeds in diameter the spinal cord, but at the time of hatching it has considerably increased in size. The ventricle is narrow, but somewhat expanded at the top and roofed in by a single layer of cells (Fig. 29, Pl. X). The white matter is confined to bands on the sides, while most of the walls consist of closely-crowded ganglion-cells. The fibres increase rapidly in number, and in larvæ of 15–20 mm. in length make up most of the substance of the hind-brain (Fig. 34, Pl. X), the cells being reduced to a layer surrounding the ventricle and scattered nuclei for the roots of the cranial nerves. At the same time, the hind-brain changes its form (compare Figs. 29 and 34, Pl. X), the dorsal region of the ventricle widening out, and the roof becoming very thin and membranous. A vascular network appears and forms a choroid plexus which projects into the cavity of the ventricle, though separated from it by the membranous roof (Fig. 13, Pl. IX). This thinning of the roof is widest anteriorly, and tapers posteriorly to a mere slit (Fig. 15, Pl. IX). There appear very early in both mid and hind-brains a series of alternate widenings and narrowings of the ventricles, giving the internal border, when seen in horizontal section, a scalloped appearance, which persists for a considerable period (Figs. 15, Pl. IX, and 23, Pl. X). In the hind-brain, some of the expansions, at least, seem to stand in relation to the roots of the cranial nerves (see Fig. 23), as Balfour has described for the vagus roots in the Selachian embryo; and they may possibly be regarded as of segmental value in the mid-brain, though this is

not likely, as only one cranial nerve, the third, has its origin in this region.

The *cerebellum* is formed, as Ahlborn has shown, by the appearance of fibres in the posterior wall of the already-mentioned dorsal fold, which marks the junction of the mid and hind-brains. This fold is found in the embryo, but I have not detected the presence of fibres in larvæ of less than 20 mm. length. This portion of the roof of the fourth ventricle (for this fold is morphologically, and at a later stage actually, a part of the roof) is, of course, not included in the thinning which accompanies the formation of the choroid plexus. The cerebellum is always very narrow (in the antero-posterior direction), but especially so in the early stages of its formation, when it is very minute. It is larger in the adult than in any larval stage.

2. *The Spinal Cord.* — The spinal cord of the lamprey is, as has long been known, remarkable for its curiously-depressed shape, which, in tranverse section, appears like that of a very much flattened bean. This peculiarity is not known to occur outside the group Cyclostomata, and it is, therefore, interesting to observe that the character is not an original one, strongly marked from the beginning, but is, on the other hand, of comparatively late appearance. In advanced embryos, the spinal cord has a greater vertical than transverse diameter; the lumen is contracted in the middle and somewhat expanded dorsally and ventrally, having thus a section somewhat like the figure 8; the fibres appear as two isolated bands along the sides (Fig. 38, Pl. XI). The roof and floor of the canal are, as in the case of the brain, formed by a single layer of cells. Shortly after hatching the fibrous bands increase very much, and extend around the entire circumference of the cord, which, however, still retains its former shape. In Ammocœtes of 7 mm. length the change of form begins to appear (Fig. 44, Pl. XI); the transverse diameter of the cord is now greater than the vertical, the bands of fibres being especially thick along the sides; the ganglion cells are reduced in number, and have commenced to extend laterally; the canal is much smaller, widening below, and reduced to a mere slit above; a slight protuberance or keel runs along the ventral median line of the cord. In the next stage, of 14 mm. (Fig. 45), these changes are carried still

further; the broadening and flattening of the cord, the reduction of the canal, the increase of the fibrous bands, and the lateral extension of the cell mass are all more clearly marked. The third stage, of 30 mm. (Fig. 46), shows the changes in the same direction more pronouncedly, especially the increase of the white matter, the broadening and depression of the mass of cells, and the diminution of the canal. In large Ammocœtes (Fig. 47), the cord has assumed the characters found in the adult. Here the cells form a very small proportion of the whole, and the canal has become circular in shape and minute in size.

Several intermediate stages between Figs. 46 and 47 might be shown, but those given are sufficient to make the process intelligible. The point to be emphasized is that the features peculiar to the Cyclostomata are of comparatively late appearance, while the early stages are in entire accordance with those of the higher vertebrates.

3. *The Cerebro-Spinal Membranes.* — Langerhans (22), Ahlborn, and others have shown that cerebral membranes, in the proper sense of the word, are not present in *Petromyzon*. The brain is encased in a soft cellular tissue with firmer internal and external layers, which the older writers named *dura* and *pia mater*. On passing from the brain to the spinal cord, certain histological differences in this tissue are noticeable, which need not be dwelt upon here. In advanced embryos and young larvæ, indifferent mesoblastic cells gradually enclose the nervous axis, part of which give rise to a dense fibrous envelope (Figs. 34, Pl. X; 44, Pl. XI), separated from the nervous axis by numerous small rounded cells. In larvæ of 20 mm. and upwards in length, branched pigment cells become conspicuous in this tissue, which at the time of metamorphosis becomes greatly increased in bulk. In young larvæ (Figs. 45–46), a distinct layer of small spindle-shaped cells may be observed surrounding the spinal cord; but I have not been able to detect this in older larvæ, nor at any stage in connection with the brain.

II. The Peripheral Nerves.

The development of the peripheral nerves is exceedingly difficult to follow accurately and continuously, both on account

of their very small size, and because in early stages the organs are so closely crowded together, and the tissues so loaded with yolk-granules, that it is often almost impossible to distinguish between them. By long-continued study, however, I have obtained an account, more or less complete, of the development of most of the nerves; but of some, such as the trochlearis and abducens, I have not been able to observe any trace whatever.

1. *The Olfactory Nerves.* — The development of the olfactory nerves is very obscure, as the nasal epithelium is differentiated at an early stage, and lies in immediate contact with the fore-brain, even before the formation of the hemispheres. In other vertebrates there is usually a space between the brain and the nasal pits, which allows the appearance of the nerve to be observed, but in *Petromyzon* there is no such space. When the nerve first appears, I cannot say; but in larvæ of about 10 mm. an appearance, perhaps deceptive, of fibres passing from the fore-brain to the olfactory epithelium may be seen. At a later stage (Fig. 11, Pl. IX), the nasal pit is somewhat farther removed from the brain, and shows the exceedingly short olfactory nerve (in Fig. 11 it is cut through obliquely). In larvæ of 22 mm. (Fig. 35, Pl. X), the nerve is very plainly shown, but the ganglion has not as yet been differentiated. The ganglia appear at a much later period, and apparently are derived from the olfactory epithelium, though I have not observed the process. With the great increase in size and complexity of the olfactory organ, which takes place at the metamorphosis, the olfactory nerves undergo a corresponding increase (Fig. 22, Pl. X). The olfactory ganglia are now very conspicuous bodies, and send off numerous bundles of fibres, which pass along the septa between the chambers. The ganglia are contained within the nasal capsule, and are connected by short nerve-trunks with the olfactory lobes. As far as I have been able to observe, the olfactory nerves are paired from the first, — a fact which has an important bearing upon the question of the primitive or secondary mode of origin of the median nasal organ characteristic of the Cyclostomata.

Shipley, as already mentioned, has described and figured a mass of cells, which he believes to be nervous, which are at first unconnected with the brain, but continuous with the nasal pit and canal, and which he suggests may give rise to either

the olfactory lobes or the ganglia. I cannot agree with Shipley in considering this tissue to be nervous, for, when examined with high powers and strong illumination, the constituent cells are seen to be polygonal epithelium cells, very different in character from the rounded ganglion cells found in the brain at this stage. They would seem to be simply a part of the thickened epithelium of the nasal involution. But be this as it may, these cells cannot give rise to either the olfactory lobes or ganglia, for they remain unchanged in position and appearance long after the formation of the olfactory lobes and nerves, and are included between these structures as a single mass. The ganglia must, of course, arise at the distal ends of the olfactory nerves.

2. *The Optic Nerves.* — These are peculiar for the very large proportion of the primary optic vesicle which goes to their formation. In other vertebrates the retina takes up nearly the whole of the primary vesicle, while the nerves are at first very short and grow out at a later stage, but in the lamprey the retina is at first exceedingly minute (Figs. 24 and 26, Pl. X) and the optic nerves correspondingly long. The cells of the hollow stalk thicken and obliterate the lumen, and then begin to develop fibres (Fig. 26), a transformation which is completed either before or shortly after the time of hatching. Throughout larval life the optic nerve remains very slender and inconspicuous, in accordance with the undeveloped condition of the eye; but at the period of metamorphosis it becomes much stouter, when the eye reaches its final stage of completion.

3. *The Oculo-Motor Nerves.* — Shipley states that the eye-muscle nerves are not developed till a late stage; a statement which is very probable in view of the rudimentary character of the eye-muscles during larval life. I have not succeeded in finding any trace of the fourth and sixth pairs in the larvæ, and, unless the ciliary ganglion be regarded as belonging to it (iii., Fig. 41, Pl. XI), none of the third pair. This ganglion appears early, though I cannot describe its mode of origin; it lies above and somewhat internally to the ophthalmic branch of the fifth.

5. *The Trigeminal Nerves.* — These nerves are very conspicuous objects in the later embryonic stages, where the large Gasserian ganglia bulge out the side walls of the head (Figs. 23 and 24, Pl. X). According to Shipley the ganglion is derived

from an epiblastic thickening immediately behind the lens invagination, a statement which I can confirm. It is, at first, apparently single, but soon divides into two parts (Figs. 39, 40, and 41, Pl. XI), one of which lies above the optic vesicle and belongs to the ophthalmic branch, while the other gives rise to the maxiliary and mandibular branches. I have not observed any connection between the Gasserian and the ciliary ganglia.

7. *The Facial Nerves.* — Much dispute has arisen of late as to the distribution of the seventh pair, and its history, therefore, needs to be examined with especial care. The facial nerve arises at about the same period as the trigeminal as an outgrowth from the hind-brain, which fuses with a thickening of the skin, the future ganglion. According to Shipley this ganglion is at first continuous with the Gasserian, but this stage I have not seen; its connection with the skin is, however, plain. The main trunk of the nerve soon divides into three branches, one of which, the *ramus recurrens*, passes backward beneath the auditory vesicle. Shipley states that in young larvæ no connection exists between this recurrent branch and the vagus, nor have I succeeded in finding it. It probably is brought about at a comparatively late stage of larval development. Of the other branches of the facial the posterior passes down between the first and second gill-clefts, while the anterior arches over and runs down in front of the first cleft, which later gives rise to the ciliated circumoral ring.

Marshall and Spencer (23) have described the formation of a secondary root and atrophy of the primary root of the seventh nerve in the case of *Scyllium*. Fig. 42, Pl. XI, shows a somewhat similar process taking place in the young *Ammocœtes*, where two distinct roots, a dorsal and ventral, connect the brain with the facial ganglion. I have, however, seen nothing which suggests an atrophy of the dorsal root, and judging from the origin of the facial in the adult, it is very unlikely that the ventral root, seen in Fig. 42, can become the permanent root of the seventh. Wiedersheim (41) and Ahlborn have shown that in *Petromyzon* the auditory nerve has a double origin, and not improbably the ventral strand passing to the facial ganglion may eventually become one of these.

Julin (see Van Beneden, 40) has stated that in *Ammocœtes* the seventh nerve supplies the first *permanent* (*i.e.*, second)

gill-cleft. Not having seen Julin's figures as yet, I do not know upon what evidence his statement rests, but I think it must be a mistake. In the adult the facial has no such distribution. Fürbringer's (14) account of this nerve in *Petromyzon* is as follows: —

"Wenig kräftiger, rein sensibler nerv. Tritt mit dem Acusticus durch eine Oeffung in die Gehörkapsel, durchbohrt den vorderen Umfang derselben schräg nach unten und vorn um dicht an der Aussenfläche derselben den

"1) Ramus recurrens abzugeben, der sich in nahezu horizontalem Verlauf nach rückwarts um die Aussenfläche der Gehörkapsel schlägt um mit dem Ramus lateralis des Vagus, nahe dessen Abgang von letzterem zu verschmelzen. Die Fortsetzung des Stammes verläuft weiter nach vorn und lateralwärts, zwischen Auge und subocularem Bogen, sich mit dem Hautaste des Ram. ext. Trigem. unter spitzem Winkel kreuzend über ihn tretend. Kurz vor der Kreuzungsstelle giebt er den

"2) Ramus posterior ab, welcher nach hinten, aussen und unten verlaufend zwischen der dorsalen und ventralen Portion des Seitenrumpfmuskels unter die Haut dringt in dieser zwischen Auge und 1. Kiemenloch verästelnd."

I have satisfied myself of the accuracy of these statements, and also that in the adult the first permanent cleft (second of the primary series) is supplied by the ninth, or glosso-pharyngeal nerve, which is in harmony with the account given by Ahlborn (2). Sagittal sections of late embryos and very young larvæ clearly show the branches of the facial forking over and supplying both sides of the first (primary) cleft. Shipley also states that this nerve is distributed to the circumoral ciliated groove, in agreement with Dohrn's description. It would be exceedingly remarkable if the older larvæ should turn out to differ so radically both from the embryo and the adult with regard to the distribution of the seventh pair of nerves, as is indicated in Julin's statements. But even admitting the correctness of his observations, there can be no possible doubt that the embryo of *Petromyzon* possesses eight pairs of branchial clefts. Huxley's account of this (18), which first directed my attention to the subject, is incorrect, as at the stage which he describes and figures the first pair of pouches has long since been converted into the ciliated groove, but in the embryo

it is perfectly clear that there is present a pair of hypoblastic diverticula immediately behind the mouth and in advance of the first permanent gill-pouches. (See my former paper (34), Fig. 38, Taf. X.) These diverticula are in every respect like those which form the permanent clefts, with regard to mesoblastic segments, arterial arch, nervous and skeletal elements, differing only in the fact that they do not seem at any time to perforate the skin. This observation has been abundantly confirmed by Dohrn (13) and Shipley. Now, if Julin's view that the first *permanent* cleft is the homologue of the spiracle in the Selachians be correct, the development of *Petromyzon* demonstrates the correctness of the hypothesis advocated by Dohrn and Beard (6), that the hyoid arch is composed of two cranial segments. But, from the evidence now at command, I cannot but conclude that the first permanent cleft is supplied, not by the seventh, but by the ninth nerve, and in consequence that the transitory cleft is the representative of the Selachian spiracle.

8. *The Auditory Nerves.*—The eighth pair of nerves opposes several difficulties to the following out of its development. Shipley's account is very brief, and is as follows: "A few fibres from the brain enter the recessus labyrinthi of the ear; these arise close to the root of the seventh, and constitute the eighth nerve." These fibres I have not seen, but they may possibly constitute the dorsal root of the acusticus. The main portion of the eighth nerve appears to arise from a common root with the seventh, and its ganglion would seem to be continuous with the facial ganglion (Fig. 39, Pl. XI); and it is not at all improbable that the ventral root seen in Fig. 42, Pl. XI, which joins the facial ganglion, is one of the roots of the auditory nerve. I have not detected any fusion of the auditory ganglion with the epiblast of the auditory involution, although it is very probable that such a fusion takes place; if so, it must occur before the ganglion is separated from that of the seventh nerve. The auditory ganglion becomes separated from the facial, and lies close against the auditory vesicle, and is in more advanced larvæ enclosed within the wall of the cartilaginous ear-capsule (Fig. 34, Pl. X), and from it fibres may be traced into the membranous labyrinth. At this stage the auditory nerve arises from a mass of cells high up in the side wall of the hind-brain, the

trunk passes downwards closely applied to the brain-wall, and, entering the ear-capsule, passes into the large and conspicuous ganglion. At this stage I cannot detect the two auditory roots so clearly shown in the adult, nor the division of the auditory nucleus in the hind-brain. On the contrary, this nucleus appears to be continuous with the nucleus of the facial, and, perhaps, even with that of the trigeminus. Only a very imperfect division of this elongated mass of cells is indicated.

9–12. *The Vagus Group.* — The large complex of nerves which arise immediately behind the auditory capsule has a somewhat curious history. The earliest stage is shown in Fig. 36, Pl. XI, where is seen a series of nerve-roots arising from the hind-brain, each one provided with a ganglion, and all connected by means of a longitudinal commissure. Owing to the fact that the hind-brain, even in the earliest stages, is overlapped by the mesoblastic somites, the first of which is placed immediately behind the auditory vesicle, instead of being separated from it by a considerable interval, as in the Selachians, it is exceedingly difficult to say how many of these roots belong to the cranial and how many to the spinal series. The important fact to notice is that these vagus elements are exactly like the dorsal roots of the spinal nerves, and form a continuous series with those roots with which the commissure connects them (Fig. 37, Pl. XI). On account of this fact, and the position of the mesoblastic somites, one cannot well determine the limits of the vagus group. This stage is essentially the same as Balfour has figured in the Selachians; but the two series are more closely continuous, and not separated by a marked interval, as in those fishes.

In a very short time (*i.e.*, in embryos nearly ready for hatching, and in just hatched larvæ) the appearance of the vagus group has essentially changed, and a great concentration has taken place (Fig. 3, Pl. VIII, and Fig. 40, Pl. XI). The ganglion of the ninth nerve is placed most inferiorly, and somewhat above and behind this are two ganglia, both of which probably belong to the vagus. The ninth nerve has two branches, one of which passes forwards over the hyobranchial (first persistent) cleft and then downwards in front of it, and the other straight downwards between the first and second (persistent) clefts (2 and 3, Fig. 3, Pl. VIII). In the adult the

direction taken by those branches of the glossopharyngeal is somewhat, but not essentially, different from that seen in the very young larvæ; a change which is brought about by the shifting of the entire branchial apparatus posteriorly. In the embryo and young Ammocœte the hyobranchial (first permanent) cleft is situated immediately beneath the auditory vesicle (Figs. 3 and 5, Pl. VIII), while in the adult the branchial basket has become greatly extended and shifted backwards, so that the first (permanent) cleft comes to lie distinctly posterior to the ear-capsule. The nerves, of course, follow this shifting, and the anterior branch of the ninth nerve now passes downwards and somewhat backwards, instead of forwards, as in the embryo.

In the stage shown in Fig. 40, Pl. XI, there would seem to be two distinct vagus ganglia connected with the brain by three, or perhaps four, roots, and it is noticeable that these roots are relatively much more widely separated than in the older larvæ or the adult, as figured by Wiedersheim and Ahlborn. From these ganglia the branchial nerve for the six posterior gill-clefts proceeds, and shows a ganglionic swelling between each pair of clefts. Whether these ganglia contain any elements derived directly from the epiblast of the skin, I was unable to determine. The most noticeable fact about this stage in the development of the vagus group is the great concentration of its elements as compared with the stage shown in Fig. 36, Pl. IX; and later stages still further increase this concentration, and the two vagus ganglia coalesce into a single one (*i.e.*, assuming that these two ganglia really belong to the vagus, as there is every reason to believe). The lateral nerve is exceedingly small, and, as Langerhans (22) showed, peculiar in position, being placed very high up towards the median dorsal line, a peculiarity which is seen from the first appearance of the nerve. I believe I have traced the origin of this nerve from a progressive differentiation of the epiblast, beginning in the region of the vagus ganglia and proceeding posteriorly. This takes place in the later embryonic stages, but I have not demonstrated the process completely to my satisfaction. At all events, the lateral nerve is unmistakably present very shortly after hatching, a much earlier period than Shipley has supposed, as from its minute size one may very easily overlook it. It is best seen in transverse sections, and lies close to and just above the spinal

cord (Figs. 44–47, Pl. XI), running back to the end of the tail.

This account of the development of the ninth and tenth nerves is somewhat different from that given by Shipley, but the difference consists for the most part in the fact that he has not observed some of the stages. He has overlooked the stage figured in Fig. 36, Pl. XI, and says that "the ganglia of the ninth and tenth nerves would seem to arise from a mass of cells split off from the epiblast close behind the ear." Nor does he seem to have found the second vagus ganglion, or the lateral nerve. "There is no trace of the ramus lateralis even in my oldest larvæ."

Born (10) has mentioned a very interesting and peculiar connection between the vagus and the anterior spinal nerves, as occurring in the branchial region. Unfortunately I have been unable to discover how and when this connection takes place, nor could I obtain any observations upon the formation of the hypoglossus. From its appearance and mode of origin in the adult, it would seem to represent the ventral roots of the vagus group; but this is, of course, uncertain until its developmental history can be worked out.

The Spinal Nerves. — The development of the spinal nerves has been worked out by Sagemehl (31), and his account is confirmed by Shipley. My own observations agree, as far as they go, with those of the observers mentioned, and I have nothing to add to their statements. The most important fact to be mentioned is, that the dorsal roots are primarily all connected by a commissure (Fig. 38, Pl. XI), as was first described by Balfour for the Selachians, and since then observed in many other types. Shipley states that the spinal ganglia are situated opposite to the myotomes, whereas all the sections I have examined show them between the myotomes (Fig. 38). The spinal commissure, as already mentioned, is continuous with that which unites the ganglia of the vagus and glossopharyngeal; and this would seem to negative, so far at least as the post-auditory cranial nerves are concerned, the view maintained by Beard of a radical and fundamental difference between the cerebral and spinal nerves. It is true that the ganglia of the former have a different mode of origin from the latter in that they are partially derived from the external epiblast. But this process, I am in-

clined to think, is a secondary one, brought about in connection with the development of the sense-organs of the skin, which do not occur in connection with the spinal nerves, and have evidently extended from the head to the trunk. In the anurous Amphibia, as described by Spencer (38), the process has extended a step farther, and the nerve-trunk, as well as the ganglion, is split off from the epiblast, and this can hardly be regarded as the primary mode of formation. Certainly the entire resemblance of the post-auditory cerebral nerve-roots to the dorsal roots of the spinal nerves, and the connection of these cranial roots by means of a continuous commissure with the spinal nerve, is, to say the least, suggestive of a much closer homology than Beard is inclined to allow them. This suggestion is further strengthened in the case of *Petromyzon* by the apparent presence of ventral roots, represented by the hypoglossus. In the Selachians these ventral elements have been suppressed, or, perhaps, never differentiated. Beard's view rests upon the assumption that the ganglia of the cranial nerves originated simultaneously with the so-called "branchial sense-organs." But it is equally probable that these ganglia primarily arose entirely from the nervous axis, just as do the spinal ganglia, and that the sense-organs originated later in connection with the ganglia. That the ontogeny should have been abbreviated, and that the two sets of organs in most existing types should arise together, is certainly nothing strange. It does not at all follow from this that the cranial and spinal nerves are identical in plan, as indeed they clearly are not, or that the cranial nerves were originally such as the spinal nerves are now. On the contrary, Balfour's view seems much more probable, that the two classes of nerves are differentiations along diverging lines from a primitive form common to both. It may very well prove to be the case that the *post-auditory* cerebral nerves conform in the course of their development much more closely to the type of the spinal nerves than do the anterior cranial nerves, as would indeed be expected from the much greater differentiation of the organs which the anterior nerves supply.

III. THE SENSE-ORGANS.

The development of the organs of sense in *Petromyzon* offers several peculiarities, most of which are, however, simply due to

retardation, either in the time of their formation or in their subsequent differentiation. This retardation is, doubtless, to be explained by the habits of the larva, which lives buried in sand or mud, and to which the organs of higher sense, more particularly that of vision, could be of little or no service. A great increase in the size, complexity, and perfection of the sense-organs takes place at the time of metamorphosis, when the sexually complete animal seeks the clear water and abandons its subterranean habits.

1. *The Sense-Organs of the Skin.* — Beard (6), in the beautiful observations already quoted, has followed the development of the sense-organs of the skin in the Selachians, and has given a very complete account of their formation in that group. He finds that these organs arise from the epiblastic thickenings which fuse with the roots of the cranial nerves, and, partially at least, give rise to their ganglia. In the case of the lateral line, the epiblastic proliferation, which forms the lateral branch of the vagus, also develops the sensory portion of the skin. In *Petromyzon* the history of these organs is apparently quite different. Shipley was unable to observe any rudiment of these organs in connection with the thickenings of the epiblast, which assist in the formation of the ganglia of the central nerves; and, in spite of the most careful and persistent search through large series of sections taken in all three planes, I have had no better success. So far as I can make out, the serial sense-organs in the head, and those of the lateral line, arise quite independently of the cerebral nerves which supply them. Shipley has, however, placed the period of their formation much too late, saying that they had not appeared in his oldest larvæ (52 days after impregnation). I have first detected them in larvæ of about 7 mm. in length, and here only in the upper lip. Their mode of formation is very simple: a small group of epiblastic cells begins to increase in vertical height, and by pushing aside the surrounding cells, reaches the surface. The increase in height is accompanied by a decrease in breadth, so that the cells have the appearance of protoplasmic rods, which pass directly from the underlying mesoblastic dermis to the surface of the head, while the surrounding indifferent epiblast is made up of several layers of more or less flattened cells. A pit is formed, by a slight separation of the indifferent cells from

the "sense-bud," and, as the latter is somewhat constricted at the top, the opening of the pit is smaller than its interior. The organs in other parts of the head and in the trunk region I have not detected till more advanced stages; but their mode of formation would seem to be the same. The distribution of these organs in the Ammocœte and the adult is well figured by Langerhans, and the remarkably high position taken by the lateral nerve is followed by the organs of the lateral line, which are separate "sense-buds," and not a continuous line of epithelium.

It may, no doubt, be eventually proved by some more fortunate observer that these sense-organs arise in the lamprey, just as they do in the Selachians, from the epiblastic thickenings which form the ganglia of the cranial nerves. But, assuming the correctness of the account here given, which is to be regarded as the primary mode of formation, that seen in *Petromyzon*, or that shown by the Selachians? The evidence, it seems to me, points very strongly to the view that the Selachians exhibit the primitive method, especially when we remember the remarkable retardation which affects the development of nearly all the sense-organs of *Petromyzon*. For, aside from the fact that the process is much more clearly and completely known in the case of the Selachians, the fusion of the cranial nerve-rudiments with the epiblast (whether that fusion be itself a primary or a secondary process) is unintelligible except in connection with the development of the sense-organs; nor can we suppose that the latter have become differentiated from the skin otherwise than in connection with the nerves. Especially is it difficult to understand how a branch of a cerebral nerve, the lateral, came to extend itself throughout the entire length of the trunk, unless that extension were conditioned by an accompanying extension of the sensory organs which primarily belonged to the head alone. So far, I think, Beard's position is altogether reasonable and probable. I cannot follow him, however, in regarding these organs as especially branchial; they appear rather as segmental organs of the head, and their occurrence in front of the mouth by no means implies the former existence of pre-oral gill-clefts, as, indeed, Beard admits, though much of his reasoning tacitly implies the existence of pre-oral clefts.

2. *The Olfactory Organ.* — The nasal epithelium appears very early, in embryos of 17–18 days, and is formed by the increase in height of the epiblastic cells situated along the median line of the ventral surface of the head. The sensory epithelium speedily divides into two imperfectly distinguishable layers of spindle-shaped cells (Fig. 2, Pl. VIII), the superficial one being ciliated, as Shipley has shown. At this stage the olfactory organ forms a thick mass, which projects inwards beyond the level of the skin and lies in close contact with the fore-brain; posteriorly it is continuous with the pit of columnar epithelium, which will later give rise to the pituitary body. The organ is from the first single and median in position, and even the median fold, which eventually indicates a division into lateral halves, does not appear until a much later stage. The next alteration of importance is the shifting of the opening of the nasal pit from the ventral to the dorsal surface of the head. This, as already indicated, is brought about partly by the rotation of the head, which accompanies the correction of the cranial flexure, so that the nasal epithelium presents forwards instead of downwards, as before (Fig. 3, Pl. VIII; Fig. 12, Pl. IX). As far as the position of the olfactory organ itself in relation to the nervous axis is concerned, it does not materially change from this time onwards; but this process of itself would cause the organ to remain at the anterior end of the head, as in Fig. 3. The great extension and rotation of the upper lip changes the position of the nasal opening very essentially; by advancing the anterior wall of the pit, it restricts the opening to a small pore, and brings the pore to the top of the head, a considerable distance behind the front end of the lip. It is thus evident that the very peculiar and exceptional situation of the olfactory organ in the Cyclostomata has reference rather to the position of the opening than to that of the olfactory epithelium, except in so far as the latter is single and median. It is likewise clear that the opening of the nasal canal upon the dorsal surface of the head is a necessary result of the mode of development of the upper lip and the formation of the suctorial disc.

Throughout larval life the olfactory organ remains very simple and unfinished in character. The first essential change appears in larvæ of about 10 mm. in length, where a median

thickening imperfectly divides the cavity into lateral chambers (Fig. 11, Pl. IX, and Fig. 35, Pl. X). In larvæ of 43 mm. in length this median thickening of the epithelium cells has become very much thinner than that which lines the chambers, though its character as a septum is now very much more clearly indicated. This septum is for the most part made up of a mass of connective tissue covered by a thin layer of columnar epithelium, like that which lines the anterior non-olfactory parts of the nasal chambers. The two chambers are now deeper and more distinct; the sensory epithelium is thickened and is confined to the posterior and external walls of the chambers.

Shortly after the shifting of the nasal opening from the ventral to the dorsal surface of the head, a ridge-like elevation of the skin, including both dermis and epidermis, is formed around the opening. This ridge rapidly grows in height and forms a freely-projecting funnel, at the bottom of which lies the nasal pore. The funnel is a conspicuous object in sections of advanced larvæ through this region of the head. The nasal organ proper, then, of the Ammocœte may be described as a pair of imperfectly separated epithelial chambers, continued into a short and narrow canal which ends blindly, though probably still connected with the hypophysis. Langerhans' figure of a section through the olfactory organ of the Ammocœte represents a stage more advanced than any that I have been able to find in a larva; it was probably taken from a specimen just at the beginning of the metamorphosis into the adult animal. At no time is there any indication of "smell buds," such as Blaue (9) has described in the Teleosts and Amphibia, but, as in the Selachians, the sensory cells extend throughout the olfactory organ proper (which does not include the anterior wall of the chambers), and seem to have entirely displaced the intervening indifferent epiblast cells.

In the adult lamprey the olfactory organ undergoes a great increase in size and complexity; radiating septa covered with columnar ciliated epithelium (Fig. 22, Pl. X) extend from the circumference towards the centre, each septum being supplied with fibres from the olfactory ganglia. At the same time the naso-palatal canal, which throughout larval life remained exceedingly small and almost rudimentary, now becomes greatly

enlarged, both in length and in diameter. It apparently functions as an apparatus for drawing water over the sensory epithelium, and so compensates for the removal of the olfactory organ from the region of the mouth.

An accessory organ is developed from the postero-inferior portion of the olfactory involution, which is of doubtful significance. It first appears in larvæ of about 12.5 mm. in length (J. Fig. 18, Pl. IX) as a small diverticulum of epiblastic cells below the sensory cells of the olfactory epithelium. This diverticulum gradually increases in size and apparently becomes constricted off from the nasal chambers, though, possibly, it retains some connection with them (Fig. 20, Pl. IX). In this stage the posterior wall is much thicker than the anterior, and especially thickened in the median line. In transverse sections of larvæ 26 mm. long, the diverticulum is seen dividing into two lateral portions by means of a median constriction. Anteriorly, the two portions remain connected, but posteriorly they are separated and form two blind sacs. From this stage of development I have detected no change during larval life. In the adult, however, this simple rudiment has become greatly enlarged and very much more complex, forming apparently a large median gland, having many follicles which are lined by low columnar epithelial cells. This gland is contained within the cartilaginous nasal capsule at its postero-inferior portion, and is situated below the olfactory lobes (Fig. 22, Pl. X). It is best seen in transverse sections, which show that its main mass is placed between and below the olfactory lobes. I have not been able to detect any opening of this apparent gland into the chamber of the olfactory organ or into the naso-palatal canal, though such communication not improbably exists. Apparently all of the follicles are connected together.

Just what this organ represents it is difficult to decide, though to all appearances it is the representative of the organ of Jacobson. Götte has described the formation of this organ in *Bombinator* (Unke, p. 654) as a diverticulum from the nasal pit, which becomes connected with a gland formed from the epithelium of the stomodæum. In *Petromyzon* the oral epithelium has no share in the formation of the organ in question, nor does the latter at any time have any communication with the mouth. If this organ is to be identified with that of Jacob-

son, it can correspond only to the nasal portion of it, and it will be somewhat remarkable to find the organ of Jacobson so low in the vertebrate series.

Is the unpaired condition of the olfactory organ in the Cyclostomata to be regarded as a primitive or as a secondary character? I think there can be no hesitation in accepting the latter alternative. If the organ were primarily a single one, it would be impossible to account for the fact that the olfactory nerves are paired from the time of their first appearance. Were the olfactory lobes and nerves unpaired and median, there would be good grounds for considering the single nasal organ as the primitive condition, but not otherwise. The fact that the olfactory organ is unpaired at its first appearance and only in later stages exhibits a tendency towards the formation of a paired structure, is no ground for assuming that the unpaired condition is the primitive one, for such abbreviations of development are exceedingly common. Primarily, then, we have every reason to believe that the olfactory organs, like all the other organs of higher sense, were double. The reason for the coalescence of the paired nasal pits into a single median pit is, I think, to be found in the development of the type of suctorial mouth peculiar to the Cyclostomata. We have already seen that the position of the olfactory epithelium and its relations to the mouth are very similar in the early stages to the condition seen in the Selachians, but that the extension and rotation of the upper lip brings the opening of the pit to the dorsal surface of the head. This displacement and removal of the opening from the neighborhood of the mouth necessitates the enlargement of the naso-palatal canal to supply some ready means of providing the olfactory organ with a current of water. This was long ago pointed out by Johannes Müller (24): "Die Cyclostomen bedienen sich aber entweder gar nicht des Mundes zum Einathmen oder wengistens nicht beim Ansaugen, vielmehr muss dann das Einathmen und Ausathmen durch dieselben Oeffnungen der Kiemen geschehen. Da nun letztere zugleich weiter als bei den übrigen Fischen zurückweichen, bei den Myxinoiden sogar durch einen sehr grossen Raum vom Kopfe getrennt sind, so folgt dass das Athmen der Cyclostomen nur geringen oder gar keinen Einfluss auf die Erneuerung des Wassers an ihren Geruchsorganen haben könne, und daraus folgt die Nothwendigkeit eines

eigenen Ventilationsapparates des Geruchsorganes ausser dem Athemorgane. Diesen Zweck hat der Spritzsack der Petromyzon und der segelartige Ventilator am Gaumen der Myxinoiden."

The only convenient place for the unobstructed development of the naso-palatal canal is the median position it occupies, as elsewhere the great muscles of the suctorial disc and tongue would impede its action. These considerations would seem to explain the coalescence of the originally paired olfactory organs, and the transferrence of the opening of the nasal pit from the ventral to the median dorsal line of the head.

Dohrn (12) has propounded very similar views to those just given, with regard to the development of the upper lip and its effect upon surrounding organs. But he does not seem to have read my preliminary papers (32 and 33) in the "Zoologischer Anzeiger" and the "Quarterly Journal of Microscopical Science," in which these considerations were all outlined.

3. *The Eye.* — Wilhelm Müller (6) was the first to demonstrate that the eye of *Petromyzon* is developed in essentially the same manner as in the higher vertebrates. His observations leave little to be done, except with regard to the earlier stages. The first rudiment of the eye appears about the sixteenth day after impregnation, in the formation of the optic vesicles, which are slender hollow stalks of strikingly small size, the walls of which consist of a single layer of columnar epithelial cells. The vesicles are covered by a delicate layer of more or less spindle-shaped mesoblastic cells, a continuation of those which envelop the brain. About the eighteenth day (Fig. 24, Pl. X) the retina is formed by the elongation of some of the cells in the anterior wall of the primary optic vesicle, while the posterior wall becomes thinner and its cells flatter; the two walls are now in close contact, so that in the region of the retina the cavity of the primary vesicle is obliterated, though it still persists in the stalk. The great peculiarity in this mode of development consists in the very small part of the vesicle which becomes the retina, as compared with the retinal region in other vertebrates. This is no doubt for the most part due to the retardation which affects the formation of the eye, for during larval life this organ is entirely functionless; but it is also probably due in part to the fact that in the higher vertebrates the brain and organs of

the head generally exhibit an acceleration of development, inasmuch as these organs are proportionately much larger and more conspicuous in the embryo than in the adult. The minuteness of the embryonic and larval eye of *Petromyzon* is thus chiefly a secondary character.

At this stage the layer of spindle-shaped mesoblastic cells which envelopes the optic vesicle is clearly shown (those cells are omitted in Figs. 23 and 24, Pl. X, on account of the small scale on which these figures are drawn). Just after hatching, the cavity of the optic stalks becomes obliterated and fibres begin to be developed in their substance (Fig. 26, Pl. X). The lens is formed by an invagination of the epiblast (Fig. 25, Pl. X) at or about the time of hatching. This figure is copied from the one given by Balfour (Comp. Embryology, Vol. II, p. 410, Fig. 291), who very kindly allowed me to study the preparation from which the drawing was made. It represents a stage which none of my sections show. The lens is gradually separated from the epiblast, and though of very minute size, completely fills up the shallow optic cup. It is composed of long, cylindrical epithelial cells, and encloses a central cavity which is long persistent (Fig. 27, Pl. X). The posterior wall of the lens is somewhat thicker than the anterior, though not markedly so. In later stages this difference between the two walls of the lens becomes more and more evident, the posterior becoming thicker, especially in the middle, and the anterior more and more flattened. The cells of the posterior wall gradually take on a fibrous appearance and obliterate the lens-cavity, while those of the anterior wall remain as a thin layer of flattened epithelium. This change is to be observed only in full-grown Ammocœtes.

The optic cup remains very small, and to the slight growth of its outer edge is due the rudimentary character of the choroid slit; this growth is, however, sufficient to carry away the lens from its contact with the retina, and thus produce a space into which, as W. Müller has shown, there grows a process of mesoblastic cells through the choroid slit, and thus forms the rudiment of the vitreous humor. In large larvæ the eye-ball has a rather peculiar shape, being much depressed from above downwards, so that the transverse diameter considerably exceeds the vertical.

There has been much dispute as to the origin of the lens-

capsule, which Balfour (5) with Kölliker and others regard as "a cuticular membrane deposited by the epithelial cells of the lens;" a view which is mainly supported by the fact that "at the time when the lens-capsule first appears, there are no mesoblast cells to give rise to it." Whatever may be the fact with regard to the formation of this structure in other vertebrates, there can be no doubt that in *Petromyzon* a layer of mesoblastic cells is included in the eye between the retina and the lens; and this, taken in connection with Müller's statement that he has found cellular elements in the lens-capsule, would certainly seem to indicate that in this type at least the capsule is of mesoblastic origin.

The changes which take place in the optic vesicle itself during larval life are not of a radical character. In the smaller larvæ the eye is so minute that it is rather difficult to find. The anterior wall of the retina increases slowly in thickness, and several layers of cells make their appearance, which, however, are not at all clearly differentiated from each other, while the posterior wall becomes flatter, and in Ammocœtes of 20 mm. length is pigmented, thus forming the pigmented epithelium of the choroid. The various portions of the retina are very imperfectly developed in the larvæ, the rudiments of the rods and cones appearing but a very short time before the metamorphosis, and, as Langerhans has remarked, the blindness of the Ammocœte is caused not only by the deep situation of the eye and the opacity of the overlying skin, but also by the absence of percipient elements.

The mesoblastic envelops of the eye are at first very simple. A mass of mesoblastic cells in front of the lens (Fig. 27, Pl. X) arranges itself into a layer, the membrane of Descemet, which is imbedded in a layer of fibrous connective tissue, which surrounds the optic vesicle and represents the choroid; this layer is pigmented. A cornea cannot be said to be present in the larva, a thick layer of dermis separating the optic vesicle from the epidermis, which, over the eye, is entirely indifferent and like the general epidermis. Nor have I detected any beginnings of the formation of the iris beyond a slight thinning of the exterior edges of the optic cup. After the first formation of the lens, and in small larvæ, the eye lies in close contact with the epidermis (Fig. 26, Pl. X); the formation of the dermis

separates it from the epiblast, and its position becomes gradually deeper as the growth of the optic nerve and vesicle does not keep pace with the increasing thickness of the walls of the head. In Ammocœtes of 25-30 mm. in length a thick mass of loose connective tissue occurs between the dermis and the eye.

I have found none of the eye-muscles in the larvæ, though in young larvæ the first head-cavity (Fig. 39, Pl. XI) is seen lying in close contact with the optic vesicle, and probably gives rise to at least some of the muscles of the eye.

At the time of metamorphosis the eye increases enormously in size and approaches the surface; the retinal elements rapidly make their appearance, and the iris and cornea are formed. Even the adult eyes shows some peculiarities, which Langerhans has described, and into which it is needless to enter here.

4. *The Auditory Organ.* — The structure of this organ in the adult has been described by J. Müller, and latterly with great minuteness by Ketel (21) and Retzius (29). It is in many respects widely divergent from the plan exhibited in the higher vertebrates, and the homologies of its various parts are matters of great obscurity. The membranous labyrinth consists of a vestibule, two semicircular canals, and a sac-like appendix of the vestibule. The vestibule is of an irregular oval shape, with the long axis placed antero-posteriorly; it is divided by a rather deep median fold into an anterior and posterior portion, which are of similar size and appearance. This fold is seen in transverse section to form a prominence in the interior of the labyrinth, and is crossed by a similar though smaller process, which divides the vestibule into upper and lower portions. (In *P. Planeri* I have observed only doubtful indications of this second fold.) These prominences are somewhat stiffer than the other parts of the vestibule, and are covered with a thickened and sometimes many-layered epithelium, some of the cells of which are provided with stiff auditory hairs. Above each of the divisions of the vestibule lies a semicircular canal, which is but incompletely separated from the vestibule. The anterior canal arises from the lower antero-external corner of the vestibule, the posterior from the postero-external corner; the two canals pass upwards and inwards, and unite in the median dorsal line of the inner edge of the vestibule, and open into the latter by means of a common passage. The external origin of

each canal is marked by an ampulla-like expansion, which consists of three hollow diverticula of the vestibule, the canal arising from the median one. On the lower side of the vestibule, and connected with its cavity by means of a constricted opening, is the unpaired appendix, which has a symmetrical median position. The labyrinth is filled with a fluid containing otoliths. This description covers only the more obvious structures of the ear, as the details of the finer structures would be of no use for our purpose.

The homologies of the various portions of the auditory organ of *Petromyzon* are exceedingly difficult to decipher. Retzius' views are as follows: "Was nun zuletzt die morphologische Deutung der einzelnen Abtheilungen des membranösen Labyrinthes vom *Petromyzon* betrifft, so muss ich gestehen, dass die Ansichten von Ketel im allgemeinen sehr bestechend sind, und in der That viel für sich haben. Dass wir in den beiden Bogengängen den vorderen und hinteren Bogengang der höheren Fische und der übrigen Wirbelthieren vor uns haben, scheint ja von vorn herein mehr als wahrscheinlich zu sein: ebenfalls sind wohl die beiden Cristæ acusticæ nichts anderes als die Crista acus. anterior (sagittalis) und posterior (frontalis), sowie die mittleren Abtheilungen der Ampullæ trifidæ die Ampulla anterior und posterior. Die sogenannte Commissur des Bogengänge ist ebenfalls aller Wahrscheinlichkeit nach dieselbe Bildung wie bei anderen Wirbelthieren. Ob aber das Vestibulum mit seinen beiden symmetrischen Partien einen stark erweiterten Theil der Commissur anderer Wirbelthieren entspricht, lässt sich wohl vermuthen aber nicht sicher darlegen. Die Einmündungsstelle der beiden Röhren, von welchen jedenfalls eine als Aqueductus vestibuli zu deuten ist, spricht indessen dagegen, noch mehr unsicher scheint es mir aber in dem vorderen Ende der Crist. long. ant. ein Rudiment der Crist. acus. der aüsseren (horizontalen) Ampulle zu erblicken, obwohl es wahr ist, dass die Crista sowohl wie die Ampulle ungefähr an dieser Stelle zu finden wären. Dass die lange Macula acustica den vereinigten Mac. rec. utric., Mac. sacc. und Papilla lagenæ cochleæ entspricht, ist sehr wahrscheinlich; schwieriger scheint es mir aber zu sein, die obwohl nur ungefähre, Abgrenzung dieser Theile auszufinden. Dass die vordere Partie derselben der Mac. rec. utric. entspricht. ist aber höchst wahrscheinlich;

ob aber die kleine Grube am Boden des Vestibulum dem Recessus sacculi und der sackförmige Anhang der Schnecke gleichzustellen sind, will ich meines Theiles unentschieden lassen. . . . Ohne verbindende Glieder zwischen dem Gehörorgan der Petromyzonten und dem des übrigen Fische scheint mir eine Entscheidung dieser Frage sowohl wie im Ganzen die von einer sicheren morphologischen Deutung des Gehörorganes der Petromyzonten nicht möglich zu sein."

Unfortunately the development of this organ throws but little light upon these obscure homologies. The ear is the first of the sense-organs to be formed in the embryo; on the fourteenth day after impregnation (Fig. 28, Pl. X) the epiblastic cells lying near the anterior end of the hind-brain increase greatly in height, but remain single layered, and are at the same time depressed so as to form a shallow pit. The pit rapidly deepens and is cut off from the skin, forming a large oval vesicle with comparatively thin walls, and very long *recessus labyrinthi* (Fig. 29, Pl. X; Fig. 40, Pl. XI). At this stage the auditory vesicles are so large as to form conspicuous prominences on the sides of the head. Shipley has shown that about the twenty-second day "certain patches of the epithelium become higher than the others, and the cells develop each a very large cilium which projects into the cavity and bears a knob at its free end." These cilia are not shown in Calberla's preparations (or, at all events, I did not see them), but are perfectly clear in the beautiful sections which Mr. Shipley has so kindly sent me. In horizontal sections these ciliated patches are seen to be confined to the anterior and postero-internal portions of the vesicle, and apparently represent the rudiments of the *cristæ acusticæ*.

At this stage the vesicle is ellipsoid in shape, the fore and aft axis being somewhat the longer, though but slightly so. This difference becomes more and more marked with advancing development, and in larvæ 20 mm. long and upwards the transverse axis is much the shorter; the vertical axis also increases in length. When muscles make their appearance between the ear-capsule and the skin, the prominences of the head disappear, and the auditory vesicles are removed from the surface. In larvæ of 8 mm. (Fig. 30, Pl. X) there appear two processes from the outer and inner walls of the vesicle which grow toward each other. The inner process is situated higher up and just

external to the *recessus labyrinthi*. In the next stage (9 mm. Fig. 31, Pl. X) the two processes are in contact, and then coalesce, dividing the vesicle by means of an oblique partition into two chambers. In the middle of the dorsal wall the partition is incomplete, and here the two chambers communicate, as they do also at the anterior and posterior ends of the vesicle The upper chamber is, of course, the rudiment of the semicircular canals. Seen from above, the canals run obliquely from the middle of the inner edge of the vesicle to its outer corners, but with very slight curvature. In larvæ of 13 mm. a median invagination of the outer wall of the vesicle appears and forms a prominence within the cavity, dividing the vestibule into the two divisions already mentioned in the adult; in consequence of this fold the semicircular canals take on a more pronounced curvature (Fig. 32, Pl. X). At the same time the appendix is formed by a diverticulum from the vestibule; at a later stage its opening is narrowed by a constriction of the walls (Fig. 34, Pl. X). In larvæ of 22 m. length all the essential parts of the auditory organ are present, and later changes affect only the form and proportions of the parts; the capsule gradually increases in height and breadth until it assumes the form seen in the adult.

The *recessus labyrinthi* persists for a long time as a blind membranous canal (Fig. 30–32, Pl. X), the end of which lies close to the brain and outside of the cartilaginous capsule of the ear. It is plainly visible even in the larger larvæ; but I have not been able to determine which structure, if any, of the adult ear is derived from it.

The early stages of the development of the auditory organ in *Petromyzon* are altogether like those of the higher vertebrates, but the ear remains permanently on a lower plane. No trace of the horizontal semicircular canal occurs at any stage, and from the time of the first appearance of the canals the structure of the organ deviates widely from that found among other vertebrates. It is a question as to whether these peculiarities are to be regarded as primitive, or as the result of degeneration. The much greater simplification of the labyrinth in *Myxine* strongly suggests degeneracy; but, on the other hand, the entire absence of the horizontal semicircular canal may very well be a primitive feature. It is plain that the auditory organ of the

lamprey is derived from the same primitive type as that of the higher vertebrates; the type shown in the early stages, which, as already indicated, is the ordinary one. But whether in the differentiation of the ear in the lamprey it has passed through stages higher than the present condition, and more like that of the typical fishes, cannot from present evidence be decided.

The ear is the only one of the higher sense-organs which is not greatly retarded in its larval development. No such great advance occurs at the time of metamorphosis as takes place in the olfactory organ and eye. The reason of this difference is probably to be found in the fact that the possession of an organ of hearing is of value even to the larva buried in mud, while those of smell and sight could hardly be employed at all under such conditions.

Regarded as a whole, the sense-organs of *Petromyzon* do not show degeneracy, but rather a retardation of development. There are also certain minor peculiarities which appear to have been acquired within the Cyclostomatous phylum, such as the union of the nasal pits into a median unpaired organ and the development of the naso-palatal canal; the peculiar structure of the retina, as described by Langerhans and Müller; the absence of the horizontal semicircular canal; the division of the vestibule into chambers, and the presence of the auditory appendix. These characteristics are remarkable; but they cannot fairly be called degenerate, or, at least, there is no sufficient evidence for so regarding them.

SUMMARY.

(1.) The upper lip rotates through an arc of 180°, and this rotation has a great effect upon the development of the anterior organs of the head.

(2.) All parts of the brain, except the olfactory lobes, are present in the freshly-hatched larva. The brain is exceedingly small, particularly the fore and mid-brains, due, no doubt, to the undeveloped condition of the sense-organs during most of larval life. Even the hind-brain, though large in proportion to the anterior divisions, is absolutely small.

(3.) The cranial flexure is always slight, and is partially corrected by a rotation in the opposite sense.

(4.) The hemispheres arise as an unpaired solid mass, which

afterwards divides, though remaining solid; the lateral ventricles first appear in larvæ of about 14 mm. length. The olfactory lobes are formed from the hemispheres, not from special proliferations of the olfactory epithelium.

(5.) The infundibulum is formed as a diverticulum of the floor of the thalamencephalon, and is at first single, though in small larvæ it soon divides into lobus and saccus.

(6.) The epiphysis arises as in other vertebrates; it soon exhibits its character as an optic vesicle, though without a lens; a second vesicle is formed from the primary one, which enters into intimate relations with the left ganglion habenulæ. Its position is at first median, but the growth of the right ganglion habenulæ forces its point of attachment over to the left side. The acquirement of some secondary function by the epiphysis is probable.

(7.) The ganglia habenulæ are from the first unsymmetrical, the right being much the larger. The right shifts its point of attachment somewhat, and projects above the roof of the brain; the left divides into two portions connected by a fibrous stalk.

(8.) The pituitary body is derived from the epiblast of the surface of the head in close connection with the olfactory involution. This connection is regarded as secondary, and reasons are given for the belief that this organ is the rudiment of a canal once opening on the surface of the head.

(9.) The mid-brain has a considerable extent of floor. The optic lobes do not appear till late in larval life in correlation with the retarded development of the eyes.

(10.) The hind-brain undergoes few changes; the principal being the thinning of the roof, formation of the choroid plexus, and breaking up of the cell-mass into nuclei. The cerebellum is formed from the posterior wall of the dorsal fold between the mid and hind-brains; it long remains very minute.

(11.) The spinal cord in the later embryonic and early larval stages is like that of the higher vertebrates: the characteristic flattening takes place in the larva.

(12.) The peripheral nerves are developed much as in the Selachians; the olfactory nerves are originally paired, the ganglion is derived from the olfactory epithelium, though not as Shipley has described; the optic nerves are remarkable for their great length at first; the ciliary ganglion and its root arise from

the mid-brain in late embryos; the trigeminal has two ganglia formed from the skin; the facial has a ganglion formed in the same way. This nerve supplies the first temporary gill-cleft; the auditory is in part derived from the facial; the ninth and tenth nerves arise at first from several separate roots each provided with a ganglion and connected with each other and with the dorsal spinal nerves by means of a longitudinal commissure. The ninth and tenth nerves early concentrate into a complex mass. The lateral nerve is formed early, and apparently from the epiblast of the skin.

(13.) My observations on the development of the spinal nerves agree entirely with the accounts of Sagemehl and Shipley.

(14.) The epidermal sense-organs of the head and lateral line are not developed in connection with the ganglia of the cerebral nerves or with the lateral nerve, but at a later stage. This separation is regarded as a secondary process.

(15.) The olfactory organ is at first ventral in position, and is always single and median. The rotation of the upper lip brings the opening to the dorsal side of the head, and this is probably the condition which produced the coalescence of the primitively paired nasal pits. A glandular organ, resembling that of Jacobson, but having no communication with the mouth, is formed from the postero-inferior portion of the nasal involution.

(16.) The eye is formed as in other vertebrates, but is remarkable for the very small part of the primary optic vesicles which gives rise to the retina. During larval life the eye remains in an undeveloped state, the retinal elements appearing only just before metamorphosis. The lens-capsule is probably of mesoblastic origin. No cornea is present in the larva.

(17.) The early stages of the auditory organ do not differ in any essential respect from those of the higher vertebrates; the young larva first exhibits the divergences. There is no trace of the horizontal semicircular canal; the vestibule is divided imperfectly into chambers, and a median appendix is formed. The *recessus labyrinthi* persist throughout larval life, and probably in the adult. The cristæ acusticæ are marked in the young larva by patches of epithelium bearing stiff auditory hairs. The ear is relatively better developed in the larva than either of the other higher organs of sense, and does not undergo such marked changes at metamorphosis.

The next paper of this series will deal with the development of the alimentary canal and its accessory structures, of the skin, skeleton, muscles, and urinogenital system.

MORPHOLOGICAL LABORATORY,
PRINCETON, N.J., Nov. 15, 1887.

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EXPLANATION OF THE PLATES. (VIII, IX, X, AND XI.)

REFERENCE LETTERS.

I. Olfactory Nerve.
II. Optic Nerve.
III. Ciliary Ganglion.
V. Trigeminal Nerve.
VII. Facial Nerve.
VIII. Auditory Nerve.
IX. Glossopharyngeal Nerve.
X. Vagus Nerve.
1. First Temporary Gill-cleft.
2. First Permanent Gill-cleft.
3. Second Permanent Gill-cleft.
An. Auditory Vesicle.
Br. Branchial Nerve.
Ch. Notochord.
C.H. Cerebral Hemispheres.
Chr. Choroid Coat of the Eye.
Cm. Spinal Commissure.
D. Membrane of Descemet.
Ep. Pineal Gland.
F.B. Fore-Brain.
Hb. Ganglion Habenulæ.
H.B. Hind-Brain.
H.C. Head Cavity.
Hy. Pituitary Body.
I.Cm. Optic Chiasma.
In. Infundibulum.
J. Organ of Jacobson.
Ln. Lens.
L.T. Lamina Terminalis.
M. Mouth.
M.B. Mid-Brain.
N.E. Olfactory Epithelium.
N.L. Lateral Nerve.
N.P. Naso-palatal Canal.
O.G. Olfactory Ganglion,
S.C. Semicircular Canal.
S.Cm. Superior Commissure.
Sp.C. Spinal Cord.
Sp.G. Spinal Ganglia.
Tr. Trabecula Cranii.
T.V. Choroid Plexus.
U.L. Upper Lip.

Errata. Pl. VIII, Fig. 5, *Opt* points to the olfactory Epithelium, and *N.E.* to the fore-brain. Pl. IX, Fig. 14, the dotted line leading from *In* to the optic tract should be erased. Fig. 21, for *N.G.* read *N.E.*

PLATE VIII.

FIG. 1. Sagittal section through the head of an embryo *Petromyzon* of the 17th day after impregnation.

FIG. 2. Ditto of the 18th day.

FIG. 3. Ditto of freshly hatched larva (4.8 mm.). The cells in this drawing are somewhat diagrammatic.

FIG. 4. Enlarged view of the anterior portion of Fig. 3.

FIGS. 5 and 6. Other sections of the same specimen. These sections are slightly oblique; in Fig. 6, the plane of section is median at the notochord, but somewhat lateral at the nasal epithelium.

FIGS. 7, 8, and 9. Transverse sections through the fore-brain of a larva 6.8 mm. in length.

FIG. 10. Section through the fore-brain of a lārva 6 mm. long.

PLATE IX.

FIG. 11. Horizontal section through the anterior part of the head of a larva of 16 days (10 mm.).

FIG. 12. Sagittal section through head of a larva of 12 mm.

FIG. 13. Lateral sagittal section through brain of a 13 mm. larva.

FIG. 14. Median sagittal section of the same.

FIG. 15. Horizontal section through the superior portion of the brain of 22 mm. larva.

FIG. 16. The same, taken in a more ventral plane.

FIG. 17. Transverse section through the infundibulum and pituitary body of a larva of 53 mm.

FIG. 18. Sagittal section through the olfactory organ, showing the organ of Jacobson (larva of 12.5 mm.).

FIG. 19. Horizontal section through the same.

FIG. 20. The same; larvæ of 20 mm.

FIG. 21. Sagittal section in the median plane through the nasal capsule and fore-brain of adult Petromyzon. The epidermal cells are diagrammatic.

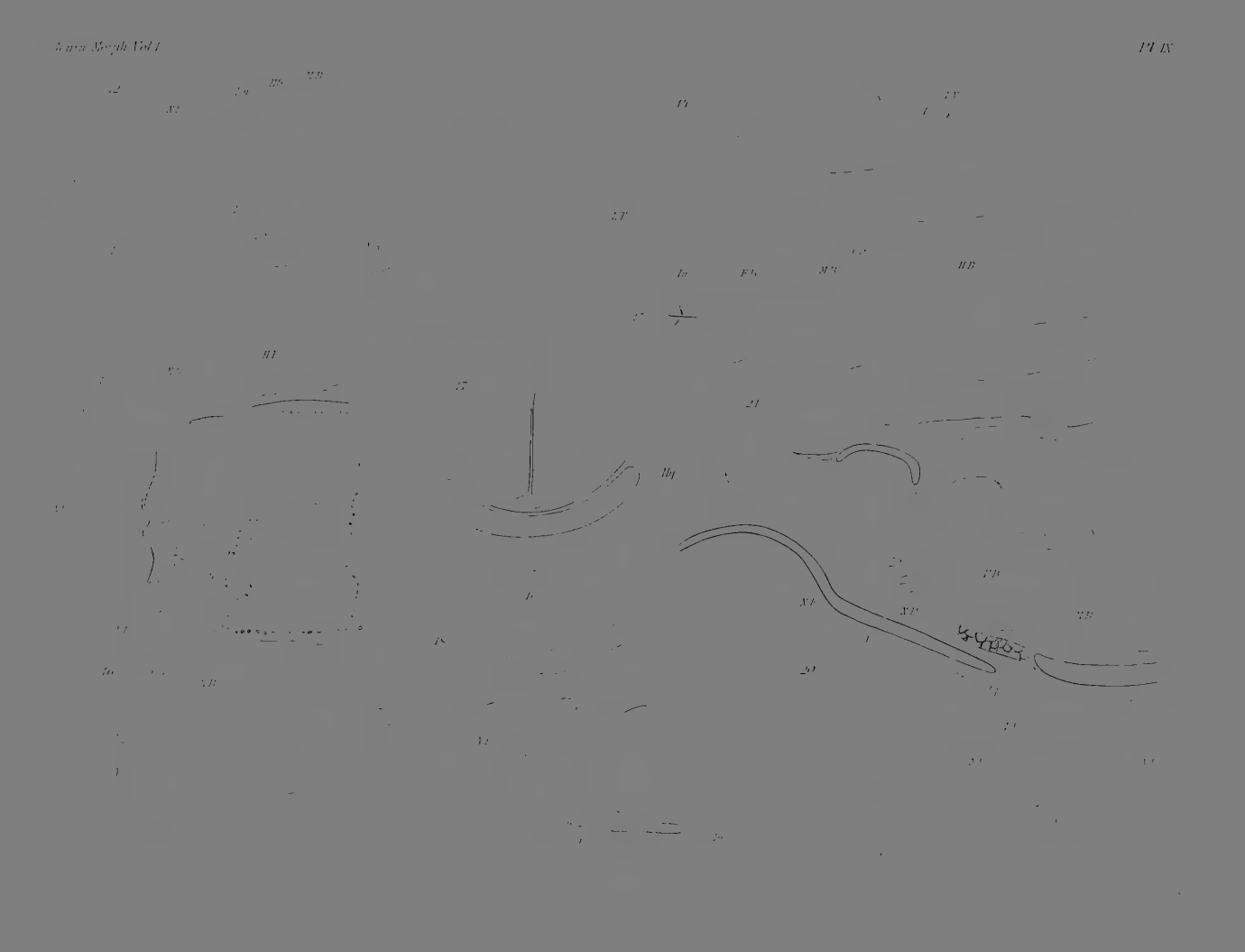


PLATE X.

FIG. 22. Sagittal section in a lateral plane through the nasal capsule of adult Petromyzon.

FIGS. 23 and 24. Horizontal sections through the anterior portion of the head of two embryos just before hatching.

FIG. 25. Ditto, larva of 4.8 mm. This section is very oblique and almost between the horizontal and transverse planes (after Balfour).

FIG. 26. Ditto; larva of 8 mm.

FIG. 27. Sagittal section in a lateral plane through the eye of a larva of 22 mm.

FIG. 28. Transverse section through the hind-brain of an embryo of the 15th day, showing the auditory involution.

FIG. 29. Transverse section through the head of a ripe embryo.

FIGS. 30–33. Sections through the auditory vesicle of Ammocœtes: Fig. 30, transverse, larva of 8 mm.; Fig. 31, larva of 14 mm.; Fig. 33, larva of 22 mm., showing the opening of the commissure into the vestibule; Fig. 33, horizontal, larva of 22 mm.

FIG. 34. Transverse section of the posterior part of the head, larva of 53 mm.

FIG. 35. Horizontal section through the olfactory organ and nerve of small Ammocœte (22 mm.).

PLATE XI.

Fig. 36. Hind-brain of 17 days' embryo; sagittal section.

Fig. 37. Ditto, 18 days' embryo; horizontal section.

Fig. 38. Spinal cord, ganglia, and spinal nerves, 17 days' embryo; horizontal section.

Figs. 39–41. Cerebral nerves and ganglia of larva, 4.8 mm. in length; sagittal section.

Figs. 42, 43. Transverse sections through the posterior part of the head of newly-hatched larvæ (exact size unknown).

Figs. 44–47. Spinal cord, vertebral column, and lateral nerves of Ammocœtes, transverse section: Fig. 44, larva of 7 mm.; Fig. 45, of 14 mm.; Fig. 46, of 30 (?) mm.; Fig. 47, of full-grown Ammocœte.

CONTRIBUTION TO THE EMBRYOLOGY OF THE LIZARD;

With especial Reference to the Central Nervous System and some Organs of the Head; together with Observations on the Origin of the Vertebrates.

HENRY ORR, PH.D.

University Fellow in Biology at Princeton, N. J.

INTRODUCTION.

THE embryology of the lizard has been investigated in parts by various authors, yet all the work done on the subject is hardly so complete as to warrant a monograph. Concerning the segmentation of the egg and the formation of the germ-layers we are indebted for our knowledge to *Balfour*,[1] *Hoffman*,[2] and *Weldon*.[3] These earliest stages of development were lacking in the material collected by me, so that my work begins about the stage where that of *Balfour* and *Weldon* ended, and deals with the early development and differentiation of some of the organs. The most comprehensive contribution hitherto made to lizard-embryology is that of *Hoffman*. *Strahl*, in a series of articles in the Arch. f. Anat. u. Phys. (Anat. Abth.) '81–87, has described the relations of the amnion and some anatomical features, but his methods prevented him from entering upon details requiring a finer histological investigation.

The material for the present investigations was collected by me while a member of the zoölogical expedition generously equipped and sent to Abaco, Bahamas, W.I., by the *Johns Hopkins University*. The specimens have been kindly identified for me by *Prof. E. D. Cope*.

[1] *Balfour*. — On the Early Development of the Lacertilia, together with some Observations on the Nature and Relations of the Primitive Streak. — *Quart. Journ. of Mic. Sci.*, Vol. XIX., N.S., 1879.

[2] *Hoffman*. — Weitere Untersuchungen z. Entw.-gesch. d. Reptilien. — *Morphologisches Jahrbuch*, XI. Bd., 1885.

[3] *Weldon*. — Note on the Early Development of Lacerta Muralis. — *Quart. Journ. of Mic. Sci.*, Jan., 1883.

The species on which most of my work has been done is *Anolis sagræi* (Dum. et Bib.), but I have also examined some stages of *Sphærodactylus notatus* (Baird) and *Liocephalus carinatus* (Gray). The embryo of Anolis develops in the uterus until about the stage represented in Fig. 2, C, Pl. XII. I found the full-sized eggs, rarely four in number, in the uterus. They have a hard white shell when deposited, and could be obtained in large numbers in the sand-filled crevices of heaps of broken conch-shells on the sea-beach. The eggs of the other two species are of a different size, with soft shells. The embryos were killed in Perenyi's fluid, and preserved in 90° alcohol. After this method of treatment I found a saffranin stain gave the best results.

Owing to the ventral curve in the head of the embryo (cranial flexure), the words "anterior" and "posterior," etc., come to have two meanings. One has regard to the entire embryo, in which sense the extreme anterior end of the embryo would be the dorsal summit of the mid-brain. The other meaning regards the organs as they would appear if the curve were rectified and the head continued in the straight line of the body axis. In this case the extreme anterior end would be the outer surface of the front median lip (anterior medullary fold) of the medullary groove. This would also be the dividing line between the dorsal and ventral surfaces. As this latter morphological meaning expresses the homologies of the parts and greatly simplifies the terminology, I shall adopt it throughout.

Except where otherwise specified, the description and figures refer to Anolis.

PART I.

General Description of Youngest Stage.

The youngest stage which I have obtained is represented entire in Fig. 1, A, and in sections in Figs. 7, A–18, A, inclusive. There are four protovertebræ. The cranial flexure is well marked, and the lateral medullary folds touch each other above the central canal from a point just behind the primary forebrain, backward as far as the lumbar region. Although the medullary folds touch each other through this distance, they are

fused together in only a short part of the middle dorsal region. Toward the tail the lumen of the central canal gradually enlarges, and finally is wide open above. Anteriorly to the apex of the cranial flexure the lateral medullary folds spread wide apart (*MF*, Figs. 8, A–12, A), but in the most anterior part of the head they curve toward each other and unite in a median anterior fold (*AF*, Fig. 13, A). By measurements of its thickness and distance from the dorsal crest of the hind-brain, it may be seen that this anterior fold is not a simple continuation of the ventral floor of the primary first ventricle, but an elevated fold continuous with the lateral folds, and enclosing the primary first ventricle anteriorly as the lateral folds enclose it laterally.

The epiblast, which is continuous with the dorsal edges of the medullary folds, is spread a certain distance laterally over the body of the embryo, and is then folded up over itself to form the amnion. The line of this fold runs parallel to the curved axis of the body and head, so that the line of the fold at the side of the head curves ventrally and slightly backward. In Fig. 13, A, the curved line of the fold is cut in two places on the left side of the head. The part of the amnion springing from the ventrally and backward curved line of the fold covers the mid- and fore-brain. The epiblast, continuous with the dorsal edge of the anterior medullary fold, is spread over the external surface of the anterior fold until just beyond the ventral edge of the latter it meets the line of the amnion fold, and recurving on itself it forms the anterior median part of the amnion (see Fig. 14, A, and its explanation). In a median sagittal section, the anterior part of the amnion cavity appears to curve around the head, and terminate with a small blunt end at that point where the mouth will first appear, — *i.e.*, the pit of the so-called mouth-involution (cf. Diagram I.). These relations of the amnion I have deduced by reconstructing the drawings of consecutive transverse sections.

The hypoblast extends as a blind sack into the head, forming the rudiment of the head intestine. Along the dorsal median area of the hypoblast arises the notochord: its origin from the hypoblast has been described by *Hoffman*. In the embryo of series A, the greater part of the notochord is distinctly developed, but in some places it still shows a more intimate connection with the hypoblast. At this stage it lies everywhere in

close contact with the hypoblast below it, and with the medulla above it. The dorsal wall of the head intestine, with its accompanying notochord, follows, therefore, the curved ventral surface of the primary mid- and fore-brain around to that point immediately ventral to the anterior medullary fold, where the hypoblast touches and fuses with the epiblast. At the base of the anterior medullary fold the notochord runs into a mass of cells (*HC*) which is continuous with the fused hypoblast and epiblast. The mass of cells seems, however, to be of hypoblastic origin, in like manner as the notochord (Figs. 11, A–15, A, Pl. XIII.).

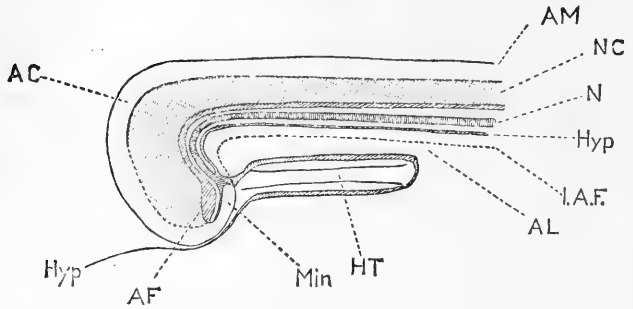
The ventral wall of the head intestine curves slightly downwards to meet the epiblast at the point of the oral fusion. In the present stage there are no traces of the branchial system: when the branchial clefts appear later, the posterior clefts are parallel to each other, and at right angles to the axis of the intestine. The two anterior clefts are not parallel, but their ventral ends are convergent. These facts seem to me explicable only as the result of a general ventral bend of the anterior end of the embryo, — a bend which appears primarily as the cranial flexure, and imparts itself secondarily to the notochord and head intestine; for, owing to the relative mass of the different parts, it would be impossible to suppose the converse method to be true.

We cannot avoid regarding as dorsal all that part of the intestine which gives rise to notochord. In this case the notochord extends as far as the oral fusion of hypoblast and epiblast; therefore this point of fusion must be regarded as the extreme anterior end of the head intestine. Owing to the curve of the dorsal wall of the intestine, its lumen appears in section as far forward as section 13, A, Pl. XIII.; but this is simply the dorsal part of the intestine, not a "pre-oral intestine," for this latter, in Anolis at least, would be impossible.

Diagram I. will make the relation of these parts more readily apparent. It will be seen that the fusion of notochord and hypoblast with the epiblast at the pit of the "mouth involution" appears to have a restraining influence on the epiblast, and to hold it in place at this point. The external or ventral wall of the "mouth involution" is simply the median part of the amnion, and arises directly on the continuation of the lateral line of the

amnion fold, — indicated by the dotted line *l, A, F.* The ventral curvature of the medullary folds gives to the "mouth involution" the appearance of being an invagination of epiblast to meet the hypoblast; but its relation to the amnion fold does not warrant such a conclusion. If we imagine the head continued forward in a straight line (which was undoubtedly its more

DIAGRAM I.



A C — Amnion cavity. A F — Anterior fold. A L — Alimentary canal. A M — Amnion. H T — Heart. Hyp — Hypoblast. l. A. F. — Lateral line of the amnion fold. Min — Mouth involution. N — Notochord. N C — Neural canal.

The dotted line running from the anterior fold to the apex of the cranial flexure indicates the part of the brain-roof which is still open.

primitive condition), then we should have the hypoblast of the head intestine extending to the anterior surface of the head, and fused with the epiblast at a point immediately ventral to the medullary fold and just dorsal to the line of the amnion fold.

Having passed the stage just described, and figured at 1, A, Pl. XII., the embryo begins to develop the various organs in rapid succession, or some apparently simultaneously. Before the primary first ventricle is completely closed anteriorly, the optic outgrowths have made their appearance (Fig. 45, Pl. XV.). Shortly after the closing of the brain, the ear appears as a dorso-lateral invagination of the epiblast. At the same time appear the hypoblastic evaginations to form the gill-clefts. At this time the mouth has not yet broken through (see series B). Shortly after the appearance of the oral opening, the condition of the head is as shown in the median sagittal section (Fig. 51, D, Pl. XV.). At this time the segmental organs are well developed; the thyroid gland, the liver, and pancreas appear. Of the outgrowths of the anterior half of the intestine, the thyroid

gland is the first to appear, and the lung the last. The head-cavities have become large, oval-shaped cavities on either side of the head, medial to the posterior part of the eye-cup (*HC*, Fig. 2, C, Pl. XII.).

Figs. 2, C, and 24, C–42, C, represent that stage in which all the embryonic organs have appeared save the lung, and the point of origin of the latter is already indicated. The development of some of these parts is given below in detail.

2. *The Notochord, Head-cavities, and Hypophysis.*

It is generally agreed that the notochord arises by a differentiation of the linear median area of the dorsal wall of the primitive intestine. The extreme anterior point of this differentiation of hypoblast into notochord still remains uncertain for most animals, and we are in doubt as to whether it remains constant in its relations to other parts in all animals, or whether it is sometimes nearer to, and again sometimes farther from, the anterior extremity of the intestine. In the frog, the intestine has the appearance of extending much beyond the anterior end of the notochord, while in the cyclostomata the converse seems to be true, and the notochord appears to extend beyond the intestine. The lizard, in this respect, seems to represent a primitive state, for here the notochord continues to the anterior extremity of the intestine, or the oral fusion of hypoblast and epiblast. The origin of the notochord has already been referred to (S. 1). In the lizard it is relatively very much smaller than in Elasmobranchs and Amphibians, and never attains such large proportions; also in relation to the growth of the other organs it becomes vacuolated much later in the lizard than in Amphibians. In advanced embryos the vacuolated part extends slightly anterior to the first vertebral rudiment; but from here forward to the region of the hypophysis it remains a long time in its primitive condition, sometimes irregularly crooked, and finally disappears, leaving as a last trace a more densely nucleated line in the tissue of the base of the rudimentary skull.

When the notochord first appears, its anterior part lies between the intestine and the brain-rudiment, touching both. Fig. 10, A, Pl. XIII., represents a section tangential to the ventral external surface of the mid-brain. In the third section, behind

10, A, the ventrally curved part of the notochord is visible (*N*, Fig. 11, A). Its anterior end fuses with a mass of cells (*HC*) which lie close to the ventral surface of the rudiment of the fore-brain. In the fourth section, behind 11, A, this mass of cells (*HC*) is seen to be fused with the epiblast (Fig. 12, A). This fusion with the epiblast is, however, continuous from side to side across the median line. Five sections behind 12, A, this mass of cells (*HC*) is seen to be also completely fused with the wall of the intestine (*AL*, Fig. 13, A). The section 13, A, passes near the inner surface of the epiblast at *Hph*. The epiblast near this point lies close against the base of the anterior medullary fold, *AF*. The epiblast at *Hph* is the roof of the hypophysis, which, appearing at this early stage, remains permanently unmoved from this point of contact with the fore-brain. Six sections farther backward, Fig. 14, A, represents a section passing near the external surface of the roof of the hypophysis (*Hph*), and cutting in its own plane the epiblast (*Ep*) which covers externally the anterior medullary fold. Five sections behind 14, A, the section 15, A, passes through the anterior curved end of the amnion cavity (*M.in*). The wall of fused epiblast and hypoblast, which separates the lumen of the intestine from the amnion cavity, shows here its thinnest point in the median line, and indicates where the mouth-opening will appear. Just posterior to this point the epiblast recurves around over the head to form the amnion, so that within five sections backward there is no epiblast on the ventral side of the body.

This manner of development of the notochord and hypophysis seems to point to some peculiar relation between the two organs. There are also other parts which are intimately related to these organs, namely, the muscular elements of the head, — primarily the head-cavities, which are developed from the mass of cells, *HC*.

The next older embryo which I have been able to examine is represented in the series of transverse sections, B. The mouth-opening has not yet appeared. The notochord in the head still lies close to the hypoblast, but the increased growth of the brain has lifted the mid-brain away from the notochord. In Fig. 19, B, is seen the ventrally curved portion of the notochord (*N*). In 20, B, — four sections farther backward, — is seen the dorsal wall of the intestine and its lumen (*AL*).

Anteriorly, at the base of the fore-brain (*FB*), appears the recurved end of the notochord (*N.*) in cross-section; and laterally from it, extending partly around the base of the fore-brain on both sides, is a double wall of cells (*CZ*), pressed close together for a distance, but separating distally to form a lumen. This lumen is enclosed by the distal union of the two walls. The cavity is round, excepting a conical point at the place where the two walls separate; so that proceeding toward the notochord the lumen gradually disappears, though the walls remain distinct as far as to the notochord. An examination of the whole series shows the structure in question to be a rod-like body with a distal hollow enlargement. Meeting each other at the median line, the two parts enclose and fuse with the anterior extremity of the notochord. The hollow enlargement is the head-cavity. As I am unacquainted with any name for such a median connection of the head-cavities and notochord, I shall hereafter refer to it as the coelenteric zone.[1]

In the embryo of series B, that connection of the notochord and rudiment of the coelenteric zone with the epiblast has disappeared. In the embryo of series A, the cell-mass (*HC*) at the anterior end of the notochord was fused with the epiblastic roof of the hypophysis, and not completely separated from the hypoblast. In the present stage, however, the separation from the hypoblast is complete. Section 21, B (six sections behind 20, B), shows also that the separation of the notochord and the coelenteric zone from the hypophysis is complete, for here nothing but a thin band of the general interstitial tissue separates the intestine from the fore-brain anteriorly to the coelenteric zone. The second section behind this shows in tangential section the epiblastic roof of the hypophysis (*Hph*, Fig. 22, B) As in the preceding stage, it is in close contact with the base of the fore-brain. Three sections farther backward, Fig. 23, B, shows the oral fusion of the hypoblast and epiblast, also the laterally open cavity between the oral fusion and fore-brain, which becomes the cavity of the hypophysis. In Figs. 22, B,

[1] *Marshall* (On the Head-Cavities and Associated Nerves of Elasmobranchs. *Quart. Journ. of Mic. Sci.*, Vol. XXI., N.S., 1881) has described homologous and nearly similar relations as existing in the Elasmobranchs. *Van Wijhe* (Ueber d. vorderen Neuroporus, etc., Zoöl. Anzeig., pp. 683, 684) supposed, for theoretical reasons, that some such connection must exist or have existed.

and 23, B, it will be seen that the ventral flexure of the brain causes an angle to be formed between the ventral (or morphologically anterior) surface of the head and the external surface of the oral fusion and lower jaws. The apex of this angle of surfaces is seen in the line of epiblast, *Ed* and *Hph*, in Fig. 22, B. In 23, B, the space (*M.in*) enclosed by the angle is seen. The apex of the angle, therefore, extends from side to side of the head in nearly a straight line. As the brain and lower jaw increase in size, the angle becomes more and more acute. In Fig. 48, D, this angle (*M*) is shown in a lateral, longitudinally vertical section. In the median line, posterior to the apex of the angle, the mouth-opening breaks through. The lower jaw, thus released in the middle, curves outward, and, overlapping the epiblast anterior to the apex of the angle, it begins its growth forward, which eventually brings its median part to the nasal tip of the head.

Between the apex of the angle referred to and the dorsal edge of the mouth-opening there remains a small portion of the posterior epiblastic surface of the angle. This small portion of epiblast forms the posterior wall of the hypophysis; it is visible in Fig. 49, D, under the end of the notochord and posterior to the space *M*, and also in 50, D, — a slightly lateral section (*Hph*). Owing to the lateral twist of the head of the embryo, it is impossible to make a truly median, longitudinally vertical section; although section 49, D, passes through the median plane at the notochord, it cuts also the lateral boundary of the mouth-opening just behind the space *M* (Fig. 49, D). (I have omitted to reproduce this posterior wall of the hypophysis in the reconstructed section of Fig. 51, D.) As soon as the hypoblast separates from, and sinks down from the notochord, the characteristic appearance of the hypophysis becomes much more pronounced, and appears as pictured in Fig. 47. Here the hypoblast blends indistinguishably with the posterior wall of the hypophysis. The cavity of the hypophysis has meanwhile become enclosed laterally by the tissue at the sides of the hypoblastic mouth (*M*, Fig. 22, B) growing forward to form the upper jaw. The expansion of the head-cavities may possibly help to cause this effect. The later condition of the hypophysis may be seen in Figs. 33, C, and 34, C. Here the brain is increased very much in size, and a ventral distension of

the lateral parts of the head has enclosed the hypophysis laterally, while its median roof has remained in its primitive position in contact with the same part of the brain where it first appeared. In the embryo of series C the hypophysis appears as a lateral, slit-like depression in the roof of the mouth. Later stages show that the growth of the surrounding parts gradually constricts the opening of the hypophysis to the mouth. The hypophysis appears then as a laterally distended body with extremities turned slightly upward, according with the curved surface of the brain, against which it lies.

The head-cavities having first appeared as already described, become large oval cavities, with walls of a distinct, compact, epithelial nature. Their position and relations may be seen at *HC*, in Figs. 2, C, 34, C, 35, C, 36, C, and 48, D. The nerves of the third pair enter their dorsal posterior walls (*CZ*, Fig. 34, C). At a late period in the duration of the head-cavities, at that end of each cavity nearest the mouth, a transverse constriction appears in the median wall, as if the cavity were to be divided into two,—one smaller anterior and one larger posterior cavity. Whether such a division really takes place I have been unable to prove, for very soon after the appearance of the constriction the whole cavity seems to disappear, its walls being converted into muscle. The constriction is interesting, as corresponding with a similar condition of the first head-cavities in the elasmobranch fishes.[1]

The coelenteric zone continues to exist for a considerable time, uniting the anterior ends of the head-cavities; it is still visible in stage C (*n III*, Fig. 34, C). Figs. 43, C, and 44, C, Pl. XV., represent two successive sections in the region of the coelenteric zone of the embryo figured at 2, C. In Fig. 43, C, a solid band of cells (*CZ*) connects the head-cavities (*HC*) above the hypophysis (*Hph*); and in the posterior section (44, C) is seen the anterior tip of the notochord (*N*) joining in the middle with the band (*CZ*). I have not discovered any later trace of the coelenteric zone. Immediately after this period it seems to become absorbed.

The preceding statements describe what seems to me to be the most common manner of development of the anterior end

[1] *Balfour.*—A Monograph on the Development of Elasmobranch Fishes. London, 1878.

of the notochord and the coelenteric zone in the embryos which I have examined; but as it is usual for degenerate rudiments of organs to present variations, so also the anterior tip of the notochord and the coelenteric zone apparently follow the general rule. To follow the lines of development of these variations would, of course, be impossible; but the description of a few stages will make clear the chief variations. In an embryo slightly older than that of series B, and in which the mouth-opening has broken through, I found the notochord anteriorly widely separated from the hypoblast. Only the anterior tip retains its fused connection with the roof of the hypophysis. The notochord, for a short distance from the tip backwards, is very crooked. Laterally from the tip of the notochord extends the coelenteric zone, also connected for a distance with the roof of the hypophysis. The lumen of the head-cavities extends into the coelenteric zone very near to the median line, as is shown by a sagittal section (47, Pl. XV.), two or three sections from the notochord. Other older embryos show that the coelenteric zone has entirely disappeared, while the anterior tip of the notochord still remains fused with the roof of the hypophysis. This condition I found in one embryo older than that of series C, and in another so far advanced that the head-cavities had entirely disappeared.

It might be supposed that in some cases the roof and posterior wall of the hypophysis are formed from the hypoblast, and that therefore the tip of the notochord has simply remained connected with the cell-layer from which it originated; but on this hypothesis it would be difficult to imagine an adequate reason why the tip should be bent out of line to retain this connection. The appearance of the hypophysis in all the embryos is so similar, that there is no reason to suppose a variation in its origin. In one instance I have shown it to be derived from the epiblast; this agrees with what has been found by authors on the embryology of other vertebrates. In the present case, the first appearance of the tip of the notochord, the coelenteric zone, and head-cavities is in the form of a small mass of cells, apparently budded from the hypoblast. This mass is fused with the epiblast. In some individuals the notochord and coelenteric zone separate from the epiblast at the same time, though retaining connection with each other. In other individuals the coe-

lenteric zone separates from the epiblast much earlier than does the notochord, and disappears; while the notochord remains a long time connected with the epiblast or hypophysis.

3. *The Alimentary Canal.*

The alimentary canal in the youngest stage examined by me (series A) has already been described. I need only add that in the region of the mouth it appears laterally compressed, while behind the mouth it is dorso-ventrally compressed. Behind the region of the mouth the lumen appears everywhere of equal size as far back as the yolk-sack. No traces of gill-pouches have appeared.

The oral fusion of epiblast and hypoblast appears very early in the lizard. *Hoffman* has described it as present in an embryo with only two somites. *Balfour* has pictured the two layers in contact in this region in an elasmobranch embryo, of which the medullary groove is only a slight depression. It seems probable, therefore, that in these cases no mesoblast ever develops between the other two layers at the point where the oral fusion appears. If this supposition be true, the mouth-opening of the lizard presents a method of origin very different from that of the gill-clefts.

The gill-cleft rudiments first appear as paired pouch-like protrusions from the dorso-lateral parts of the alimentary canal (series B). They grow toward the epiblast, pushing through the mesoblast, which at first entirely surrounded the alimentary canal. The first and second clefts are the first to acquire an external opening. Then follow in order the third and fourth clefts (Figs. 30, C, and 31, C, Pl. XIV.). Behind the fourth there appears later a fifth rudiment, for which alone I have never detected any external opening. In longitudinal-horizontal section there may be seen in some embryos small rounded swellings of the epiblast on the lateral posterior corners of the gill arches. These were at first suggestive of gills, but are perhaps simply remnants of the breaking through of the epiblast. That part of the alimentary canal from which the gill-clefts open is, comparatively, extremely large, and may be supposed to indicate its condition at the time when it was functionally active, as part of the respiratory system.

On the ventral surface of this large gill chamber, intermediate to the regions of the first and second clefts, appears the first rudiment of the thyroid gland (Fig. 31, C). In horizontal section its outline is a circle. It is a compact thickening of the wall of the gill chamber, and its cells lie in a radiate position. Immediately ventral to it is the fork of the ventral aorta. In a later stage this round thickening of cells has become depressed in the centre, so that it has a lumen slightly constricted at its opening to the gill chamber. Later it appears entirely separated from the wall of the alimentary canal, and without lumen. Finally it assumes its usual shape and position, with its thin median part lying ventrally across the trachea, a little in front of the separating bronchial tubes.

After the breaking through of the primitive mouth, as already stated, the lower jaw, overlapping the primitive anterior surface (anatomical base) of the brain, grows toward the nasal tip of the head. The progress of this growth may be seen in Fig. 51, D, where the jaw is a little in advance of the hypophysis; and Fig. 63, F, Pl. XVI., where the jaw extends beyond the optic chiasma.

The lumen of the gill chamber gradually decreases posteriorly until, just behind the region of the fifth cleft-rudiment, the ventral half of the lumen seems to be obstructed by a transverse wall. Dorsally the lumen continues, but so small that a single one of its limiting cells would suffice to fill it. From this transverse, obstructing wall (*Tr*, Fig. 36, C) is developed the lung rudiment. First appears a small tube growing out posteriorly and parallel with the intestine. This tube soon divides into two similar tubes, which continue their growth backward, though separating laterally. All these tubes are provided with a lumen, and show the same columnar endothelium as the intestine. A distal expansion of these lateral tubes finally leads to the growth of two large bladder-like sacks. At one period, when these sacks occupy relatively the entire space intended for the lungs, they possess simple large oval cavities without any reticulation. They have, however, small ridges on their internal surface through which run blood-vessels. These ridges soon increase in size, and, extending into the cavity, cause the reticulation.

Behind the origin of the trachea, and opposite the posterior

end of the heart, the liver and pancreas make their first appearance. They grow out toward the right side of the embryo as hollow diverticula of the intestine; and are almost opposite each other, the liver tending ventrally and the pancreas dorsally (Fig. 40, C, Pl. XV.). The liver is the first to assume a glandular appearance. It projects into the venal sinus, behind the heart, so that its relations to the circulatory system are about the same as those described by *Shipley*[1] in Petromyzon.

In Anolis, although the walls of the neurenteric canal appear distinct in my youngest stage, yet the lumen makes its appearance at a rather late period. The alimentary canal extends a very short space behind the allantoic diverticulum, and then bending upward, its lumen becomes that of the neurenteric canal. This region presents the usual features of the fusion of the three germ-layers. Ventrally, just behind the allantois there exists in an early stage a median elongated thickening of the epiblast. A solid mass of cells extending from the intestine is fused with this thickening anteriorly. This is the spot where the cloaca appears later; and, in view of the recent researches on the subject, I should judge it to be the last trace of the blastopore. The neurenteric canal remains open a comparatively long time. There is still a trace of it in embryos in which the tail extends behind the cloaca more than half the length of the trunk. Fig. 58, Pl. XV., represents a section through the tip of the tail of such an embryo. The section seems to be not exactly transverse. In the centre is seen the neurenteric canal (*NeC*); at the right are a few cells of mesoblast; and at the left a fused mass of mesoblast and hypoblast (*MHp*). Above the latter, and next to the ectoderm, is a small round body (*N*), which, when followed forward, approaches a central position, and is found to be the notochord. This appearance is very peculiar, and seems to indicate a very irregular and rudimentary condition. I have examined this part in only a few embryos at this stage, but found no two exactly alike. A little farther from the tip of the tail (Fig. 57) the medulla (*Md*), notochord (*N*), and caudal intestine (*AL*) become well defined. The caudal intestine sometimes shows a lumen, and sometimes is only a cord of cells, though with indications of a lumen. The meso-

[1] *Shipley.* — On some Points in the Development of Petromyzon Fluviatilis.— *Quart. Jour. of Mic. Sci.*, Jan., 1887.

blast (*Mp*) is also distinctly defined, with its usual shape dorsally but uniting ventrally below the intestine. Proceeding farther forward, the caudal intestine invariably becomes a solid cord, which gradually disappears. The condition of the tail at this point is shown in Fig. 56, which represents a cross-section just behind the middle length of the tail. From these facts I conclude that the caudal intestine continues to grow in the neurenteric region, even after its anterior part behind the anus has atrophied. The atrophy occurs from before backward, and for a time the proximal end seems to atrophy about as fast as the distal end grows.

4. *The Mesoblast and Primitive Kidney.*

The origin of the mesoblast has been described by the authors referred to in the introduction. I will only refer to a few points in its development. The origin of the head-cavities, which are homologous with the body-cavity, has already been described. The segmentation of the mesoblast into somites occurs from before backward, — the first somite appearing at just the distance behind the ear that would equal the space occupied by one somite. About the time of the segmentation of the mesoblast the dorsal part forming the somites becomes separated from the ventral part, which incloses the permanent body-cavity. On each side the walls of the body-cavity — the somatopleure and splanchnopleure — meet dorsally at an acute angle. The apex of this angle becomes divided off by a longitudinal constriction, and appears at first to form a continuous rod of cells more or less fused dorsally and ventrally with the adjacent mesoblast. This rod later becomes segmented. It is the "intermediate cell-mass," or rudiment of the Wolffian bodies, and has been described by *Weldon* in Lacerta muralis. In an embryo with four somites the parts of the mesoblast are crowded on each other, and are not very distinctly defined. In the posterior region of an older embryo, owing to the greater size of the body, the parts referred to are separated from each other and distinctly marked. In an embryo with nine somites the unsegmented mesoblast, slightly anterior to the region of the neurenteric canal, has not divided into dorsal and ventral parts. A little farther forward this division is taking place, and the "intermediate cell-mass" appears as pictured by *Weldon* (Figs. 15 and 17 of *Weldon's*

article). Some of my sections show, also, that a part of the lumen of the body-cavity is enclosed by the intermediate cell-mass. When the constriction is completed, the lumen at first projecting into the intermediate cell-mass has disappeared. Farther forward the intermediate cell-mass in cross-section appears round, and is completely removed from the protovertebræ, though remaining in contact and fused with the wall of the body-cavity. Still farther forward this fusion disappears, and the intermediate cell-mass is segmented into the Wolffian bodies. The Wolffian bodies are much elongated, and extend in a dorso-lateral direction to near the epiblast, where they unite with the segmental duct. A lumen is visible in the most anterior of the Wolffian bodies, uniting with the lumen of the segmental duct. The gradation of all these changes is apparent in the consecutive sections from behind forwards.

Haddon[1] has recently summarized the facts and reasons for considering the segmental duct as a product of the epiblast. As most of the literature on this subject referred to by him, has been inaccessible to me, I give but a brief account of what I have found in this respect. It is best illustrated in the same embryo of nine somites, in which I have described the development of the Wolffian bodies. Near the region of the neurenteric canal, opposite that part of the unsegmented mesoblast which has not yet divided into a dorsal and a ventral part, there appears a small linear thickening of the epiblast. This thickening is the same on each side, and lies horizontally and a little above the level in which the intermediate cell-mass is to appear. Posteriorly this epiblastic thickening fades away; but in the direction of the head it becomes more marked, and appears in cross-section as a distinct semicircular clump of five to eight cells adhering to the epiblast. (In one embryo in which the epiblast is stained darker than the mesoblast, the cells in question took the deeper stain.) A little farther forward this thickening of the cells becomes gradually separated from the epiblast, and lies as a solid cord about midway between the epiblast and the rudiment of the Wolffian body. Still farther forward the cord of cells acquires a lumen, and lies in contact with the Wolffian body. It is now easily recognizable as the seg-

[1] *Haddon.* — Suggestion respecting the Epiblastic Origin of the Segmental Duct. — *Scientific Proceedings of the Royal Dublin Society* (read Feb. 16, 1887).

mental duct. Its size increases anteriorly, until in the body segments behind the heart-region it unites with the most anterior, and accordingly the oldest, Wolffian bodies, as above described. Later stages show that each Wolffian body becomes a much convoluted tubule, with the median end of its lumen separated by a thin membrane from a protruding pocket of the aorta, while distally its lumen connects with that of the segmental duct, which opens into the cloaca.[1] (See series B.)

In stages about the time the egg is laid may be seen the method of development of the mesoblast of the tail. In transverse sections through the anterior region of the large neurenteric canal, and just in front of it, the intestine presents a vertically oval lumen, with a wall of distinct columnar cells. In the dorso-lateral parts of the wall the cells are much elongated, and extend their free spindle-shaped ends outward. A band of similarly shaped cells on each side connects this part of the wall of the intestine with the corner of a triangular solid mass of mesoblast, which lies dorsal to the intestine between the neural tube and the epiblast. Aside from the cells just mentioned no others appear between the hypoblast and epiblast. A few sections farther forward this cell-proliferation ceases, and the mesoblast unites ventrally.

This derivation of the caudal mesoblast corresponds to a very general method of origin of the mesoblast, from the dorso-lateral walls of the alimentary tract. This appears also to be the method of origin of the head-cavities.

5. *The Circulatory System.*

The circulatory system of the lizard in these early stages presents but few peculiarities of difference from the accounts given of other lower vertebrates. In general it agrees with the description given by *Shipley* of the same system in Petromyzon.

In the space between the oral fusion and the opening of the head-intestine into the yolk-sack the splanchnopleures fold in-

[1] It was not at first my intention to treat of the primitive kidney in this paper. When I became impressed with the significance of what I described above it was too late to add new figures to my plates, which had already been sent to the lithographer. I hope to publish, at another time, figures illustrating what I have here described.

ward below the intestine, and unite the apical surfaces of the folds in the median line just ventral to the intestine. A little distance below this another similar in-folding takes place; and thus arises a tubular lumen enclosed by the splanchnopleures, between the head-intestine and the hypoblastic wall of the yolk-sack. Fig. 16, A, shows this organ in process of formation: *HT* represents the lumen of the tube, — eventually the lumen of the heart. Owing to the twist of the embryo the growth is very indistinct on one side. When the tube is completely enclosed, it becomes separate from the splanchnopleures above and below it. These, however, remain continuous with each other across the median line, above and below the tube. Later, the growth of the somatopleure and epiblast, encircling the anterior ventral part of the body, separates this tube from that layer of the splanchnopleure which is adjacent to the yolk-sack. The ends of the tube open to the spaces between the hypoblast and mesoblast. As the tube grows in size it folds over on itself and forms a ventral loop, assuming at the same time the appearance and functions of a heart (see Fig. 59, B, and series C). The pericardial cavity is by this time very large, extending forward to the lower lip of the mouth. Enclosed courses have also appeared for the circulation of the blood. The blood-vessels, for the greater part of the body, may be traced out in series C. At first, all the blood from the heart passes through the mandibular arteries which divide where the ventral trunk leaves the pericardial cavity. At this time none of the posterior arterial arches have appeared. The mandibular arteries empty into two dorsal arteries. Anterior to the confluence of the mandibular arteries with the dorsal arteries the latter are continued (with decreased lumen) forward into the head as the carotids. In the region of the Wolffian organs the two dorsal arteries become a single median trunk, but, again, in the tail appear as two distinct arteries. This paired condition of the dorsal arteries is interesting, from the fact that previous to the appearance of any walls to the blood-vessels, the blood-corpuscles are found throughout the length of the body in the paired spaces on either side of the notochord, these spaces being completely separated by the medulla, notochord, and hypoblast.

The cardinal veins are continued in unbroken course forward

as far as the eyes. Just anterior to their confluence in the ductus cuvieri the cardinals give off on each side a vein that runs backward immediately dorsal to the Wolffian organs, and supplies each of these organs with a small vein running between the convolutions of the tubule.

6. *The Central Nervous System.*

The central nervous system in the earliest stage of my material has been already referred to. Its chief features may be seen in the transverse serial sections represented in Figs. 7, A, and 18, A, inclusive. In general appearance it is a long tube, anteriorly enlarged and curved ventrally. For a short distance at both ends the tube is open dorsally, toward the middle the walls meet dorsally, and in the middle region they are fused. In the posterior open part of the tube the wall is a single layer of columnar cells. Farther forward the lateral parts gradually become much thicker, and the nuclei increase in number; the cells no longer extend from surface to surface of the wall, but spindle-shaped cells appear internally. This condition obtains throughout the anterior part of the tube. The floor of the tube, however, remains permanently a single layer of cells. Owing to the laterally compressed condition of the tube, this arrangement is in some places difficult to observe, but it is well marked in the floor of the brain. The floor may thus be distinguished from the anterior medullary fold, which corresponds with the lateral walls.

At this stage may be seen the three anterior swellings of the tube which correspond to the hind- mid- and fore-brain. The latter is the part that is open dorsally. This opening extends from the division of mid- and fore-brain to the dorsal edge of the anterior medullary fold. In the lateral walls of this primary fore-brain appear internally small depressions (*Op*, Fig. 9, A), which are the first indication of the optic outgrowths.

The lateral walls of the neural tube are largest in the head, and taper gradually toward the posterior end of the body. In the fore-brain the lateral walls appear to grow most rapidly, so that by the time they fuse dorsally they enclose in diameter the largest part of the neural canal. The anterior medullary fold does not partake in this growth. Its growth ceases at an early

period, and it seems to take no part in the dorsal enclosure of the fore-brain.

If we imagine a middle longitudinal axis running through the neural tube of this earliest stage, its anterior part would have a marked ventral and slightly posterior curve,[1] and would end at a median point on the inner surface of the anterior fold. The growth of the lateral walls continues chiefly in a dorsal direction, so that before their dorsal fusion takes place the lateral folds are about twice as high from the floor as is the anterior fold. This I have ascertained by a series of horizontal sections parallel to the axis of the fore-brain in a slightly older embryo of *Sphærodactylus notalus*. The dorsal growth of the fore-brain brings its middle longitudinal axis into such a position that its anterior end at the time of the dorsal fusion is a point about the dorsal edge of the anterior fold. For convenience sake I shall consider the axis as ending exactly at this spot. Fig. 45, Pl. XV., represents a section through the fore-brain of an embryo of sphærodactylus slightly older than the embryo of series A. This section runs just dorsal to the longitudinal middle axis of the fore-brain, and parallel to the same. It is also symmetrically horizontal to the back of the embryo; but this part is assymmetrical because of the lateral twist of the head. The fore-brain is not yet dorsally enclosed; a narrow slit (*o*) extends from the dorsal edge of the anterior fold to the division between fore- and mid-brain. The sections of this series which are ventral to the longitudinal middle axis show that part of the lumen of the fore-brain which is inclosed anteriorly by the anterior fold. The latter, at this stage, is of the same appearance and thickness as the lateral folds. Later, the floor of this part of the brain becomes the infundibulum. The rudiments of the optic vesicles (*Opv*) are comparatively very large. In the figure, the vesicle of only one side is seen; on the other side the section passes dorsal to the vesicle. The distal end of the vesicle bends slightly in a posterior direction. In the dorsal half of the fore-brain — *i.e.*, that part above the middle axis and the edge of the anterior fold — the anterior walls of the two optic vesicles are separated from each other by the narrow slit *o*. This slit, therefore, extends the entire dorsal length of the primary fore-brain, and down the anterior surface to a point between the rudiments

[1] The lateral twist of the head is here not taken into account.

of the eyes. This region would correspond in a much older embryo (Fig. 51, D), as nearly as can be estimated, to the space between the constriction of the brain, *c*, and the point *og*, which lies directly between the two hollow optic stalks. In embryos a little older than that of Fig. 45, but much younger than that of 51, D, there is a linear median fusion of the dorsal and part of the anterior wall of the fore-brain with the epiblast. Everywhere else the brain is separated from the epiblast. The latter consists of a single layer of cubical cells. This fusion extends exactly through the region of the slit above described, and indicates the line of the closure of the fore-brain. Along the middle region of the fusion is a linear external depression, — the last remnant of the slit. The fusion is perhaps more complete at its ends, but its general appearance has led me to the conclusion that the entire slit closed nearly simultaneously.

In view of what I have found to be the case in the embryos examined by me, I am at a loss to account for the statements of *Hoffman* in this respect. He has figured a median longitudinal, vertical section of the entire head of an embryo of Lacerta, in which in the middle dorsal region of the primary fore-brain there is a pore-like external opening of the ventricle, the adjacent brain-wall being continuous with the epiblast. This, he says, is the last point of the enclosure of the brain. The lateral twist of the head would make it impossible to obtain any such complete median vertical section in all my embryos of this stage. In my youngest specimen the morphologically vertical plane of the head is at an angle of about 45° to the vertical plane of the trunk. In later stages, sections parallel to the vertical, longitudinal plane of the trunk and hind-brain, but slightly to one side, would cut the slit (*o*) in the fore-brain obliquely, so that it would present in each section the appearance of a pore.

Shortly after the complete closure of the brain and its separation from the epiblast, its vertical median section appears as represented in the reconstructed section of Fig. 51, D. The epiblast is a thin membrane of flattened cells, and lies close to the roof of the brain. The roof of the hind-brain is also a thin membrane. There is no dorsal constriction between the hind-brain and mid-brain, but they are separated by a marked lateral constriction. The mid- and fore-brain are separated by an encir-

cling constriction in the region *c*. The condition of the cranial flexure is here well illustrated. In the curved floor of the mid-brain (*MB*) there are peculiar transverse folds disappearing up the sides of the brain, — as though the curvature of the brain had compressed its floor into folds. This seems to preclude the hypothesis that the curvature is caused by a lack of growth in the floor of the brain.

In the middle of the anterior wall of the fore-brain, between the optic stalks, is a small transverse groove (optic groove, *og*). It has a very slight lateral extension, appearing only in about four sections. The position of the groove between the optic stalks seems to correspond to the anterior end of the slit above referred to, and to the dorsal edge of the anterior fold. Though I have not been able to trace out exactly and prove such an origin for the groove, yet I think there is little room for doubting that it is the last trace of the union of the lateral medullary folds, above the anterior fold. There seems to be no other plausible explanation for the presence of such a groove in this particular spot. This groove also appears in amphibian embryos. *Goette* has pictured it in Bombinator, and I have observed it in the frog and Amblystoma. In a median vertical section of an embryo of Amblystoma, just ventral to the groove begins an enlarged thickening of the brain-wall, which extends toward the hypophysis, and there thins out into the thin floor of the brain in the infundibular region. The position of this thickening in relation to the hypophysis characterizes it as the anterior fold. I cannot determine the relation, in this embryo, of the groove to the closure of the brain, as the latter has already taken place.

The eye is the first of the sense organs to develop, the second and third being respectively the ear and olfactory organs. The term "outgrowth" hardly conveys the full significance of the first appearance of the eye rudiment. In Fig. 45 it will be seen that the walls of the optic vesicles are parts of the lateral brain-wall, which remain in their primitive contact with the epiblast, while the other parts have sunk inward from the epiblast. This inward-sinking is continued until every part of the central nervous system is separated from the epidermis by the mesoblast, and only the eyes retain their original contact. At a later period mesoblastic tissue enters the eye-cup by the choroid slit,

to form the vitreous humor; but this cannot be said to separate the eye from the epiblast. A proximity to the epiblast is a fundamental necessity for an organ of sight, and this has undoubtedly been one of the chief formative motives in the early growth of the eye. Figs. 45 and 46, Pl. XV., represent two early stages of the growth of the eye, the sections being cut nearly horizontal, and parallel to the longitudinal axis of the primary fore-brain. In Fig. 45 the optic vesicle extends laterally, with a slight posterior bend at the distal end. In Fig. 46 the greater part of the vesicle extends in a posterior direction, so that what is at first the posterior wall becomes later the median wall. This wall may be distinguished from the anterior wall, as the postero-median wall does not touch the epiblast. About this time the distal expansion of the vesicle makes the stalk appear as though dorso-ventrally constricted, the lumen being round. The postero-median wall develops a heavy pigment on its inner surface (toward the vesicle). If we presume the existence of an ancestral form with eyes, the outer surface of whose head corresponded with the present inner surface of the brain, then these pigmented spots would seem to indicate the position of those primitive eye-spots, which, as the brain became enclosed by the sinking in of the middle part of the head, first faced anteriorly and then laterally. On this supposition, the anterior parts of the brain-wall, thus brought between the primitive eye-surface and the epiblast, would, being nearest the surface, tend to assume the optic functions, and the primitive eye would thus degenerate. This idea is suggested by the fact that in the embryonic eye the anterior or external wall continues to grow and differentiate, while the postero-median wall atrophies. The general features of the development of the eye are so well known that I will here refer only to a few points. An examination of Figs. 45, 46, and series C and D, will show that the optic organs are not ventral outgrowths, as they appear to be in the adult brain, but are lateral outgrowths from the extreme anterior region of the primary fore-brain, so that the anterior walls of the optic stalks are in the same plane with the anterior surface of the fore-brain. The optic vesicle becomes compressed by the development of the lens, so that its walls touch, and it takes the shape of a double-walled cup, with an unclosed slit (choroid slit) at its anterior end immediately external to the distal end of the

optic stalk. The relation of these parts and the development of the lens is illustrated in the figures just referred to.

The first development of the ear (*E*) and the olfactory organs (*Na*) is shown in the figures 59, B, Pl. XVI., and 41, C, Pl. XV. Both organs appear first as thickenings of the epiblast, which gradually become hollow, rounded depressions. The ear becomes later constricted off from the epiblast as a hollow spherical body, with its median wall touching the hind-brain (Fig. 24, C). At the same time its nerve reaches it from before. The nasal thickening receives its nerve before there is any appearance of a depression (41, C). The depression, which appears later, always remains externally open.

By the time the above-described changes have taken place, the brain has reached the stage of development which is illustrated in series C (Figs. 2, C, and 24, C, to 42, C). The line *P–s*, Fig. 2, C, indicates the plane of the sections. This series shows the rudiments of the anterior ten nerves of the head, with the exception of the fourth and sixth, which have not yet appeared. There is still no trace of nervous fibres in any part of the brain or spinal cord. The hind-brain (Figs. 24, C, to 29, C) is a slightly curved tube, with a triangular lumen much widened anteriorly, while posterially it blends into the spinal cord. The sides are thick, meeting almost at an angle ventrally; dorsally they become thinner, and from their edges a thin membraneous roof is spread almost flat across the top. Section 30, C, shows the narrow part between the hind-brain and mid-brain. From this point forward the mid-brain extends as a rounded swelling, and is cut almost transversely. The third nerve-pair springs from it ventrally. In Fig. 35, C, appears the infundibular region of the fore-brain (*FB.*). A slight elongated constriction separates the mid-brain from the fore-brain; this is seen in Figs. 38, C, and 39, C. This constricted region becomes the thalamencephalon, and in Fig. 42, C, is seen the dorsal constriction (*c*) marking off the region of the thalamencephalon from the dorsal swelling which later divides into the two lobes of the secondary fore-brain (*FB'*). Sections of more advanced embryos, showing the later growth of the brain, are figured at 6, Pl. XII., 52, E, Pl. XV., and 62, F, and 63, F, Pl. XVI. The explanations of the plates will show their relations to the parts just described.

Previous to the stage represented in series C, there have

appeared in the lateral walls of the hind-brain and the region of the thalamencephalon a number of symmetrical constructions, giving the walls in horizontal, longitudinal, sectional an undulated appearance. *Kupffer*[1] has given a short account, without figures, of a similar condition in embryos of osseous fishes, but did not discover the relation of these parts to the cranial nerves. *Kupffer* has called these parts "Medullar-falten;" but as the word "medullary folds" has another English meaning, I have adopted for them the word "neuromeres." The general appearance of the neuromeres is shown in the sections of the hind-brain in series C. Each neuromere is separated from its neighbors by an external dorso-ventral constriction, and opposite this an internal sharp dorso-ventral ridge, — so that each neuromere (*i.e.*, one lateral half of each) appears as a small arc of a circle. The constrictions are exactly opposite on each side of the brain. Fig. 6, Pl. XII., shows the arrangement of the cells in the neuromeres at a very early stage. The elongated cells are placed radially to the inner curved surface of the neuromere. The nuclei are generally nearer the outer surface, and approach the inner surface only toward the apex of the ridge. On the line between the apex of the internal ridge and the pit of the external depression, the cells of adjoining neuromeres are crowded together, though the cells of one neuromere do not extend into another neuromere. This definition of adjacent neuromeres presents, in some sections, the appearance of a septum extending from the pit of the external depression to the summit of the internal ridge (*Spt*). This septum may be nothing else than those parts of cell-walls which form the boundary line of the neuromeres, and which are made conspicuous by lying in a straight line. Of the neuromeres in the hind-brain, five are distinctly marked with the above characteristics, and are of equal extent in length. The most anterior of these gives off (on each side) from its dorsal half a mass of ganglion cells, constituting the root of the fifth nerve. The second gives off ventrally, and at a much later period, the sixth nerve. The third neuromere gives off, in a manner similar to the first, the single ganglionic root of the seventh and eighth nerves. The fourth neuromere gives off no nerve, but the

[1] *Kupffer.* — Primare Metamerie des Neuralrohrs der Vertebraten, Sitzing d. Math', — physische Classe vom 5 Dec., 1885, München.

space lateral to it is occupied by the auditory vesicle, so that the fourth might be considered the original neuromere of the auditory nerve. The fifth neuromere gives off, also dorsally, the root of the ninth nerve. Immediately posterior to the fifth neuromere is another similar swelling, which, however, is not separated by so definite a ridge and depression from the posterior neural tube into which it blends. This swelling gives off, dorsally, the tenth nerve; so we are justified in calling it a neuromere. Anterior to the neuromere of the fifth pair of nerves, the lateral brain-wall continues forward in a long curve to the constriction marking the rear boundary of the mid-brain. At this constriction there is a slight external depression and internal ridge, thus giving to the region of the long curve (in a very young embryo) the appearance of a neuromere nearly three times as long as the posterior neuromeres. There is no nerve for this neuromere, unless we suppose that primitively some relationship existed between it and the fourth nerve, which arises at a very much later period in the region of the anterior limiting constriction. I know of no evidence to support such a view. At the time of the earliest development of the neuromeres which I have described, the mid-brain appears to be simply an enlarged neuromere. Anterior to the mid-brain in the region of the thalamencephalon are two neuromeres, which are similar to the characteristic neuromeres of the hind-brain, except that they never give off any nerves. Fig. 6, Pl. XII., shows these neuromeres in a more advanced embryo; anterior to them is the swelling of the secondary fore-brain. As the nerve-fibres in the brain begin to develop, the constrictions marking off those neuromeres, posterior to the division of mid- and hind-brain, gradually disappear. There are no neuromeres behind the tenth nerve. As the embryo approaches the time of hatching, the cartilaginous dorsal arches of the vertebræ seem to cause a regular constriction of the medulla; but at this time the dorsal and ventral fissures have appeared, and there are no ridges in the small central canal. This different appearance and apparently different origin do not allow any very direct homology of these parts of the medulla with the neuromeres of the brain. *Balfour* described certain internal swellings of the lateral wall of the hind-brain of elasmobranch embryos: "Swellings of the brain towards the interior of the fourth ven-

tricle are in connection with the first five roots of the vagus and the glossopharyngeal root, and a swelling is also intercalated between the first root of vagus and the glossopharyngeal root." In his figure (Fig. 5, Pl. XVI., *l c*) there are no external marks of these divisions, and the "swellings" lie opposite the nerve-roots, while in the region between the nerve-roots there are internal depressions. In the lizard, on the contrary, in the region between the nerve-roots are internal ridges. The two conditions are thus very different; but possibly younger elasmobranch embryos might show a connection between these "swellings" and neuromeres.

As has already been mentioned, the greater number of the cranial nerves appear during the stages of development described in the preceding paragraphs. By examining a transverse section of the neural tube of the young embryo it will be seen that the lateral outlines are for a distance almost straight, and then meet dorsally and ventrally in rather sharp curves. In the region of the dorsal curve, on each side, the cells point laterally and ventrally from the inner to the outer surface, while the straight part of the wall has its cells directly transverse. It is in this region of the latero-dorsal curve that the cells which form the nerve-ganglion are proliferated from the neural tube. Without observing the actual moving process, it is a difficult point to decide; but all my sections lead me to believe that the cells are proliferated throughout the entire dorsal curve, retaining their connection with the tube, however, only at the lower end of the curve, which thus remains the permanent point of exit of the dorsal nerve-root. I find nothing to support the view that the permanent attachment of the dorsal nerve-root is secondary to the formation of the ganglion. The origin of the dorsal nerves in the brain seems not so simple as in the spinal cord, but is apparently of the same nature. Their earliest appearance, as observed by me, is figured at Fig. 5, Pl. XII.; a few cells of the ganglion of the fifth nerve lie adjoining the dorso-lateral part of the hind-brain, midway between two neuromeric constrictions. Excepting the optic, the third, and the sixth, the first nine pairs of nerves of the brain are exclusively of dorsal origin, *i.e.*, they first arise from the dorsal parts of the lateral brain-walls. The optic nerve has been in part described, and I shall again recur to it. The third and sixth pairs of nerves have a ventral

origin nearly similar to the ventral roots from the spinal cord. Their point of exit will be described later. The first pair of nerves spring laterally from the anterior dorsal (nasal) tip of the primary fore-brain, and run a very short distance direct to the nasal thickenings of the epiblast, in which they end. The nerves of the third pair arise from the mid-brain ventrally, in the middle region of the cranial flexure, and pass directly to the dorsal posterior walls of the head-cavities. The fourth pair arises at a time much later than the stages on which this work is based, and is intimately related to the later histological differentiation of the brain. The fifth nerve, arising as already noticed, passes forward, dividing into a dorsal and a ventral branch. The latter loses itself in the denser tissues which give rise to the structures of the jaws. The dorsal branch (ramus opthalmicus) passes forward dorsally to the head-cavity, and ends close to the dorsal surface of the eyeball; later it gives off a branch to the anterior wall of the head-cavity. The sixth nerve passes directly from its ventral origin in the hind-brain to the ventral posterior wall of the head-cavity. The seventh nerve passes down behind the first gill-cleft. The eighth passes to the anterior wall of the auditory vesicle. The ninth nerve passes down behind the second gill-cleft. The tenth nerve passes to the median wall of the first somite, which lies dorsally intermediate to the second and third clefts. Shortly after its first appearance a dorsal longitudinal commissure may be seen extending from this nerve backwards through the region of about four somites. This longitudinal commissure is irregularly attached to the brain-wall by a number of small fibrous roots. Distally a few rudiments of nerves may be seen extending from it a short distance. Two ventral nerve-roots unite, at this later stage, with what I first described as the tenth nerve. These nerves, together with the commissure, represent the rudiment of the vagus. I have not followed their development in detail. Behind the vagus-rudiment all the nerves present the typical features of spinal nerves.

It is worthy of notice that between the neuromere of the fifth nerve, which sends a branch down behind the mouth-opening, and the neuromere of the seventh nerve, which passes down behind the first gill-cleft, there is an intermediate neuromere. Also between the neuromere of the seventh and the neuromere of the ninth nerve, which passes down behind the second gill-

cleft, there is another intermediate neuromere. Therefore if we consider the visceral arches as indicating the metameres of the head, the latter do not correspond to the neuromeres of the brain.

The fibrous elements of the central nervous system appear first in the stages succeeding that of series C. The fibres seem to be formed from the contents of the cells, by the breaking up of the cell-nucleus and absorption of the wall; or in other cases by the attenuated prolongation of the distal pole of the cell. The first method seems to be the case with the lateral longitudinal fibres; but I have not been able to follow it with certainty, and cannot say whether it is preceded by a polar attenuation. My sections, however, show the breaking up of the nucleus, and the gradual disappearance of the cell-walls. The second method is the case with the fibres forming the continuous transverse ventral commissure. The cells internal to the lateral longitudinal band of fibres give off long filaments from their distal poles, which run ventrally to form the transverse commissure.

The first fibres to appear are a thin, superficial band of longitudinal fibres, in cross-section, extending around the ventral lateral corner of the neural tube. From this corner springs the ventral nerve-root, and the greater part of the fibrous band lies dorsal to this nerve-root. Shortly after the appearance of the ventral band a similar superficial band of longitudinal fibres appears on the dorsal lateral corner, with its lower edge at the point of exit of the dorsal nerve-root. In the spinal cord these two bands are at first very distinctly separate; but in the head they blend into a single band lying superficially, — lateral in the hind-brain and ventro-lateral in the mid- and fore-brain. The appearance of these bands in the spinal cord is represented at *vL* and *dL* in Fig. 66, Pl. XVI. The same figure shows how the distal poles of the cells internal to the longitudinal bands bend downward, sending out fibres which run around the ventral surface of the tube to the opposite lateral wall. These fibres form the transverse ventral commissure. They appear at the same time with the dorsal longitudinal band. The floor of the central canal is still a single layer of columnar epithelial cells. These cells appear to take no part in the formation of the fibres of the ventral commissure, which lies closely attached to the floor of the central canal. The fibres of this commissure seem

to be simply attenuated polar outgrowths connecting the ganglion-cells of one lateral wall with the ganglion-cells of the other. In longitudinal section it may be seen that these fibres crossing the ventral surface of the tube run parallel with each other, and exactly at right angles to the long axis of the tube. I have not been able to find any trace of their bending horizontally to join the course of the longitudinal fibres. Whatever connection may take place between the transverse and longitudinal fibres, seems to occur only through the mediation of ganglion-cells. The ventral transverse commissure is continuous through the body-region, the hind-brain and mid-brain, as far forward as the ventral angle caused by the cranial flexure just in front of the exit of the third nerve-pair. Anterior to this angle is the infundibular region, where no nerve-fibres appear. The ventral commissure, in its upward lateral course, cuts off from the main mass a small bundle of ganglion-cells, which are marked *gc* in Fig. 67. From this continuous bundle of ganglion-cells spring the fibres which enter the ventral nerve-roots. The ventral lateral longitudinal band of fibres as it grows in thickness gradually extends upward until it meets and blends with the dorsal band, leaving only the small spaces where the fibres of the dorsal nerve-roots pass out from the main mass of ganglion-cells. Neither the longitudinal nor the transverse fibres enter the nerve-roots. In the cranial as well as in the spinal region the fibres which enter the nerve-roots pass from the neighboring ganglion-cells through the lateral band to the roots.

Fig. 67, Pl. XVI., represents a stage in the development of the spinal cord later than that of Fig. 66. In Fig. 67 it will be seen that the lateral bands have coalesced, and their continued growth causes on each side a ventral protuberance (*pt*). These protuberances gradually extend in a ventral median direction, and form the lateral walls of the ventral fissure. The ventral commissure remains the roof of the ventral (anterior) fissure. In this way the ventral commissure comes to lie eventually just below the middle of the cord. The dorsal fissure arises by the median compression of the lateral walls of the central canal, and the atrophy of the roof of the latter, so that at a slightly later stage there may be traced a straight line dividing the lateral halves of the cord, and running from the dorsal surface down to the remnant of the central canal. The remnant of the

canal has a small round lumen, and is entirely surrounded by a columnar epithelium. It looks as if the compression of the walls had caused the edges of the original epithelium of the floor of the canal to curve upward and unite dorsally, thus enclosing a small lumen.

Fig. 67 also shows a few elongated cells isolated in the fibres of the middle lateral region of the ventral commissure. The fate of these cells I have not traced. The bundle of cells from which spring the ventral roots (*ar*) has increased in size and is more definitely marked. This bundle of cells, the ventral (anterior) gray column, gradually disappears on entering the hind-brain, and is not found in any region of the brain anteriorly. In no part of the brain is there a ventral extension of the lateral bands of fibres, like that which forms the ventral fissure of the cord. The transverse and longitudinal fibres, however, are conspicuous in the brain. The ventral edge of the longitudinal band is very distinctly marked. Between the ventral edges of the two longitudinal bands extend the transverse fibres, which run upwards laterally at right angles to, and apparently interlacing with, the longitudinal fibres. The definite endings of the transverse fibres of the brain I have not been able to find. Fig. 65, Pl. XVI., shows a part of a transverse section through the mid-brain, cutting the roots of the third pair of nerves. This section shows the ventral transverse fibres (*TF*), and, as has been before intimated, is just a little behind their anterior limit of extension. The section also shows the ventral edge of the longitudinal band (*LF*). Just on the edge of this band, fibres from the internal ganglion-cells pass outward, constituting the root of the third nerve. This method of origin, though not exactly similar, is at least homologous with that of the ventral spinal nerve-roots. The sixth nerve originates in exactly the same relations to the transverse and longitudinal fibres as does the third nerve. In the case of the sixth nerve, however, the fibres of the nerve-root pass out in two or three separate bundles, which unite first external to the brain surface in the cellular nerve rudiment.

Although the lateral longitudinal bands of fibres in the fore-brain possess the same fundamental features as in the posterior parts, yet their relations to certain commissures make it more convenient to treat of the fore-brain separately. As has been

said, the ventral transverse commissure does not appear in the fore-brain. The main part of the lateral band, following the curve of the cranial flexure, passes along the ventral lateral wall of the fore-brain, and appears on the anterior surface of the fore-brain immediately ventral to the optic stalks. Fig. 52, E, Pl. XV., shows a section of the fore-brain cut at right angles to its anterior surface and immediately ventral to the optic stalks. The lateral band (*LF*) bends slightly dorsal to the plane of this section, and then, entering the plane again, passes in it around the anterior surface of the brain (*LF''*). At *LF''* the lateral longitudinal fibres unite, blending together so that this whole system may be described as a band of generally parallel fibres extending across the anterior surface of the central nervous organ and running posteriorly along its sides. At the stage represented in Fig. 53, E, there is only one other band of fibres in the region of the primary fore-brain, and that is the posterior commissure (*PCs*). Its general appearance is as if, on entering the fore-brain, a part of the fibrous mass had split off dorsally, and instead of following the curve of the cranial flexure these fibres continued across the dorsal surface of the brain just anterior to the mid-brain. In Fig. 52, E, the posterior commissure is cut obliquely near its point of separation from the lateral band. In Fig. 53, E (a section dorsal and anterior to 52, E), is shown the posterior commissure crossing the dorsal surface of the brain. Fig. 54, E, shows the first rudiment of the pineal eye, which arises a little distance anterior to the posterior commissure. Fig. 55 shows a section from an embryo of about the same stage as that of series E. This passes through the hypophysis (*Hph*) and fore-brain parallel to the anterior surface of the latter, cutting also the optic stalk (*O.st*), (cf. figures of series D). Immediately ventral to the optic stalk is seen the lateral band of fibres — in cross-section — just posterior to their meeting on the anterior surface of the brain. Outside the region of the bands above described no trace of fibres can be found in the brain at this stage; nor have any fibres appeared yet in the optic stalks. By comparing this description of the main band of longitudinal fibres with the description of earlier stages, it becomes evident that the main band of fibres follows the course of the primitive lateral and anterior walls of the medullary groove or tube. The band occupies a position

corresponding to the external surface of these walls. In the spinal cord this identity of parts is at once evident; but in the head the dorsal dilatation of the primitive tube to form the swellings of the brain has left the band in a position relatively more ventral.

In the stages immediately succeeding that which has just been described, the fibres of the optic nerve make their appearance. *Hoffman* has stated that the fibres of the optic nerve form first on the ventral wall of the optic stalk. This is true according to a general and rather inexact terminology; but it is on the morphologically anterior wall of the optic stalk that the fibres first appear. The fibres of the opticus develop in a manner very different from the fibres of all the other nerve-roots. They are not polar elongations, nor do they originate in the internal ganglion-cells of the neighboring brain-wall. They are formed next the external surface of the anterior wall of the optic stalk and the lateral wall of the optic cup, in the same manner as the fibres of the lateral bands are formed. The fibres appearing on the anterior wall of the optic stalk are continuous with the dorsal edge of the anterior and lateral band above described, so that the fibres of the band appear to continue outward along the stalk. Fig. 61 shows a horizontal section through the optic stalk at right angles to the anterior surface of the primary forebrain. As the stalk does not lie exactly in a horizontal plane, the section cuts it obliquely. The stalk still has a lumen (*l*), and on its anterior wall (*op''*) are the nerve-fibres. There is no trace of fibres on the posterior wall (*op'*). At this stage I could not trace any decussation of the fibres from the opposite sides, — probably because the fibres run so nearly parallel. Fig. 60 represents a section through the anterior part of the eye-cup and part of the optic stalk, in the same plane as Fig. 61. This section shows the fibres *NF* developing on the anterior wall of the stalk (*op''*) and on the lateral wall of the eye-cup (*Ey'*). The fibres in the eye-cup do not spread equally in all directions, but extend chiefly in a posterior direction, which is the direction of the long axis of the cup. The lateral wall of the optic cup has increased very much in size; while the median wall has become very thin, and is in some places a mere membrane. The corresponding walls of the optic stalk are affected in a similar manner. The anterior wall, as it develops the

fibres, increases in thickness. The posterior wall develops no fibres, but gradually becomes thinner. At a period when the two walls are still distinct, though the lumen has been obliterated by the growth of the anterior wall, the posterior wall is a thin layer of flattened cells, connecting the membraneous wall of the eye-cup and the cellular, non-fibrous brain-wall immediately dorsal to the lateral band. This thin layer of flattened cells is entirely free from fibres, and in stages a little older it has disappeared, excepting a wedge-shaped cellular projection of the brain-wall immediately posterior to the point where internally a conical depression marks the original median opening of the stalk-lumen (*l*), (cf, Fig. 61). The dorsal and ventral division of the anterior and posterior walls of the optic stalk corresponds originally with the line along which the lateral and median walls of the optic cup unite.

During the period of the development of the optic-nerve fibres and the formation of the chiasma, the brain undergoes considerable change. The parts of the brain already present become more pronounced, and the lobes of the secondary fore-brain appear. These changes are illustrated in Fig. 63, F. Anteriorly this section passes to the right of the fold which separates the ventricles of the two lobes (see description of figure). The dorsal anterior part of the primary fore-brain becomes dilated into a small, rounded swelling (*FB''*, Fig. 6). The anterior surface of this round dilatation extends to a point just dorsal to the optic stalks, where its boundary is marked by a constriction. A little later a small median groove appears in the roof of the swelling, dividing it into lateral halves. From this time on, each half continues its growth separately, leaving the originally slight groove as a deep cleft separating the two. Bearing in mind the foregoing descriptions, it is evident, from a comparison of Fig. 51, D, with Fig. 63, F, that the secondary fore-brain is a dorsal and not an anterior outgrowth. In this latter stage (63, F) the increase of the cranial flexure has brought the infundibular region relatively much nearer the floor of the hind-brain. The secondary fore-brain is shown at its largest vertical and longitudinal diameters in this figure.

Fig. 62, F, represents a section of the same series as 63, F, and in a parallel plane, but passing through the lateral wall of the brain. This section shows the lateral longitudinal band of

fibres (*LF*) passing forward to meet the opposite band just vertical to the chiasma (*Ch*). A comparison of the distance between the ventral parts of the fore- and hind-brain in the two sections (62, F, and 63, F) will give an idea of the transverse curve of the ventral surface of the brain. Owing to this curve and the anterior diminution of the basal breadth of the brain, the lateral band passes out of the plane of section 62, F, before reaching the anterior surface of the brain.

Before the last trace of the lumen of the optic stalk has disappeared, the decussation of the fibres of the optic nerves becomes apparent. The fibres of the dorsal edge of the lateral band, as they pass across the anterior surface of the brain towards the root of the optic stalk on the other side, bend dorsally, and running along the anterior wall of the stalk spread over the lateral wall of the eye-cup. Where the fibres from opposite sides meet, those from each region resolve themselves into four or five flattened bundles, and cross in such a way that between each two bundles of one nerve lies a bundle from the opposite nerve. I have searched in vain for any traces of fibres running from the lateral band into the optic nerve of the same side. None such appear. At this time the chiasma lies within the general superficial surface of the brain, its flat outer surface corresponding with the surface of the brain, while its inner surface is rounded. The ventral edge of the chiasma is at first continuous with the dorsal edge of the anterior band (*LF*), but later a wedge-shaped growth of cells pushes in between the two edges. Fig. 64, F, is an enlarged view of the region of the chiasma in the same section that is pictured at 63, F. Here the chiasma (*Ch*) and the anterior band are seen still touching each other, while at the same time they are marked off from each other by a wedge-shaped protrusion of the cells of the brain-wall. Later this wedge-shaped protrusion increases in size, and completely separates the two parts in the median line. By following the courses of these two fibrous parts in the consecutive sections between sections 63, F, and 62, F, it is found that the anterior band (*LF*) is perfectly continuous with the lateral band (*LF*, Fig. 62, F), and that the bundles of the chiasma coming from the opposite optic stalk also unite and blend with the lateral band; while the bundles from the other lateral band run into the optic

stalk of this side. In later stages the chiasma comes to lie outside the surface of the brain. At that time, in transverse sections the anterior band is very distinct, and is completely separated from the chiasma in the median line; but laterally the optic fibres from the chiasma run into the lateral continuations of the anterior band, *i.e.*, the lateral bands.

In Fig. 62, F, may be seen the posterior commissure (*PCs*) in lateral section. This is the only commissure of the brain which is complete at this stage. Its fibres are continued dorsally in a broad band lying superficially across the roof of the brain. At the anterior edge of this band is the rudiment of the pineal eye. The latter has acquired a lumen, but is otherwise unchanged from the condition shown in Fig. 54 E. Anterior to the pineal eye, the roof of the thalamencephalon is a thin layer of cubical cells; and just behind the lobes of the secondary fore-brain it shows a number of small, round, gland-like outgrowths. About half-way between the posterior commissure and the optic chiasma is a small band of fibres extending dorsally from the lateral band and ending in a point. (This is represented in the drawing, but not lettered.) None of these fibres cross over the roof of the brain. From its position, I judge this pointed band to be the rudiment of the superior commissure described by *Osborn* in several amphibia, and by *Shipley* in *Petromyzon*. About half-way between the rudiment of the superior commissure and the chiasma lies another band of fibres, extending dorsally into the anterior wall of the secondary fore-brain. At this stage I find no trace of these fibres uniting across the median line with fibres from the band of the opposite side; but they undoubtedly form, later, the anterior commissure. In embryos of the frog and Amblystoma, at a stage apparently corresponding to this stage of Anolis, I find the general features of the lateral and anterior bands, and their relation to the optic fibres, to be about the same as above described; but in the amphibian embryos the fibres which run dorsally from the lateral bands, just behind the optic stalks, unite across the anterior surface of the brain, forming a superficial commissure a short distance dorsal to the optic stalks; therefore I think there can be no doubt that the corresponding fibres in the brain of the lizard are the rudiments of the anterior commissure.

Whether the fibres of these dorsal commissures are continuations of the lateral longitudinal fibres, I have not been able to determine with certainty. The commissural fibres spread wider apart on entering the region of the lateral bands, so that their appearance is easily deceptive; nevertheless, many of them, perhaps the majority, continue downwards, crossing for a distance the lateral longitudinal fibres nearly at right angles, but not appearing ventral to the lateral bands. Their ultimate endings I could not find. The superficial position of these three commissures, — anterior, superior, and posterior, — their similar connections with the lateral bands, and their relation to the constrictions of the brain, suggest at this period a striking homology between them.

Before hatching, the brain of the lizard undergoes great histological changes, as well as changes in the relative size and position of its parts. The most remarkable of these changes is the great development of white matter, and the change in the character of the comparatively few remaining ganglion-cells. The tissues assume the complex appearance of the adult brain-tissues. Through these changes I have not followed the fate of the lateral and anterior bands. Their position remains up to about the time of hatching, still occupied by white matter, but this is not distinguishable from adjacent parts; and the courses of the fibres appear very complicated,— an appearance which is perhaps increased by the greater irregularity in the surface of the brain. The fibres of each optic nerve can be traced along the opposite wall of the thalamencephalon, where they gradually become indistinguishable, a large bundle of them apparently passing to a ganglionic kernel in the lateral wall of the thalamencephalon. The anterior and posterior commissures are at this time well marked, for they constitute the largest bundles of parallel fibres. The fate of the above-described rudiment of the superior commissure I have not discovered.

Although it is not my intention at present to follow out the later stages in the development of the brain, yet I wish to call attention to a feature of the secondary cranial flexure, which falls partly in the embryological period with which I have dealt. As seen in Fig. 2, C, Pl. XII., the hind-brain has a slight dorsally convex curve. The future roof of the mouth from the hypophysis to the nasal tip lies nearly at a right angle to the

long axis of the hind-brain. In the fully developed individual this part of the mouth-roof lies nearly in the horizontal plane of the long axis of the body, and the primary cranial flexure has meanwhile increased. It is necessary, therefore, as the anterior part of the head approaches the straight line of the main body-axis, that the secondary cranial flexure should form a curve or angle of about ninety degrees. The manner of this bending is illustrated in Figs. 3 and 4, Pl. XII. In the region of the bend, the roof of the hind-brain is so thin that it offers comparatively no resistance to the mechanical changes of position of the lateral walls. Anteriorly and posteriorly, however, the walls are held in position by the thickened roof. Fig. 4 shows how the hind-brain (*HB*) bends ventral-wards, the ear being just behind the apex of the bend. Fig. 3 shows how, at the same time, the dorsal edges of the lateral walls are distended laterally at the apex, so that the roof of the ventricle presents the appearance of a rhomboid. If we conceive a pliable body of the same shape as the hind-brain, it is evident that by undergoing a similar bending it would present the same appearance. In the hind-brain the bend continues to increase. The posterior and anterior walls of the rhomboid approach each other, the median angles dividing the lateral walls becoming more obtuse, while the lateral angles become more acute. Finally, the two anterior and the two posterior walls lie approximately in two transverse straight lines and nearly touch each other. The reduced lumen presents the form of a cross. By this process the region of the elongated neuromere between the mid-brain and fifth nerve-pair, becomes the region of the cerebellum. The fifth nerve-pair springs from the lateral angles produced by this bend of the hind-brain. The ear, which now occupies a somewhat greater space, touches the mid-brain. The hind-brain has naturally continued its growth during this period, but its principal changes of form are produced according to the mechanical laws of flexion.

In some amphibian and teleost[1] embryos which I have examined, in various stages of early development, the process of the secondary cranial flexure seems to be very much abbreviated or almost obliterated. The first appearance of it consists in lateral angular outgrowths, or distensions of the walls in the

[1] I am indebted to *Mr. C. Earle* for kindly allowing me to examine his preparations of the different stages of the embryos of *Ctenolabrus*.

anterior part of the hind-brain. Thus, the lumen at this point acquires the same appearance of a cross as in the late stages of the lizard. The process of formation of this part in the lizard explains the angular outgrowths in these other embryos, and the lizard may be considered to represent in this regard the more primitive method of development.

PART II.

Certain phyllogenetic considerations give the development of the lizard a peculiar interest. The results of general morphology indicate that the elasmobranchs present a relatively primitive type of vertebrates,[1] distinct from the other lower forms in the fact that the latter have been more modified by degeneration and peculiar specialization. The development of the elasmobranchs appears also to be very primitive. The embryological development of the Teleostei seems to be partly abbreviated, and otherwise peculiarly modified and changed from a primitive condition. The same is true in part for the Cyclostomata and Amphibia.[2] The embryology of the lizard, which is probably next to the lowest form of amniota, resembles more closely the embryology of the elasmobranchs than that of any other forms of the icthyopsida. Moreover, the fact that the lizard has retained one organ — the pineal eye — in a condition much less degenerate than in all other living vertebrates shows that the lizard may be in some respects a very primitive form, in regard to this part of the nervous system, — even more primitive than the elasmobranchs. That other parts of the central nervous system would also be in a primitive condition seems a permissible deduction; but at the same time the retention of the pineal eye in the lizard shows how complicated is the subject of "higher and lower forms," and the determination of what is primitive, and also with what care one should receive deductions in this regard which are drawn from the supposed position in the phyllogenetic relationship. The history of the hypotheses in this connection, during the last decade, shows that we should not expect to find all the differ-

[1] *Gegenbaur.* — Das Kopfskelett der Selachier.

[2] Cf. *Gegenbaur's* review of *Goette's* "Entw.-gesch. d. Unke" in *Morphologisches Jahrbuch.* Vol. I.

ent organs in one animal to be primitive in the same degree. But wherever we find an organ in a condition which gives a clue to its original significance, and reasonably explains the largest number of variations of the organ in other animals, so that we may consider the variations as secondarily derived from the first condition, — such a condition would then deserve the attribute "primitive."

With this view in mind, my own work has naturally led me to a consideration of the different hypotheses as to the origin of vertebrates, and what bearing the evidence before me had on the theories. The comparison of my own results with those of other investigators suggested also explanations of some features generally obtaining in the embryological period with which I have dealt. I do not wish, however, to be understood as considering these theories or explanations in any way perfect or ultimately satisfactory. The discussion of theories of such wide bearing does not properly belong in scientific description; and for this reason, and partly for the sake of clearness, I have reserved these speculations for a separate part.

The hypothesis of the origin of the central nervous system from a pair of dorsally converged nerve-trunks is well known. It has received the approbation of *Balfour* and *Haeckel;* and *Hubrecht* also, for a time, supported it by forcible arguments. The value of the hypothesis lies therein, that it points out the Platyhelminthes as the group of invertebrates from which, on the one hand, by the dorsal convergence of the lateral nerves may be derived the vertebrate nervous system; and, on the other hand, by the ventral convergence of the lateral nerves may be derived the nervous system of *Huxley's* sub-kingdom annulosa. This theory has been strongly combated by the theory brilliantly expounded by *Dohrn* and his followers, — that the vertebrates are descended from annelid worms, accompanied by a process of inversion through which the ventral side became the dorsal side. Some of the arguments in favor of this latter theory concern the body-cavity and the distinct segmentations of the body; also in the annulosa the yolk-mass closes in on the dorsal side. The body-cavity is a fundamental anatomical feature in many animals, and in different groups its origin seems to vary, so that it is not necessary to

suppose that it appeared at one time in one smaller group, from which it has been inherited. The same is true of the segmentation of the body, and is illustrated in the incipient rudimentary segmentation shown by *Hubrecht* to exist in the Nemertina. The aggregation of a yolk-mass is an embryonic feature, and appears much modified in closely allied forms. We must suppose that the character of a type is due to selection acting chiefly on older individuals, at least after the period when a yolk-mass is present. When the character of the type has been determined, the nutritious matter of the embryo seems simply to collect at that spot where it interferes least with the development of the important organs. In view of the variability of the yolk-mass in the vertebrata and annulosa, there seems to be no reason for supposing a connection in this regard between the two groups; no more than for supposing a connection between the so-called placenta of Salpa and the placenta of Mammals. While the above objections to the annelid-inversion theory have merely a negative force, there are certain positive objections, which are met with great difficulty. The first of these relates to the homologizing of the vertebrate brain with the œsophageal nerve-ring, and the supposition that the œsophagus once passed through the vertebrate brain. At one time this objection appeared to be met with the aid of the pituitary body and the pineal gland, but more recent researches have closed this means of escape and failed to open plausibly any other. The connection of epiblast and notochord ventral to the brain, which I have described, adds new strength to the objection, and makes it seem insurmountable. The second objection relates to the inversion of position. We cannot suppose here a lateral turning over after the gradual manner in which it is supposed that in certain forms of the echinodermata the vertical axis gradually became the longitudinal horizontal axis, and of which intermediate forms remain. The supposition that the lateral sides of the worm became dorsal and ventral leads to the conception of a form that is incompatible with our knowledge of the symmetry of animals and the locative relation of their paired organs to the line of the force of gravity. On the other hand, if we suppose the change to have been sudden, we meet with equal difficulty in conceiving a form of such peculiar instability that it could at once turn itself upside down, and place,

relatively in a new position, organs which had arisen in their old position through a long course of phyllogenetic development. It seems, also, that peculiar changes of environment must be supposed in order to have made this sudden change of position permanent. Again, it might possibly be supposed that the ancestral worm gradually became insensible to, and unaffected by, the force of gravity; so that in motion and rest the morphological axes of his body bore no relation to the direction of gravity. But the influence of gravity on animals is so universal, that on the last supposition we are almost forced to imagine a temporary suspension of gravity. Perhaps the greatest objection to the annelid-inversion theory, no matter what method the inversion may be supposed to have followed, is the absence of intermediate forms, or traces of them. The most plausible methods — those of gradual, lateral, or longitudinal inversion, or gradual shifting of the organs — seem the most likely to have left intermediate forms or traces of them.

The theory of the dorsal approximation of lateral nerves has the advantage of being less specialized in its application. It is not dependent on such difficult tasks as showing how the œsophagus passed through the brain, or how a worm-like form became inverted. It can also refer to a general intermediate form. As a rule, those biological theories which are based on a few far-reaching facts, and allow greater scope for processes of which we are ignorant, but which are from analogies imaginable, are more trustworthy than those theories having perhaps equivalent facts, but which are yet absolutely dependent on detailed complicated processes of which we are equally ignorant, and which are less easily imaginable. The annelid theory has been skilfully adapted to explain certain particular details of vertebrate anatomy, — for instance, a relation of the vermicular locomotory appendages with setæ, to the paired and unpaired fins with their rays and spines. Such explanations give the theory a false appearance of strength; but it must be remembered that, until the main stumbling-blocks of the theory are cleared away, such explanations are comparatively valueless as support for the theory, and add almost nothing to our knowledge of the probabilities. The special application of the dorso-lateral nerve-theory has been attempted in no such degree as the above instance. Its possibilities of explanation remain

chiefly to be tried. *Hubrecht*,[1] however, made valuable efforts in this direction, and indicated some of the possibilities.

The Nemertina have afforded the best basis for the application of the theory of the paired origin of the ventral nerve-cord, and also of the dorsal nerve-cord. *Gegenbaur*[2] says: "Although in most of them the longitudinal trunks run along the lateral edge of the body (imbedded within the muscular layers), in others (Oerstedia) they approach one another ventrally, and are distinguished by swellings at the joints, where nerve-branches are given off. *This is in anticipation of the future development of ventral ganglia*, the elements of which are already present in the longitudinal trunks. The ventral approximation of the longitudinal trunks shows us how the central nervous system got its ventral position, which becomes further developed by the formation of ganglia." *Hubrecht*[3] has found that there is a "sheath of ganglion-cells which uninteruptedly accompanies these trunks from their origin in the cephalic lobes down to the extremity of the tail in all genera without exception." *Hubrecht* has given reasons for considering the genus Carinella the most primitive of the nemertine group. Of the lateral nerve in this genus he says: "It is not surrounded by nerve-cells, as these form only an external coating to it. . . . This cellular portion in *Carinella* is also of a less compact nature than in those of more differentiated genera, and is everywhere in direct contact with the epidermoidal tissue." Here, then, we find a general resemblance to certain forms of the embryological development of the vertebrate medulla, in which each lateral half shows in cross-section a lens-shaped swelling of epidermoidal cells, on the inner surface of which, later, the longitudinal nerve-fibres develop. A glance at several different forms will make this clearer. *Balfour* (Comp. Embryol.) has given a figure, after *Kowalevsky*, of an amphioxus larva, in which the medullary plate is distinctly thinner along the median line. The same author (Elasmob. Fishes) has pictured the median thinner portion of the medullary plate of elasmobranch embryos with

[1] See *Hubrecht's* papers in recent volumes of the *Quart. Journ. of Mic. Sci.*

[2] *Gegenbaur.* — Elements of Comparative Anatomy. Translat.

[3] *Hubrecht.* — Zur Anat. und Physiol. des Nervensystems der Nemertinen. Kön. Akad. d. Wiss. Amsterdam — Researches on the Nervous System of Nemertines. — *Quart. Jour. of Mic. Sci.*, 1880.

about the same thickness as the general epiblast. Again (Comp. Embryol.), he has pictured the chick at eighteen hours with an epiblast of a single layer of cells, which only on each side of the median longitudinal portion is thickened to a double layer. In his paper, to which I have referred in the introduction, *Balfour* represents a somewhat similar condition as existing in Lacerta muralis, as does also *Weldon*. *Scott* and *Osborn*[1] figure the same in the newt, and I have observed it in Amblystoma. The most striking illustration, however, is that of Bombinator igneus, which is also very carefully described by *Goette*.[2] Few other authors have referred to this appearance in their text. It has apparently been usually considered that the medullary groove and canal were at this stage the primary feature, to which the nature of the walls was of secondary importance; yet it is more natural to consider the walls as primary and the lumen as incidental. *Goette* describes a double symmetrical rudiment of the nervous system. In the figure of the round cross-section of the embryo, the lateral halves of the rudiment appear as lens-shaped thickenings of the "nervous layer" of the epiblast. These thickenings are separated by a median portion of exactly the same appearance as the parts of the nervous layer adjoining the lateral edges of the thickenings. In the head the paired thickenings separate farther laterally, to unite as a transverse curved thickening in the most anterior part of the medullary plate. I give here, in his own words, *Goette's* explanation of this appearance: —

"Es lässt sich alsdann nicht verkennen, das der vom Axenstrange auf das obere Keimblatt ausgeübte Druck die Ursache für die ursprüngliche bilaterale Anordnung der Anschwellung desselben in ihrem mittleren Abschnitte ist. Aus der folgenden Entwickelung ergibt sich aber, dass damit keine wirkliche Doppelanlage im oberen Keimblatte gegeben ist. Denn indem jene Seitentheile auf Kosten der übrigen Ausbreitung der Grundschicht deutlicher anschwellen, nimmt dass sie über dem Axenstrange verbindende Mittelstück im Verhältniss zu jenen dünnen peripherischen Theilen an Machtigkeit zu, offenbart

[1] *Scott* and *Osborn*. — On some Points in the Early Development of the Common Newt. — *Quart. Jour. of Mic. Sci.*, Vol. XIX., N.S., 1879.

[2] *Goette*. — Entwickelungsgeschichte der Unke. Bombinator Igneus.

sich also als zu der gesammten Anschwellung der Grundschicht gehörig (Taf. III., Fig. 62)." P. 157.

Even in the rather compressed parts of the amphibian egg this pressure of the notochord is hypothetical. The existence of this originally bilateral arrangement of the nervous thickenings, in so many different forms, under different embryonic conditions, casts strong doubt on the explanation by pressure. It is also a questionable method of morphological reasoning, by which the original significance of a part is first explained by deductions from its ultimate condition. *Goette*, in his description of a later stage, refers to the subject again as follows: —

"Im Rumpftheile sind die seitlichen Anschwellungen soweit zusammengerückt, das sie als zwei mit ihren Rändern unmittelbar zusammenhängende Bäuche (Medullarplatten) erscheinen. Im Kopftheile, welcher sich viel langsamer und in geringerem Masse zusammenzieht, bleiben die seitlichen Anschwellungen mehr auf den Rand der Axenplatte beschränkt, während ein nach Breite und Dicke ansehnliches, nach unten konkav gebogenes Mittelstück die ursprüngliche Einheit der ganzen Platte gegenüber ihrer Entwickelung aus scheinbar getrennten Seitenhälften im Rumpftheile hervorhebt." P. 165.

This "Mittelstück" is the same part that I have described in the lizard as the anterior medullary fold. So far from disproving the bilateral separateness of the nerve-thickenings, it seems to me to strengthen that view. If there were no anterior union of the lateral nerve-thickenings, we should have in this case a nervous system without a homologue; but an anterior commissure is characteristic of the Platyhelminthes, and this affords ground for a comparison. I have shown in the lizard that the first nerve-fibres appear to develop in longitudinal bands along the internal surface of the lateral nerve-thickenings, and are likewise continuous with each other anteriorly. If we suppose a change in the chronological order of development, whereby the nerve-fibres should appear during the earliest growth of the lateral and anterior thickenings and before these latter had formed a tube, then we should have an appearance very similar to that described in Carinella, except that Carinella has the nerve-trunks lateral, while in the vertebrate embryo they are subdorsal or dorso-lateral. In the head, so great a similarity of the nervous systems of these two forms disappears, but the main

feature — the anterior union of the paired trunks — remains. In both cases this anterior union of the paired trunks lies dorsal to the mouth, and in the vertebrate embryo it is also dorsal to the attachment of the notochord to the epiblastic hypophysis.

I have drawn the above comparison because it seemed to me to suggest a possible explanation of the primary condition of the nerve-fibres in the nervous systems of Amphibia and Reptilia, — in other groups observations on this point seem to be lacking, — a condition that appears later either to degenerate or to become so modified that it loses its primitive significance. The continuous band of transverse ventral fibres which I have described seems to be a secondary result of the close approximation of the lateral parts of the nervous system, and apparently is not derived from the median epiblast connecting the two parts. There are a number of minor points which seem to accord with this explanation, but it is unnecessary to enter into perhaps useless detail until the main features shall have been more widely and strictly tested.

Independent of any theory as to their origin, we have the fact of the primitive lateral-longitudinal and anterior band of nerve-fibres entering into peculiar relations with the optic organs. The condition which I have described suggests an explanation. The idea that the posterior or median wall of the optic vesicle may once have performed the function of sight has already been mentioned. Supposing this to have been the case, we would have, then, in each of the paired optic vesicles an organ homologous with the pineal eye of Lizards[1] and Petromyzon;[2] that is, there would be in each case a continuous part of the brain-wall lying between the true optic surface and the source of light. As the paired vesicles arise primarily within the region of the primitive longitudinal fibres, these fibres coming from behind would probably have run completely around the longitudinal periphery of the vesicle, and continued their course anteriorly to the lateral band of the opposite side. In this way the posterior median wall of the vesicle would be covered with fibres on its median surface, and, as is usual in simpler eyes, the cellular elements would lie between the nerve-fibres and the

[1] *Spencer.* — Pineal Eye in Lacertilia. — *Quart. Jour. of Mic. Sci.*, Vol. XXVII., N.S.

[2] *Beard.* — The Parietal Eye in Fishes. — *Nature*, July 14, '87, No. 927., Vol. 36.

light. The lens being developed in the epiblast would do away with a lenticular growth in the outer anterior wall of the vesicle. The outer anterior wall, as has been stated, is covered externally with fibres. If, going a step farther, we suppose the cellular elements of the outer anterior wall, being nearer the source of light, to have gradually assumed the optic function, we should then have an eye in which the nerve-fibres lay between the cellular elements and the light. The posterior median wall of the vesicle, having become useless, would degenerate, together with its nerve-fibres and the fibres connecting it with the brain along the posterior wall of the optic stalk. On the other hand, that part of the longitudinal fibres supposed to have been present on the outer anterior wall of the vesicle would remain as the optic nerve, and unite with the brain and anterior band of fibres along the anterior wall of the optic stalk. The origin of the remarkable conditions peculiar to the eyes of vertebrates is an extremely difficult subject to explain. The above explanation seems to me to accord best with the main facts, as I have found them in the lizard.

The cranial flexure is an almost universal feature in vertebrate embryos, and is of a nature so striking that it must have attracted the attention of all embryologists. One explanation of this feature generally given is, that it is due to unequal growth in the dorsal and ventral halves of the brain. But it remains a mystery what purpose this unequal growth subserves, and why it should cause such a peculiar curve in the entire anterior end of the embryo, — a curve which must later be rectified in part by the secondary flexure. I have remarked upon and figured certain transverse folds in the floor of the brain, where the flexure is greatest, which discountenance this theory as the sole explanation of the facts. Another explanation is, that the early and great development of the brain makes it necessary that it should partly roll itself together in order to accommodate itself to the space allowed it for storage. This seems hardly to explain why the curve should be almost universally of the same character, or why it should bend out of line the notochord and intestine. It is, moreover, improbable that a feature so fundamental in determining the shape of the adult brain and head should have been acquired simply to accommodate the wants of an early embryonic condition. The lateral twist of the head may be ac-

counted for by this latter theory, but the twist is only necessitated by the cranial flexure, and all traces of it disappear in later stages.

Even the combination of these two explanations does not account altogether satisfactorily for the peculiarities of the cranial flexure. While they perhaps play a considerable rôle, there seems to be another primary element. The probabilities deduced from our knowledge of the facts point strongly to a primitive paired condition of the central nervous system. This is indicated by the facts which have previously been referred to, by deductions from the principles of phyllogeny, and by the paired condition of the adult cerebro-spinal system. This paired condition has been a recognized principle in nearly every theory of the origin of vertebrates.[1] It is best illustrated in Bombinator, where there are the two bands of thickened epiblast running dorso-laterally along the back, spreading apart in the head, and uniting anteriorly in a graceful curve. In all cases where we find a nervous system originating in the epidermis, and then, by sinking into the body, removing itself entirely from the external surface, we are forced to conclude that the primary motive is to protect the increasing delicacy of the nervous organ from the rough stimulus of contact with external objects. There is no reason to suppose a different primary motive for this process in vertebrates. The thinner median epiblast connecting the thickened bands is carried inward with the thickened parts, — probably owing to its relatively small size and its intimate association with the commissures connecting the lateral neural parts. The tendency of the lateral parts of the epiblast to unite above the neural parts is such that they carry with them toward the median line the lateral edges of the neural thickenings. Thus is formed first the medullary groove, to which so much attention has been paid, and finally the central canal. Why this canal should have remained open is utterly unknown, but it probably serves, or served, only a secondary purpose. During the period in which these changes take place the neural thickenings are the densest and most unyielding of the tissues of the

[1] *Hubrecht* ("Quar. Jour. of Mic. Sci.," March, 1887) describes a small median dorsal nerve in nemertines, and suggests its homology with the spinal cord. In view of the bilateral symmetry of the spinal cord, the attempt to derive it from a nerve so small and specialized seems hardly an advance toward the solution of the problem.

body. Observing thus the nature of these paired thickenings, and the curve in which they unite anteriorly, the question arises, May they not, to a certain extent, be influenced by the simple mechanical laws of flexion; and may not the anterior downward-bending be in part the result of the gradual approximation of the lateral edges of the paired thickenings? The following is *Goette's* description of these parts: —

"Am Schwanzende ist die Tiefe der Medullarfurche gering; in der Mitte des Rückens und beim Übergange in den Kopftheil nimmt sie merklich zu, indem die Rückenwülste in dem Masse als die ursprüngliche Rückenfläche einsank, sich heben. Bis zur Mitte des Kopfs flacht sich die Medullarfurche weider ab, indem die Hirnplatte an der Knickungsstelle gewissermassen hervorgedrängt, die Erhebung und Umwälzung der Wülste zurückgehalten wird. In der vorderen Kopfhälfte erheben sich die Wülste wieder bis zu ihrer vorderen bogenförmigen Vereinigung, wo ihre Umwälzung zugleich am stärksten ausgebildet, der Grund der umschlossenen Einsenkung am meisten in die Tiefe gedrückt ist. Jener hervortretende mittlere Theil der Hirnplatte verdeckt aber den Eingang zu der davor und darunter entstandenen Tasche und lässt die Richtung und Ausdehnung derselben, mithin die starke Umbiegung der Hirnplatte leicht übersehen. Da nun die Rückenwand des Embryo während der bisher geschilderten Entwickelung in ihrem Dickendurchmesser sich nicht wesentlich verändert, also ihre Axe sich der Oberfläche analog verhält, so kann man an dem medianen Umrisse der letzteren die Umbildung der ursprünglichen halbkreisförmigen Rückenaxe verfolgen. Wenn diese Bogenlinie in zwei gesonderten Abschnitte sich gerade streckt, d. h. mit den betreffenden Sehnen zusammenfällt, so müssen diese beiden geraden Linien unter einem Winkel zusammenstossen, die ganze ursprüngliche Linie ein Knie bilden." Pp. 169–170.

A small strip of paper, if not too stiff, will afford a means of comparing the above description with the same manner of bending a pliant body of a somewhat similar shape.

This is illustrated in Diagram II. At A is represented a flat strip with its ends (*s*) held parallel. By approaching the upper edges of these ends until they touch in a straight line, the form B is produced, — *a* representing the anterior curve. If now the lateral edges of the two stems be moved upward toward each

other in the median line, the primary inner (shaded) surface becomes the external surface, while the anterior curve (*a*) bends as seen at C. The relations of the surfaces in the region of the curve, in C, are not the same as found in the embryo, for the primary outer surface still faces outward. If, however, the upper edge of the anterior curve be held inward at the same time that the two ends are brought flat together, a form is produced similar to that represented at D. Making allowances for the

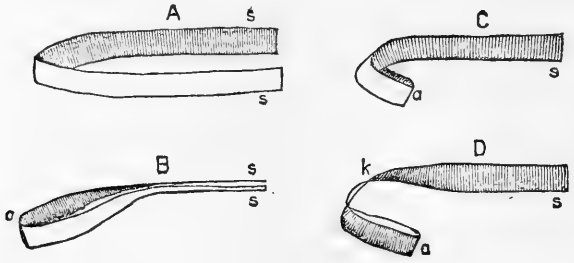


DIAGRAM II.

difference in texture and in the surrounding media, the form D permits a comparison with *Goette's* description of the neural growths in Bombinator. At *k* is seen the "Knickungsstelle" and "hervortretende Hirnplatte," and at *a* is the "Tasche" to which he refers. Could the primitive lateral and anterior bands of nerve-fibres which I have described in the nervous system of the lizard be removed entire, their form would correspond almost exactly with the form D. The supposition that the cranial flexure may have been partly caused in the manner just described, agrees with the fact that in the amphibian and lizard embryos the primary cranial flexure forms during the period of the closing in of the medullary groove. I judge, from *Balfour's* description, that this is also the case in the elasmobranch embryo. In other accounts relating to this subject, the attention not having been directed to this point, the descriptions of it are too obscure to admit of certainty. I have previously referred to mechanical influences in the secondary cranial flexure.

The connection of the notochord with the hypophysis in the lizard is a point worthy of mention. The exact relations of the anterior end of the notochord have rarely been traced out in all the early stages of development, and it is possible that more ex-

tended investigations of this part might throw light on the nature of the notochord. *Shipley* has recently described in Petromyzon the anterior end of the notochord as "being in contact with the posterior end of the nasal invagination." *Hubrecht*[1] has ingeniously suggested the derivation of the notochord from the proboscidean sheath of nemertines. If this view be accepted, the connection of the notochord and hypophysial invagination would apparently add weight to it, especially since *Heape*[2] has shown that at one period the notochord is tubular in the mole, and that its anterior end is also at first fused with the epiblast.

In conclusion, I wish to add, as apology for having entered the field of speculation, that in so doing my desire has been simply to call attention to points that seem worthy of future investigation.

[1] *Hubrecht.* — On the Ancestral Form of the Chordata. — *Quart. Jour. of Mic. Sci.*, Vol. XXIII., N. S. 1883.

[2] *Heape.* — The Development of the Mole (Talpa Europea). — *Quart. Jour. of Mic. Sci.*, Vol. XXIII., N. S. 1883.

EXPLANATION OF PLATES.

INDEX-LETTERS.

A. Artery.
AF. Anterior medullary fold.
AL. Alimentary canal.
Am. Amnion.
Ao. Aorta.
ar. Anterior spinal nerve root.
CC. Canalis centralis of the medulla.
c. Dorsal constriction in the region of the thalamencephalon.
Ch. Chiasma of optic nerves.
CS. Choroid slit.
[1] *CZ.* Coelenteric zone.
d. Dorsal point of union of medullary folds.
dL. Dorsal longitudinal fibres of the medulla.
E. Ear.
Ed. Epidermis.
Ep. Epiblast.
Eph. Epiphysis.
Ept. Epithelial wall.
Ey. Eye. *Ey''.* Outer wall of eye-cup. *Ey'.* Inner wall of eye-cup.
FB. Fore-brain. *FB'*, rudiment of secondary fore-brain, and *FB''*, of thalamencephalon.
g, Ganglion of spinal nerve.
gc. Anterior gray column.
HB. Hind-brain.
HC. Head-cavity.
Hep. Liver-rudiment.
Hph. Hypophysis.
HT. Heart. *HTa.* Atrium. *HTv.* Ventricle of heart.
Hyp. Hypoblast.
in.FB. Infundibular region of fore-brain.
LF. Longitudinal fibres of walls of the medullary tube; *LF''*, the same uniting anteriorly.
LJ. Lower jaw.
M. Mouth.
MB. Mid-brain.
m.b. Muscle-bud.
Md. Medulla.
Mes. Mesoblast.
MF. Medullary folds.
MHp. Mass of indistinct mesoblast and hypoblast.
M.in. Mouth involution.
Mp. Muscle somites.
N. Notochord.
Na. Nasal thickening of epidermis, with rudiment of *N* olfactories adjoining.
Ne.c. Neurenteric canal.
NF. Nerve-fibres.
NM. Neuromeres. *NM'.* Neuromeres of fore-brain.
[1] *nIII, nV, etc.* Third, fifth, etc., cranial nerves. nV_1, nV_2. First and second branches of the fifth nerve.
o. External opening of the primary first ventricle.
Œ. Œsophagus.
og. Optic groove.
Op. Wall of optic vesicle. *Op'* posterior and *Op''* anterior wall of optic stalk.
Opv. Optic ventricle.
O.st. Optic stalk with lumen.
Pan. Rudiment of pancreas.
PC. Pluero-pericardial cavity.
PCs. Posterior commissure.
P–S. Plane of section.
PT. Pleuroperitoneal cavity.
pte. Ventral downgrowths of spinal cord.
PV. Protovertebræ.
So. Somatopleure of mesoblast.
Sp. Splanchnopleure of mesoblast.
Spt. Neural septa.
T. Tail.
TF. Transverse ventral fibres.
TH. Thalamencephalon.
Thg. Thyroid gland.
Tr. Rudiment of tracheal tube.
v. Vein.
vL. Ventral longitudinal fibre.

[1] In Fig. 34, C., the index-letters *CZ* should stand in the place of *nIII*, and *nIII* should be in the place of *CZ*.

Wb. Wolffian body.
Wd. Wolffian duct.
wHC. Wall of head-cavity.
x. Break in the embryo.
y. Lateral nerve-cells giving rise to transverse fibres.
I, II, III, etc. Hyoid and following gill-clefts.
Iv'. Primary first ventricle.
IIv. Ventricle of mid-brain.
IIIv. Ventricle of hind-brain.

Where a number of figures represent sections, or the entire view, of the same individual embryo all those figures have the same letter affixed to their numbers.

All figures of sections have been drawn with the Abbey camera lucida and a Zeiss microscope, — *Z. 2. A.* means, Zeiss ocular 2 and objective *A*, etc.

PLATE XII.

FIG. 1, A. — Young embryo viewed from beneath, in which the medullary folds (*MF*) have coalesced only in the dorsal region. The tail (*T*) is broken at *x*. *Iv*^{1}, primary first ventricle. *MB*, mid-brain, *N*, notochord. *PV*, pro tovertebræ. *Mes*, unsegmented mesoblast.

FIG. 2, C. — Embryo much more advanced than Fig. 1, A. *FB*, *MB*, *HB*, fore-, mid-, and hind-brain. *HT*, heart. *Ey*, eye. *HC*, head-cavity. *Hph*, hypophysis. *NM*, neuromeres of the hind-brain. *E*, ear. *I*, *II*, *III*, hyoid and two succeeding gill-clefts. *M*, mouth. *P–S*, Plane of section for series of figures 24, C — 44, C.

FIG. 3 and FIG. 4. — Dorsal and side view of head of an embryo, showing secondary cranial flexure in the hind-brain with the concomitant distending of the walls. *HB*, hind-brain. *E*, ear. *TH*, thalamencepalon.

FIG. 5. — Left wall of the medullary tube in the region of the fifth and sixth nerves, shown in longitudinal-horizontal section. *n V*$_{1, 2, 3}$, not of the trigeminus nerve. *NM*, neuromeres. *Spt*, lines of division between the neuromeres. (These lines have not been strongly enough marked in the plate.) (*Z. z. D.*)

FIG. 6. — Horizontal section dorsal and parallel to the axis of the primary fore-brain; showing the neuromeres (*NM*) of the thalamencephalon (*TH*). *FB''*, secondary fore-brain. *MB*, Mid-brain. This embryo is further advanced than that of series C. (*Z. z. A.*)


I.M
MB
MF
PV
Mes
T
Ov
5
MB
TH
MB
MF
Hp
HB
HB
MB
FB
E
E
2 v
HS
Hyp
HC
5
P
XV
Sm
FB
I
E
Hy
XM
Z
f
H
HT
Sp
S
XV
N
nV
6
Sp
XX
XB
Hy
Sp
Sp
Sp
ED
TH
XV
XV

PLATE XIII.

FIGS. 7, A, 8, A, 9, A. — Sections through the head of the embryo figured at 1, A, cut transversely to the main long axis of the embryo. Fig. 7, A, shows the medullary folds (*MF*), meeting to enclose the cavity of the mid-brain, *o*, opening to the primary first ventricle. Fig. 8, A. — Section passing through the middle height of the canal connecting the primary first ventricle (*Iv'*) with the ventricle of the mid-brain (*IIv*). *Ep*, epiblast; *d*, dorsal point of union of the medullary folds. Fig. 9, A. — Section passing through the floor of the canal connecting the primary ventricle of the fore-brain with that of mid-brain. *Op*, hollowed part of the wall of fore-brain, which becomes later the optic vesicle. *Mes*, mesoblast. (*Z. 4. A.*)

FIGS. 10, A, 11, A, 12, A. — Series A, continued: sections in the same plane. 10, A. — Section touching the ventral outer surface of the brain, tangential to the curve of the ventral surface caused by the primary cranial flexure. *A*, artery. 11, A, 12, A. — Sections passing through the anterior curved portion of the notochord (*N*). *IIIv*, ventricle of hind-brain. *HC*, rudiment of head-cavity. (*Z. 4. A.*)

FIGS. 13, A, 14, A, 15, A. — Series A, continued: sections in the same plane, through the head in the region of the blind end of the alimentary canal and the mouth. *AL*, alimentary canal. *N*, notochord. *AF*, unpaired anterior medullary fold. *Am*, Amnion. *Ep*, epiblast. *HC*, part of rudiment of head-cavity fused with epiblast and wall of the alimentary canal. *Hph*, rudimentary roof of hypophysis. *Hyp*, hypoblast. *Mes*, mesoblast. *M.in*, mouth involution. *So*, somatic layer of mesoblast. *Sp*, splanchnic layer of mesoblast. *Ep*, in Fig. 14, A, is the epidermis covering externally the anterior medullary fold; it is also the proximal wall of the mouth involution; the distal wall of the mouth involution is formed by the amnion folds Am' (Fig. 14, A) bending outward and forward, and finally meeting, as in Fig. 15, A, about Am'. (*Z. 4. A.*)

FIGS. 16, A, 17, A, 18, A. — Series A, continued: sections in the same plane, through the regions of the heart (*HT*), of the opening of the head intestine to the yolk-sack, and of the middle of the back. *CC*, canalis centralis of spinal cord. Other letters the same as in preceding figures. (*Z. 4. A.*)

FIG. 19, B. — Series B is cut transversely to the long axis of the body. The embryo is older than that of series A; the embryo figured in series B having nine protovertebræ. The section (19, B) passes through the anterior curved part of the notochord (*N*), and through the head-cavities (*HC*), whose walls (at *CZ*) are prolonged towards the anterior end of the notochord. *HB*, hind-brain. *FB*, fore-brain. *A*, artery. (*Z. 2. A.*)

FIG. 20, B. — Section through the anterior end of the alimentary canal (*AL*). Other letters the same as in 19 B and the rest of the series. (*Z. 2. A.*)

FIGS. 21, B, 22, B, 23, B. — Sections in the region of the first visceral (hyoid) cleft (*I*). *Ed*, epidermis forming the roof of the mouth involution. *IIph*, the point where the hypophysis is formed. *M*, hypoblastic mouth. *M.in*, epidermoidal mouth involution between fore-brain and lower jaw. *E*, ear. (*Z. 2. A.*)

PLATE XIV.

FIGS. 24, C, 39, C. incl. — The figures represent, in the order of succession, a series of sections at almost regular intervals, through the head and anterior part of the body of the embryo figured at 2, C, Pl. XII. They are cut in the plane denoted by the line P–S (see Fig. 2, C) at right angles to the sagittal plane of the embryo. This same series is continued in Figs. 40, C, 41, C, and 42, C, on Plate XV. *v*, denotes the cardinal veins, uniting at *v* (Fig. 38, C) in the ductus cuvieri. *v'*, marks the hepatic veins in Fig. 40, C, which are continued from the mesenteric and vitelline veins, *v'*, Fig. 41, C.

For the other parts see Register of Index-letters. (*Z. 2.* obj. 1 inch, Browning.)

Plate xv.

Figs. 40, C, 41, C, 42, C. — See explanation of Plate XIV.

Figs. 43, C, 44, C, showing coelenteric zone (*CZ*) and anterior end of the notochord (*N*). These sections are immediately consecutive. *A*, artery. *Ept*, epithelial lining of hypophysial invagination (*Hph*). *HC*, head-cavity. *wHC*, wall of head-cavity. (*Z. 2. D.*)

Fig. 45. — Frontal section through the brain of young embryo of Sphærodactylus, parallel to the brain-axis anterior to the cranial flexure. (This embryo is more advanced than that of series A.) The assymmetry is due to the twist of the embryo. *MF*, medullary-folds. *Mes*, mesoblast. *MB*, mid-brain. *FB*, fore-brain. *Opv*, optic vesicle, already pointing backward, the wall of the same (*Op*) lying immediately under the epiblast (*Ep*). *o*, external opening of the primary first ventricle at the anterior end of the neural axis. (*Z. 4. A.*)

Fig. 46. — Part of section through the fore-brain and eye in the same plane as Fig. 45. *L*, lens. *Opv*, optic ventricle. *O.st*, lumen of optic stalk. *wFB*, wall of fore-brain. (*Z. 2. A.*)

Fig. 47. — Sagittal section through the head of young embryo, immediately to one side of the median sagittal plane. *Ep*, epiblast. *in.FB*, infundibular region of fore-brain. *HC*, proximal end of head-cavity. *Hph*, hypophysial invagination. *Hyp*, hypoblast. *LJ*, lower jaw. *M*, mouth. (*Z. 4. A.*)

FIGS. 48, D, 49, D, 50, D. — Three sections, cut nearly in a sagittal plane, of an embryo slightly younger than that of series C.

FIG. 48, D, cuts the optic stalk (*O.st*) outside of the fore-brain; shows the mandibular arch (*A*); also the head-cavity; and passes through the median vertical plane at the thin points of the brain-roof where the mid-brain joins the fore-brain on one side and the hind-brain on the other. Fig. 49, D, passes through the median vertical plane at the point where the notochord touches the infundibular region of the fore-brain (*FB*). Fig. 50, D, passes through the median vertical plane at the extreme anterior dorsal region of the fore-brain, and also at a point low down in the hind-brain (*HB*). *I*, dorsal end of the hyoid-cleft. *og*, optic groove. For the other parts see Register of Index-letters. (*Z. 2. A.*)

FIG. 51, D. Diagrammatic median sagittal section of the embryo represented in series D (reconstructed from 22 drawings of consecutive sections). This figure shows the ventricles of the fore-, mid-, and hind-brain (*FB*, *MB*, *HB*), the first two separated by a lateral constriction posterior to *c*. It also illustrates the primary cranial flexure, the position of the notochord (*N*), and the first rudiment of the thyroid gland (*Th*).

FIG. 52, E. — Section passing through the anterior-dorsal wall of the mid-brain (*MB*) forward through the anterior wall of the primary fore-brain (*FB*) at right angles, immediately ventral to the eye-stalks. It shows nerve-fibres belonging to the main system of longitudinal fibres (*LF*) uniting anteriorly (*LF''*). Dorsal-wards are seen in oblique section the fibres of the posterior commissure (*PCs*). (*Z. 2. A.*)

FIGS. 53, E, 54, E. — Sections in the same plane as 52, E, cut a little farther forwards. Fig. 53, E, shows the posterior commissure (*PCs*) where its fibres pass around the dorsal summit of the brain, immediately in front of the mid-brain. Fig. 54, E, shows the first rudiment of the epiphysis (*Eph*). (*Z. 2. A.*)

FIG. 55. — Transverse section through the primary fore-brain, passing through the hypophysis (*Hph*) and the optic stalks (*O.st*). *LF*, cross-section of longitudinal fibres passing ventral to the eye-stalks. (*Z. 2. A.*)

FIGS. 56, 57, 58. — Transverse sections through the tail of an advanced embryo. Fig. 56 shows a section about the middle of the tail, Fig. 57, a section near the tip, and Fig. 58, a section just at the tip. *A*, artery. *Md*, medulla. *N*, notochord (in 58 remarkably pushed away from its usual central position). *AL*, rudiment of the caudal intestine. *vv*, veins. *mp*, muscle somites. *Mes*, mesoblast. *MHp*, indistinguishable mass of mesoblast and hypoblast. *Ne.c*, neurenteric canal. (*Z. 4. A.*)

PLATE XVI.

FIG. 59, B. — Transverse section of the young embryo of series B, showing the heart (*HT*) and the first formation of the ear (*E*). *AL*, alimentary canal. (*Z. 2. A.*)

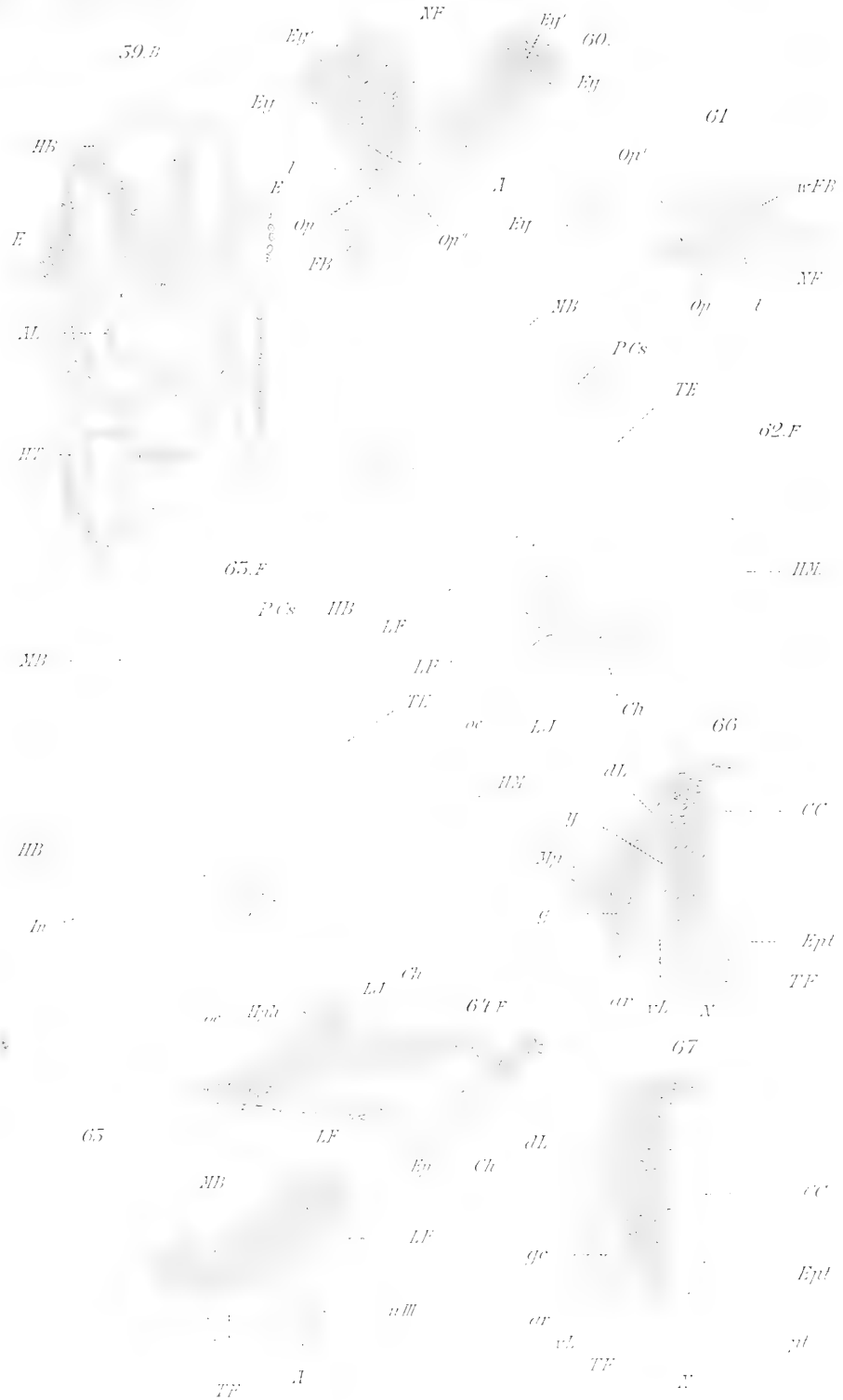
FIG. 60. — Section at right angles to the anterior surface of the fore-brain, through the eye and optic stalk of an embryo much more advanced than that in Fig. 2, C. *Ey'*, inner wall of the eye-cup. *Ey''*, outer wall of the eye-cup. *A*, artery entering the choroid slit. *Op''*, anterior wall of optic stalk, with nerve-fibres (*NF*) which run into the inner surface of the eye-cup. *Op'*, posterior wall of optic stalk. *l*, lumen of optic stalk. *FB*, wall of fore-brain. (*Z. 4. A.*)

FIG. 61. — Section in the same plane as Fig. 60, through the optic stalk where its lumen communicates with that of the fore-brain. *wFB*, wall of fore-brain. For other letters see Fig 60. (*Z. 4. A.*)

FIGS. 62, F, 63, F, 64, F. — Slightly oblique sagittal sections through the head of an embryo in which the paired lobes of the secondary fore-brain (*HM*) have appeared. Section 62, F, passes through the right lateral wall of the brain, cutting through the ventricles where they are most distended laterally. Section 63, F, shows the connection of all the ventricles. A line drawn through the hypophysis and posterior commissure would lie in the median sagittal plane of the embryo and in the plane of section; that part anterior to this imaginary line diverges very slightly to the right of the median plane. Fig. 64, F, represents, more highly magnified, the optic region of section 63, F. *Ch*, chiasma. *Ep*, epidermis. *HB*, hind-brain. *HM*, hemispheres. *LF*, longitudinal nerve-fibres. *LJ*, lower jaw. *œ*, œsophagus. *PCs*, posterior commissure. *Hph*, hypophysis. *In*, infundibulum. *TH*, thalamencephalon. 62, F, 63, F (*Z. 2.* Obj. 2 inch, Ross), 64, F. (*Z. 4. A.*)

FIG. 65. — Transverse section through the ventral wall of the mid-brain (*MB*), showing the exit of the third pair of nerves (*nIII*). *LF*, longitudinal fibres. *TF*, transverse fibres. (*Z. 4. A.*)

FIGS. 66 and 67. — Transverse sections through the spinal medulla at an earlier (66) and a later (67) stage of development. *ar*, anterior nerve-root. *CC*, canalis centralis. *dL*, dorsal longitudinal fibres. *Ept*, epithelial floor of canalis centralis. *g*, ganglion of posterior nerve-root. *gc*, anterior gray column. *Mp*, muscle-plate. *N*, notochord. *pt*, ventral downgrowth of the lateral walls of the medulla, which enclose later the anterior fissure. *TF*, transverse fibres, which unite with the prolonged distal ends of the nerve-cells in the region marked *y*. *vL*, ventral longitudinal fibres. (The ventral longitudinal fibres in Fig. 66 are not quite so conspicuous in the plate as they are in the section.) (*Z. 4. A.*)


NF
Ey'
Ey
59.B
60.
Ey
Ey
61
HB
Op'
I
E
A
wFB
E
Op
Op''
Ey
FB
NF
MB
Op
I
M
PCs
TE
62.F
HT
65.F
HM
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THE FŒTAL MEMBRANES OF THE MARSUPIALS: THE YOLK-SAC PLACENTA IN DIDELPHYS.

DR. HENRY F. OSBORN,

PROFESSOR OF COMPARATIVE ANATOMY, PRINCETON COLLEGE.

THE observations[1] upon the advanced condition of the fœtal membranes of *Didelphys Virginiana*, which are here recorded, were made during the spring of 1887, before I learned of the complete success attending Selenka's experiments in the artificial rearing of this marsupial. After my own repeated failures in similar experiments, I can admire the perseverance and skill shown by Dr. Selenka, which have finally been so amply rewarded. Through the kindness of Dr. John A. Ryder, I have lately received a copy of Part I. of Selenka's valuable memoir upon the Development of *Didelphys*.[2] This terminates with the early history of the *Amnion*, but from the outline of the whole period of development, given upon page 108, it is evident that Part II. will complete the full developmental history. For this reason I will not enter so thoroughly into the subject as I should otherwise have done, but must embrace the opportunity which new material affords of correcting some speculative views which I advanced in an earlier paper.[3]

In the spring of 1883 I procured an opossum with the young in the mid-period of uterine development and established the following facts: (1) That while the allantois is still a small, non-vascular sac, the yolk-sac is closely applied, by both surfaces, to a considerable area of the subzonal membrane, thus forming a vascular disc bounded by the *sinus terminalis*, and this disc has a loose attachment to a definite area of the uterine epithelium. (2) That the portion of the subzonal membrane

[1] These were first communicated to the Biological section of *the American Association for the Advancement of Science*, August, 1887.

[2] Studien über Entwickelungeschichte der Thiere, von Dr. Emil Selenka; 4te Heft, 1te Hälfte, Das Opossum. Wiesbaden, C. W. Kreidel, 1886.

[3] Observations upon the Fœtal Membranes of the Opossum and other Marsupials, by Henry F. Osborn. — *Quart. Jour. Mic. Science*, July, 1883.

attached to the yolk-sac is at this period elevated into numerous hollow villi.[1] Later, I procured for comparison embryos of *Halmaturus* and of another undetermined species, possibly *Phascolarctos*, and observed that the subzonal membrane attached to the vascular portion of the yolk-sac was in each case covered with rudimentary villi, and in the specimen last named these villi covered vascular papillæ. The membranes were in such a torn condition that the relations could not be pre-

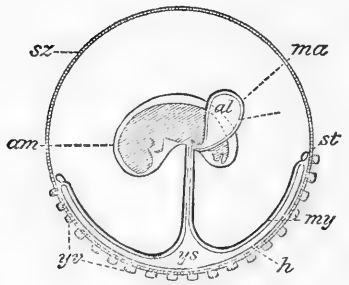


Figure 1.—Didelphys Virginiana. Relations of the fœtal membranes at the mid-period of development.

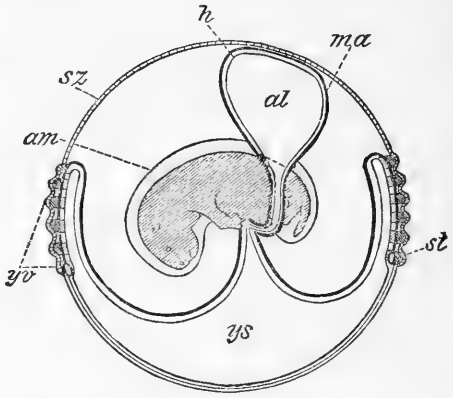


Figure 2.—Australian Marsupial, undetermined. Collection, Cornell University. Membranes at a later period. The relations are somewhat conjectural.

Abbreviations for all the text woodcuts: *Al*, cavity of allantois. *ys*, cavity of yolk-sac. *am*, amnion. *ma*, *my*, mesoblast of allantois and yolk-sac. *h*, hypoblast. *yv*, low temporary villi of the subzonal membrane. *sz*, subzonal membrane. *sz'*, amœboid area of subzonal membrane. *st*, sinus terminalis. *av*, permanent allantoic villi.

cisely determined. In the supposed *Phascolarctos* embryo (loc. cit., Fig. 2, text, and Fig. 5, Plate XXXIII.) the allantois was, however, seen to be attached to the smooth subzonal membrane. "A number of low villi were discovered upon it (*i.e.*, the subzonal membrane) without the aid of a glass; they were distributed over an area to which a highly vascular portion of the yolk-sac was adherent, which was, however, just *without* the

[1] Haddon, in his *Introduction to the Study of Embryology*, p. 89, has slightly misunderstood my account of the *Didelphys* membranes, when he speaks of "vascular villi" covering the yolk-sac. I intended to make it clear that *vascular* villi had not been observed in *Didelphys*, but conjectured that they would be found at a later period.

limits of the *sinus terminalis*." The subzonal membrane covering these low villi consisted of squamous cells, readily detached and exposing a vascular papillus beneath. The position of these villi with reference to the *sinus terminalis* was wholly different from that observed in *Didelphys*, as they were situated between the *sinus terminalis* and the umbilical stalk, while in *Didelphys* they were observed in the distal area circumscribed by the *sinus terminalis*. This difference was explained subsequently by Caldwell's investigations, which showed that in *Phascolarctos* and *Halmaturus* the vascular and non-vascular areas of the yolk-sac are not applied to each other with the obliteration of the cavity of the sac, as in *Didelphys* (see cut). In the light of present evidence the inference drawn at the time from these facts, that we had here a rudimentary yolk-sac placenta covered with villi physiologically similar to those of the true allantoic placenta, appears to have been insufficiently founded.

Shortly after this, Caldwell,[1] not having seen my paper, independently described the fœtal membranes of *Phascolarctos* and *Halmaturus* from his fine series of embryos procured in Australia.

In these the allantois and yolk-sac were found to be extensively applied to the subzonal membrane; but, unlike *Didelphys*, the cavity of the yolk-sac was widely open and the non-vascular portion of the subzonal membrane, overlying the hypoblast, *i.e.*, beyond the area of the mesoblast and *sinus terminalis*, was composed of amœboid cells which were attached to the uterine epithelium. "This attachment is caused by the growth of the cells of the subzonal membrane immediately outside the *sinus terminalis;* the cells of the subzonal membrane begin to enlarge and become amœboid. They throw out pseudopodia-like processes, which fit in between the cells of the uterine epithelium, and serve to attach the blastodermic vesicle to the uterus; this attachment is entirely non-vascular, and is the sole means by which the vesicle is attached to the uterus" (p. 657). No villi were observed at any period, and "there is no vascular connection developed in any stages of the development between the embryo and the uterine walls." The embryo is nourished

[1] Quarterly Journal of Microscopical Science, Vol. XXIV., October, 1884. "On the Arrangement of Embryonic Membranes in Marsupial Animals."

by the nutritive fluids secreted by the utricular glands, which open separately by small pores. The author did not, however, suggest in what manner this nutritive fluid is taken into the fœtal circulation. There is now no doubt from the substantial agreement between Caldwell's and Selenka's observations and my own, hereafter described, as to the character of the false chorion in the marsupials, that the villi, which I observed at the mid-period, do not continue to develop, but are transitory structures, which wholly disappear, in *Didelphys* at least, before the close of embryonic life. In fact, both Caldwell and Selenka,

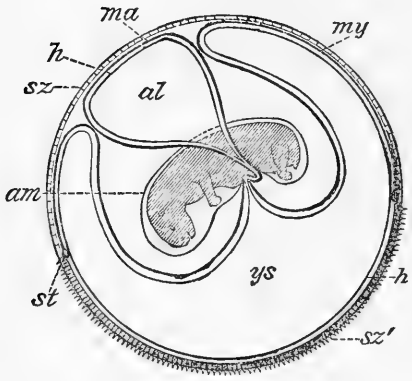


Figure 3. — *Phascolarctos cinereus.* Relations of the fœtal membranes at an advanced period. After Caldwell.

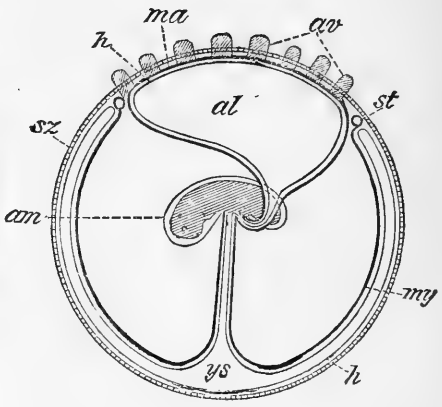


Figure 4. — Fœtal membranes of the rabbit at an early period. After Kölliker.

so far as the observations of the latter have been published, have failed to confirm my observations of the presence of villi at the mid-period. I myself failed to detect any such structures in several marsupial embryos, which Caldwell kindly allowed me to examine in his collection. I am unable to explain this discrepancy at present, unless by the fact that the villi are very transitory structures, which appear and disappear in a period intervening between those which have been obtained by Selenka and Caldwell. However, I by no means wish to retract the observations made in my former paper in regard to the presence of the villi, and I believe they will eventually be confirmed; but the inferences which were drawn from them, I freely admit, are now proved to be untenable.

In the following description there are necessarily gaps in the determination of the various embryonic layers of which the fœtal membranes are composed, which can only be filled by the observation of the stages intermediate between those of the mid and final periods of uterine life. This, I trust, will be done by Selenka. The same obtains of the physiology of that portion of the chorion which is not attached to the uterine wall, and of the fate of the allantois.

About March 1st a number of female opossums were brought into the laboratory. In one, the young had been recently born, transferred to the pouch, and then probably destroyed by the mother at the time of capture. The nipples were drawn out and the mammary-glands distended, but the pouch was empty. Both uteri were laid open, and the fœtal membranes were found crowded into the uterine orifices of the vaginæ, which indicates that they had been detached from the embryos in the uterus itself. No membranes were found in the vaginæ. A second female was then examined. The pouch had been distended and moistened for the reception of the young by the mother, but the nipples were sessile. The uteri contained nine embryos each, which by size and weight were obviously in the latest stage of development. The free cavity of the uterus was filled with a clear, thick fluid, which coagulated rapidly when the alcohol was admitted. The condition of the mucous lining of the uterus at this stage is very interesting, extensive changes having taken place since the mid-period of development. As described in my earlier paper, the inner wall of the uterus at the mid-period presented one or two long parallel furrows faintly defined upon its ventral surface, in which the embryos were ranged in a single row, with the portion of the subzonal membrane to which the yolk-sac is attached lying fixed in the furrow, the remainder of the membrane lying free in the dorsal cavity.

At this advanced period the mucous lining is reflected into prominent folds, which in general have a circular arrangement, greatly increasing the surface area, and serving to enfold the large discs of the *yolk-sac placentæ*. The interior of these folds is richly vascular, and the epithelium covering them is altogether similar to that of the non-reflected surfaces (Fig. 4). The walls of the uterus, in section, show the following rela-

tions (Fig. 3). Overlying the muscular coat is the connective tissue coat, which sends numerous septæ into the mucous coat. The latter is richly vascular, containing numerous blood-vessels, some of which immediately underlie the inner layer of columnar epithelium, *ep* (see Fig. 4, *ub*). The utricular glands are interspersed with numerous irregular cells, the lumina decreasing in diameter towards the inner surface. The most remarkable feature of the uterus is the production of the mucous coat into numerous folds (*utf*, Figs. 1, 3, 4), which in many cases partially separate the closely crowded embryos from each other. These folds contain all the glandular and vascular structures of the main mucous coat. The smaller blood-vessels frequently form the core of the secondary folds (*ub*, Fig. 4), and are thus separated by a single layer of columnar cells, *ep*, from the yolk-sac placenta. At other points the ducts of the utricular glands, *d*, *d*, closely underlie the inner epithelial coat, *ep*. I have not observed the openings of these glands. Fig. 4 represents a typical secondary fold.

The general arrangement of the embryos in the uterus is in two rows facing each other, as shown in Fig. 1, drawn after the removal of two of the embryos. The bodies lie obliquely across the long axis of the uterus, with the heads and tails of opposing pairs often alternating, as shown in Fig. 2, the ventral line being turned towards the cavity, the dorsal line towards the wall of the uterus. It follows from this that the yolk-sac placentæ are usually turned over the back (see yp^5, yp^7, and yp^8); but this disposition is frequently altered by the fusion together of the chorions of two or three embryos. As already observed, the embryos are partially separated from each other, and enclosed by the folds of the mucous membrane. Numerous and extensive changes have taken place in the fœtal membranes since the mid-period of development. The proximal portion of the yolk-sac is constricted into a wide flattened stalk conveying the umbilical artery and veins. The distal portion forms a large circular disc, *yp*, bounded by the *sinus terminalis*, *st*, which represents the folding double of the distal portion of the yolk-sac against the subzonal membrane to form the yolk-sac placenta. This disc is somewhat cup-shaped, the *sinus terminalis* forming the rim. Its upper surface is thrown into a number of minor folds, and covered with elongated cells with

amœbiform processes (Fig. 4, *amb*), which are closely applied to the lining epithelium of the uterine wall. Fig. 4 represents a section of the rim of one of the yolk-sac placentæ which was removed. Fig. 3 represents a more extended section, upon a less enlarged scale, of the edge of one of the yolk-sac placentæ which has been partially detached from the epithelium. Over the central depressed region of the vascular disc the cells to some extent lose their amœbiform character. These cells immediately overlie a thin layer of small cells, usually two deep, *mhy;* these, probably, represent the mesoblastic elements of the yolk-sac — the hypoblast having been absorbed. This layer is interspersed with blood-vessels, *fb*. As the attaching cells are reflected around the *sinus terminalis* they gradually lose their amœbiform character until, upon the lower surface of the disc, they pass into the simple elongated cells of the subzonal membrane. This and the adjoining amœbiform cells are probably derived from the epiblastic layer of the subzonal membrane. This under layer is attached only at scattered points to the yolk-sac placenta, and is reflected to the stalk of the placenta, where it forms part of the investment of the umbilical stalk and allantois.

So far as I have observed, the upper surface of the yolk-sac placenta is the only portion of the fœtal membranes which attaches to the wall of the uterus. The condition of the *allantois* is difficult to determine at this advanced period. According to Selenka it remains distinct from the subzonal membrane throughout (p. 110), and its blood-vessels diminish in size towards the close of fœtal life. In transverse sections of the membranes, between the navel and yolk-sac placenta, I find the separation of the allantois from the subzonal membrane is almost complete, although at one or two points there is quite an extensive union. The walls of the allantois are much folded, and the interior is partially filled with a stroma or network in which are suspended numerous blood corpuscles, which indicate that the allantoic vessels empty into vascular sinuses. The stalk of the yolk-sac is apparently flattened upon itself, with the obliteration of its primitive cavity at this point. The subzonal membrane surrounding this area (*al*, Fig. 2) consists chiefly of flattened cells, but in some places they are columnar.

The yolk-sac placentæ, as shown in Fig. 1, lie nearest the

uterine wall, while the remaining surface of the chorion lies nearer to the centre of the uterus, but is, nevertheless, in close contact with the folds of the uterine walls (see Fig. 1, e^5, utf^2). There is one fact which seems, however, to demonstrate that the entire nutritive and respiratory function at this advanced period devolves upon the yolk-sac placenta; this is, that in some cases, where two or three healthy embryos are found united by the fusion of the chorions, the yolk-sac placentæ are free and intact, while the entire remaining membrane surfaces of the three embryos are tightly twisted into a single cord, in which only the large vessels of the vascular discs can be distinguished. In other cases, where such coalescence has not taken place, the allantoic region of the chorion is often widely extended.

The arrangement of the attaching cells over the vascular area and of the flattened subzonal cells over the non-vascular area in *Didelphys* is directly the reverse of what obtains in *Phascolarctos* and *Halmaturus*, as observed by Caldwell, and renders it probable that other marsupials will show still further variations in the growth of the yolk-sac placentæ, while they may retain in common the area of attaching cells over some portion of the yolk-sac. The disposition of the blood-vessels in the fold of the uterus, as represented in Fig. 4, indicates that the attaching cells have functional relations directly with the maternal circulation as well as indirectly through the medium of the utricular glands. However, having once been near the fire of inferential biology, I will leave the question of the physiology of the yolk-sac placenta in the able hands of Selenka.

EXPLANATION OF PLATE.

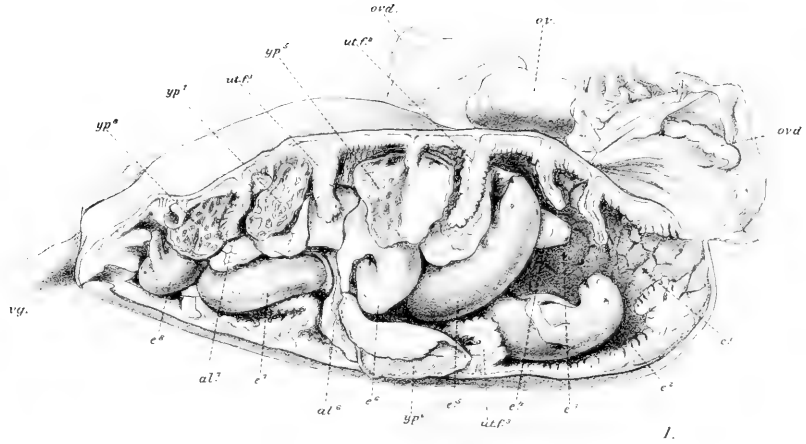
al. Region of the chorion, containing the allantois and stalk of the yolk-sac.
amb. Amœboid attaching cells, covering yolk-sac placentæ.
ua. Umbilical arteries.
bc. Blood corpuscles.
d. d. Ducts of utricular glands.
e. Embryos.
ep. Epithelium, forming inner lining of uterine wall.
fb. Fœtal blood-vessels of yolk-sac placenta.
m. hy. Mesoblast and hypoblast (?) of yolk-sac.
ov. Ovary.
ovd. Oviduct.
s. Septa from muscular into mucous coat of uterus.
st. Sinus terminalis.
sz. Subzonal membrane.
ub. Utricular blood-vessels.
ut. f. Folds of uterine wall.
ut. Muscular wall of uterus.
vg. Vagina.
yp. Yolk-sac placenta.

FIG. 1. — Right uterus of *Didelphys*, with a portion of the wall removed, showing the arrangement of the embryos, the folds of the uterine wall, and the series of yolk-sac placentæ. Two embryos, e^1 and e^3, have been removed. The specimen as drawn is now preserved in the Morphological Laboratory. The drawing represents twice the natural size.

FIG. 2. — Two *Didelphys* embryos in which the yolk-sac placentæ are free, while other portions of the chorion have coalesced with each other and with the *amnion* of one of the embryos, *e.* The second embryo e' has lost its amniotic investment.

FIG. 3. — An enlarged section of a portion of the entire uterine walls with one of the folds and the edge of one of the yolk-sac placentæ *in situ*. The drawing is semi-diagrammatic. The thicker portions of the placenta, *yp*, represent areas where the attaching cells are cut obliquely.

FIG. 4. — Camera drawing of the edge of one of the yolk-sac placentæ with a subjacent fold of the uterine wall. Enlarged about 350 diameters.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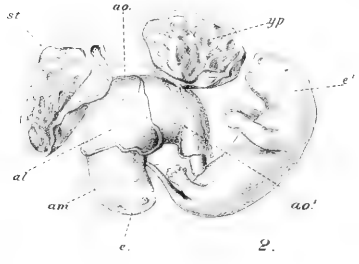
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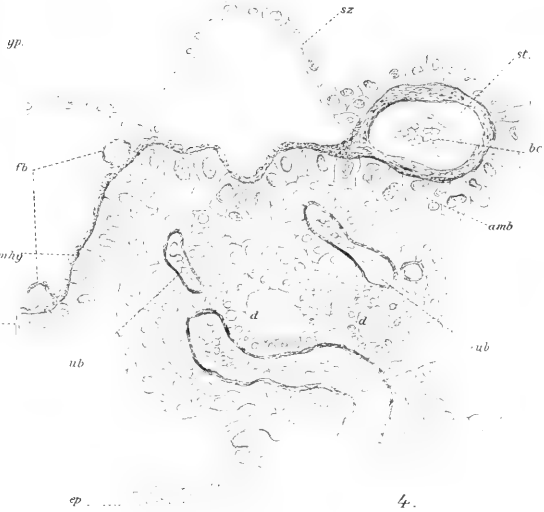
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SOME OBSERVATIONS ON THE MENTAL POWERS OF SPIDERS.

GEORGE W. AND ELIZABETH G. PECKHAM.

INTRODUCTION.

THE differences of structure between a man and a spider are so numerous and profound that he who infers the mental state of a spider from a given action should not be in haste to make positive statements and broad generalizations. A critical study of many of the current anecdotes concerning animal intelligence would prevent their use as data for comparative psychology, at least until after their confirmation by competent observers. Up to that time they have, as Romanes says, only the value of suggestions. How far, for example, are "personal preconceptions" responsible for both facts and inferences in Dr. Brookes' assertion that *Epiblemum scenicum* "has been sometimes seen in the act of instructing its young ones how to hunt"? and also that "whenever an old one missed its leap, it would run from the place and hide itself in some crevice, as if ashamed of its mismanagement"?[1] After having observed spider after spider building a new web on the eve of a storm, how shall we explain the statement, that "when a storm threatens, the spider, which is very economical with its valuable spinning material, spins no web, for it knows that the storm will tear it in pieces, and waste its pains, and it also does not mend a web which has been torn; if it is seen spinning or mending, on the other hand, fine weather may be generally reckoned on"?[2] This would be, no doubt, the wisest way for spiders to act under the circumstances, and Dr. Büchner is in very illustrious company when he — unconsciously, of course — orders the actions of such simple creatures in full accord with the higher reason.

Lange has well said that the core of all the numerous cau-

[1] Bingley's *Animal Biography*, Vol. III., p. 455.
[2] Romanes' *Animal Intelligence*, p. 211.

tionary measures of the scientific method lies just in the neutralizing of the influence of the observer's subjectivity. The subjective element cannot, of course, be eliminated; but the observer should keep facts and inferences separate, and should, in addition, state the particular action, among the many, which is the external sign of the mental state which he believes to be proved by the experiment. Lange's words on the subject are worthy of immortal memory: —

"Where external observation shows us primarily only movements, gestures, and actions, the interpretation of which is liable to error, we may, nevertheless, carry out a comparatively very exact procedure, since we can easily subject the animal to experiments, and put it into positions which admit of the most accurate observation of each fresh emotion, and the repetition or suspension, as we will, of each stimulus to a psychical activity. Thus is secured that fundamental condition of all exactness; not, indeed, that error is absolutely avoided, but certainly that it can be rendered harmless by method. An exactly described procedure with an exactly described animal can always be repeated, by which means our interpretation, if it is due to variable bye-conditions, is at once corrected, and at all events thoroughly cleared from the influence of personal preconceptions, which have so great a share in so-called self-observation."[1]

We have felt that it might properly be demanded of us that we give the generic and specific names of every spider experimented upon, and also that we so describe our methods that the experiments can be repeated by any one who desires to test the validity of our conclusions.

Our rule has been not only to repeat an experiment many times, but to repeat it under as many different conditions as possible. The histologist often finds it necessary to adopt complicated and tiresome methods in order to demonstrate a single fact. So, also, we have found that to learn anything of the mental processes of spiders the way is long and beset with difficulties. To use the words of Ribot: "Many of these investigations, we shall see, pertain to very modest questions, and it is probable that the partisans of the old psychology will find the work too great for results so small. But those who give allegiance to the methods of the positive sciences will not com-

[1] *History of Materialism*, Vol. III., p. 178.

plain of this. They know how much effort the smallest questions require; how the solution of small questions leads on to the solution of great ones, and how barren of results it is to discuss great problems before the small ones have been solved." [1]

SENSE OF SMELL.

Our experiments on the sense of smell in spiders extended over two summers. Many of them were performed by each of us separately, that one might detect the mistakes of the other. Our usual plan was to hold a slender grass rod, eight inches in length, in such a position that one end closely approached the spider, noting what effect, if any, was produced, and then to dip it into whatever scent we were using, hold it in the same position, and again note the effect. We tested them in this way while at rest in the web, while stalking their prey, while feigning death, and under various other conditions.

The scents used were some essential oils, cologne, and several kinds of perfumes. Acetic acid, vinegar, and like materials were avoided on account of their irritating action upon the integument.

Our first experiments were upon some tame Attidæ that had taken up their abode with us. They were fearless little creatures, always ready to jump upon a finger, to catch the gnats that we offered them, or to drink from a spoon. They were quick to respond to any test of their sense of smell.

For example, an *Astia vittata* ♂ (var. niger) was placed upon a table and the end of a clean rod was held just in front of him. He promptly leaped upon it as he had been in the habit of doing with our fingers, and after a moment's pause leaped again to some other object, whence he was returned to the table. This trial was repeated with the same result. The end of the rod was then dipped into oil of peppermint and placed as before. The spider instantly raised his first legs and palpi and waved them in the air, this being the usual position for threatening or defence. After standing in this way for two minutes he turned slowly and walked to a little distance. Soon, however, he returned and took up his former position in front of the rod, remaining again for two minutes, but not repeating the

[1] *German Psychology of To-day*, p. 14.

movements of the legs and palpi. A second time he walked away and came back, but this time he came so close as to touch the oil with one leg, whereupon he hurried away, evidently in distress. Half an hour later we found him with his legs drawn in, looking very miserable, but when the oil of peppermint was held three inches away he immediately came to it and stood near it for about a minute, when it was removed.

We next tested a *Philæus militaris* ♂, placing him on a table and using oil of peppermint, but holding the rod, at first clean and then wet with the oil, behind him and just over the extremity of the abdomen. When the clean rod was used he remained perfectly quiet; but when it approached him wet with the peppermint, he raised his first legs and palpi very high, and moved them up and down, turning from side to side, and trying to reach the rod, which was kept behind him.

Another male of the same species was experimented upon in the same way, excepting that cologne was substituted for oil of peppermint. This one also made no response to the clean rod; but when the cologne was held behind him he stood with his head and first legs erect for several minutes, and then turned and tried to reach it.

A female of *Astia vittata* gave the following results: When the clean rod approached her from in front she paid no attention to it; when the oils of lavender and cedar were used she raised her head and backed away; when oil of cloves was used she raised her first legs and palpi and struck at it, but when peppermint was used she became greatly excited, dancing about with her legs and palpi raised, and finally leaped upon the rod.

A little colony of *Drapetisca socialis*, which we found on the wall of a smoke-house, was now made the subject of a series of experiments. An empty bottle was held near ten individuals in succession. They remained quiet. An open bottle of oil of peppermint was then used in the same way. All indicated that they noticed it, at first by moving their legs, and afterward by walking away. A dry cork was held near six individuals. They paid no attention to it. A cork wet with oil of wintergreen was substituted, when they acted as they had with the peppermint. The corks and bottles were held sometimes behind and sometimes in front of them. Both males and

females were represented among the spiders experimented upon. At another time ten individuals were tested, at first with a clean match, and then with a match dipped into oil of lavender. The results were in all respects like those given above.

We next turned our attention to the orb-weavers. To procure good material we made a trip to a neighboring swamp, and captured half-a-dozen large and handsome specimens of *Argiope riparia*. These we set free in a wire-enclosed porch, which, by the following morning, was ornamented by several of the interesting webs peculiar to this species.

Our first trial was made with a female, while she hung in the usual position in her web. The end of a clean rod was held for some moments just in front of her. There was no response. The rod was then dipped into oil of lavender and held as before. The end of the abdomen was immediately jerked upward, and the first legs were moved from side to side. After an interval of ten minutes the lavender was held just above the end of the abdomen; it was again lifted, and a moment later the tips of the third and then of the first pair of legs were rubbed, one at a time, between the falces and the palpi.

Turning to another female of this species, we held a clean rod near the hind legs. There was no response. The rod was then dipped into essence of heliotrope, and held as before. One of the legs of the third pair was immediately moved, the tip being rubbed between the palpi and falces, as in the preceding instance; and similar movements of some of the other legs followed. The same movements took place when the heliotrope was held in front of the spider.

Finding a female of *Epeira strix* with her head and most of her body hidden under the silken covering within which spiders of this species commonly remain during the day, we brought a rod, at first clean, and then wet with oil of lavender, near the tip of the abdomen. There was no response in the first instance, but in the second the spider quickly retreated within the covering, so as to be entirely out of sight.

A female of *Epeira labyrinthea*, while hanging in her web, was gently touched with a rod which had shortly before been dipped into essence of heliotrope, but which was quite dry. She instantly seized the rod, and went vigorously to work to bind it

up, as though it had been a fly. After she had worked at it for two minutes, and had wrapped it up very thoroughly, it was taken away as gently as possible, whereupon she began to put the tips of her legs between the palpi and falces. We have seen a male of this species go through the same motions, after catching an ant in his web, and then losing it. So decided a response to the dry heliotrope seeming to show an unusual sensitiveness, the rod was again dipped into heliotrope, and held, as soon as it was dry, first, near a male, and then near three females of *labyrinthea*. All jumped at it and grasped it, seeming puzzled, but returned to the web without binding it up.

We now took a clean, unscented rod, and with it gently touched, in turn, the five spiders already experimented upon. Each of them clasped it, examined it for a moment, and then returned to the web, their action being not very unlike that of the second, third, fourth, and fifth spiders experimented upon with the dried heliotrope, but showing less excitement of manner.

The dried heliotrope was then again offered to the first spider. She began to bind it up as before; then stopped and rubbed her palpi violently up and down upon it for some time; then rubbed it hard with all her legs, excepting the fourth pair; then again with her palpi. She seemed to be trying to get something off. After five minutes, while she was still at work in this way, the rod was removed.

It may be noted, in connection with this experiment, that we have repeatedly noticed, among spiders of the same species, great differences of degree in their sensitiveness to odors.

The position of the organ of smell in spiders is unknown. It has been generally supposed that it existed in the palpi, although Robineau-Desvoidy located it in the mandibles.[1]

[1] "As to spiders, it is not certainly known whether, and to what extent, they share in the sense of smell. Robineau-Desvoidy (1842) said that their sense of smell is very well developed and localized in the mandibles, but Perris placed them in the lowest rank of arthropods; though he remarks on the sensibility of their palpi to smells." — A. S. Packard's abstract of Kraepilin's criticisms on the works of writers on the olfactory organs of arthropods. *Am. Nat.*, Vol. XXI., p. 182.

"Perris accorde aux palpes une faible olfaction à courte distance. Il fait remarquer que les aranéides, les seuls articulés qui n'ont pas d'antennes, paraissent avoir l'olfaction tout à fait rudimentaire. . . . Enfin Perris et Comparetté croient que les palpes servent à l'olfaction à côté des antennes." — Auguste Forel. *Sensations des Insectes*, II. *Recueil Zoölogique Suisse*, Tome IV., No. 2, pp. 190, 192.

Hoping to throw some light on the matter, we made a few experiments to determine whether spiders deprived of their palpi would respond to a test of their sense of smell.

Taking two females of *Argiope riparia* that had shown themselves sensitive to cologne, heliotrope, oil of lavender, and a perfume called Chinese bouquet, we carefully removed their palpi and replaced them in their webs. They spent some hours in rubbing the tips of their legs over the wounded parts, but by the following morning appeared quite comfortable, and breakfasted, with appetite, upon some grasshoppers with which we provided them.

We tested the first one, after the usual check with a clean rod, by holding a rod wet with cologne at first in front of her, and then at the posterior end of the abdomen. In the course of twenty-five minutes there was no more decided response than an occasional slight jerk of the abdomen or a faint movement of the legs. Oil of lavender was then held in front of her. Instantly the legs contracted and their tips were rubbed, one at a time, upon the falces.

Passing to the second and offering the clean rod without response, we tested her with heliotrope and Chinese bouquet. To each she responded quickly by jerking the abdomen and rubbing the tips of the legs over the falces.

To sum up our work on the sense of smell, we made, in all, two hundred and twenty experiments. We found three species (*Argyrocpeira hortorum*, *Dolomedes tenebrosus*, and *Herpyllus ecclesiasticus*) that did not respond to the tests. In all other cases it was evident that the scent was perceived by the spiders. This they showed in different ways, — by various movements of the legs, palpi, and abdomen, by shaking their webs, by running away, by seizing the rod and binding it up with web as they would an insect, and in the case of the Attidæ, by approaching the rod with the first legs and palpi held erect; but whether in the way of attacking it, or, as it sometimes seemed, because the smell was pleasant to them, it is impossible to say.

We add a list of the species experimented upon: —

Epeira infumata Hentz, Epeira insularis Hentz, Epeira strix Hentz, Epeira labyrinthea Hentz, Epeira bombycinaria Hentz, Cyllopodia cavata Hentz, Cyclosa conica Menge, Argiope riparia Hentz, Argyroepeira hortorum Hentz, Tetragnatha

laboriosa Hentz, Theridion blandum Hentz, Theridion unknown, Drapetisca socialis Menge, Linyphia communis Hentz, Linyphia mandibulata Emerton, Astia vittata Hentz, Philæus militaris Hentz, Hasarius hoyi Peckham, Phidippus rufus Hentz, Philodromus duttoni Hentz, Xysticus gulosus Keyserling, Thomisus piger Hentz, Misumena unknown, Herpyllus ecclesiasticus Hentz, Micromata carolinensis Hentz, Agelena naevia Bosc.

HEARING.

Our first experiments in this direction consisted in shouting, clapping our hands, and whistling close to spiders which were at rest in their webs. They gave no sign of hearing anything. We felt, however, that this was not enough to warrant us in concluding that they were deaf, since there is nothing in the habits of these spiders that would lead them to make any active response to loud noises, even supposing they did hear them. *A. vittata*, when standing on a finger, jumped to one side when "*bang*" was shouted in a loud voice, with the head turned away; and when we whistled, it stood on the tip of its abdomen with its head held high. With this exception we failed to discover, by these means, anything about the hearing of spiders.

Fortunately a better method was suggested to us by the experiment of Mr. C. V. Boys with a tuning-fork on the garden-spider.[1]

We began a new series of experiments by sounding three tuning-forks near a large female of *E. strix* as she stood in the centre of her web. Two of the forks, A and C, were small, while B was large. The spider did not notice the two small forks, but when the large one was sounded she raised her first legs almost vertically, holding them as though ready to ward off an attack, and looking much like a boxer in an attitude of defence. The B fork was again sounded, and again the legs were raised. As a control experiment the fork, when not in vibration, was brought into the same relation to the spider. No notice was taken of it. The fork was again sounded, and held behind and above her cephalothorax. She extended her legs as before. The experiment was repeated with the fork still. She paid no attention to it. The fork was sounded and

[1] *Nature*, XXIII., pp. 149–150.

brought to one side of her, when she not only moved the first legs, but also the leg of the second pair on the side toward the fork. It would tire the reader unnecessarily were we to describe the check experiments that were made after each observation, but we felt their importance, and never failed to make them; in fact, our check experiments were more numerous than our direct ones.

Later on tests similar to those given above were made on a smaller spider, a female of *E. labyrinthea*, as she stood in her web. In this instance she responded to all the forks, A, B, and C.

Second and third large individuals of *E. strix* acted as the first had done, responding to the large fork, but not to the small ones. On the other hand, five small individuals of *strix* were much excited by the small forks. Subsequent observation left no room for doubt that the large spiders, with few exceptions, only attended to the sound produced by the large forks.

To show the results of our experiments and also the way in which we worked we quote from our notes.

July 14. — Held the big fork, in vibration, over a large male of *E. insularis*, an inch and a half away. He threw up his first legs, making frantic efforts to reach it. When the fork was removed he settled down quietly in his web. This was repeated ten times, always with the same result. A female of this species acted as the male had done, but seemed less excited by the vibrations. Unless the fork was sounding neither spider paid any attention to it.

July 18. — Held the large fork, in vibration, near a female of *E. infumata* standing quietly on a wire screen. She did not move. Repeated the test with the fork, at first vibrating and then still, ten times without result. She was then placed in the web of another spider, and the B fork was brought near her as she stood there. She appeared frightened, and at once threw up the first and second pairs of legs. The fork was next held behind and to one side, so that she could not see it; but she seemed to hear it, since she turned toward the fork and almost fell backward in her efforts to reach it. The fork was now held in front of her again, when she moved her legs as before. This experiment was repeated many times with like results. To hear the fork when she could not see it evidently excited her

more than to both hear and see it. The presence of the fork, when not in vibration, brought no response, nor did rapidly moving it to and fro in front of her attract her attention.

August 13. — The large fork, in vibration, was held near a female of *A. riparia*. She at once gave the usual sign that she heard it. It was next held behind her, and entirely out of her sight, when she quickly turned in the direction of the sound.

August 14. — Tried a new species, a young *Phillyra mammeata* Hentz. When the vibrating C fork approached she lifted first one, and then the other, of the anterior legs.

Were it necessary, we could cite a great many similar experiments which had like results, to show that certain spiders indicate that they hear a vibrating tuning-fork by characteristic movements of the legs. Another set of spiders, however, manifested their perception of the sound in a different way. With these the approach of a vibrating fork seemed to cause greater alarm, making them drop from the web and keep out of sight for a longer or shorter time. However, after one of these spiders had been subjected to the experiment several times, it would, instead of dropping, raise its legs in the manner described above.

For example, when the vibrating C fork approached a female of *E. labyrinthea* as she stood in her web, she fell. This was repeated eleven times, the spider falling each time, but at the twelfth she merely raised her first legs.

A few days after this experiment we found a more excitable spider of the same species. Not until she had fallen out of the web twenty-two times, at the approach of the fork, could she restrain the impulse to drop. It was apparent, however, after the seventh or eighth time, that she was less startled by the sound than at first, since the distance that she fell and the period of time that elapsed before she returned to the web grew shorter and shorter in the later experiments. At first she fell fifteen or eighteen inches, and remained at the end of her line for several minutes, while toward the last she fell only an inch or two, and immediately ran back to the web. After the twenty-second trial she only held up her legs as the fork approached. Finally, completely worn out and disgusted, she retreated to a neighboring branch, drew in her legs, and remained sullenly unresponsive to all further attempts.

We shall now give a series of notes which describe an attempt to teach a very interesting and docile little female spider of the species *Cyclosa conica* Menge to listen composedly to the vibration of the tuning-fork. We first saw her on July 18, when we marked her with a spot of scarlet paint, that there might be no question of mistaken identity; and by the time that we lost her, a month later, we had come to have a very friendly interest in all that concerned her. Her web was about five feet from the ground, in the branches of a cedar-tree. Across it was stretched a line of bits of rubbish, dead insects, and cocoons, and in the middle of this stood the little spider, bearing so close a resemblance, in color and shape, to the other parts of the line that she was almost indistinguishable. So perfect was the mimicry, that even after we had visited her day after day for weeks, we frequently thought, at the first glance, that our spider was gone.

Her record stands as follows: —

July 18. — *C. conica* fell from the web three times when the vibrating C fork was held one inch away. Further efforts failed to move her.

July 19. — Used the B fork. She fell five times in succession, — only short distances the fourth and fifth times, — after which she would not leave the web.

July 20. — She fell nine times before becoming accustomed to the C fork; the last three times she dropped only two or three inches, and hung at the end of the line.

July 21. — After falling six times she paid no attention to the sound.

July 22. — After falling six times became accustomed to the sound, and would not leave the web.

July 24. — A day having elapsed without a lesson she fell eleven times before becoming accustomed to the sound.

July 25. — Dropped from her web six times as the fork was held near; after that, paid no attention to it.

July 26. — Dropped only five times before becoming accustomed to the vibration.

July 29. — Dropped seven times, and then became indifferent.

July 31. — Dropped eleven times before refusing to move.

August 1. — Dropped seven times, and then remained undisturbed by the sound.

August 2. — She dropped fifteen times, and then refused to

move. We left her for fifteen minutes, and, then returning, sounded the fork near her five times without making her move. She probably remembered her former experience and profited by it.

August 3. — Her memory proved short. She dropped eleven times before remaining quiet, as the fork approached. Moreover, she was very slow about returning after each fall, so that it took a much longer time than usual to teach her to pay no heed to the sound.

August 4. — She seems to be in better mood to-day. After the seventh trial she gave no sign of hearing the fork.

August 5. — Education begins to affect her character. When the fork was sounded she seemed startled, and ran up a little way on the band of rubbish, but quickly returned to the centre. This she did a second time, but to nine subsequent trials, the fork being held both behind and in front of her, she gave no response.

August 6. — We could not make her move, though we sounded the fork nine times.

August 7, 4 P.M. — Eight attempts failed to move her in the least. 6.30 P.M. — Made eleven trials, with the same result.

August 8. — Sounded the fork near her fifteen times, but she did not move.

August 9. — Seven trials; the spider remained perfectly quiet.

August 10. — She has spun herself a new web inside the circumferential lines of the old one, preserving the *débris* in its original position. (This is the fourth web she has spun since we found her on July 18.) The fork was sounded ten times, but she paid no attention to it.

August 11. — She seemed more nervous. At the first trial she dropped two inches; at the second and third, she fell about a quarter of an inch, and immediately ran back. Five subsequent efforts failed to move her.

August 12. — In the morning we made nine, and in the afternoon eight, trials with the fork. The spider gave no sign that she heard anything.

August 14. — A day and a half having passed without a lesson, the spider was somewhat startled at the approach of the fork, falling a very short distance the first time it was sounded, but after that remaining imperturbable.

August 15, 10 A.M. — Sounded the fork near the spider ten times. She would not move. 5 P.M. — Made nineteen trials, with the same result.

August 16. — The fork was sounded twenty times in the morning and twenty in the afternoon without disturbing her.

August 17. — While the fork was sounded close to her eleven times she stood immovable in the centre of the web.

August 18. — The web was tenantless. Our little *conica* has probably fallen a prey to some bird or wasp.

As the habit of falling from the web is almost the only safeguard of these spiders in times of danger, the instinct must be of immense importance to them. Taking this into consideration, it seems remarkable that one of them should so soon have learned the sound of the vibrating fork, and should have modified her action accordingly.

In all essentials our results agree with those of Mr. Boys, who says: "If, when a spider has been enticed to the edge of the web, the fork is withdrawn, and then gradually brought near, the spider is aware of its presence and of its direction, and reaches out as far as possible in the direction of the fork; but if a sounding-fork is gradually brought near a spider that has not been disturbed, but which is waiting as usual in the middle of the web, then, instead of reaching out toward the fork, the spider instantly drops — at the end of a thread, of course." [1]

A few experiments were made to determine where the organ of hearing is located, but we can offer nothing positive on this question. It seems probable that the auditory apparatus is but little specialized. Possibly it is spread over a considerable portion of the epidermis.

Finding that *E. strix* and *E. labyrinthea* were very sensitive to the tuning-fork, we removed both palpi from an individual of each of these species. They seemed a good deal disturbed by the operation, and retreated to the tents near their webs. On the next day, when the fork was sounded near them, there was no definite indication that it was heard. On the second day they each responded once; and on the third, they seemed to have entirely recovered, and responded eight or ten times in succession. We afterward removed the palpi from several

[1] *Loc. cit.*, p. 149.

specimens of *strix*, *labyrinthea*, and *insularis*. All seemed able to hear perfectly well without these organs. We also found that the palpi play no essential part in the building of the web, since all these spiders constructed normal webs after their palpi were removed. This confirms, to some extent, the conclusions of Plateau,[1] though his further statement that "these appendages are to be placed in the category of useless organs" seems to be scarcely warranted.

We made an effort to determine how far the first and second pairs of legs subserve the sense of hearing, by removing them, and noting the results. We first removed, at the coxæ, the two anterior legs of a female of *E. insularis*. She soon built a good web, and when, two days later, the B fork was sounded near her, she promptly threw up her second pair of legs in the characteristic way.

Some days later we caught a large female of *A. riparia* that had lost her first pair of legs and also the left leg of the second pair. She was placed in the enclosed porch, and by the next day had built a good web, which lacked, however, the zigzag line down the centre, which is characteristic of the web of this species. (Two other specimens of *A. riparia* that had lost their palpi, also omitted the zigzag.) The remaining leg of the second pair was then removed, leaving the spider with only the posterior two pairs. She was now offered a fly, which she quickly seized and devoured. After her repast the B fork was sounded near her, when she attempted to lift the third pair of legs, but only partly succeeded. Several trials gave similar results. The fork was next held well behind her, when she slowly turned toward the sound.

So far we had experimented only upon orb-weavers. We now used the tuning-fork with half-a-dozen species of different groups, making ten or twelve trials with each spider. None of them gave the least indication of hearing anything. These unresponsive species were: *Herpyllus bilineatus* and *ecclesiasticus* Hentz, *Pardosa pallida* Emerton, *Pirata minutus* Emerton, *Lycosa nigroventris* Emerton, and *Dolomedes tenebrosus* Hentz. It struck us as remarkable that, while all the Epeirids responded promptly, being evidently alarmed by the sound of the tuning-fork, the spiders that make no web, on the contrary, gave not

[1] *American Naturalist*, April, 1887, p. 384.

the slightest heed to the sound. This may, perhaps, be partially explained by the difference in the feeding habits of the two groups.

MATERNAL EMOTIONS.

The only tender feeling that can be attributed to spiders is the affection for her offspring manifested by the female; except, perhaps, in the case of a few species where the male and female live together in the same web, in conjugal happiness.

Our observations on this subject necessarily included other mental states beside the emotions, and for the sake of convenience we shall consider, under this head, the various sensations, perceptions, and manifestations of memory met with in this set of experiments.

We endeavored to estimate the strength of the maternal feeling in our spiders by removing their cocoons and then noting with what degree of eagerness they sought to regain them; and also by determining for how long a time they would remember the cocoons if they were separated from them.

We selected for study the Lycosidæ, spiders that keep the egg-sack attached to the spinnerets, and carry the young about on their backs for a certain length of time after they leave the cocoon. We thought that the lengthening of the period of infancy, during which the female cares for her young, might — as in the case of monkeys and man — produce a greater development of the maternal instinct than in other species of spiders where the eggs receive little or no attention from the parent after she has deposited them.

On July 15, 1886, we found a female *Pirata piraticus* Clk. carrying her cocoon. While we were taking the egg-sack away from her she seized it with her falces several times and tried to escape. After we had finally accomplished its removal she seemed very much affected by its loss, and searched about in all directions to find it. In an hour and a half we returned it to her, when she immediately took it between her falces, and after a slight delay passed it back to the under side of her abdomen, where she fastened it. It was again removed and not returned to the spider for three hours. She did not seem so ready to receive it as in the first instance, but after a little hesitation took it up and carried it off.

On the following day we kept the cocoons away from three spiders of the same species for thirteen, fourteen and a half, and sixteen hours, respectively. All remembered them and took charge of them when they were returned. From the same spiders we again removed the cocoons, keeping them, this time, for twenty-four hours. The spiders again picked them up. There seem to be individual differences in the depth of feeling experienced by these spiders, since one female of this species utterly refused to take back her cocoon, after an interval of twenty-four hours.

We repeated the experiment with the three Lycosids mentioned above. Their cocoons were kept away from them for twenty-four hours, and then restored. Two of them refused to resume their maternal duties, seeming not to recognize their cocoons; the third, after hers had been placed in front of her seven times, slowly resumed charge of it, but with none of the eagerness before displayed.

In the following summer, on July 14, we took the cocoon from a female of *Lycosa* (sp.?) She recollected it and promptly took it up after having been separated from it for one day. We kept the eggs away from a second individual of this species for forty-three hours. When it was restored she had apparently forgotten all about it, since, although she touched it five times with her legs, and we four times placed it directly under her, not until the fifth time did its presence recall her to a sense of duty. She then very slowly and languidly took it up and attached it to the usual place. From another individual of the same species we kept the cocoon forty-eight hours; but the little spider could not remember so long, and, although we worked long and patiently to make her recollect, she would have nothing more to do with it.

Pardosa pallida Emerton was also separated from her cocoon for forty-eight hours. We tried for thirty minutes to make her take it back, but failed. She held it under her legs and palpi five times, several seconds at a time, seemingly feeling of it, and then left it.

Notwithstanding many efforts we never found a spider among the Lycosidæ that was constant in her affection for so long a period as forty-eight hours. A female of *Clubiona pallens* Hentz, however, remembered her eggs for this length of time,

and, when they were returned to her, spun a web over them in the corner of the box in which they were placed. Of all the spiders that we experimented upon, the little *Theridium globosum* Hentz had the best memory for her cocoon. We took her from her web and returned her to it after fifty-one hours. She at once went to the eggs and touched them with her legs. She then left them to improve her house, every now and then running back to see if they were safe. After she had arranged her household to her satisfaction she settled down near them.

Several species of Attidæ and Thomisidæ did not remember their cocoons for twenty-four hours; yet these spiders, although they do not carry the egg-sack about with them, remain near it for from fifteen to twenty days.

SENSE OF SIGHT.

We were much surprised in the earlier experiments to find how entirely the Lycosidæ depended upon touch in finding their cocoons. We had almost concluded that their sense of sight was but little developed. To quote from our notes: —

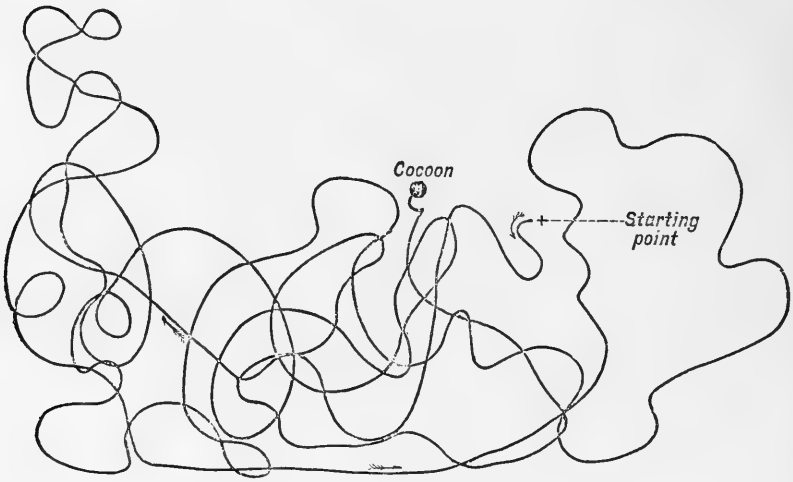
July 9. — Took the cocoon from *P. montanus* Emerton. She seemed much disturbed, and hunted around very eagerly; several times she passed close to it, and her eyesight must have been dull, or she would have seen it. At last she chanced to touch it with one leg, when she at once perceived it and laid hold of it with her falces. After a little we repeated the experiment, but now the cocoon was suspended just high enough to allow her to pass beneath, without touching it. She ran about seemingly very anxious to find it, but although she several times passed directly under the cocoon she did not discover it.

During the past summer we took away the eggs from a number of these spiders, and, placing them upon paper from one to two inches away from their cocoons and looking toward them, traced along the course they ran in looking for them. The tracing on the next page illustrates the persistency of the spider in hunting for her egg-sack, and how little she was aided by the sense of sight in recovering it.

The spider, *Pardosa pallida*, was some ten minutes in making the various turns before she reached the cocoon. Three times she came very close, and when, at last, she found it, she had

already turned away, but accidentally touching it with her leg, returned and seized it.

To further test how far they depended upon sight for finding the cocoon we removed the egg-sacks from two specimens of *P. montanus*, and, after coloring them scarlet and letting them get thoroughly dry, placed them on a board near the mothers. Both approached them several times, but, until they came into



ROUTE FOLLOWED IN FINDING COCOON,
BY
PARDOSA PALLIDA.

actual contact with them, did not take them up. The instant, however, that they were touched, they were seized by the mothers, and treated as affectionately as they had been before they were colored. We repeated this experiment five times, with the same results.

Professor Auguste Forel, in discussing the sight of arthropods, says that insects furnished with only simple eyes see but a short distance, more distinctly when an object is in motion, very imperfectly when it is at rest. Regarding spiders, he remarks that if you take up one of the ground-spiders (probably one of the Lycosidæ) carrying its eggs, and remove the cocoon to a distance of two or three inches, she will hunt about for it, and have the greatest difficulty in finding it. He also observes that jump-

ing-spiders (Attidæ) only perceive their prey when two or three inches distant.[1]

When our spiders came within a fifth of an inch of their cocoons, and yet gave no sign of seeing them, we were almost ready to agree with Professor Forel; but a little further consideration convinced us that we were judging them too hastily.

Let us go over the history of the egg-sack. In forming it, the spider first makes the upper part, and then, holding the openings of the oviduct against it, forces out the eggs. After the eggs are laid she uses the spinnerets, and possibly the posterior legs, to complete the cocoon. When it is finished she bends down the end of the abdomen, and attaches it there by lines of web. All this time she has probably not seen it once; and she never will see it, since it remains on the under side of the body until the young spiders come out, when they attach themselves to her back.

Now, bearing in mind that a perception is the interpretation of a sensation in terms of past experience, what is there in the experience of this spider to enable it to make a mental synthesis of the sensation of sight, with those other qualities of the cocoon which are learned by touch? The spider doubtless saw the cocoon, but could only recognize it as such through the medium of the sense of touch.

That this explanation is the correct one is made more probable by the fact that those spiders that not only touch their cocoons, but also see them, evidently depend on sight in recognizing them, if they are removed.

We took the cocoon from *Theridion blandum* Hentz, and placed the spider in a bottle three inches high. After she had settled herself in the upper end, we dropped the cocoon into the bottom of the bottle. She immediately descended, picked it up, and returned to her former position. We repeated this experiment several times.

Theridion frondeum Hentz was placed under an inverted tumbler, four inches high. While she was standing on the upper surface of the glass the edge was lifted and the cocoon pushed under. She at once lowered herself, seized it, and took it up to

[1] *Sensations des Insectes*, I. *Recueil Zoölogique Suisse*, Tome IV., No. 1, pp. 18, 19.

the top of the glass. This experiment was repeated at least ten times.

It is evident that these spiders recognized their cocoons by means of the sense of sight. It was not that the Lycosidæ could not see their eggs at a distance of a fifth of an inch, but rather that they could not perceive them unless they came into contact with them.

While experimenting on the color-sense of spiders, we have frequently, while feeding our captives, seen them stalk their prey at a distance of five inches; and we have repeatedly held the active jumping-spider, *Astia vittata*, on one finger, and allowed it to jump on to a finger of the other hand, gradually increasing the distance up to eight inches. As the distance increased the spider paused longer before springing, gathering its legs together to make a good ready.

We have twice seen a male of this species chasing a female upon a table covered with jars, books, and boxes. The female would leap rapidly from one object to another, or would dart over the edge of a book or a box so as to be out of sight. In this position she would remain quiet for a few moments, and then, creeping to the edge, would peer over to see if the male were still pursuing her. If he happened not to be hidden she would seem to see him, even when ten or twelve inches away, and would quickly draw back; but in case he was hidden behind some object she would hurry off, seeming to think she had a good chance to escape.

The male, in the meantime, frequently lost sight of the female. He would then mount to the top of the box or jar upon which he found himself, and, raising his head, would take a comprehensive view of the surrounding objects. Here he would remain until he caught sight of the female, — which he often did at a distance of at least ten inches, — when he would at once leap rapidly after her.

The ocelli of some spiders, then, enable them to see objects at a distance of at least ten inches.[1]

[1] We quote, in this connection, some observations of Hentz, in which he speaks of the sight of spiders as being acute: "This very common spider (*Marptusa familiaris* Hentz), almost domesticated in our houses, by its habits, deserves a longer notice than others. It dwells in cracks around sashes, doors, between clapboards, etc., and may be seen on the sunny side of the house, and in the hottest places, wandering in search of prey. It moves with agility and ease, but usually with a cer-

COLOR-SENSE.

Spiders are often so brilliantly colored that their being endowed with a well-developed color-sense seems *à priori* probable. Hoping to decide this question, which, as yet, had not been attempted, we began a series of experiments in the summer of 1886. The details of our method we omit, since the results were entirely unsatisfactory. In the following summer, however, we hit upon a plan of procedure which gave us the desired data.[1]

We had worked, during the first year, on species that are found in exposed places, or even in direct sunlight; but in our

tain leaping gait. The moment, however, it has discovered a fly, all its motions are altered; its cephalothorax, if the fly moves, turns to it, with the firm glance of an animal which can turn its head; it follows all the motions of its prey with the watchfulness of the falcon, hurrying its steps or slackening its pace, as the case may require. Gradually, as it draws near to the unsuspecting victim, its motions become more composed, until, when very near, its movements are entirely imperceptible to the closest observation, and, indeed, it would appear perfectly motionless, were it not for the fact that it gradually draws nearer to the insect. When sufficiently near, it very suddenly takes a leap, very seldom missing its aim. I saw one, however, make a mistake, for the object which it watched was only a portion of the wing of an hemipterous insect entangled in a loose web. It took its leap and grasped the wing, but relinquished it immediately, apparently very much ashamed of having made such a blunder. This proves that the sight of spiders, though acute, is not unerring." — *Spiders of the United States*, p. 56.

Also, Bingley says of the jumping-spider: "If it sees a fly at the distance of three or four yards, it does not run directly to it, but endeavors, as much as possible, to conceal itself till it can arrive near; and then creeping slowly up, and but seldom missing its aim, it springs upon the insect's back, and it is then almost impossible for the fly to effect an escape. But if, before the spider gets to it, the fly takes wing, and fixes upon another place, it whirls nimbly about, and still keeps its eyes upon it, in order to commence a fresh attack." — *Animal Biography*, Vol. III., p. 455.

While it is probably an exaggeration to speak of a spider seeing an object at a distance of three or four yards, it would scarcely have been possible for the writer to make such a statement if the spider he had been watching had been able to see only at a distance of three or four inches.

[1] Mr. Wallace, in *Tropical Nature*, p. 238, remarks that "the fact that the higher vertebrates, and even some insects, distinguish what are to us diversities of color by no means proves that their sensations of color bear any resemblance whatever to ours. An insect's capacity to distinguish red from blue may be (and probably is) due to perceptions of a totally distinct nature."

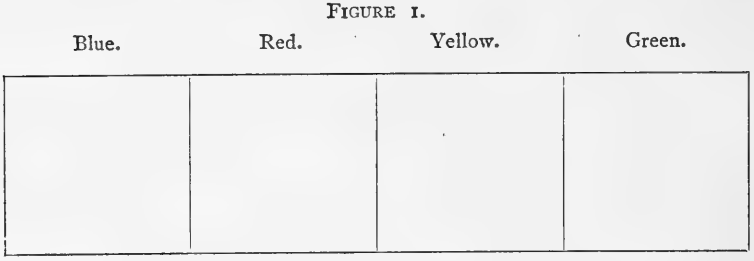
It is true enough that the mental states of men and insects must differ greatly, but if we are to comprehend the sensations of insects at all, we must do so by thinking of them in terms of the only conscious states that we know anything about, namely, our own. For this reason we shall assume that when a spider distinguishes red from blue, the best conception of its feelings will be attained by reference to our own sensations under similar circumstances.

second attempt we turned to the spiders that are found during the day, running among dead leaves or hiding under stones or wood.

In July last we constructed two cages in the following manner: On a base of wood we placed a row of four pieces of differently colored glass, each four inches square, held upright by slender nails on the inner and outer sides of each piece; and parallel to this, four inches away, a similar row. The ends and roof were formed by squares of glass which matched in color the parts of the sides which they touched. Thus we had a cage sixteen inches long by four inches wide, and four inches high, formed of four differently colored compartments all opening freely into each other. The cages were placed on a table in a covered porch with the wall of the house to the east, while the south, west, and north sides were exposed to the light.

On July 18 we placed a spider (*Lycosa nigroventris* Emerton) in a cage with the order of colors as in the following diagram: —

FIGURE 1.



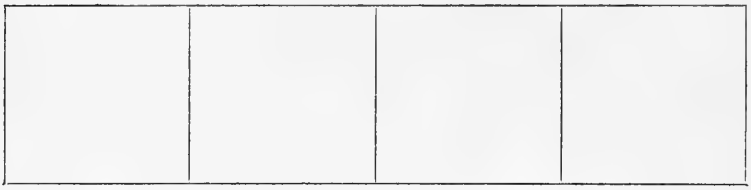
After walking about for a time, remaining in no spot more than a few moments, it at last settled down in the green compartment at one end of the cage, where it remained without change of position for several hours. Being satisfied that it had given up all efforts to escape, we now began our experiments by lifting off the green roof-plate, and gently driving the spider into the blue compartment at the other end. (Hereafter, to avoid circumlocution, we shall designate the compartments by their colors, — red, blue, green, and yellow.)

After an interval of thirty minutes it was found in the red. It was driven into the yellow, but after fifty minutes was again found in the red. It was then driven into the green, where it remained for half an hour, when it was driven into the blue. It

again moved to the red. We proceeded in this way, driving it into the various compartments, and crediting it, each time, with the color it had settled in, until its account stood as follows: Red 16, yellow 5, blue 2, green 2.

The order of the colors was then changed as follows: —

FIGURE 2.



After every three or four experiments the cage was brushed out to remove any web-lines that the spider might have formed.[1]

We now allowed the spider more time in which to choose its color, and had better results. The record for July 21 and 22 stood as follows: Red 21, yellow 4, blue 1, green 2.

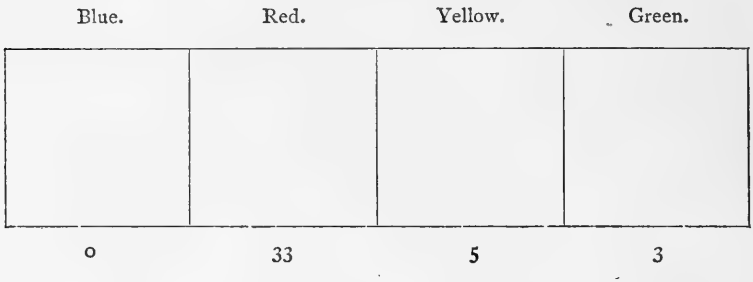
On July 18 we had placed another species of Lycosa (unfortunately this spider and a second specimen of the same species escaped before we identified them) in the second cage. After it had become accustomed to its new conditions we kept a record of its performances as before, with the following result: Red 16, yellow 2, blue 2, green 0. This spider escaped on July 21. The second specimen did not seem to have as marked color sensibilities as the first, but the record of this one, also, shows a preference for the red: Red 16, yellow 6, blue 3, green 2.

On July 31 a second specimen of *L. nigroventris* ♂ was placed in the cage, and, though very restless, soon settled down in the red. The cage was carried into the direct sunlight, and the spider driven into the blue, at the end; but after a moment it turned around and went back to the red. After ten minutes it was driven to the opposite end into the green compartment. Again it came back to the red. The record for the day was: Red 10, yellow 0, blue 0, green 1. The cage was overturned that evening, and the spider escaped.

[1] All the species used in the color experiments make no web, but stalk their prey.

On August 2 a third specimen of *L. nigroventris* was placed in the cage. Three days' experimenting showed that this spider had stronger preferences than any of the others. The cage was arranged as follows: —

FIGURE 3.



The record for each color is given just below the line.

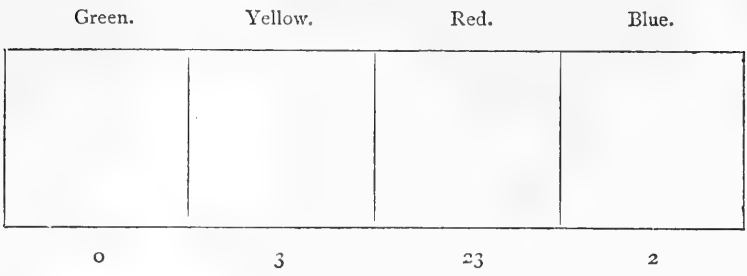
It seemed quite apparent that this animal preferred red, since it returned to this color thirty-three times out of forty-one. Still the experiment was open to the objection that it was temperature rather than color that determined the spider's movements. To test this we carefully covered the eyes of this specimen with paraffine. After having satisfied ourselves that it could not see, we put it back into the same cage. The color now produced no effect. It remained quiet in whatever compartment it was placed until it was driven out. It was once placed in the blue, with its eyes as close as possible to the red square, but it showed no inclination to enter, although this color had before proved so attractive. When taken out the spider was still blind. The record of this experiment was as follows: Red 6, yellow 6, blue 6, green 5.

A fourth specimen of *L. nigroventris* ♀ gave similar results: Red 11, yellow 2, blue 0, green 0.

The record of a fifth specimen of the same species (a male) was: Red 12, yellow 3, blue 0, green 1.

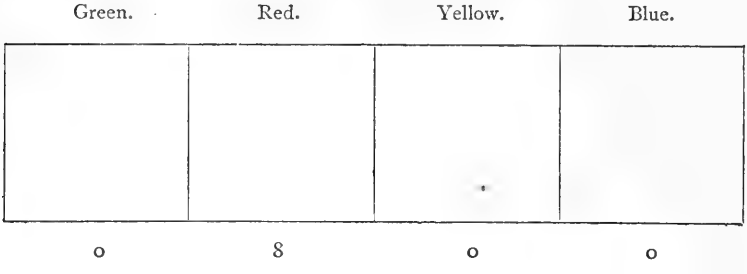
Our last experiment was with a large Lycosa (*L. nidicola* ♀, Emerton) which we found under a stone on August 18. The arrangement of the colors and the preferences were as follows: —

FIGURE 4.



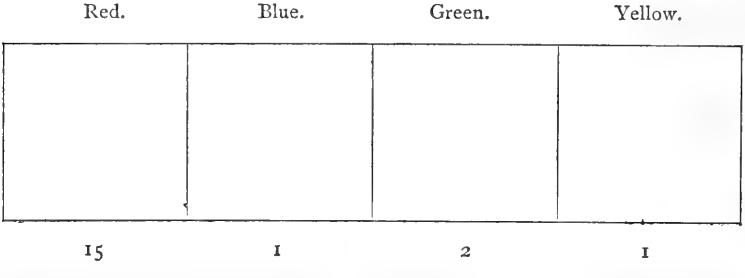
We then changed the colors, transposing the red and yellow, with this result: —

FIGURE 5.



All the colors were then changed, and the following record obtained: —

FIGURE 6.



The following table gives the facts for all the experiments with all the spiders: —

Red 181.
Yellow 32.
Blue 11.
Green 13.

The preference of our spiders for red seemed to be much more marked than that of Sir John Lubbock's ants; and the spiders had not so positive a dislike for blue. In Lubbock's experiments, on one occasion there congregated under a piece of red glass 890 ants, under yellow 495, under green 544, and under blue 5.

These experiments seem to be conclusive as to the existence of a color-sense in certain spiders.

FEIGNING DEATH.

Wishing to discover what spiders would do in the way of feigning death, we subjected them to various alarming conditions, in the hope of getting an exhibition of this interesting habit. We began with a female of *Astia vittata* that had been caught in a sweep-net. She was prevented from escaping by lightly striking the cloth of the net underneath the spot upon which she was standing. In this way, as often as she attempted to run or jump out of the net, she was knocked over and baffled. At first she immediately jumped up and made another attempt to escape; but at the eleventh repetition of the blow she fell upon her back and remained motionless, with legs outstretched, for half a minute. The next time she was knocked over she fell in the same position, and did not move for ten minutes. After that she kept quiet for only half a minute at a time. Several other species of this family behaved in the same way when subjected to like annoyances, but we never again saw an Attus lie quiet for so long a time as ten minutes.

A very active female of *H. ecclesiasticus* was placed upon a table and lightly knocked and brushed about. She jumped up as soon as she fell, excepting in one instance, when she lay on her back with legs outstretched for half a minute.

A female of *Micromata carolinensis*, a species which runs with great rapidity, was treated in the same way. She appeared much alarmed, but when she fell always struggled to her feet without an instant's pause, and again endeavored to escape.

A great many more tests were made with running and jumping spiders; but in no case did they show that the spider would feign death, nor, indeed, that it had even acquired a habit of keeping still when alarmed. They were only reduced to quiet

after much buffeting about, when they probably lay still from sheer confusion.

Thinking that less active spiders would be more likely to develop a habit of keeping quiet as a means of escaping danger, we next tried some experiments with the Epeiridæ. A pretty little female of *E. bombycinaria* was softly touched as she hung in the web; she dropped two feet and then swung to a neighboring branch, where she crouched motionless for three minutes. Being again gently touched, she fell to the ground, with her legs outstretched, and then quickly drawing them in, remained clinging in a very inconspicuous heap to a blade of grass. Here she stayed motionless for one hour, when she was placed in a bottle, carried into the house, and, still keeping perfectly quiet, was shaken out upon a table. After two minutes she was pushed about with the end of a glass rod, and then her legs were lifted one by one with a needle. She seemed so lifeless that we began to wonder if we had been watching a dead spider, after all. We finally touched her with the point of a needle; but at the first suggestion of a prick she ran rapidly away. She was knocked over as she ran, and remained motionless just as she fell, resting on the cephalothorax, with all the legs drawn closely in, excepting one, which was slightly extended. She did not look like a live spider, nor yet like a dead one, nor like anything else, excepting, perhaps, a bit of bark or a small lump of dirt. She lay thus, without a perceptible quiver, for more than two hours and a half, and then suddenly ran away. She was reduced to quiet several times after this, but was less patient, and endured no more handling. She did not usually lie still just as she fell, but deliberately gathered up her legs in such a way that they were undistinguishable from each other and from her body.

Shortly after this, while walking in the woods at dusk, we caught a large female of *E. infumata*. She was put into a tumbler, and left until the following morning, when one of us, upon going to look at her, exclaimed that she was dead. Her legs were drawn up and bent, and she looked stiff and dry. She was handed from one to another of those present. Her demise was duly regretted, and her wonderful protective coloring was remarked upon. She was then put back into the tumbler. An hour later, much to our astonishment, we found

her moving about alive and well. As we were, at this time, experimenting upon the color-sense, she was placed in one of the boxes of colored glass described above, and at intervals of one hour, during the day, was moved from the section in which she had settled to another. Every time that this removal was made she fell stiffly on to her side, drawing her legs in, and remained thus for about three minutes. In experimenting with her afterward we found that when knocked about on a table she would stay in the position in which she fell, although this was often a very uncomfortable one. She showed no sign of life when rolled about, but jumped up at the least prick of a needle. She never remained quiet for more than twenty-seven minutes, and never absolutely motionless for so long a time as this, there being slight quivering movements of the legs and palpi at intervals of three or four minutes.

We had thus far found no spider that would endure bad treatment without showing signs of life; but in our next experiment we were more successful. A female of *E. insularis*, when rolled about on the table, acted a good deal as *infumata* had done, but had no such rigid, lifeless appearance. When she was knocked or touched with the point of a needle there was a convulsive twitch of the legs, though she seemed to be trying to keep quiet. The first time that she was pricked so as to puncture the skin she remained motionless, but at the second puncture she ran. On experimenting afterward with both males and females of this species we met with similar results, once finding an individual that did not run until the skin had been punctured five times. When the needle entered the skin there was usually a twitch of the legs, which seemed to show that sensation was present. Outside of this species we found no spider that would endure a puncture of the skin without running away, and we rarely found one that would keep quiet while being handled.

We now made a series of experiments with Epeiridæ under more natural conditions, alarming them as they hung in their webs, and noting whether they feel, how long they kept quiet, and whether they were absolutely or only comparatively still. Some selections from our notes will best show what the experiments were, and how they resulted.

July 25. — Experiments were performed with four females of *E. strix* as they hung in their webs.

Number one shook her web with sharp jerks when a branch to which it was attached was moved; and did the same when she was lightly struck, eight times in succession, with a glass rod.

Number two, when touched, dropped to the ground and lay on her back, with her legs drawn in, for ten minutes. She then moved, but upon being touched again became quiet. Tried to pick her up, when she ran a few steps and then drew in her legs and kept still. Repeated this several times.

Number three, when touched, ran to the circumference of her web and hid under a branch. On being touched again she dropped to the ground and remained quiet for ten minutes, then began to run back and forth on the grass, and after fifteen minutes settled under a branch which touched the ground. Had she lost her way?

Number four, when touched, did not run nor drop, but huddled her legs together and hung, apparently lifeless, for three minutes, then ran to a neighboring branch.

July 26. — Finding a female of *strix* in her web we held a vibrating tuning-fork near her, when she dropped to the ground. The tuning-fork was then applied to her web, when she ran quickly up, probably under the impression that a fly was struggling there.[1] Repeated this with six individuals of *E. strix* and *E. labyrinthea* with the same result. Then tried holding the vibrating tuning-fork just over the spiders as they lay motionless after dropping from the web. They did not move.

When a male of *Tetragnatha grallator* was touched, as he stood in the web, he ran to a branch and there stretched himself out. In this position he was almost indistinguishable, as his color was exactly like that of the branch to which he clung. When the branch was gently shaken, instead of keeping quiet, he ran a little way and then stretched out again; and this he repeated, stupidly betraying his presence as often as the branch was touched.

[1] Mr. Boys, in the experiment before referred to, found that after a spider has been made to drop, by bringing a tuning-fork near it, "if the fork is made to touch any part of the web, the spider is aware of the fact, and climbs the thread and reaches the fork with marvellous rapidity."

July 27. — Four experiments were made with females of *E. bombycinaria* as they hung in their webs.

Number one, when touched, shook her web violently; when touched again she dropped to the ground and remained still ten minutes. She then ran among the grass and was lost sight of. At the end of thirty-five minutes she had not returned to her web. Had she lost her way?

Number two, when lightly touched, dropped two feet, resting motionless on a twig. After twenty-five minutes she ran straight up to the web.

Number three, when touched, ran to a twig and crouched there with the first and second legs of one side extended on the web. After forty minutes she ran back to the centre.

Number four, when touched, ran to a twig and crouched there, but kept one leg of the fourth pair extended on the web. After fifteen minutes she was touched with the end of a pencil, when she started on a rapid run. As often as she paused she was again touched. She ran and swung from place to place, stopping every moment to crouch on twigs; when swinging she gathered up her legs. Her idea seemed to be to keep quiet and inconspicuous. There was no suggestion that she thought of feigning death.

July 29. — A female of *C. conica*, at the approach of a vibrating tuning-fork, dropped nearly to the ground and waited some minutes before returning to her web; but after this had been repeated several times the fork ceased to be alarming, and she paid no attention to it.

A male of *T. laboriosa*, when caught in the hand and then dropped, fell on his side and remained quiet half a minute. He was dropped again, and while he lay motionless first one leg was pinched and then a second and a third. He remained quiet. His abdomen was then pinched, when he ran away.

August 1. — A female of *Cyllopodia cavata* stood on a line leading to her little triangular web. When touched she ran to the nearest branch, and, dropping an eighth of an inch, gathered her legs together and remained hanging, without perceptible motion, for four hours. As she hung there, swinging in the wind, she looked much more like a bit of dry leaf than like a spider.

A male of the same species was caught in a sweep-net. On

being shaken he suspended himself from a fold of the cloth, remaining quiet at first two minutes, and after a second shake, three hours and a quarter. When touched he ran away. Here we have an instance of one of our best feigners keeping quiet for hours while holding on to a line by a muscular effort.

In two of these experiments it seemed probable that the spider was unable to retrace its way to the web. This suggested the idea that the habit of keeping still after dropping must not only help a spider to avoid detection, but must also make it more certain of finding its way home after the danger is over. There would thus be a double advantage in absolute quiet.

It must be remembered that as a spider drops, it spins a line of web which forms a straight path backward to the starting-point; but as soon as the spider moves, the line adheres first to one twig or blade of grass and then to another, and its way home is thus rendered indirect.

Bearing this point in mind in our subsequent observations we were soon convinced that if a spider kept quiet after dropping, it could easily return to the web by means of its line; whereas, if it moved only a very little, it became confused and either lost its web entirely or only regained it after a lengthy search.

For example, we found, one evening, a female *labyrinthea* spinning her web in a cedar-tree, and made her drop by bringing a vibrating fork near her. She paused on a branch two feet below the web, and remained quiet for four minutes; she then changed her position, moving about half an inch. After this she was perfectly still for twenty minutes. At the end of that time she began to climb up and down over the branches, with her first legs extended, apparently hunting for the line leading to her web. She occasionally swung off from a branch for a little way, and then returned to it. After forty-five minutes she seemed to become discouraged, and crouched down on a twig, where she remained for over an hour, when she was replaced in the web, and immediately went to work to complete it.

We made another female of this species drop from her partly completed web. She stopped on a branch, and, after keeping perfectly quiet for one minute, changed her position (probably to one of greater comfort), moving about a quarter of an inch. After keeping still for five minutes more, she started to go back to her web; but it soon became evident that she had lost her

line. She began to search for it, stretching out her first legs, and running about over the branches. After hunting for twenty-five minutes she touched a strand leading to the web, and ran to it, taking up her work just where it had been interrupted.

Both of these spiders were spinning their webs at the usual time, — toward nightfall, — and, had they not regained them, would probably have gone without their suppers, and perhaps their breakfasts and dinners the next day. Any interruption in the food-supply must be in a high degree detrimental, and we therefore incline to the opinion that we have here an important factor in the development, at least among orb-weavers, of the habit of lying motionless after dropping out of the web.

It seems probable that the habit of keeping quiet in time of danger is better developed in adult than in young spiders. In the few experiments that we have made on this point the young spiders neither remained motionless so long, nor endured so much handling while keeping still, as the old ones. Thus, the adult *E. bombycinaria* will frequently lie motionless for hours; but in working with three young spiders of this species, we never saw them keep still for more than half a minute at a time.

Our experiments on this subject numbered two hundred and ten. They were made upon spiders from nineteen different genera.

The consideration of the meaning of the so-called habit of feigning death may be appropriately prefaced by the following quotations from Darwin and Romanes: —

"Animals feigning, as it is said, Death — an unknown state to each living creature — seemed to us a remarkable instinct. I agree with those authors who think that there has been much exaggeration on this subject: I do not doubt that fainting (I have had a robin faint in my hands) and the paralyzing effects of excessive fear have sometimes been mistaken for the simulation of death. Insects are most notorious in this respect. We have amongst them a most perfect series, even within the same genus (as I have observed in Curculio and Chrysomela), from species which feign only for a second and sometimes imperfectly, still moving their antennæ (as with some Histers), and which will not feign a second time however much irritated, to other species which, according to De Geer, may be cruelly

roasted at a slow fire without the slightest movement — to others, again, which will remain motionless as much as twenty-three minutes, as I find with *Chrysomela spartii*. Some individuals of the same species of Ptinus assumed a different position from that of others. Now it will not be disputed that the manner and duration of the feint is useful to each species, according to the kind of danger which it has to escape; therefore there is no more real difficulty in its acquirement, through natural selection, of this hereditary attitude than of any other. Nevertheless, it struck me as a strange coincidence that the insects should thus have come to exactly simulate the state which they took when dead. Hence I carefully noted the simulated positions of seventeen different kinds of insects (including an Iulus, Spider, and Oniscus), belonging to the most distinct genera, both poor and first-rate shammers; afterwards I procured naturally dead specimens of some of these insects, others I killed with camphor by an easy slow death; the result was that in no one instance was the attitude exactly the same, and in several instances the attitude of the feigners and of the really dead were as unlike as they possibly could be."[1]

Romanes, after some discussion of the habit of feigning death in higher animals, goes on to say that Professor Preyer "ascribes the shamming dead of insects to the exclusive influence of kataplexy. . . . Now, I think it is not at all improbable that 'kataplexy' may have been of much assistance in originating, and possibly also in developing, this instinct. . . . But I desire it to be particularly noted that I only adduce this speculation, as it were, parenthetically. I think with Preyer that the shamming dead of insects is a phenomenon in which the principles of hypnotism are probably concerned. But if so, I regard these principles only as furnishing the materials out of which natural selection has constructed this particular instinct."[2]

There seem to be no reasonable grounds for thinking that spiders have any idea of simulating death, since only about once in fifty times is their attitude when motionless from alarm like that which they take when dead. The point at issue, then, is

[1] Darwin's *Essay on Instinct;* Appendix to *Mental Evolution of Animals*, by G. J. Romanes, p. 363.

[2] *Mental Evolution in Animals*, pp. 308–309.

whether alarm may cause them to fall into a kataplectic state in which they will endure bad treatment without showing any sign of pain.

Duncan, "On Instinct," says that spiders while feigning death "will suffer themselves to be pierced with pins and torn to pieces without discovering the smallest signs of terror," and Darwin refers to De Geer as saying that some insects may be cruelly roasted at a slow fire without the slightest movement. Out of the species with which we experimented we found one which would endure a moderate amount of pricking with a needle, and a second which did not move when its legs were pinched. Beyond this there was no stoicism under anything that approached bad treatment, although a few species allowed themselves to be handled without showing signs of life. We do not believe that any spider which came under our observation ever fell into a kataplectic condition. Our reasons for this disbelief may be formulated as follows: —

As a usual thing the spiders did not become motionless as soon as they were alarmed, but only after a preliminary arrangement of their legs, which tended to make them inconspicuous.

During the time that they were quiet they frequently were not absolutely motionless, there being not only slight quiverings of the terminal joints of the legs, but also slight changes of position.

When a vibrating tuning-fork was brought into contact with the web of a spider which, upon being alarmed, had dropped to the ground, and was lying motionless, it quickly ran up the line, apparently not being able to resist the inclination to secure its supposed victim.

While lying motionless in time of danger they were not insensitive to pain, and would seldom endure even a gentle touch without running.

The gist of the matter is, that certain Epeiridæ, when alarmed, drop from the web and remain quiet for a longer or shorter time, their concealment being greatly assisted by the protective coloring which is present to some extent in nearly all of them. This amounts to nothing more than that when another spider runs to a place of safety, an Epeirid drops a greater or less distance (in the case of *C. cavata* only an eighth of an

inch) to a place of safety; both then remain quiet, unless disturbed, in which case the first spider trusts to its powers of running, while the Epeirid often (but not invariably) finds its best chance of safety in keeping quiet unless it is actually abused; the habit of keeping quiet also insuring the spider's safe return to its web when the danger is over. There is no need to call in "kataplexy" to explain the origin or development of a habit which can be so easily accounted for by natural selection alone.

We hold, then, that without question Darwin's explanation of the habit of lying motionless is the true one. It is the result of natural selection, and has been acquired by different species in different degrees, according to its usefulness in their various modes of life. Thus we find it in its greatest development among the comparatively sluggish Epeiridæ, whereas it is badly developed or lacking in the running and jumping spiders, which are able, as any one who has pursued them will testify, to move with astonishing rapidity.

MISTAKES OF SPIDERS.

We found spiders much less clever than we had supposed them to be in regard to the recognition of their cocoons. We several times endeavored to deceive them by offering a bit of cotton rolled into a ball instead of their eggs, but without success, so that our spiders proved a degree more intelligent than the one deceived in this way by Dugès;[1] but, although they were too discriminating to take the cotton, a little pith-ball led them entirely astray.

When we took the cocoon from a specimen of *P. pallida*, and offered her in its place a pith-ball, she at first refused it, although it was several times so placed that it touched her. On comparing the pith-ball with the cocoon, however, we found that it was three times as large. When we reduced its size, and again offered it to the spider, she took it between her falces, and in a few minutes attached it to her abdomen. As far as we could see, the bit of pith gave her as much satisfaction as her eggs.

We found that when the cocoons were nearly of a size one

[1] Romanes' *Mental Evolution in Animals*, p. 382.

mother would take the cocoon of another — although of a different genus — just as quickly as she would her own. On one occasion we gave the cocoon of *Pardosa pallida* to *Lycosa* (sp.?), and in a few minutes removed it from this spider to give it to *Pirata piraticus*, and the foster-mothers seemed fully as devoted to the eggs as the real mother.

To test still further their general intelligence, we took the outer coat from a cocoon of *pallida* and slipped it over a lead shot of the same size, but at least three or four times as heavy. There was so little of the cover left after the operation that we could scarcely perceive any difference between it and an uncovered shot. We offered it to the spider, and, much to our surprise, she at once seized it, and, after a good deal of trouble, succeeded in fastening it to the under side of the abdomen. Our impression that we had entirely demolished the cover in getting it over the shot must have been an error, since otherwise she could scarcely have attached it by means of the lines of web. The load was so heavy that the spider could only with great difficulty, and moving very slowly, walk up the side of a board. While transferring her to another box the shot, from its weight, fell from her abdomen, and she spent over thirty minutes, working with all her might, in fastening it on again. She had taken only five or six steps when it again fell off, and she then carried it about between the falces and the third pair of legs. We next endeavored to induce a second specimen of this species to take a plain shot, but all our efforts failed. We then took away the web-covered shot from the first specimen, and offered her in its stead the plain shot, but this she stubbornly refused, so that after a little we returned to her the web-covered shot, which she took back with every evidence of tender emotion.

Having satisfied ourselves that a Lycosid had not sufficient intelligence to distinguish between a pith-ball or even a heavy shot and its own cocoon, we made some experiments to determine whether it had intelligence enough to choose its cocoon if we offered the cocoon and the pith-ball together. To test this we placed the two side by side. The spider, approaching from one side, first touched the pith-ball and at once seized it with her falces; but as she moved away one of her anterior legs came into contact with the cocoon. In this position she remained quiet for a minute or two, and then dropping the pith-ball she took up her

cocoon and moved away with it. On the next day, when we placed the two in front of her, she again happened to meet the pith-ball first, and, as before, took it up at once. This time she ran off with it, and it was some time before we could manage to place the cocoon just in front of her; but as soon as we succeeded, and her legs touched it, she stood still, and within a few minutes dropped the pith-ball and took up her eggs.

It is evident, from these observations, that this spider, when allowed a choice, will select the cocoon rather than the pith-ball; but in the absence of the cocoon will content herself either with a pith-ball or a web-covered shot. The fact that a spider will carry about so comparatively heavy an object as a lead shot instead of its cocoon certainly argues a poorly developed muscular sense.

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